Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Beverly Victoria Robinson Date

The role of *Drosophila* CPEB protein, Orb2, during asymmetric neural stem cell division

By

Beverly Victoria Robinson

Doctor of Philosophy

Graduate Division of Biological and Biomedical Science

Genetics and Molecular Biology

_____

Dorothy A. Lerit, PhD

Advisor

_____

Gary J. Bassell, PhD

Committee Member

_____

Victor Faundez, MD, PhD

Committee Member

_____

Peng Jin, PhD

Committee Member

_____

William G. Kelly, PhD

Committee Member

Accepted:

_____

Kimberly Jacob Arriola, PhD

Dean of the James T. Laney School of Graduate Studies

_____

Date

The role of *Drosophila* CPEB protein, Orb2, during asymmetric neural stem cell division

By

Beverly Victoria Robinson
B.S., Florida Institute of Technology, 2015

Advisor: Dorothy A. Lerit, PhD

An abstract of a dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in the Graduate Division of Biological and Biomedical Science,
Genetics and Molecular Biology
2022

Abstract

Centrosomes serve as the primary microtubule-organizing centers of most cells, and centrosome dysregulation affects a range of cellular processes including cell division, cell-cell communication, cell motility, and intracellular transport of molecular cargo. Centrosomes attain diversity of function by altering their microtubule-nucleating activities through the cyclic shedding, recruitment, and rearrangement of a protein-rich lattice, called the pericentriolar material. Nevertheless, the mechanisms that centrosomes employ to modulate rapid and dynamic changes to pericentriolar material are still poorly understood. Identifying such mechanisms of centrosome regulation would not only expand our understanding of this versatile organelle, but also inform new therapeutic options for diverse, devastating, centrosome-related diseases. Developmental diseases such as microcephaly, congenital heart disease, and polycystic kidney disease are all associated with centrosome genes and manifest symptoms in the brain, heart, lungs, skin, eyes, kidneys, etc. Similarly, cancers associated with aberrant centrosome function have a poorer prognosis than those that do not. Thus, identifying mechanisms regulating centrosome activity may help inform the pathogenesis of human diseases. To understand disease pathogenesis associated with aberrant centrosome function, a basic understanding of the molecular mechanisms instructing pericentriolar material recruitment is required.

Toward this end, this thesis investigates a previously undefined mechanism of centrosome regulation: post-transcriptional regulation. Using neural stem cells from the *Drosophila* larval brain, we identified the conserved RNA-binding protein Orb2 as an important regulator of centrosome activity. *Drosophila* larval neural stem cells are a powerful model for studying centrosome biology, as the two centrosomes are differentially regulated both spatially and temporally. Differential recruitment of the pericentriolar material to the two centrosomes within a single interphase neural stem cell results in drastically different levels of microtubule-nucleating activity. This functional asymmetry ensures the segregation of differential fate determinants through proper alignment of the spindle pole along an invariant apical/basal axis, ultimately leading to asymmetric stem cell division. In support of Orb2 directing centrosome activity, *orb2* loss generates a population of interphase neural stem cells with symmetrized, active centrosomes. Neural stem cells lacking *orb2* are susceptible to mitotic spindle defects, despite maintaining cellular polarity. We show Orb2 is required cell autonomously to support centrosome asymmetry and maintain neural stem cell homeostasis. In addition, *orb2* null brains are microcephalic, and Orb2 functions outside of neural stem cells to regulate brain size. We propose Orb2 plays opposing roles in centrosome regulation, possibly through the translational regulation of multiple mRNA substrates. These data support a novel paradigm of centrosome control in which activity is modulated by RNA-binding proteins. Using the insights gleaned from this work, we can build a more comprehensive understanding of the cellular mechanisms that regulate centrosome activity, which may inform the pathogenesis of human disorders arising from aberrant centrosome activity.

The role of *Drosophila* CPEB protein, Orb2, during asymmetric neural stem cell division

By

Beverly Victoria Robinson
B.S., Florida Institute of Technology, 2015

Advisor: Dorothy A. Lerit, PhD

A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in the Graduate Division of Biological and Biomedical Science,
Genetics and Molecular Biology
2022

Acknowledgements

I would first like to thank my advisor, Dr. Dorothy A. Lerit for adopting me into her lab as her first graduate student mentee. As an ambitious new mentor and a role model for all women in STEM - Dorothy pushed for scientific excellence. I am grateful for the opportunities and lessons I learned while in the Lerit Lab. I would also like to thank Dr. Victor Faundez who has also been an exceptional and influential mentor to me and has always supported, encouraged, and believed in me. I am so incredibly fortunate to have found a place within the Cell Biology Department – thank you so much for having me these last five years. I would like to sincerely thank my other committee members: Drs Gary Bassell, Peng Jin, and Bill Kelly; my former RayBiotech mentors: Drs Bill Edens, Valerie Jones, Jarad Wilson and Will Naclerio; and my amazing undergrad advisor Dr. Dick Turner. They have been an incredible support network for me. Each one of them has been my cheerleader and has supported me from the beginning through to here and I'm sure beyond. These individuals are truly wonderful and inspirational people who I will always look up to greatly. Together they have molded me into the scientist I am today, and their teachings will continue to influence the scientist I become. Thank you.

I would like to thank my lab mates; especially Drs. Pearl Ryder, Junnan Fang, and Hala Zein-Sabatto, who all let me ask those naive questions you do as a grad student without any judgement; my work spouses (future Drs.) Elias Castro, Lauren Lym, and Jovan Brockett, who were always willing to get coffee and chat; and my mentees Dipen Mehta and Joey Buehler who were always quick to remind me that I can do science and I can do it well. Together they would attend my presentations ensuring I always had a supportive face in the crowd. They listened to me and let me vent when experiments (or life) weren't going how I wanted them to go. They cheered me up when grad school got hard and made sure to know I was never alone. They were always amazing, and they will remain my friends for a lifetime without question.

Outside of lab I am lucky to have made life-long friends with Janise and Chris Kuehner. They were always right there to celebrate my success, encouraged me to rest when I was burnt out and reminded me to get up again after falls. I know they both will be a part of my family forever and I thank them for their unwavering support. And of course, I must thank my other GDBBS colleagues, Athena Russell, Dylan Holder, Juan Rodriguez, and Kun Lin (who I miss dearly every day).

Most importantly, I thank my family. Their love and support have been never-ending. I could call any one of them on my way home from a lonely day in the lab and my day would be instantly brighter. My parents, Vicki, and Gene Petilli celebrated every win with me and commiserated with me on every loss. My siblings Connor, Nicole and Michelle Petilli kept our family group chat poppin' and assured me they would still be around to do proper, annoying sibling things if I was unable to find success in grad school. My sweet (fur) baby Niko, who listened to every practice seminar and occasionally added his own contributions to written work. But most importantly, I must thank my incredible husband, Hunter Robinson. Without you, I could not have accomplished this. You kept me level-headed when I wasn't thinking rationally, you encouraged me to stand up for myself when I was being disregarded, you pushed me to keep going when you knew I still had gas left in the tank and carried me when I was out. Most important, you reminded me every day that I am worthy of the love you give me unconditionally. I love you.

Table of Contents

List of Figures and Tables

List of Abbreviations

aa	amino acid
ACD	asymmetric cell division
AI	asymmetry index
ana2	*anastral spindle 2*
apc/c	Anaphase-promoting complex/cyclosome
αpkc	Atypical protein kinase C
ARC	Activity-regulated cytoskeleton associated protein
Asl	Asterless
asp	*abnormal spindle*
Baz	Bazooka/Par-3
BC	biologic complexity
BDNF	Brain-derived neurotrophic factor
BDSC	Bloomington *Drosophila* Stock Center
Brat	Brain tumor
βTub	β-Tubulin
BTZ	Barentsz
C-Nap1	centrosomal Nek2-associated protein 1
C-term	carboxyl (COOH) terminus
CaMKII	Calcium/calmodulin-dependent kinase II
CC3	cleaved caspase 3
CDC42	Cell division control protein 42 homolog
Cdk	cyclin dependent kinase
cen	*centrocortin*
Cep135	Centrosomal protein 135kDa

CMOS	complementary metal oxide semiconductor
Cnb	Centrobin
Cnn	Centrosomin
CNS	central nervous system
CPE	cytoplasmic polyadenlation element
CPEB	cytoplasmic polyadenlation element binding protein
CPSF3	Cleavage And Polyadenylation Specific Factor 3
DDX6	DEAD-box helicase 6
DIPOT	DRSC integrative ortholog predication tool (https://www.flyrnai.org/diopt)
Dlg1	Discs large
DPN	deadpan
DRSC	*Drosophila* RNAi Screening Center
Eg5	Kinesin-Related Motor Protein Eg5
eIF4	Eukaryotic translation initiation factor 4
EJC	exon-junction complex
ERK	extracellular signal-regulated kinase
FDR	false discovery rate
FLP-FRT	Flipase - FLP recombinase recognition target
FMRP	Fragile X mental retardation protein
fzr	*frizzled*
γ-TuRC	γ-Tubulin ring complex
GDC	Genomic Data Commons (https://gdc.cancer.gov/)
GMC	ganglion mother cell
GO	gene ontology (http://geneontology.org/)
γTub	γ-Tubulin
HPO	human phenotype ontology (https://hpo.jax.org/)
insc	*inscrutable*
khc-73	kinesin heavy chain 73
KS test	Kolmogorov-Smirnov test
l(2)gl	Lethal giant larvae
mad2	*mitotic arrest deficient 2*
Magoh	Mago Homolog
MAGUK	membrane-associated guanylate kinase
Mira	Miranda
MOPDII	microcephalic osteodysplastic primordial dwarfism type II
MT	microtubule
MTOC	microtubule-organizing center
Mud	Mushroom body defective/NuMA
N-term	amino (NH_2) terminus
NCI	National Cancer Institute
Nek2	NIMA-related kinase 2
NIMA	Nimrod A
Nin	*Ninein*
NSC	neural stem cell
nt	nucleotide
OMIN	online Mendelian inheritance in man (https://www.ncbi.nlm.nih.gov/omim)
ORPHA	rare disease code from orpha.net (https://www.orpha.net/consor/cgi-bin/index.php)
PABP	Poly(a) binding protein
PAR-CLIP	photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation

Par-Complex	Complex containing Baz/Par-3, Par-6, αPKC
PARN	poly(A)-specific ribonuclease
PBS	phosphate buffered saline
PCM	pericentriolar material
PCNT	Pericentrin
Pins	partner of inscrutable
Plk	polo-like kinase
PLP	Pericentrin-like protein
Polo	Polo kinase
Pon	partner of numb
PP2A	protein phosphatase 2A
Pros	Prospero
θ	angle
Rbm8a	RNA binding motif protein 8A
RBP	RNA-binding protein
RNP	ribonucleoprotein
ROI	region of interest
RPS6	ribosomal protein S6
RRM	RNA recognition motif
SAC	spindle assembly checkpoint
sas-4	*spindle assembly abnormal 4*/*CPAP*
Scrib	Scribbled
sdp-2	*spindle defective 2*
SIM	structured-illumination microscopy
Stau	Staufen
TACC3	Transforming Acidic Coiled-Coil Containing Protein 3
TBST	tris-buffered saline, 0.05% Tween-20
UAS	upstream activating sequence
UTR	untranslated region
Wdr62	WD repeat domain 62
wor	*worniu*
WT	wild-type

Chapter 1. Understanding microcephaly through the study of centrosome regulation in *Drosophila* neural stem cells

This manuscript has been published as

Robinson BV, Faundez V, Lerit DA. Understanding microcephaly through the study of centrosome regulation in *Drosophila* neural stem cells. Biochem Soc Trans. 2020 Oct 30;48(5):2101-2115. doi: 10.1042/BST20200261. PMID: 32897294; PMCID: PMC7606523.

B.V.R. Wrote the original draft and conducted the exploratory bioinformatics performed in the introduction. All authors edited the manuscript.

1.1. Abstract

Microcephaly is a rare, yet devastating, neurodevelopmental condition caused by genetic or environmental insults, such as the Zika virus infection. Microcephaly manifests with a severely reduced head circumference. Among the known heritable microcephaly genes, a significant proportion are annotated with centrosome-related ontologies. Centrosomes are microtubule-organizing centers, and they play fundamental roles in the proliferation of the neuronal progenitors, the neural stem cells (NSCs), which undergo repeated rounds of asymmetric cell division to drive neurogenesis and brain development. Many of the genes, pathways, and developmental paradigms that dictate NSC development in humans are conserved in *Drosophila* melanogaster. As such, studies of *Drosophila* NSCs lend invaluable insights into centrosome function within NSCs and help inform the pathophysiology of human microcephaly. This mini-review will briefly survey causative links between deregulated centrosome functions and microcephaly with particular emphasis on insights learned from *Drosophila* NSCs.

1.2. Introduction

Microcephaly is a neurological condition characterized by an abnormally small cerebral cortex and a head circumference that is more than two standard deviations below the population mean (Allanson et al. 2009). The characteristic small head of microcephalic individuals may manifest as the sole developmental phenotype, as in primary or non-syndromic microcephaly. Alternatively, microcephaly may present in conjunction with other comorbidities, also known as syndromic microcephaly. Those comorbidities include but are not limited to intellectual disability, epilepsy, eye abnormalities, short stature, etc. as observed in diverse human syndromes, such as primary recessive autosomal microcephaly, microcephalic osteodysplastic primordial dwarfism

type II (MOPDII), Seckel syndrome, etc. (clinical manifestations of microcephaly reviewed in (Pirozzi, Nelson, and Mirzaa 2018; Passemard, Kaindl, and Verloes 2013)).

Not surprisingly, microcephaly is extremely genetically heterogenous. The human phenotype ontology (HPO) shows an association of the microcephaly phenotype (term HP:0000252) with more than 1400 diseases and 960 genes, with new causative genes routinely being discovered (Kohler et al. 2019; Lee et al. 2020). Intellectual disability, which is defined as an individual with an IQ score below 70 (Semichov 1984), is observed in ~50% of microcephaly cases and represents the most frequent microcephaly comorbidity (Passemard, Kaindl, and Verloes 2013; Dolk 1991; Watemberg et al. 2002). HPO shows an association of the intellectual disability phenotype (term HP:0001249) with more than 2700 diseases and 1560 genes (Kohler et al. 2019; Lee et al. 2020). The American Psychiatric Association defines intellectual disability as a neurodevelopmental disorder characterized by significantly limited intellectual functioning that begins in childhood ('Neurodevelopmental Disorders' 2013). As both microcephaly and intellectual disability arise directly from aberrant neurodevelopment, it is not surprising that the two gene data sets have ~45–75% overlap **(Figure 1.1A; Supplemental Table S1.1 and Supplemental Table S1.2)**. Using Enrichr gene analysis to focus on gene ontology (GO)-cellular components reveals that both the microcephaly gene data set and intellectual disability gene data set are significantly enriched for genes annotated with the centrosome (**Figure 1.1B–D**; yellow bars (Chen et al. 2013; Kuleshov et al. 2016)). In fact, the number of genes annotated with at least one of the significantly enriched centrosome-related ontologies (centrosome (GO:0005815), microtubule-organizing center (GO:0005813), and spindle pole (GO:0000922)) represent ~10% of each phenotype data set **(Figure 1.1A; Supplemental Table S1.1)**.

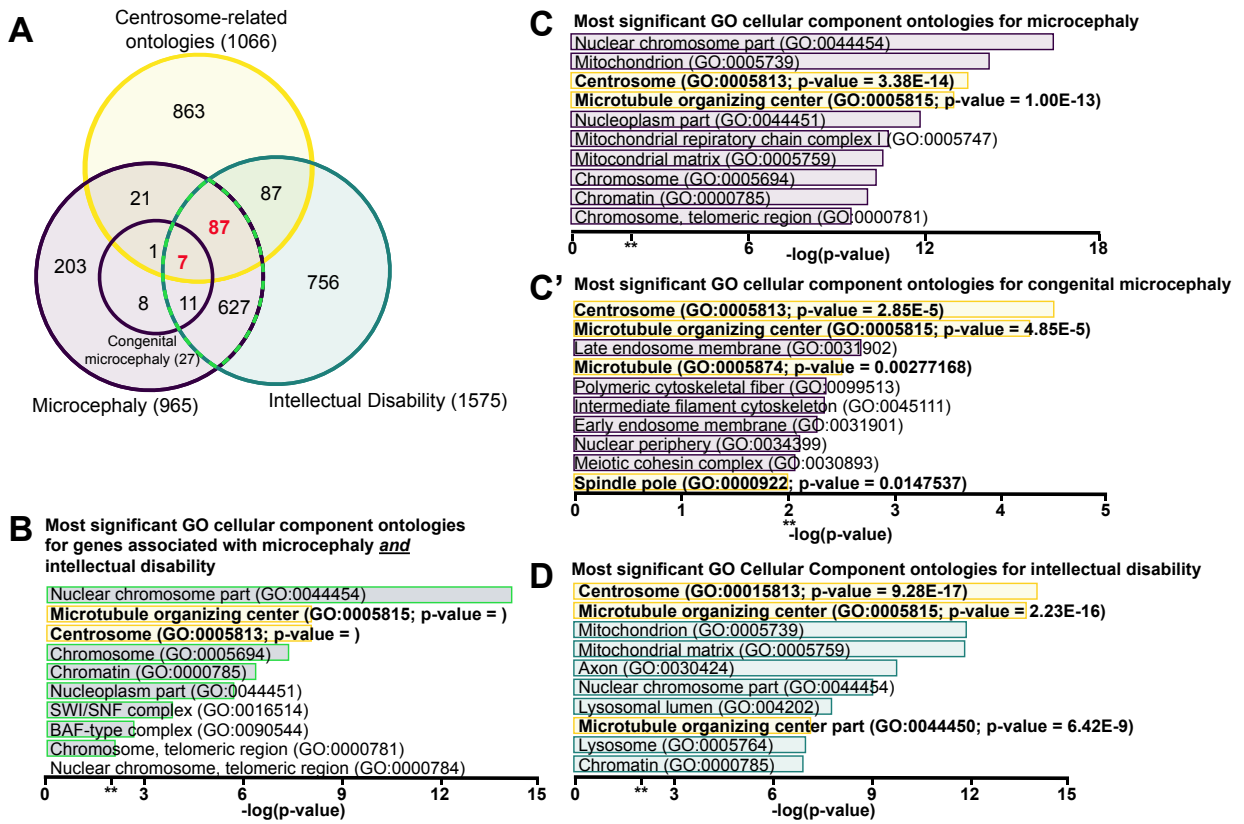


Figure 1.1 - Microcephaly associated genes are significantly enriched with centrosome genes.
(A) Venn-diagram depicts curated gene lists and overlap indicates the number of common genes present within each data set. A gene list curated by HPO indicates 1575 human genes are associated with intellectual disability (term HP:0001249; blue circle) and 965 human genes are associated with microcephaly (term HP:0000252; purple circle). Of these genes, 27 are also associated with congenital microcephaly (term: HP:0011451; purple circle inset). A gene list generated by combining all genes annotated with the following centrosome-related cell component ontology IDs curated by the Gene Ontology Resource: centrosomes (GO: 0005813), microtubule-organizing centers (GO: 0005815), and spindle pole (GO:0000922) contains 819 unique genes (yellow circle). The microcephaly phenotype and intellectual disability phenotype share 732 genes (dotted green outline). This overlap accounts for ~75% of the microcephaly data set and ~45% of the intellectual disability data set, indicating neurodevelopmental convergence between the neuroanatomical and behavioral phenotypes. Of the genes associated with microcephaly, 96 overlap with genes annotated with centrosome-related ontologies. Of the genes associated with intellectual disability, 152 overlap genes annotated with centrosome-related ontologies. The microcephaly and intellectual disability data sets share 78 common centrosome-related genes (red), representing ~10% of the shared disease genes, indicating enrichment of the centrosome and centrosome-related cell components with both diseases. **(B–D)** Bar graphs show the most significant cellular components enriched in each data set as determined by Enrichr. P-values are displayed for centrosomes and centrosome-related cellular components (bolded text). (B) GO-cellular component analysis reveals that the centrosome and microtubule-organizing center are enriched among genes overlapping with both the microcephaly and intellectual disability phenotype data sets. **(C)** GO-cellular component analysis reveals that the centrosome and microtubule-organizing center are among the top five significantly enriched cellular components in the microcephaly gene data set. **(C′)** GO-cellular component analysis reveals that the centrosome and microtubule-organizing center are the most significantly enriched cellular components in the congenital microcephaly gene data

set. **(D)** GO-cellular component analysis reveals that the centrosome is the most significantly enriched cellular component in the intellectual disability gene data set; **, P = 0.01.

Enrichments in centrosome genes are also noted in other microcephaly comorbidities. For example, genes associated with epilepsy (term HP:0001250) and eye abnormalities (term HP:0000478) are significantly enriched for centrosome annotation (P-value = 2.13 × 10−07 and P-value = 3.17 × 10−15, respectively). These observations highlight the prevalence of centrosome genes in microcephaly and some of its most frequent comorbidities.

Taken one step further, 30% of the genes associated with congenital microcephaly, defined as overt microcephaly at or before birth (term HP:0011451), are ontologically linked to the centrosome. Indeed, the centrosome and related microtubule-organizing center terms represent the two cellular components with the highest significant ontological enrichment for congenital microcephaly associated genes (**Figure 1.1C′**; yellow bars, P-value = 2.85 × 10−5 and **Supplemental Table S1.2**; (Chen et al. 2013; Kuleshov et al. 2016; Ashburner et al. 2000; Mi et al. 2019; The Gene Ontology 2019)). This ontological analysis highlights the importance of centrosome regulation for normal brain developmentphology, and function. Consequently, centrosome-related microcephaly genes have been studied in depth (recently reviewed in (Bond et al. 2005; Degrassi, Damizia, and Lavia 2019; Marthiens and Basto 2020; Mochida 2009; O'Neill, Schoborg, and Rusan 2018; Homem, Repic, and Knoblich 2015)). Not only genetic deficiencies point to the centrality of centrosome-dependent mechanisms to microcephaly but also infectious agents like Zika, whose cardinal pathology is microcephaly, interfere with centrosome-related mechanisms (Shah et al. 2018; Link et al. 2019; Devakumar et al. 2018).

Centrosomes are membrane-less organelles composed of two cylinder-shaped centrioles surrounded by a rich protein-matrix of pericentriolar material (PCM) and function as the

microtubule-organizing centers of most animal cells. Centrosomes are responsible for preserving the genome during cell division and templating the primary cilia in quiescent cells (Nigg and Raff 2009). The levels, composition, and organization of PCM oscillate in conjunction with the cell cycle, and these oscillatory behaviors dictate the microtubule-nucleating activity of centrosomes (Palazzo et al. 2000; Khodjakov and Rieder 1999). As cells enter mitosis, centrosomes duplicate and recruit PCM, a process called centrosome maturation. Following mitotic exit, centrosomes shed PCM and each daughter cell inherits a single centrosome. The processes of centrosome duplication and maturation are tightly regulated (as reviewed in (Nigg and Holland 2018; Nigg and Stearns 2011; Fu, Hagan, and Glover 2015)). Deregulation of centrosome number or activity manifests in developmental diseases, including congenital heart disease, ciliopathies (e.g. Bardet–Biedl syndrome), and microcephaly — the focus of this review (Dieks et al. 2014; Lorenzo-Betancor et al. 2018; Blacque and Leroux 2006). These links to human disease underscore the importance of understanding centrosome function and regulation.

A significant cause of microcephaly is the depletion of the neural stem cells (NSCs) required for neurogenesis (Barkovich et al. 2012). NSCs are the progenitor cells of the nervous system, and they undergo asymmetric cell division to yield one self-renewing stem cell and a daughter cell fated to differentiate into neurons or glia (Rao, Carpenter, and Vemuri 2012). Centrosomes are critically important for NSC divisions. Centrosomes contribute to NSC polarity, engineer the bipolar mitotic spindle, and establish the invariant apical–basal cell division axis (Lesage et al. 2010).

This mini-review will briefly survey causative links between deregulated centrosome functions and microcephaly with particular emphasis on insights learned from *Drosophila* NSCs. We will outline two crucial centrosome-dependent functions that are disrupted by different human

microcephaly genes in asymmetrically dividing *Drosophila* NSCs. First, we will provide an overview of the intimate connection between centrosomes and polarity. We will review seminal studies outlining the importance of centrosome activity both in polarity establishment and asymmetric cell division. We will focus on the bidirectional communication of centrosomes and polarity factors and discuss the consequences following the disruption of this communication. Second, we will examine centrosomes at the spindle poles. We will focus on how disruption of centrosome number and activity affects spindle morphogenesis and NSC division. Finally, in addition to these established centrosome functions, we will also speculate on putative contributions of post-transcriptional mechanisms to centrosome regulation, as the ability of centrosomes to execute rapid transitions in composition and function remains incompletely understood.

1.3. *Drosophila* as a model to uncover cellular mechanism of NSC divisions

Drosophila NSCs offer valuable insights into the fundamental cell biological mechanisms underlying microcephaly. Many of the genes implicated in human microcephaly are conserved in *Drosophila* (**Supplemental Table S1.1** and **Supplemental Table S1.2**), and the loss of the some of these homologous genes can result in similar microcephaly phenotypes (Jana et al. 2016; Ramdas Nair et al. 2016; Singh, Ramdas Nair, and Cabernard 2014; Thornton and Woods 2009). Indeed, several human microcephaly genes were originally identified in *Drosophila* from centrosome studies (Sunkel and Glover 1988). Notable similarities in human and *Drosophila* neurodevelopment further strengthen the utility of *Drosophila* to study neurodevelopmental disorders, such as microcephaly. For example, mammals and *Drosophila* share common progenitor lineages, their neuronal progeny undergo a regulated progression of fate determination, and many of the transcription factors that coordinate neuronal specification are conserved (Homem, Repic, and Knoblich 2015). Finally, both mammalian and *Drosophila* NSCs share

conserved polarity determinants and exhibit biased centrosome inheritance during asymmetric cell division through similar intrinsic mechanisms using conserved molecules (Li, Wang, and Groth 2014; Knoblich 2010; Wang et al. 2009).

In mammals and *Drosophila*, centrosomes are critical for normal neurodevelopment by supporting NSC proliferation and orienting the direction of asymmetric cell division (Homem, Repic, and Knoblich 2015). *Drosophila* larval NSCs are a powerful model system to study paradigms of centrosome regulation in the context of neurodevelopment. In the developing *Drosophila* central brain, the NSCs are numerous, relatively large, close to the surface, rapidly dividing, amenable to a variety of imaging platforms, and genetically tractable. These unique features allow for the discovery of mechanisms underlying NSC centrosome regulation, many of which are conserved in mammals.

Live imaging studies revealed the two centrosomes within *Drosophila* NSCs do not recruit PCM synchronously; they undergo asymmetric centrosome maturation (**Figure 1.2**; *Wild-type asymmetric cell division*; (Rusan and Peifer 2007; Rebollo et al. 2007)). Centrosomes are inherently asymmetric due to the varying age of their centrioles; an older (mother) centriole serves as the template for the formation of the younger (daughter) centriole. The daughter centrosome remains active, that is, recruits PCM and forms a microtubule aster, throughout the cell cycle and anchors to the apical cortex. In contrast, the mother centrosome is transiently inactivated during interphase and migrates throughout the cell until mitotic onset, at which point it anchors to the basal cortex and both centrosomes undergo mitotic maturation and rapidly assemble the bipolar spindle (**Figure 1.3**, *Wild-type interphase*) (Reina and Gonzalez 2014). While centrosome asymmetry is not necessary for asymmetric cell division (Januschke et al. 2013; Lerit and Rusan 2013), it is required for the non-random segregation of the daughter centrosome to the stem cell

and the mother centrosome to the differentiating cell (Conduit and Raff 2010; Januschke et al. 2011). Moreover, loss of centrosome asymmetry can compromise centrosome segregation, leading to centrosome numeracy anomalies (too many or too few inherited by the NSC) and resulting in spindle morphology defects, such as multipolar or monopolar spindles (Lerit and Rusan 2013; Marthiens, Piel, and Basto 2012). By informing the basic cell biology of asymmetric cell division in *Drosophila* NSCs, these studies have revealed insights into the pathophysiology of microcephaly.

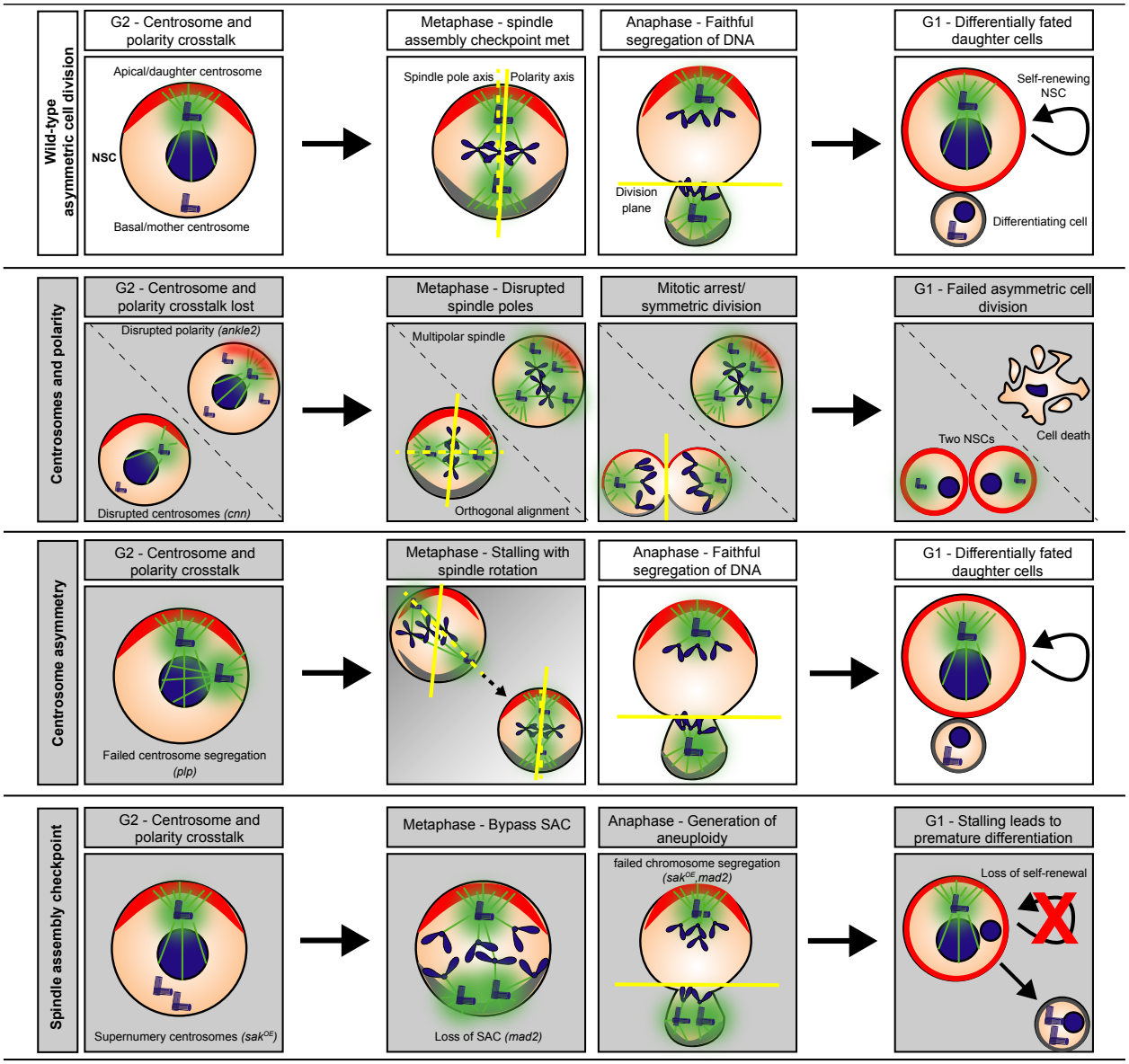


Figure 1.2 - Multiple centrosome-dependent cellular mechanisms are disrupted by homologous human microcephaly genes.

Cartoons depict the process of NSC proliferation in control (*wild-type*; top row) versus various mutant conditions. Asymmetric cell division defects are highlighted with gray-filled boxes. NSCs (peach circles) are oriented along the apical–basal axis with the apical polarity markers (red arc) and basal polarity determinants (gray arc) shown. Top row: During wild-type asymmetric cell division, two centrosomes (light blue cylinders) are present in late interphase. The apical centrosome is an active microtubule-organizing center with rich levels of PCM (green), while the basal centrosome is inactive (no PCM). Just prior to the onset of mitosis, cortical basal polarity (gray arc) is established. During metaphase, the spindle pole axis (dotted yellow line) aligns along the polarity axis (solid yellow line); both centrosomes are fully mature/active by this point. During anaphase, the chromosomes and polarity markers are segregated, and the cell divides along the division plane (yellow line). This asymmetric cell division generates one larger self-renewing stem cell (red outline) and one smaller differentiating cell (gray outline). 2nd row: *Centrosomes and polarity*. In either centrosome (e.g., *cnn*) or polarity (e.g., *Ankle2*) mutants, resultant defects include centrosome amplification with spindle morphogenesis defects or randomized spindle pole

alignment, leading to failed asymmetric cell division. These errant divisions lead to cell death or symmetric cell divisions (two NSCs). 3rd row: *Centrosome asymmetry.* Although centrosome phenotypes are observed in interphase (note the two active centrosomes), NSCs mutant for centrosome asymmetry genes rotate misaligned spindle poles before the onset of anaphase (gray → white gradient) and then resume normal asymmetric cell division (white boxes). Not shown, some stem cells missegregate their centrosomes, resulting in too many or too few centrosomes, which may compromise NSC survival. Bottom row: *Spindle assembly checkpoint*. NSCs mutant for both centrosome genes and components of the SAC generate aneuploid NSCs, which undergo premature differentiation, essentially depleting the NSC pool.

Despite the intriguing observation of biased centrosome inheritance, the functional consequences of these inheritance patterns have yet to be identified in *Drosophila*. In contrast, randomization of centrosome inheritance in the mouse neocortex led to neural progenitor depletion and premature differentiation, suggesting that the biased inheritance of the mother centrosome by the progenitor cells helps maintain their position and stem-ness (Wang et al. 2009). These findings are linked to the biased inheritance of the ciliary remnant, which remains attached to the mother centriole and promotes efficient cilia formation upon mitotic exit (Paridaen, Wilsch-Brauninger, and Huttner 2013). Functions of biased centrosome inheritance in *Drosophila* await discovery.

1.4. Centrosomes and polarity

The asymmetric division of the NSCs achieves the segregation of the apical versus basal-localized cell fate determinants, a process coupled to NSC polarization (Broadus, Fuerstenberg, and Doe 1998; Vessey et al. 2012; Freeman and Doe 2001; Cabernard and Doe 2009). Apical cortical polarity is established during late interphase/prophase and is distinguished by the localization of the Par-complex, defined by Bazooka (Baz)/Par-3, Par-6, and atypical protein kinase C (αPKC), which then recruits the adapter protein Inscuteable (Insc) (Loyer and Januschke 2020). Insc interacts with and recruits Partner of Inscuteable (Pins), which contains GoLoco motifs required to associate with the heterotrimeric G-protein subunit Gαi (Schaefer et al. 2001; Yu et al. 2000). The primary function of Pins/Gαi is to align the bipolar mitotic spindle along the apical–

basal polarity axis via interactions with Mushroom body defective (Mud), the *Drosophila* ortholog of NuMA (Siller, Cabernard, and Doe 2006; Izumi et al. 2006; Bowman et al. 2006).

Conversely, basal polarity is established after apical polarity. Localization of the cell fate determinants Numb, Prospero (Pros), Brain tumor (Brat), and Staufen (Stau) to the basal cortex is mediated by the adapter proteins Miranda (Mira) and Partner of Numb (Pon) and the tumor suppressors Lethal giant larvae (L(2)gl), Discs large (Dlg1), and Scribble (Scrib) (Caussinus and Hirth 2007; Gonzalez 2007; Ohshiro et al. 2000; Peng et al. 2000; Albertson and Doe 2003; Shen et al. 1998). Restriction of the apical and basal domains is achieved largely through inhibitory phosphorylation events by αPKC (Atwood and Prehoda 2009; Betschinger, Mechtler, and Knoblich 2003).

While localization of the Par-complex to the apical cortex represents the upstream step in NSC polarization, there is also a partially redundant microtubule-dependent pathway that contributes to polarity (Siegrist and Doe 2007, 2005). Therefore, centrosomes functioning as microtubule-organizing centers contribute to the cell-intrinsic functions that ensure polarity establishment (Doe 2008; Betschinger and Knoblich 2004). A requirement for centrosomes in the establishment of basal cortical polarity, for example, was demonstrated by genetically removing centrioles. *sas-4*, the *Drosophila* ortholog of the human microcephaly gene CENPJ, is essential for centriole assembly. Removal of *sas-4* results in a depletion of centrosomes over time, permitting the examination of centrosome requirements in various tissues. Homozygous *sas-4* adults are morphologically normal, yet partially inviable due to ciliary defects that impair locomotion and feeding. In larval NSCs, loss of *sas-4* did not alter apical polarity, as Insc localization was unaffected. However, in a subset of NSCs, the basal adapter protein Mira failed to localize, consistent with the ideas that apical polarity can proceed normally through the centrosome-

independent Par/Insc pathway and that centrosomes contribute to aspects of basal polarization. It is interesting to note that while acentrosomal microtubule spindles permit bipolar spindle formation and chromosome segregation in *sas-4* mutants, ~50% of *sas-4* NSCs show spindle alignment errors and some NSCs divide symmetrically, supporting a role for centrosomes in efficient asymmetric cell division (Basto et al. 2006).

The microtubule-dependent pathway requires astral microtubules, the plus-end-directed microtubule motor kinesin heavy chain 73 (Khc-73), and Dlg1, a membrane-associated guanylate kinase (MAGUK) protein, to recruit the Pins/Gαi complex to the apical cortex. NSCs lacking *insc* fail to localize the Par-complex to the apical cortex, yet they retain the ability to recruit Pins, Gαi, and Dlg1. Microtubule depolymerization results in a dose-dependent decrease in Pins/Gαi apical cortical localization in *insc* mutants, demonstrating a role for microtubules to polarize the NSC cortex. Genetic ablation of astral microtubules validated these findings (Siegrist and Doe 2005). To ensure mitotic spindle orientation, the Pins/Gαi complex interacts with the NuMA-related Mud protein (Siller, Cabernard, and Doe 2006; Izumi et al. 2006; Bowman et al. 2006). Taken together, these data highlight the importance of centrosome-nucleated microtubules for NSC polarization and invariant spindle orientation (**Figure 1.2;** *Centrosomes and polarity*).

Indeed, there is significant cross-talk between centrosomes and NSC polarity. When apical polarity is disrupted in *pins* mutants, the apical centrosome is initially competent to nucleate astral microtubules, but is unable to maintain apical centrosome identity throughout interphase (Rebollo et al. 2007). Likewise, when polarity is blocked, as in *Ankle2* mutants or NSCs exposed to the Zika virus protein NS4A, which interacts with ANKLE2 protein, numerous centrosome phenotypes are observed, including centrosome amplification and misaligned spindle poles (**Figure 1.2**; *Centrosomes and polarity*) (Shah et al. 2018; Link et al. 2019). Disruption of the *Ankle2* pathway

generates microcephalic *Drosophila* larvae due to impaired polarization, reduced NSC divisions, excessive apoptosis, and a reduction in NSCs (Yamamoto et al. 2014). We speculate that the centrosome phenotypes also contribute to increased apoptosis (**Figure 1.2**; *Centrosomes and polarity*). In some cases, errant spindle morphogenesis leads to a failure to satisfy the spindle assembly checkpoint (SAC) and results in p53-mediated cell death (Fong et al. 2016). Live imaging mitotic progression in *Ankle2* vs control NSCs may further inform mechanisms of *Ankle2*-dependent microcephaly. Nonetheless, this work highlights the interplay between cortical polarity establishment and centrosome function, and loss of either axis can have devasting consequences on *Drosophila* and/or human brain development.

Genetic mutants and pharmacological experiments reveal that disruption of the cross-talk between centrosome activity and polarity cues results in deleterious consequences to asymmetric cell division. While loss of neither the microcephaly gene CDK5RAP2/*centrosomin* (*cnn*) nor the NuMA-related mud gene impairs polarization, mitotic spindle orientation becomes randomized (**Figure 1.2**; *Centrosomes and polarity*) (Cabernard and Doe 2009; Siller, Cabernard, and Doe 2006). Similarly, when astral microtubules are lost in *asterless* (*asl*) or *anastral spindle 2* (*ana2*) mutants, or by treatment with microtubule antagonists, the polarity axis is no longer invariant (Rebollo et al. 2007; Januschke and Gonzalez 2010; Wang et al. 2011). When microtubules are destabilized using colchicine, centrosomes shed their PCM, migrate freely through the cell, and polarity is lost. Restoration of microtubule nucleation through UV-inactivation of colchicine, however, permits reactivation of the centrosome and the formation of a new polarity axis along a random axis dependent upon centrosome position, suggesting that the centrosome is responsible for the maintenance of the invariant orientation of the polarity axis (Januschke and Gonzalez 2010). In summary, centrosome microtubule-nucleating activity and cortical polarity are

intimately linked through multiple, nonlinear pathways throughout interphase. Disruption of this centrosome-polarity cross-talk impairs asymmetric cell division. That being said, not all centrosome genes affect polarity but are still implicated in human microcephaly through other cellular processes.

1.5. Is centrosome asymmetry dispensable in *Drosophila* NSCs?

While loss of some microcephaly associated genes results in a similar phenotype in *Drosophila*, others do not. For example, loss of *Drosophila spindle defective 2* (*spd-2*) (Giansanti et al. 2008; Dix and Raff 2007), *cnn* (Vaizel-Ohayon and Schejter 1999), *centrosomal protein 135kDa* (*Cep135)*/*bld-10* (Blachon et al. 2009; Mottier-Pavie and Megraw 2009), or *pericentrin-like protein* (*plp*) (Lerit and Rusan 2013; Martinez-Campos et al. 2004) severely impairs centrosome function and NSC divisions, yet does not yield a microcephalic phenotype. Nevertheless, these genes, as well as *polo kinase* (*polo*) and *centrobin* (*cnb*), are critical for centrosome asymmetry in interphase NSCs (Ramdas Nair et al. 2016; Singh, Ramdas Nair, and Cabernard 2014; Januschke et al. 2013; Lerit and Rusan 2013; Conduit and Raff 2010; Gambarotto et al. 2019; Li and Kaufman 1996). *WD repeat domain 62* (*wdr62*) is also required for centrosome asymmetry; however, *wdr62* mutant flies are microcephalic. Nonetheless, the microcephaly phenotype associated with *wdr62* loss is likely due to prolonged cell divisions, not centrosome asymmetry (Ramdas Nair et al. 2016; Chen et al. 2014).

Centrosome asymmetry is established through both positive and negative interactions. To generate spatial and temporal asymmetries, proteins that promote the recruitment/stability of PCM are enriched on the apical centrosome, such as Polo, Cnn, Spd-2, and Cnb (**Figure 1.3**, *Wild-type interphase*). Loss of one of these centrosome activators results in a stem cell with symmetrical

centrosomes that act in a basal-like centrosome manner (**Figure 1.3**, *middle row*) (Ramdas Nair et al. 2016; Singh, Ramdas Nair, and Cabernard 2014; Januschke et al. 2013; Conduit and Raff 2010; Januschke et al. 2011; Gambarotto et al. 2019). The microcephaly gene *Ninein* (*Nin*)/*Bsg25D* is also asymmetrically localized to centrosomes when overexpressed, but appears dispensable for normal centrosome function (Zheng et al. 2016), suggesting that not all asymmetrically localized proteins act directly on centrosome regulation. Conversely, PLP and Plk4/SAK, which promote PCM shedding, are enriched on the basal centrosome. When either of these proteins are lost, the resulting stem cell has two symmetrical centrosomes that act in an apical-like centrosome manner (**Figure 1.3**, *bottom row*) (Lerit and Rusan 2013; Gambarotto et al. 2019). Conversely, overexpression of a centrosome activator such as Cnb, which is normally only enriched on the apical centrosome, generates symmetrically apical-like centrosomes (Lerit and Rusan 2013). Additionally, overexpression of SAK also generates symmetrical centrosomes, however, these centrosomes are inactive (Gambarotto et al. 2019). Intriguingly, Cep135, which is also required to promote the down-regulation of the basal centrosome, is uniformly distributed on apical and basal centrosomes. However, loss of Cep135 also up-regulates the activity of the basal centrosome, suggesting that Cep135 likely interacts with an asymmetrically regulated protein in order to generate these spatial asymmetries (Singh, Ramdas Nair, and Cabernard 2014). Although microcephaly is not observed in *Drosophila* mutants lacking most of these centrosome asymmetry genes, they do present with many mitotic defects. For example, defects in centrosome segregation, spindle orientation, and centrosome number are consistently observed when two symmetrical centrosomes are present (**Figure 1.2**; *Centrosome asymmetry*).

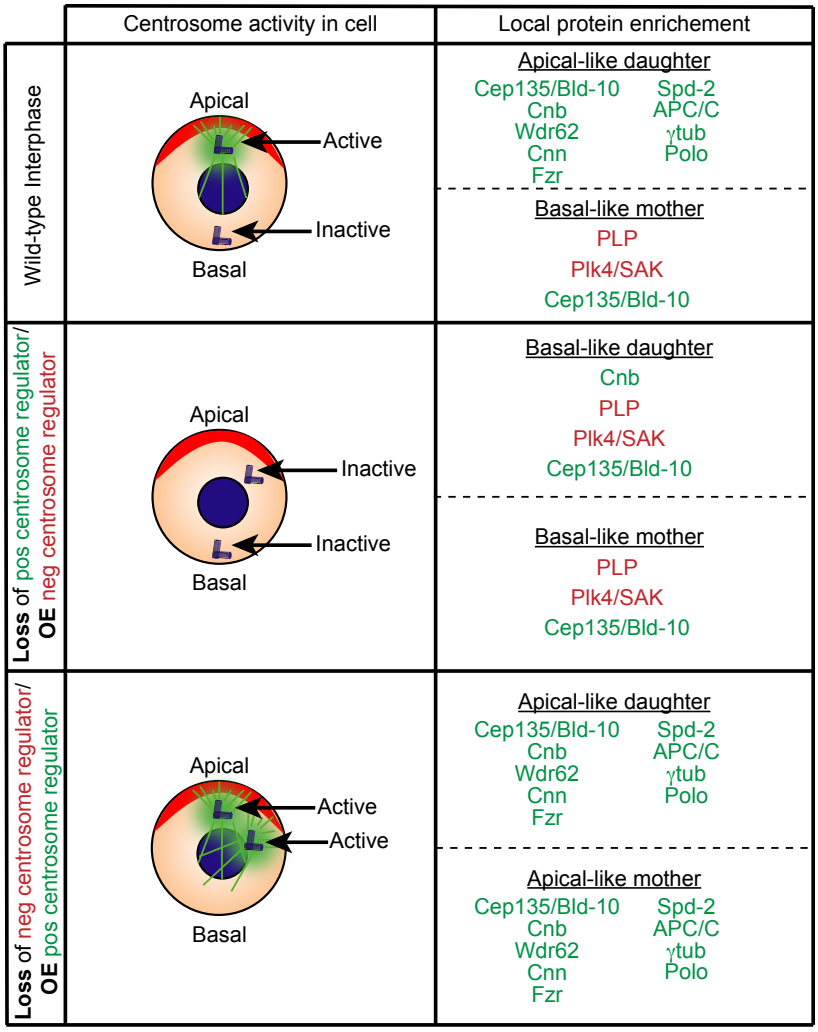


Figure 1.3 - Asymmetric protein localization directs different centrosome activity levels in interphase NSCs.

Normally, wild-type interphase NSCs exhibit asymmetric centrosome activity levels. Top row: In normal cells, the apical, daughter centrosome has high levels of PCM (green cloud) surrounding the centrioles (light blue cylinders), and it nucleates microtubules (green lines). Proteins enriched on the apical centrosome include those that promote microtubule nucleation (local protein enrichment; apical-like daughter, green font). Conversely, the mother, basal centrosome has little to no PCM. Proteins localized on the basal centrosome frequently have negative centrosome-regulating activities (basal-like mother, red font). Centrosome activity level becomes symmetrical when centrosome regulator genes are lost or overexpressed. Middle row: Loss of a positive regulator of centrosome activity (e.g., Cnb) or overexpression of a negative centrosome regulator of centrosome activity (e.g., SAK) leads to two inactive, basal-like centrosomes during interphase. Bottom row: Conversely, Loss of a negative regulator of centrosome activity (e.g., PLP) or overexpression of a positive regulator of centrosome activity (e.g., Cnb) results in two active, apical-like centrosomes during interphase.

In humans, heritable microcephaly is most commonly associated with mutations in ASPM (Nicholas et al. 2009). Loss of the *Drosophila* homolog *abnormal spindle* (*asp*) also results in microcephaly, as well as centrosome segregation and spindle orientation defects similar to the defects observed in centrosome asymmetry mutants (Schoborg et al. 2015). Expression of a full-length *asp* transgene recues brain size and microtubule defects in *asp* mutants. In contrast, expression of an N-terminal asp fragment or a full-length transgene lacking a domain required for interaction with Calmodulin (*asp*ΔIQ) rescues the microcephaly phenotype without rescuing the spindle morphology defects, suggesting the bent, unfocused spindles typical of *asp* mutants are insufficient to cause microcephaly — other mechanisms are at play (Schoborg et al. 2015). Given that both centrosome asymmetry mutants and animals rescued of *asp*-dependent microcephaly have morphologically wild-type brains, it appears that *Drosophila* neurogenesis is resistant to certain perturbations of centrosome activity to which human neurogenesis may be more sensitive. Increased sensitization to microcephaly may arise in mammals, for example, because of additional microtubule-dependent functions, such as neuronal migration, required for cell positioning in the developing, stratified neocortex (Bond and Woods 2006).

1.6. The SAC as a microcephaly fail-safe

Another intriguing hypothesis that we favor as to how *Drosophila* NSCs are able to resist failed asymmetric cell division involves the SAC. The SAC prevents misaligned or errant spindle poles (e.g. bent, monopolar, or multipolar microtubule spindles) from continuing through mitosis. This checkpoint is active in the presence of unattached kinetochores. Once all kinetochores are stably attached to microtubules, the cell cycle stall is lifted and the cell can proceed into anaphase (Lara-Gonzalez, Westhorpe, and Taylor 2012). We favor a mechanism in which the spindle orientation defects resulting from centrosome asymmetry loss are corrected prior to anaphase due

to the ‘fail-safe’ action of the SAC. In many of these mutants, spindle orientation is defective and slight mitotic stalling is observed (Ramdas Nair et al. 2016; Cabernard and Doe 2009; Gogendeau et al. 2015). Is this due to a delay in satisfying the SAC? Through live imaging, the disorientated spindles can be seen to correctly orient themselves prior to anaphase (Singh, Ramdas Nair, and Cabernard 2014; Januschke et al. 2013; Lerit and Rusan 2013; Siller, Cabernard, and Doe 2006), strongly suggesting a connection to the SAC. A pressing question in centrosome-regulated neurodevelopment is, therefore, what happens to centrosome mutants without this likely fail-safe?

Mitotic slippage occurs when components of the SAC are compromised, thereby allowing abnormal mitoses to proceed, typically resulting in chromosomal missegregation and genome instability (Rieder and Maiato 2004). Likewise, the requirement for proper centrosome regulation and activity to maintain genomic stability has been previously reviewed (Lerit and Poulton 2016). Although *sas-4* mutants lack centrosomes, they proceed through larval neurogenesis and develop an average-sized brain (Basto et al. 2006). However, if the SAC is bypassed through loss of *mad2*, the resulting *sas-4*, *mad2* double-mutant is microcephalic (Poulton, Cuningham, and Peifer 2017). It is important to note that *mad2* mutant NSCs divide normally (Buffin, Emre, and Karess 2007), highlighting that the microcephaly phenotype is due to a combination of the loss of centrosomes as well as loss of the SAC.

Centrosome amplification coupled with loss of the SAC also results in microcephaly. Centrosome amplification can arise from repeated rounds of centrosome duplication, failed centrosome segregation during cytokinesis, or failed cytokinesis (Godinho, Kwon, and Pellman 2009; Godinho and Pellman 2014). Overexpression of the master kinase regulating centriole duplication, SAK, results in centrosome amplification (Bettencourt-Dias et al. 2011; Habedanck et al. 2005; Kleylein-Sohn et al. 2007). When coupled with loss of the SAC through depletion of

mad2, the resulting NSC divisions are significantly error-prone and genetically unstable (**Figure 1.2**; *Spindle assembly checkpoint*). Loss of *mad2* paired with overexpression of SAK (*mad2*;SAKOE) causes aneuploidy as a consequence of lagging chromosomes/failed DNA segregation and cytokinesis failure. Brains that develop from *mad2*;SAKOE larva have fewer NSCs and are microcephalic, highlighting the critical role of centrosomes in maintaining genome integrity during cell division (Gogendeau et al. 2015). It is important to note that these NSCs still stall in mitosis, perhaps due to redundancy within the SAC. The loss of NSCs in these aneuploid models is not due to an increase in apoptosis or necropsy, but rather premature differentiation (Gogendeau et al. 2015). Overexpression of cell differentiation factors can also induce premature differentiation (Cabernard and Doe 2009); therefore, the extra chromosomes resulting from failed chromosome segregation may contribute to premature differentiation. Although only a few microcephaly genes have been tested in the *mad2* background, others, such as *cnn*,*mad2* double-mutants, do show aneuploidy (Buffin, Emre, and Karess 2007), suggesting that the SAC is a fail-safe that prevents microcephaly in many of these models (**Figure 1.2**; *Spindle assembly checkpoint*).

1.7. Emerging roles of post-transcriptional control in preventing microcephaly

In the mammalian brain, defects in NSC proliferation, differentiation, and neuronal migration contribute to microcephaly and other neurodevelopmental disorders. Essential to these processes is the precise control of gene expression. While understanding the contributions of post-transcriptional regulation in brain development is an emergent field, many RNA-binding proteins implicated in diverse processes, including RNA editing, splicing, export, localization, translation, and turnover, are associated with microcephaly (Lennox, Mao, and Silver 2018). Likewise, recent work in *Drosophila* highlights post-transcriptional regulation of *dpn*, *pros*, and *Myc* mRNAs is

important for neurodevelopment (Komori et al. 2018; Samuels, Arava, et al. 2020; Samuels, Jarvelin, et al. 2020). In mammalian models, haploinsufficiency of three core exon-junction components (EJC; Magoh, Rbm8a, and eIF4a) results in microcephaly associated with prolonged progenitor cell cycles leading to progenitor loss, neural depletion, and increased rates of apoptosis (Pilaz et al. 2016; Silver et al. 2010; Mao et al. 2015; Mao et al. 2016). Intriguingly, pharmacologically stalling NSC mitotic progression is sufficient to phenocopy these responses (Pilaz et al. 2016; Mitchell-Dick et al. 2019). Although centrosomes are unaffected in EJC mutants (Pilaz et al. 2016), these studies raise the possibility that other mutations that alter mitotic progression, perhaps by altering the post-transcriptional regulation of centrosome genes, could similarly impair neurodevelopment.

The idea that post-transcriptional control of centrosome genes may influence neurodevelopment is supported by recent work highlighting the alternative splicing of *Nin*. Gene expression profiling uncovered alternatively spliced variants of the microcephaly gene *Nin* differentially expressed in mammalian progenitors versus neurons (Zhang et al. 2016). *Nin* localizes to the mother centriole and promotes its maturation and is conserved in mammals and *Drosophila* (Zheng et al. 2016; Delgehyr, Sillibourne, and Bornens 2005). Zhang et al. found the Nin protein product encoded by the progenitor-enriched isoform localized to centrioles, whereas the neuronal variant remained cytoplasmic. Ectopic expression of the neuronal *Nin* variant led to premature differentiation and depletion of the neuronal progenitors (Zhang et al. 2016). These data reveal that alternative splicing generates variants of a centrosome gene that are differentially localized (centrosome versus cytoplasm) and expressed (progenitor versus neuron). Moreover, these findings provide a link between post-transcriptional regulation via alternative splicing to centrosome asymmetry within neural progenitors, as Nin localizes to the mother centriole.

Interestingly, differential expression of Nin-orthologous Bsg25D isoforms was also noted in *Drosophila* NSCs versus neurons (Berger et al. 2012), although these variants await functional characterization. Alternative splicing coupled with differential expression may contribute to the regulation of other centrosome genes and influence neurodevelopment.

The mechanisms that regulate the spatial and temporal regulation of centrosome asymmetry throughout NSC asymmetric cell division remain incompletely understood. One intriguing hypothesis is that these rapid transitions in composition and organization are mediated, in part, by post-transcriptional mechanisms, which may include RNA localization and/or local RNA translation. For example, mRNAs of several centrosome genes, including *Bsg25D* mRNA, localize near centrosomes within syncytial *Drosophila* embryos (Lecuyer et al. 2007). For a comprehensive review on the relationship between RNA localization and centrosomes, we refer the reader to (Ryder and Lerit 2018). We speculate that mRNAs encoding positive or negative regulators of centrosome maturation may be preferentially enriched, locally translated, or stabilized at the apical versus basal NSC centrosome. Supporting this possibility, local translation of centrosome genes was recently reported in non-neuronal contexts (Bergalet et al. 2020; Sepulveda et al. 2018). We surmise that differential localization, translation, and/or stability of centrosome genes within NSCs would profoundly affect neurodevelopment and that dysregulation of these processes would likely contribute to pathogenic phenotypes, including microcephaly.

Mutations in several RNA-binding proteins, which often bind the 3′-untranslated regions (UTRs) of their target RNAs, are associated with human microcephaly (Lennox, Mao, and Silver 2018). Some of these microcephaly associated RNA-binding proteins are ontologically associated with centrosomes (**Supplemental Table S1.1** and **Supplemental Table S1.2**). Likewise, a mutation in the 3′UTR of the human microcephaly gene MECP2 has also been identified in a

patient with microcephaly (Coutinho et al. 2007). Expanded use of whole-genome sequencing (as opposed to exome sequencing) of microcephaly patients may uncover additional causative mutations within UTRs. Moreover, these studies strongly suggest that mutations in RNA-binding proteins that impinge on centrosome gene regulation, or mutations within centrosome gene regulatory motifs (e.g. UTRs), likely also contribute to microcephaly.

While hundreds of RNA-binding proteins are expressed in the mammalian neonatal brain, only a handful are functionally characterized and most RNA targets await discovery (Lennox, Mao, and Silver 2018). As centrosome dynamics throughout the cell cycle clearly play a fundamental role in brain development, and RNA-binding proteins also contribute to the dynamic processes regulating neurodevelopment, whether disruption of RNA-binding proteins leads to dysregulation of centrosome activity represents a key unexplored mechanism of microcephaly. We predict that *Drosophila* models will continue to serve as valuable tools to address some of these critical questions. We are only just beginning to understand the mechanisms that govern centrosome regulation, and regulation by RNA-binding proteins is an intriguing paradigm to explore.

1.8. Summary

NSCs are neural progenitors required for neurogenesis that undergo asymmetric cell division along an invariant apical–basal polarity axis. Centrosomes are microtubule-organizing centers that orient and engineer the mitotic spindle required for NSC divisions. Deregulation of centrosome activity impairs multiple aspects of NSC divisions, including polarization, spindle orientation, spindle morphogenesis, and faithful segregation of the genome. Consequently, genetic lesions in centrosome genes represent the astounding majority of causative mutations associated

with congenital human microcephaly. Studies in *Drosophila* NSC models have proved invaluable for the discovery of microcephaly genes and their pathophysiology, particularly with respect to centrosome function and regulation.

1.9. Acknowledgements

We thank members of the Lerit laboratory for insightful comments and discussions. This work was supported by the Orphan Disease Center grant CDKL5–19-102–01 to V.F. and NIH grants T32GM008490 (B.V.R.), 1RF1AG060285 to V.F., and 5K22HL126922 to D.A.L.

1.10. Supplemental Information

Genes with centrosome-related ontologies shared among microcephaly and intellectual disability HPO datasets				
Human gene symbol	***Drosophila* homolog**	***Drosophila* indicator**	**Other ontologies**	
			RNA-Binding (GO:0003723)	SAC (GO:0071173)
AAAS	Aladin	CG16892		
ABCA2	CG6052	CG6052		
ARFGEF2	Sec71	CG7578		
ASPM	asp	CG6875		
B9D1	B9d1	CG14870		
BRCA2	Brca2	CG30169		
BUB1B	Bub1	CG14030		✓
C2CD3	CG32425	CG32425		
CAMK2B	CaMKII	CG18069		
CC2D1A	l(2)gd1	CG4713		
CCDC88A	Girdin	CG12734		
CDC42	Rac1	CG2248		
CDC45	Cdc45	CG3658		
CDC6	Cdc6	CG5971		
CDK10	Pitslre	CG4268		
CDK5RAP2	cnn	CG4832		✓
CDK6	Cdk4	CG5072		
CDKL5	CG7236	CG7236		
CENPJ	Sas-4	CG10061		
CEP135	Cep135	CG17081		
CEP290	cep290	CG13889		
CTNNB1*	arm	CG11579		
DDX11	CG11403	CG11403	✓	
DDX3X	bel	CG9748	✓	
DPF2	tth	CG12175		
DYNC1H1	btv	CG15148	✓	
DYNC1I2	sw	CG18000		
FLCN	BHD	CG8616		
HNRNPU	CG30122	CG30122	✓	
IFT140	rempA	CG11838		
IKBKG	key	CG16910		
ITGB6	mys	CG1560		
KIF11	CG32318	CG32318		
MAPRE2	CG15306	CG15306		
MCPH1	MCPH1	CG42572		
MKS1	Mks1	CG15730		
NDE1*	nudE	CG8104		
NIN	Bsg25D	CG14025		
PAFAH1B1	Lis-1	CG8440		
PCNA	PCNA2	CG10262		
PCNT	Plp	CG33957		
PLK4	SAK	CG7186		
RAD21	vtd	CG17436		
RAD51	spn-A	CG7948		
RTTN	ana3	CG13162		
SASS6	Sas-6	CG15524		
SKI	Snoo	CG34421		
SLC1A4*	Eaat2	CG3159		
SMAD4	Med	CG1775		
SMC1A	SMC1	CG6057	✓	
SMC3*	SMC3	CG9802		
SNAP29	Snap29	CG11173		
STAG1	SA-2	CG13916		
STAG2	SA-2	CG13916		
TBCD*	TBCD	CG7261		
TMEM67	CG15923	CG15923		
TUBGCP4	Grip75	CG6176		
TUBGCP6	Grip163	CG5688		
WDR62	Wdr62	CG7337		
XRCC2	Xrcc2	CG6318		

Supplemental Table S1.1 – List of microcephaly genes with centrosome ontology.

Table lists the 106 microcephaly genes (OMIM and ORPHA IDs curated by HPO) showing annotated associations with centrosomes. Bolded genes are associated with congenital microcephaly. Note that many of these human microcephaly genes also have *Drosophila* orthologs.

Supplemental Table S1.2 - Complete gene lists associated with microcephaly and intellectual disability phenotypes.

Excel file available as supplement file. Each table lists the complete gene list associated with the indicated disease phenotype. Human disease annotations (OMIM and ORPHA identifiers (IDs)) are listed. Highlighted rows indicate genes with centrosome or centrosome-related ontological annotation (GO:0005813, GO:0005815, and GO:0000922). When present, identifiers for *Drosophila* orthologs are listed; #N/A, not available.

(Tab 1; *Microcephaly genes*) All genes curated by HPO associated with the microcephaly phenotype (term HP:0000252). Asterisks mark genes associated with congenital microcephaly. (Tab 2; *Intellectual disability genes*) All genes curated by HPO associated with the intellectual disability phenotype (term HP: 0001249). Additional genes not reflected in the HPO dataset curated by recent publications are annotated with the corresponding footnotes: 1-Harripaul et al(Harripaul et al. 2018), 2-Hu et al(Hu et al. 2019) and 3-Santos-Cortez et al(Santos-Cortez et al. 2018).

(Tab 3; *Both microcephaly and ID genes*) All common genes listed in both the microcephaly and intellectual disability datasets.

(Tab 4; *Centrosome-related ontologies*) All genes from the HPO microcephaly or intellectual disability datasets annotated with one or more of the significantly enriched centrosome-related GO cellular component ontologies (centrosome (GO:0005813); microtubule-organizing center (MTOC; GO:0005815); and spindle pole (GO:0000922). Check mark indicates GO annotation.

Chapter 2. Introduction to centrosome regulation and thesis rationale

This chapter has been written and edited by

Robinson BV & Lerit DA

B.V.R. Wrote the original draft, conceptualized and constructed all figures, and conducted the 3' UTR analysis of centrosome targets in section 2.2.1 and the cancer primary site comparison performed in section 2.2.2. All authors edited the text.

2.1. Introduction

Chapter One highlights how centrosomes utilize their microtubule-nucleating activity to direct asymmetric cell division in polarized, neural stem cells and how disruption of this centrosome function can result in devastating neurodevelopmental defects with other comorbidities. Centrosome dysfunction can result in syndromes that affect multiple organ systems both before and after human development because they serve as the primary microtubule organizing centers of most animal cells. Thus, centrosomes are responsible for de novo assembly (nucleating), organizing/focusing, and anchoring of microtubules, one of the major cytoskeleton components of all Eukaryotic cells (Brinkley 1985). Microtubules build cellular structures, such as the mitotic spindle and cilia, required for key cellular process, such as division, polarity establishment and maintenance, directed cell migration, and ciliogenesis (Bornens 2012). Loss of centrosome regulation; therefore, disrupts numerous cellular activities in a variety of different cell types. This is perhaps best highlighted in multi-systematic ciliopathies, such as Bardet-Biedl syndrome, Joubert syndrome, and Merkle syndrome. All three diseases are genetically heterogeneous with mutations in multiple centrosome genes leading to any one of these syndromes. For example, all three are also associated with lesions in the centrosome gene *cep290* (OMIM: **610142;* (Sayer et al. 2006)), highlighting the diversity of phenotypic symptoms that can occur from a singular dysregulated centrosome gene (OMIMs: #615991, #610188, #611134 respectively; (Leitch et al. 2008; Bachmann-Gagescu et al. 2015; Frank et al. 2008)). Individuals affected with these syndromes may manifest symptoms in one or many organs including the brain, eyes, kidneys, liver, and skeleton (Braun and Hildebrandt 2017). Furthermore, as centrosomes are tasked with preserving the fidelity of the genome during mitosis, post-developmental conditions resulting from aneuploidy, such as tumorigeneses, are unrefutably linked with aberrant centrosome

function (Brinkley and Goepfert 1998; Duesberg 1999; Lingle and Salisbury 1999; Badano, Teslovich, and Katsanis 2005; Lerit and Poulton 2016; Basto et al. 2008). Although centrosome dysfunction underlies many devastating, multisystem human diseases, the paradigms that regulate the centrosome's activity are still poorly understood. By understanding the basic mechanisms of centrosome regulation, we aim to elucidate a general pathophysiological process of how these varied, centrosome-related syndromes arise. Knowledge of general centrosome regulatory mechanisms would inform therapeutic targets, which may hold the ability to treat diverse symptoms in a variety of human diseases. As mentioned in Chapter One, an emerging paradigm of conserved, spatial, and temporal centrosome regulation is the post-transcriptional regulation of centrosome RNAs, predicted to coordinate the expression of their cognate proteins. Post-transcriptional regulation likely contributes to the rapid and dynamic changes in centrosome activity. Identifying conserved master regulators, such as RNA-binding proteins, which regulate centrosome RNAs would likely expand our understanding of human diseases.

This chapter will briefly introduce the centrosome, its regulation, and summarize recent evidence supporting the hypothesis ossicilations in centrosome activities are mediated by post-transcriptional RNA regulation. First, we will outline the duplication cycle of the centrosome and how centrosomes undergo rapid, cell cycle-dependent changes in structure and compostion. Second, we will review seminal studies outlining the importance of RNA at the centrosome. We will focus on recent implications of post-transcriptional control of the mRNAs encoded by centrosome-specific genes. Lastly, we will review and speculate on the role of Cytoplasmic Polyadenalyation Element Binding (CPEB) family proteins in centrosome regulation.

2.1.1. Centrosome structure

Centrosomes were first identified as cellular organelles independently by Walther Flemming and Edouard Van Beneden in the late 1870s (Flemming 1875; Van Beneden 1876). Nearly a decade later, a student of Van Beneden, Theodor Boveri, discovered centrosomes organize the bipolar spindle within mitotic cells of fertilized, nematode *Parascaris equorum* (Boveri 1887). Recognizing its importance in cell division, Boveri termed this organelle the "centrosome" which means "center of the cell" (Boveri 1888, 1895). Subsequently, Boveri and others delineated the basic organization of the centrosome (**Figure 2.1**; (Bloodgood 2009; Gall 2005)). The centrosome, which is remarkably conserved between species (Bettencourt-Dias 2013; Carvalho-Santos et al. 2011; Bornens and Azimzadeh 2007), is a membrane-less organelle composed of two morphologically distinct structures: the centrioles and the pericentriolar material (Bornens et al. 1987; Glover, Gonzalez, and Raff 1993; Brinkley 1985; Brinkley and Cartwright 1971; Wu and Akhmanova 2017). Centrioles are microtubule-based, barrel-shaped structures located at the centrosome center and are surrounded by pericentriolar material. Throughout the 20th century, electron microscopy revealed the nanostructure of the centrioles (Alvey 1985; Rattner and Phillips 1973; Vorobjev and Chentsov Yu 1982; Pepper and Brinkley 1977). Although variations exist, most centrioles share 9-fold radial symmetry of microtubule bundles arranged to generate proximal-distal polarity. The centriole of cycling human somatic cells is a good representation of the typical vertebrate centriole. In cycling human somatic cells, each centriole is ~500 nm in length, ~250 nm in diameter and is composed of triplet microtubule bundles (Fu, Hagan, and Glover 2015). Within one centrosome, the two centrioles are connected by fibers extending from the proximal ends. While flexible to allow for intra-centriolar movement, these fibers hold the two centrioles in an approximate orthogonal orientation, such that only one pool of the centriole proximal end marker Centrosomal Nek2-associated protein 1 (C-Nap1) can be

resolved through immunofluorescence for most of the cell cycle (Tsou et al. 2009). Centriole structure is reviewed in depth in (Breslow and Holland 2019; Tischer, Carden, and Gergely 2021; Bornens et al. 1987). The structure of the pericentriolar material is harder to discern using electron microscopy, as it appears as a dense cloud, indicating a high concentration of macromolecules, but otherwise providing limited detail.

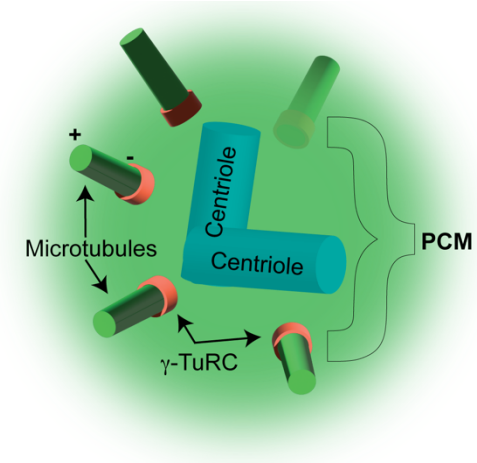


Figure 2.1 – The basic centrosome organization
Centrosomes are composed of two, cylinder-shaped centrioles (blue) connected in an approximate 'L'-shape at their proximal ends embedded in the center of a protein-rich matrix called the pericentriolar material (PCM; green cloud). γ-Tubulin ring complexes (γ-TuRCs; orange) nucleate microtubules (green tubes) such that the minus ends (-) are stabilized and focused towards the centrosome and the plus ends (+) elongate into the cytosol.

Although difficult to discern specific pericentriolar material architecture, studies show the microtubule nucleator, γ-Tubulin, is recruited to the pericentriolar material as part of the γ-Tubulin ring complex (γ-TuRC; (Farache et al. 2018; Raff, Kellogg, and Alberts 1993)), which nucleates microtubules, organizes microtubule protofilaments into a characteristic 13-fold radial microtubule, and polarizes microtubule organization with minus ends embedded in the γ–TuRC and plus ends radiating into the cytosol (Oegema et al. 1999; Moritz et al. 2000; Aldaz et al. 2005; Kollman et al. 2010). Using a variety of proteomic techniques including purification of

centrosomes coupled with mass spectrometry and large genetic screens coupled with imaging, hundreds of evolutionary conserved proteins were identified as pericentriolar material components (Andersen et al. 2003; Balestra et al. 2013; Dobbelaere et al. 2008; Goshima et al. 2007; Muller et al. 2010; Keller and Marshall 2008; Jakobsen et al. 2011; Mahen and Venkitaraman 2012). Importantly, the macromolecule-rich pericentriolar material was found to contain not just scaffolding and enzymatic proteins, but also RNA-containing ribonucleoproteins (RNPs;(Rieder 1979; Pepper and Brinkley 1980; Dippell 1976; Heidemann, Sander, and Kirschner 1977; Hartman, Puma, and Gruney 1974).

Within the last ten years, super resolution microscopy provided insight on the structure of the pericentriolar material. This work revealed the pericentriolar material is both highly structured in concentric rings of specific size, and highly dynamic with components changing and reorganizing throughout the cell cycle (Fu and Glover 2012; Sonnen et al. 2012; Lau et al. 2012; Gartenmann et al. 2017; Lawo et al. 2012; Mennella et al. 2012). For example, using amino terminus (N'-) and carboxyl terminus (C'-) specific antibodies to PCNT, ASPM, and CDK5RAP2, structured-illumination microscopy (SIM) resolved these proteins not only localize in specific regions of the pericentriolar material, but also localize with a specific orientation with in the pericentriolar material (Mennella et al. 2012; Lawo et al. 2012). Nevertheless, although hundreds of proteins and several RNA transcripts localize to the pericentriolar material, we have only just begun to define how each one fits structurally within our new understanding of centrosome architecture (Galletta et al. 2016; Nazarov et al. 2020; Feng et al. 2017). In general, structural biology abides by the same fundamental principle of building architecture, e.g., "form follows function." Therefore, one of the ways centrosomes transition between cellular processes (i.e., nucleating the spindle pole used for chromosome segregation during mitosis versus the scaffolding

used for intracellular transport during interphase) is through the structural and compositional rearrangement of the pericentriolar material. This idea is strongly supported by the finding that mitotic pericentriolar material exists in a structurally different conformation than interphase pericentriolar material (Lawo et al. 2012; Mennella et al. 2012). But how do variances in pericentriolar material abundance, composition, and organization contribute to centrosome microtubule-nucleating activity? Further, how do centrosomes execute rapid transitions in pericentriolar material conformations to build a microtubule array that accommodates the current cellular needs? Although we are far from being able to answer these outstanding questions completely, defining one paradigm in which centrosomes build and change their architecture within cells can begin to provide these missing insights on centrosome dynamics.

2.1.2. The centrosome cycle and inherent asymmetries

Boveri's initial examination of the centrosome and its vital role in cell division nearly one and a half centuries ago led him to postulate aberrant mitosis could result in chromosomal abnormalities with the potential to promote uncontrolled cellular division (Boveri 2008). In support of this, centrosome aberrations frequently generate aneuploidy (Sir et al. 2013; Debec 1978; Pihan et al. 1998), and cells that survive aneuploidy can become cancerous (Williams et al. 2008; Weaver et al. 2007; Rao et al. 2005; Gemble et al. 2022). Additionally, centrosome amplification, a type of centrosome aberration in which more than two centrosomes are present in a single cell, is associated with a poorer cancer prognosis (Chan 2011; Ghadimi et al. 2000) and is sufficient to initiate tumorigenesis in flies (Basto et al. 2008). To ensure both DNA replication and centrosome replication occur only once during the cell cycle, and to preserve the mitotic fidelity of chromosome segregation, cell cycle progression is tightly linked to the centrosome cycle (Firat-

Karalar and Stearns 2015; Stearns 2015). This is highlighted in part by the nature of the centriole biogenesis pathway, which like the DNA duplication pathway is "semiconservative" and therefore introduces inherent asymmetries (Nigg and Stearns 2011; Sullenberger et al. 2020). To simultaneous regulate both pathways, the same enzymatic proteins act as the master regulators in the signaling cascades of both cycles (Knockleby and Lee 2010; Malumbres and Barbacid 2007; Sluder 2005). For example, waves of Cyclin E-cyclin dependent kinase 2 (Cdk2) and Cyclin A-Cdk1/2 activity promote the initiation of both DNA replication and centrosome duplication (Matsumoto and Maller 2004; Ferguson and Maller 2010; Pascreau, Churchill, and Maller 2011; Moroy and Geisen 2004). Similarly, the DNA replication protein Geminin, which prevents reduplication of DNA in S phase, is present on the centrosome throughout the cell cycle, except for G1 when the centrioles need competency to replicate (Wohlschlegel et al. 2000; Tachibana et al. 2005; Lu et al. 2009). This suggests the removal of Geminin during late mitosis is an early licensing step for the subsequent replication initiation of both DNA and centrosomes within cycling cells. The enzymatic proteins and their corresponding substrates that regulate cell cycle progression have been studied in depth, providing insights into the parallel, and equally complex centrosome cycle (Keck et al. 2011; Malumbres and Barbacid 2007; Mierke 2020; Conduit, Wainman, and Raff 2015; Darling et al. 2017).

To understand centrosome regulation at the cellular level, we must relate how these cell cycle-dependent asymmetries arise as each one represents a new spatial and temporal variable to consider when investigating the centrosome at the functional level. We will briefly outline the key steps of the centrosome cycle (**Figure 2.2**), highlighting where asymmetry is introduced into the pathway.

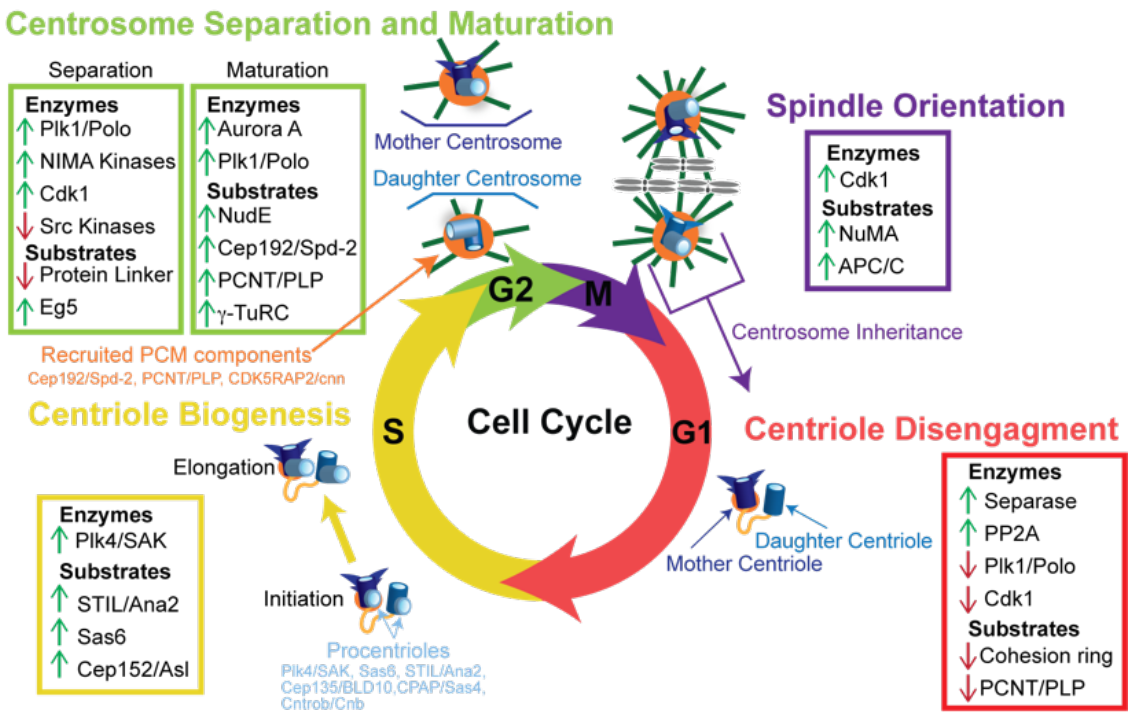


Figure 2.2 - The centrosome cycle introduces inherent asymmetries
In cycling human somatic cells, the centrosome duplication cycle is tightly linked to the cell cycle (G1, S, G2, M colored arrows). Each step of the centrosome cycle is colored and paired with a corresponding colored-box listing key kinases and their substrates (Human/Fly orthologs) that promote centrosome cycle progression. The proteins involved in each step are listed next to a green up arrow if activity/ phosphorylation is increased or a red, down arrow if activity/concentration is decreased. Key scaffolding proteins recruited during that stage are shown. Following mitosis and cytokinesis (purple), each daughter cell inherits one centrosome composed of two centrioles: an older, "mother" centriole (dark blue) and a younger, "daughter" centriole (light blue). Centriole disengagement (red) happens around the M-G1 transition (purple to red arrows), mediated by the phosphatase PP2A and kinase Plk1. Pericentriolar material (orange) is shed from both centrioles and the daughter centriole disengages from the mother centriole. The centrioles remain connected through a protein linker at their proximal ends (orange). During S-phase, centriole biogenesis (yellow) happens in two steps. First, initiation of a new procentriole (grey) forms orthogonally to each centriole on the proximal side at the site of Plk4 phosphorylation. The procentrioles then recruit scaffolding proteins, elongating throughout S-phase. In G2-M (green to purple), the protein linker connecting the two centrioles is degraded by Plk1 phosphorylation, separating the two centrosomes. Molecular motors, such as Kinesin-Related Motor Protein Eg5, separate the centrosomes from each other. As they separate, phosphorylation by Aurora A promotes the rapid recruitment of pericentriolar material, called centrosome maturation, allowing the centrosomes to nucleate microtubules (green rods).

Following mitosis, the centrosome will undergo a duplication process defined by three key events: centriole disengagement, centriole duplication, and centrosome maturation (Nigg and Holland 2018). Centriole disengagement occurs around the same time as the mitotic-G1 transition.

Immediately following mitotic exit, the majority of pericentriolar material disassociates from the centrosome. This occurs from both biochemical and physical changes of the centrosome architecture. Biochemically, the signaling cascade is initiated by Polo-like kinase 1 (Plk1)-dependent phosphorylation of both Pericentrin (PCNT) and cohesion, coupled with Protein phosphatase 2A (PP2A)-dependent dephosphorylation of both Plk1 and Cdk1 pericentriolar material targets (Pagan et al. 2015; Enos et al. 2018). This change in phosphorylation state of the pericentriolar material induces the Separase-mediated, physical cleavage of the cohesion fibers connecting the two orthogonal centrioles. Physical forces exerted through the nucleated microtubules onto the centrosome during cytokinesis also contribute to pericentriolar material dispersion (Enos et al. 2018). As a result of the physical loss of both pericentriolar material and the cohesion ring, the two centrioles lose proximal centriole "engagement", which is the physically close, orthogonal orientation of the proximal ends of the two centrioles (Tsou et al. 2009; Schockel et al. 2011). The centrioles are now considered "disengaged" and each proximal end can be resolved independently by immunofluorescence of the proximal end cap protein C-Nap1 (Tsou et al. 2009). Although no longer engaged, the centrioles remain physically connected through a protein-rich linker composed of key proteins such as Rootletin (Bahe et al. 2005). Highlighting the inter-cycle communication that occurs between the centrosome cycle and the nuclear cycle, mammalian cells undergo G0/G1 arrest following removal or damage of the centrosome. Following centrosome disruption either through the physical removal (surgically or through laser ablation) or functional blockage through siRNA-mediated knockdown or chemical inhibition of centrosome proteins, mammalian cells fail to enter S-phase of the nuclear cycle (Hinchcliffe et al. 2001; Srsen et al. 2006; Mikule et al. 2007; Wong et al. 2015). Additionally, studies suggest that the inherent centrosome disassembly and depletion in mice and rat cardiomyocytes promotes their

characteristically post-mitotic state, suggesting a need for centrosome activity in repeated mitotic divisions of regenerating tissue (Ng et al. 2020; Zebrowski et al. 2015). Taken together, these studies highlight the tight linkage between the downregulation of centrosome activity following mitosis and the ability for a cycling cell to progress through G1. In contrast, pericentriolar material shedding is executed by the activities of protein phosphatases and microtubule-dependent pulling forces (Magescas, Zonka, and Feldman 2019; Enos et al. 2018; Mittasch et al. 2020).

Centriole biogenesis, the second step in the centrosome cycle, occurs simultaneously with S-phase of the nuclear cell cycle. This stage is marked by the formation of a procentriole at the proximal end of both centrioles (Avidor-Reiss and Gopalakrishnan 2013; Azimzadeh and Marshall 2010; David 2011). Polo-like kinase 4 (Plk4) activity serves as the master kinase for initiation of centriole duplication. Following phosphorylation of scaffolding proteins STIL/Ana2, Sas6, and Cep152/Asl, Plk4 autophosphorylates, resulting in its degradation and preventing centriole initiation from occurring repeatedly in the same cycle (McLamarrah et al. 2018; Dzhindzhev et al. 2017; Dzhindzhev et al. 2014; Aydogan et al. 2018). The phosphorylated scaffolding targets recruit other centriole proteins including CPAP/Sas4, Cep135/Bld-10, and Centrobin to the growing procentriole. This protein recruitment stabilizes the tubulin dimers that compose the microtubules of the centriole, allowing it to elongate (Dong 2015).

During G2, Plk1/Polo and Nek2 kinases, prompt the next stage of the centrosome cycle, centrosome separation (Mardin et al. 2011). Centrosome separation involves the degradation of a protein linker connecting the original mother and daughter centrioles. Among the proteins required for centrosome cohesion are C-Nap1 and Rootletin (Bahe et al. 2005). Upon Plk1/Polo phosphorylation, Nek2 localizes to the centrosome and promotes centrosome disjunction (Mardin et al. 2011). No longer connected, the G2 cell now has two, distinct and complete centrosomes.

Both centrosomes migrate to opposing poles via the coordinated action of two opposing molecular motors, Eg-5 and dynein (Ferenz et al. 2009).

Concomitantly, the duplicated centrosomes undergo centrosome maturation, whereby the centrosomes recruit the robust levels of pericentriolar material necessary to organize the bipolar mitotic spindle. There are hundreds of centrosome proteins within the pericentriolar material. Key among these, the centrosome maturation process includes the recruitment of the scaffolding proteins Cep192/Spd2, PCNT/PLP, and CDK5RAP2/Cnn. Centrosome maturation requires Polo-dependent phosphorylation of Cnn, Spd2, PLP, and Centrobin (Conduit et al. 2014; Alvarez-Rodrigo et al. 2019; Lee and Rhee 2011; Januschke et al. 2013); thus, Polo is a master regulator of centrosome maturation required for the rapid recruitment of pericentriolar material to centrosomes, required to support formation of the bipolar mitotic spindle.

During anaphase, the dividing parental cell will segregate one copy of the genome and one centrosome to each daughter cell. Just as the DNA received by the daughter cell is composed of one template strand and one new strand, the centrosome in this newly born daughter cell is composed of one “older” (mother) centriole and one “younger” (daughter) centriole. Therefore, the centrosome is inherently asymmetric as the two centrioles are not the same age (Reina and Gonzalez 2014). This asymmetry is reflected in structure and function. Structurally, the mother centriole is associated with higher levels of pericentriolar material and has distinct appendages on its distal end (Tischer, Carden, and Gergely 2021). Comparatively, the centriole component Centrobin is found in the daughter centriole, but not the mother (Januschke et al. 2011; Zou et al. 2005; Jeong et al. 2007). Functionally, the mother centriole can anchor microtubules, convert to a basal body, and has competency to duplicate. The daughter centriole will not gain competency to duplicate until the two centrioles are disengaged during G1 and will not gain the distinctive

appendages until mitosis (Matsuo et al. 2012; Bowler et al. 2019). Thus, a full centrosome cycle takes one and a half cell cycles to complete.

Although the canonical centrosome maturation cycle occurs in most mitotic cells, asymmetrically dividing stem cells exhibit an alternative centrosome maturation cycle and a distinct intrinsic cortical polarity establishment (**Figure 2.3A**; (Siegrist and Doe 2005)). As discussed in Chapter One, *Drosophila* neural stem cells asymmetrically regulate their two centrosomes during interphase such that only the apical centrosome, which contains the daughter centriole, recruits pericentriolar material, while the other centrosome containing the mother centriole is transiently inactivated until mitotic onset (**Figure 1.3**; (Rusan and Peifer 2007). This functional asymmetry permits the daughter centrosome to contribute to the polarization of the apical cortex necessary for segregation of the stem versus ganglion mother cell fate determinants (Januschke and Gonzalez 2010). Centrosomes also contribute to basal cortex polarity, as *Drosophila* neural stem cells lacking centrosomes (*sas-4* mutants) lose the distinctive basal Mira crescent (**Figure 2.3B**; (Basto et al. 2006)). Differential rates of centrosome maturation may also facilitate bipolar spindle pole alignment along the invariant apical/basal polarity axis to ensure proper segregation of the cell fate determinants (**Figure 2.3C**; ((Egger et al. 2007; Malerod et al. 2018; Rebollo et al. 2007; Januschke et al. 2013)). Numerous studies have identified that asymmetric centrosome microtubule-nucleating activity and cortical polarity are intimately linked through multiple, nonlinear pathways throughout interphase. Disruption of this centrosome-polarity cross-talk impairs asymmetric cell division, further highlighting the importance of asymmetric centrosome maturation in stem cells. (**Figure 1.2**; (Robinson, Faundez, and Lerit 2020)).

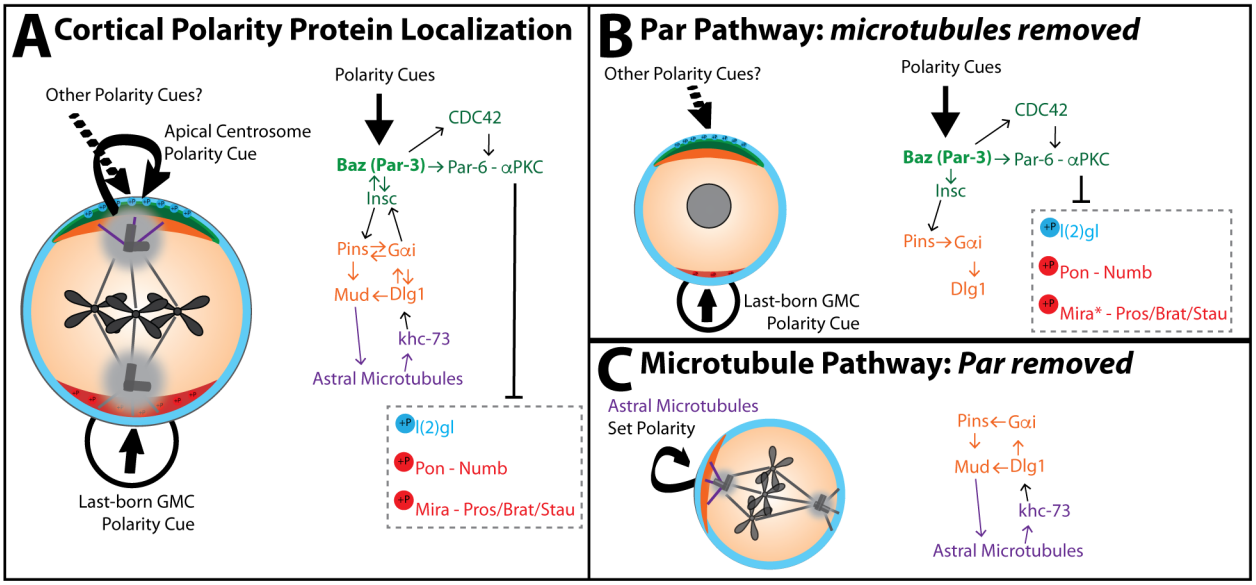


Figure 2.3 - Cortical polarity in *Drosophila* neural stem cells
(**A**) Multiple, nonlinear pathways set the distinct apical-basal cortical polarity required for segregation of cell fate determinates in *Drosophila* larval neural stem cells. Intrinsic polarity cues such as the placement of the apical centrosome and extrinsic polarity cues such as the location of the last-born differentiating daughter cell recruit Baz/Par3, marking the initial establishment of the invariant cortical polarity axis. Cortical polarity is further set and maintained through recruitment of apical cortical proteins (green) notably the Par-complex (Baz/Par-3, Par-6, αPKC). Basal cortical polarity proteins (red) are excluded from the apical cortex by αPKC-mediated phosphorylation of the adapter proteins Pon and Mira. Apical-specific l(2)gl (blue) phosphorylation contributes to this signaling cascade. Baz also recruits microtubule anchoring proteins (orange) which anchor and stabilize the astral microtubules (purple) of active centrosomes (grey cylinders) to the apical cortex. Differential centrosome maturation rates thus contribute to centrosome inheritance as the only active centrosome at this point in the cell cycle is the centrosome containing the daughter centriole. (**B**) When microtubules are inhibited (either chemically or genetically), most cortical polarity remains unaffected. In *sas-4* mutants however, which completely lack centrosomes, basal cortical polarity is reduced. (**C**) Cortical polarity is also set through a microtubule-dependent pathway. When the Par-complex is removed, the active centrosome is sufficient to set a new polarity axis. This axis however is not invariant, highlighting the need for the Par-complex in translating polarity cues to proper spindle orientation. Pathways shown are adapted from (Siegrist and Doe 2005)

Centrosome asymmetry is also required for normal centrosome segregation following asymmetric cell division (Lerit and Rusan 2013). Given the invariant alignment of the daughter centrosome at the apical cortex and the mother centrosome at the basal cortex, the daughter cell fated for stem cell self-renewal will always inherent the daughter centrosome whereas the daughter cell fated for differentiation will always inherent the mother centrosome (Januschke et al. 2011; Conduit and Raff 2010). Such biased centrosome inheritance is observed in other models of

asymmetrically dividing cells. For example, similar to the *Drosophila* neural stem cell, the human neuroblastoma NuMa+ cell retains the daughter centrosome (Izumi and Kaneko 2012). Conversely, budding yeast, mouse neural progenitor cells, and *Drosophila* male germline stem cells will preferentially inherit the daughter centrosome (or spindle pole body, as in yeast) (Pereira et al. 2001; Wang et al. 2009; Yamashita et al. 2007). Therefore, centrosome asymmetries persist generationally in dividing stem cells. While some propose biased centrosome inheritance may influence cell fate decisions, this hypothesis remains to be directly tested (Wang et al. 2009; Shinohara et al. 2013; Paridaen, Wilsch-Brauninger, and Huttner 2013).

2.1.3. RNA localization to centrosomes

As early as the 1960s, independent laboratories identified RNA at the centrosome in diverse model systems including *Ilyanassa*, *Spisula*, *Drosophila*, *Xenopus*, zebrafish, and mammalian cell lines (Hartman, Puma, and Gruney 1974; Heidemann, Sander, and Kirschner 1977; Alliegro, Alliegro, and Palazzo 2006). More recently, specific transcripts were localized to centrosomes using high-throughput RNA localization screens (Lecuyer et al. 2007; Chouaib et al. 2020; Safieddine et al. 2021; Kwon et al. 2021; Blower et al. 2007). Some of these transcripts, such as the centrosomal transcript *pcnt* of zebrafish embryos, are packaged, localized to, and translated within the centrosome pericentriolar material as polysome-containing RNP-complexes (Sepulveda et al. 2018). Polysome-containing RNP-complexes frequently represent sites of active RNA-translation (Hieronymus and Silver 2004). Translational machinery, including ribosomal-subunit 6 (RPS6) and eukaryotic translation initiation factor 4E (eIF4e) co-localize with centrosomes in human HeLa cells, further implicating centrosomes as sites for local protein

synthesis (Chouaib et al. 2020; Blower et al. 2007; Lerit 2022). However, the biological function for the localization and translation of RNAs at the centrosome is not well defined.

We postulate three possible functions for RNA localized to centrosomes (Ryder and Lerit 2018). First, the transcripts may be localized as passive cargo to ensure segregation during division. Established examples of this paradigm are frequently observed in asymmetrically dividing cells that need to segregate different cell fate determinants to each daughter cell. For example, the active localization of *loDpp* transcript to the centrosome closest to the animal pole during the third embryonic cleavage of the mollusk, *Ilyanassa obsolete* ensures the *loDpp* transcript will be within the cytoplasm of the micromere daughter cell. As *loDpp* encodes a developmental pattering molecule, its inheritance into the micromere-differentiating daughter cells and depletion in the macromere-stem daughter cells ensures its patterned expression later in development (Lambert and Nagy 2002). Second, the transcripts may play a structural role not yet described. Lastly, the transcripts may be localized and subsequently translated at the centrosome to generate spatial protein enrichments that directly contribute to centrosome activity.

The functional coupling of transcript localization and local translation is a ubiquitous, conserved paradigm allowing for the fine-tuning of cellular processes (Buxbaum, Haimovich, and Singer 2015; Jung et al. 2014; Ryder and Lerit 2018). For example, neurons require RNA localization and local translation to direct the remodeling of dendritic spine morphology during learning and memory; mRNAs encoding dendritic spine remodelers, including *β-actin*, *activity-regulated cytoskeleton associated protein* (*ARC)*, *calcium/calmodulin-dependent kinase IIα* (*CaMKIIα)*, and *brain-derived neurotrophic factor (BDNF)*, localize to dendrites (Dynes and Steward 2012; Tongiorgi, Righi, and Cattaneo 1997; Tiruchinapalli et al. 2003; Ohashi and Shiina 2020; Miller et al. 2002). Following synaptic stimulation, local translation promotes the

enrichment of these remodelers at the activated dendritic spine (Sutton and Schuman 2006; Leal, Comprido, and Duarte 2014). Consequently, dendritic spine size and shape is altered from activities of local protein enrichments (Yoshihara, De Roo, and Muller 2009; Hayashi and Majewska 2005; Yuste and Bonhoeffer 2004). Therefore, localization and local translation of dendritic mRNAs allows for the rapid, specific, and dynamic transition of dendritic spine morphologies in response to extracellular signals (Nishiyama 2019; Nakahata and Yasuda 2018; Hafner et al. 2019). We speculate the rapid, specific, and dynamic engineering of centrosome-nucleated microtubule arrays in response to inter- or intracellular stimuli is facilitated through a similar paradigm.

Theoretically, by coupling localization and local translation of mRNAs that encode centrosome proteins at the centrosome, the centrosome could execute rapid, specific, and dynamic transitions of pericentriolar material organization in response to cellular signals. In support of a centrosome regulatory pathway that couples centrosome transcript localization and translation, many of the RNAs localized to the centrosome encode known centrosome regulator proteins (Lecuyer et al. 2007; Bergalet et al. 2020; Ryder, Fang, and Lerit 2020; Safieddine et al. 2021; Sepulveda et al. 2018; Chouaib et al. 2020). For example, Pericentrin (PCNT) is a key scaffolding protein of the pericentriolar material, and its recruitment to the centrosome promotes pericentriolar material expansion and centrosome activation (Doxsey et al. 1994). Conversely, Pericentrin-like Protein (PLP), the *Drosophila* PCNT ortholog, is enriched on the inactive, mother centrosome of interphase *Drosophila* neural stem cells and loss of *plp* results in two active centrosomes, indicating an ability to act as a centrosome repressor as well (Lerit and Rusan 2013; Martinez-Campos et al. 2004). Post-transcriptional regulation of *PCNT* would provide a means of fine-tuning PCNT/PLP protein levels, permitting differential centrosome regulatory functions.

Intriguingly, *PCNT* transcripts localize to centrosomes in multiple species, including human, zebrafish and *Drosophila* (Lecuyer et al. 2007; Sepulveda et al. 2018; Chouaib et al. 2020; Safieddine et al. 2021; Ryder, Fang, and Lerit 2020; Zein-Sabatto and Lerit 2021). Moreover, a recent finding indicates that translation of *PCNT* during its localization to the centrosome supports PCNT enrichment and pericentriolar material expansion (Sepulveda et al. 2018). As little is known about the post-transcriptional regulation of centrosome RNAs, further insights are required to inform how RNA localization and local protein synthesis modulate centrosome maturation.

2.2. Evidence of post-transcriptional regulatory elements at the centrosome

Post-transcriptional gene regulation is usually mediated by *trans* elements, such as RNA-binding proteins, that recognize and bind *cis*-elements, such as specific RNA motif sequences or structures, of target RNAs (Glisovic et al. 2008; Gomes and Shorter 2019). RNA-binding proteins modulate every stage of RNA processing including, alternative splicing and polyadenylation, poly(A) tail length, nucleocytoplasmic shuttling, subcellular packaging, transport, and localization, as well as the repression/initiation of both degradation and/or translation of the bound target RNA (Gebauer et al. 2021). For example, the RNA-binding protein eIF4a3 binds a structural RNA motif and complexes with MAGOH, RBM8A, and Barentsz (BTZ) to form the exon-junction complex (EJC), a well-characterized RNP. Other auxiliary proteins then interact with the EJC core proteins and promote gene expression through multiple stages of mRNA processing including splicing, mRNA export, translation initiation and nonsense-mediated decay (Boehm and Gehring 2016). Other RNA-binding proteins, such as Egalitarian and Staufen can interact with motor proteins like Dynein to promote movement of target RNA cargos throughout the cell (Schieweck et al. 2021; Broadus, Fuerstenberg, and Doe 1998; McClintock et al. 2018). Still other RNA-binding proteins exhibit only an intermediate affinity and/or promiscuity for the *cis*-element motif

they bind, as other factors such as protein-protein interactions, post-translational modifications, and RNA structure subsequently add another layer of regulation. By binding with intermediate affinity, RNA-binding proteins can change the specificity or regulation of target RNAs ultimately fine-tuning gene expression to specific cellular contexts. For example, the RNA-binding protein Pumilio is unphosphorylated in quiescent fibroblast cells. Following stimulation by growth factors, Pumilio is phosphorylated which increases its affinity to Pumilio Recognition Elements containing mRNAs, like *p27*. Increased RNA affinity and increased Pumilio binding reduces *p27* mRNA stability, ultimately resulting in a decrease of the p27 protein allowing the cell to exit its quiescent state and enter S-phase (Kedde et al. 2010). RNA-binding proteins are key regulators of RNA metabolism in all cells; and the versatility of RNA-binding proteins has previously been reviewed in depth (Blencowe et al. 2009; Darnell and Richter 2012; Gebauer et al. 2021; Gerstberger et al. 2014).

2.2.1. CPE sites, known *cis*-elements and how they relate to centrosomal RNAs

To identify potential RNA-binding proteins that may contribute to centrosome regulation, we first analyzed the 3' UTRs of human and *Drosophila* RNAs that encode known centrosome proteins to mine for enrichment of RNA binding protein motifs implicated in RNA localization or translational control. As there are hundreds of centrosome proteins, we narrowed our search to RNAs in which the cognate protein has known roles in establishing centrosome asymmetry in *Drosophila* neural stem cells (**Figure 1.3**; (Lerit and Rusan 2013; Gambarotto et al. 2019; Singh, Ramdas Nair, and Cabernard 2014; Januschke et al. 2013; Ramdas Nair et al. 2016)), as these RNAs would be likely targets of post-transcriptional regulation. Intriguingly, 8/9 of the *Drosophila* transcripts and 9/9 of the human ortholog transcripts contained at least one, but often repeated,

variations of cytoplasmic polyadenylation elements (CPEs) (**Figure 2.4; Supplemental Table S2.1**), suggesting CPEB (CPE binding) proteins bind these RNAs. We examined the following consensus and nonconsensus CPE motifs: canonical $UUUU_{(n)}AU$ (green star), $UUUU_{(n)}A$ (green), $UUUU_{(n)}C$ (yellow), $UUUU_{(n)}G$ (red) in our analysis of centrosome RNAs (**Figure 2.4**; (Pique et al. 2008; Fox, Sheets, and Wickens 1989; Hake, Mendez, and Richter 1998; Hake and Richter 1994; Stepien et al. 2016; McGrew et al. 1989)). It is interesting to note, the asymmetry gene without any CPE sites in its 3' UTR (*cep135*) does not display asymmetric Cep135 protein levels between the two centrosomes in interphase, unlike the other asymmetry genes (Singh, Ramdas Nair, and Cabernard 2014). Namely, Cnb, Cnn, Frizzled (Fzr), Wdr62, Spd-2, and Polo protein products are enriched on the neural stem cell apical/daughter centrosome, while PLP and SAK protein products are enriched on the basal/mother centrosome (PLP, SAK) (**Figure 1.3**; (Januschke et al. 2013; Conduit et al. 2014; Conduit and Raff 2010; Meghini et al. 2016; Lerit and Rusan 2013; Gambarotto et al. 2019)).

Gene	*Drosophila* (Top Transcript)			Human (Bottom Transcript)		
	Transcript ID	**3' UTR Length (nt)**	**CPE sites**	**Transcript ID**	**3' UTR Length (nt)**	**CPE sites**
	1. Drosophila Transcript 3' UTR 2. Human Ortholog Transcript 3' UTR — nts; CPE: canonical $UUUU_{(n)}AU$, $UUUU_{(n)}A$; CPE: $UUUU_{(n)}C$, $UUUU_{(n)}G$; Scale					
Cep135	FBtr0303375 Cep135-PB	123	0	ENST0000042247 CEP135-202	1108	22
	1. 2. — 25 nt					
Cnb	FBtr0072842 Cnb-PA	149	3	ENST00000380262 CNTROB-201	66	1
	1. 2. — 10 nt					
cnn	FBtr0087703 Cnn-PA	758	9	ENST00000360190 CDK5RAP2-202	360	2

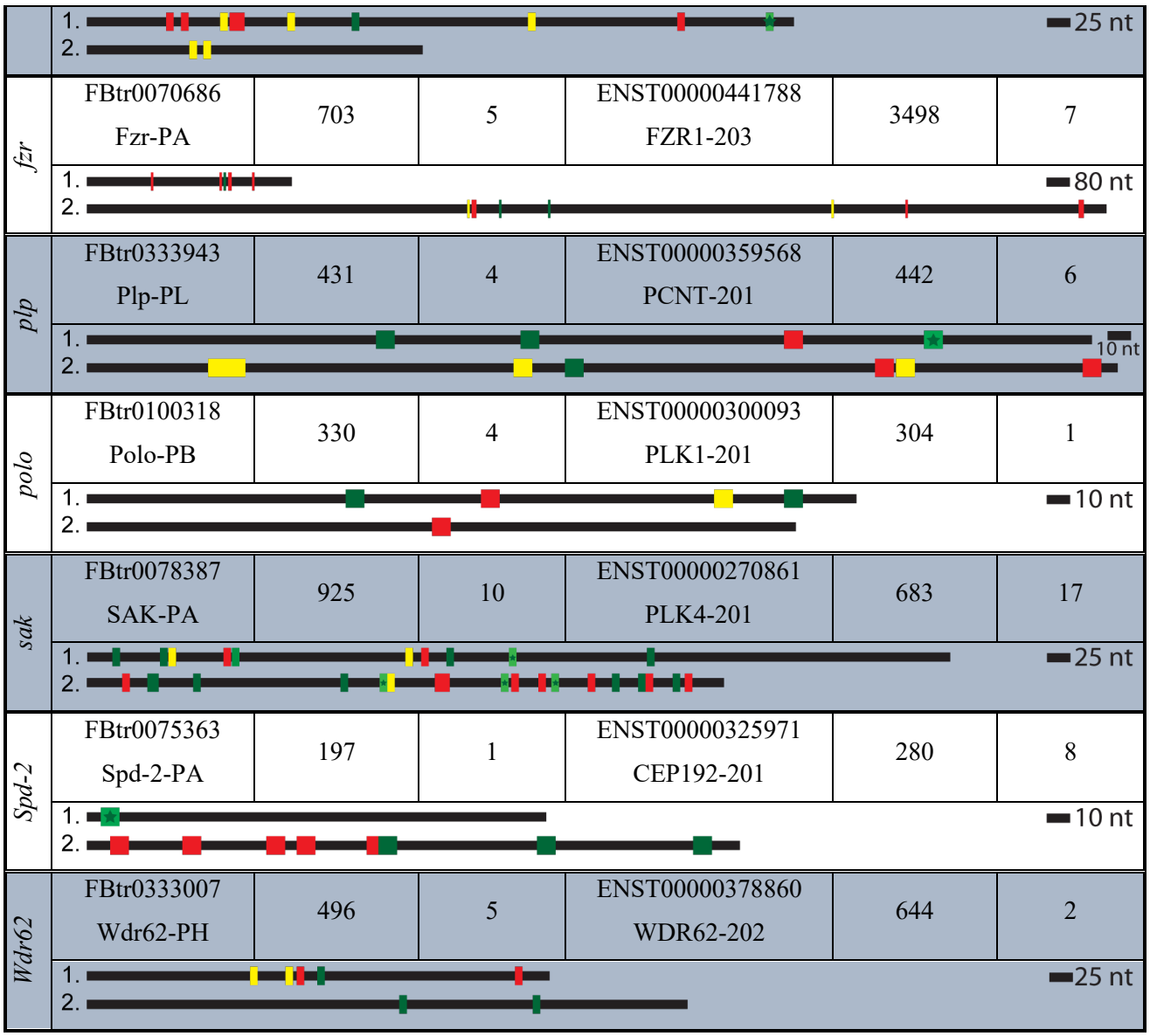


Figure 2.4 – CPE sites in human and *Drosophila* centrosome asymmetry genes
Both consensus cytoplasmic polyadenlation element (CPE) sites (green with star) and nonconsensus CPE sites (dark green, yellow, and red) are found within the 3' UTR (grey) of nine *Drosophila* (transcript 1.) and human ortholog (transcript 2) centrosome asymmetry genes (in alphabetic order; *cep135, cnb, cnn, fzr, plp, polo, sak, spd-2, wdr62*). Isoform identifiers, 3' UTR length in nucleotides (nt), and number of CPE sites are shown. All 3'UTR sequences are listed in **Supplemental Table S2.1**.

2.2.2. CPEB RNA-binding proteins, the *trans*-element associated with CPE sites

CPE sites are bound by CPEB proteins, a conserved family of RNA-binding proteins with an ordered C-terminus containing two RNA Recognition Motifs (RRMs) and a Zinc-finger binding domain termed the CPEB-domain (Fernandez-Miranda and Mendez 2012). Based on phylogenetic

analysis, CPEB proteins can be divided into two subfamilies. Mammalian CPEB1 and *Drosophila* Orb are both in the first CPEB subclass. These proteins function predominantly in oogenesis and embryonic development (Bally-Cuif, Schatz, and Ho 1998; Barr et al. 2019; Chang, Tan, and Schedl 1999; Tan et al. 2001). Comparatively, the second subclass contains three mammalian CPEB proteins, CPEB2-4, whereas *Drosophila* have only one, Orb2 (**Figure 2.5**; (Ivshina, Lasko, and Richter 2014; Xu, Tyagi, and Schedl 2014)). This subclass of CPEB proteins have established roles in learning and memory and nervous system development of both mammals and *Drosophila* (Xu et al. 2012; Qu et al. 2020; Keleman et al. 2007; Si et al. 2010; Mastushita-Sakai et al. 2010). As we identified the CPE sites in the 3' UTRs of centrosome asymmetry genes responsible for asymmetric cell division during neurogenesis, we subsequently investigated Orb2 and its role in centrosome regulation.

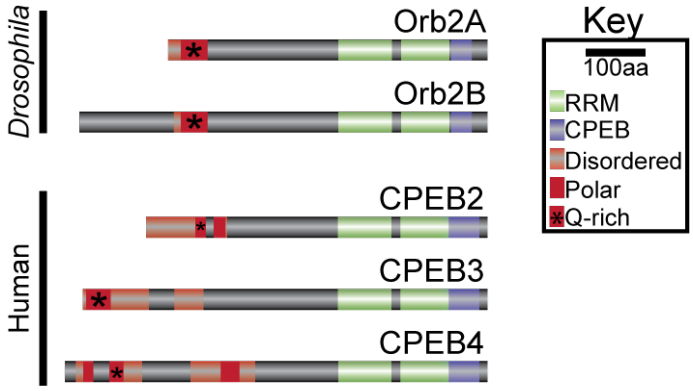


Figure 2.5 – Neuronal CPEB protein homology
Neuronal cytoplasmic polyadenylation element binding (CPEB) proteins have two isoforms in *Drosophila*. The small isoform, Orb2A, is 551 amino acids (aa; grey) and the large isoform, Orb2B, is 704aa. CPEB isoforms frequently have disordered regions (light red) in the N-terminus. Polar (dark red) and Q-rich (star) regions within disordered regions frequently have amyloid-like properties. CPEB RNA-binding domains are conserved across species. All CPEB proteins have two RNA-recognition motifs (RRM; green) and one CPEB domain (a zinc finger domain; blue) in the C-terminal of the protein.

Both classes of CPEBs direct gene expression through a variety of post-transcriptional mechanisms, but namely through the initial repression and localization of CPE site containing

transcripts (Kim and Richter 2006). CPEB proteins form different RNP complexes based on their phosphorylation state. For example, unphosphorylated CPEB forms an RNP containing both PARN and Gl2. PARN deadenylase activity outcompetes Gl2 adenylation activity, resulting in the overall repression of translation from a shortened poly(A) tail. Conversely, when CPEB is phosphorylated, PARN is dissociated from the complex, allowing Gl2 polyadenylation to elongate the poly(A) tail and functionally transitions CPEB from a repressor to an activator of translation (Kim and Richter 2006). Some CPEB proteins can also change regulatory states through the formation of functional amyloids. For example, mammalian CPEB2-3, *Aplysia* CPEB, and the *Drosophila* CPEB protein Orb2 all have a disordered N-termini capable of forming prion-like aggregates (Si et al. 2010; Sanguanini and Cattaneo 2018; Stephan et al. 2015; Hervas et al. 2021; Cervantes et al. 2016; Si, Lindquist, and Kandel 2003; Theis, Si, and Kandel 2003). Oligomerization of Orb2 is required for its switch from a translational repressor to a translational activator in *Drosophila* cells (Khan et al. 2015).

Importantly, some CPEB proteins and known CPEB-targeting kinases localize to the mitotic spindle. For example, in *Xenopus* embryos and oocytes, both the CPEB protein and its activator Eg2 localize to the mitotic spindle and centrosomes (Groisman et al. 2000; Roghi et al. 1998). Moreover, CPE sites are over-represented in RNAs cosedimenting with taxol-stabilized microtubules (Blower et al. 2007; Sharp et al. 2011), suggesting both the *cis* and *trans* acting factors occupy the same subcellular space. CPEB association with spindles was also shown in cycling human somatic cells. CPEB1 and its positive (Aurora A, Cleavage And Polyadenylation Specific Factor 3 (CPSF3), and PABP1) and negative (DEAD-box helicase 6 (DDX6), PARN, and TACC3) regulators, as well as CPEB4 and its positive regulatory factors (ERK and Cdk1) all localize to spindles (Pascual et al. 2020). Supporting the hypothesis CPEB proteins regulate

centrosome genes, Hafer et al. found *orb2* null *Drosophila* embryonic neural stem cells are unable to polarize and exhibit misaligned spindle poles during asymmetric divisions (Hafer et al. 2011). As both polarity and spindle alignment are biological processes mediated by centrosomes (**Figure 1.2**), these findings highlight shared phenotypes between CPEB mutations and centrosome genes, suggesting Orb2 may regulate centrosomes. Taken together with our preliminary identification of CPE sites in centrosomal mRNAs implicated in centrosome asymmetry, these data support a hypothesis wherein Orb2 modulates centrosome activity by acting in *trans* on CPE *cis*-elements to direct localization and/or translation of centrosome genes.

The conservation of CPE sites in the human ortholog genes suggests CPEB-dependent regulation of centrosome activities is conserved. CPEBs and centrosome genes are implicated in a variety of different cancers, which could suggest a shared pathway (D'Ambrogio, Nagaoka, and Richter 2013; Chen, Tsai, and Tseng 2016; Fernandez-Miranda and Mendez 2012; Ivshina, Lasko, and Richter 2014; Lin, Xie, and Chan 2022; Chavali, Putz, and Gergely 2014; Ganem, Godinho, and Pellman 2009). Cancer pathology, however, is both mechanistically and genetically diverse. To determine if CPEB genes and centrosome genes act in similar cancer pathways, I utilized data from the National Cancer Institute (NCI) Genomic Data Commons (GDC) harmonized database to compare phenotypic similarities across different cancer cases (Heath et al. 2021). The NCI's GDC is a public access repository of clinical and sequencing data from patient samples. Specifically, I mined data in which sequencing results indicated at least one sample in the study contained a mutation in either CPEB2-4 genes, or the 3' UTR of centrosome asymmetry genes. I then used primary cancer site as an indicator of phenotypic similarity. The resulting dataset contained over 15,000 cancer cases from 35 different primary sites across 43 studies. (**Supplemental Table S2.2**; *Tabs2,3*). Across all cases, the most enriched primary sites

representing 50% of the total cases were breast, lung, bone marrow, uterus, brain, and kidney. Comparatively, cancers with mutations in CPEB2-4 genes (360 cases) were significantly enriched in gastrointestinal, colorectal, uterus, and skin primary sites and significantly depleted in prostate, abdomen, adrenal gland, thyroid, and brain primary sites. Intriguingly, cancers with mutations in the 3' UTR of centrosome genes (952 cases) were also enriched in skin, gastrointestinal, colorectal and uterus primary sits and depleted in abdomen, adrenal gland, thyroid, and brain primary sites, indicating a phenotypic copy of the CPEB genes (**Figure 2.6**). Taken together, this exploratory bioinformatics suggests that in humans, CPEB proteins and the regulatory region of centrosome RNAs act in the same pathway and disruption of either can result in human pathologies.

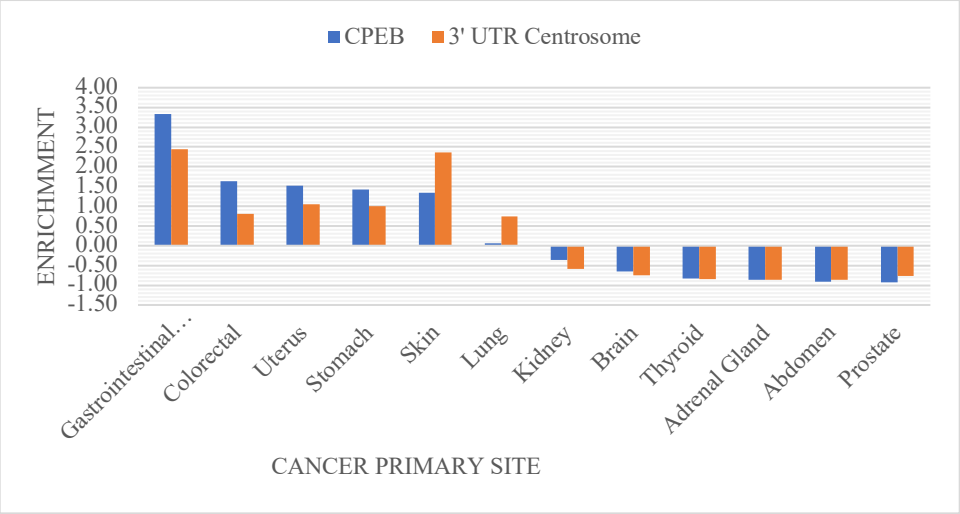


Figure 2.6 – Enrichment of primary sites containing CPEB or centrosome 3' UTR mutations
Fold-change (y-axis) of the most significantly enriched (positive) and depleted (negative) cancer primary sites (x-axis) in cancer cases with a mutation identified in a CPEB gene (blue) or in the 3' UTR of a centrosome asymmetry gene (orange) compared to all cancer cases in the studies. Data is from National Institute of Cancer's Genomic Data Commons (GDC) harmonized database (**Supplemental Table S2.2**).

2.3. Summary of thesis rationale

How oscillations in pericentriolar material are regulated throughout the cell cycle is still poorly understood, despite the fact pericentriolar material levels instruct the microtubule-

organizing activity of centrosomes (Gould and Borisy 1977). Understanding how centrosomes are regulated will increase our fundamental understanding of basic cell biology, but also inform basic principles of cellular mechanisms, which may be dysregulated in various human pathologies, such as microcephaly or cancer. Therefore, the aim of this thesis is to address the pressing biological question; "How do cells spatially and temporally regulate centrosome activities?". We hypothesize RNA-binding proteins contribute to centrosome regulation. In this thesis, we specifically examine a role for the CPEB family protein, Orb2, in regulating the centrosomes within neural stem cells of the *Drosophila* larval central brain. Our data illustrate Orb2 regulates centrosome activities cell autonomously and supports normal brain size non-cell autonomously. We will also speculate on potential Orb2 centrosome-specific RNA targets and highlight aspects of this model potentially conserved in humans.

2.3.1. Hypothesis

The central hypothesis of the following work is to determine if an RNA-binding protein contributes to the regulation of centrosome activities. Identifying an RNA-binding protein as a regulator of centrosomes would suggest a not yet identified mechanism of post-transcriptional modification of centrosome genes. Specifically, we test the hypothesis that Orb2, a CPEB family RNA-binding protein, regulates asymmetric centrosome maturation during neurogenesis of *Drosophila* larva. Given the conservation that exists between human and *Drosophila* centrosome regulation and RNA localization and local translation, the findings of this work have the potential to uncover novel paradigms of centrosome regulation, one of the first steps in identifying new therapeutics for treating human disease that arise from dysregulated centrosome activities.

2.3.2. Overview of experimental design

To determine the plausibility of a conserved CPEB-mediated centrosome regulatory pathway, we introduce a model system in which the processes of both centrosome maturation and CPEB-mediated post-transcriptional RNA modifications are conserved. The *Drosophila* genome contains a functional homolog for ~75% of human disease genes, including the centrosome asymmetry genes that encode conserved CPE sites in the 3' UTR of their mRNAs (**Figure 2.4**) and genes that encode CPEB proteins (**Figure 2.5**) (Yamamoto et al. 2014; Hales et al. 2015). Additionally, *Drosophila* have a rapid life cycle and are less complex than vertebrate models, making them both genetically tractable and amenable to imaging. These features allow us to manipulate the cellular and molecular nuances of CPEB-mediated RNA regulation in vivo enabling us to quantify the consequences, if any, of specific genetic deviations on centrosome regulation. In particular, the centrosome cycle is distinctive, conserved, and well-documented within *Drosophila* larval neural stem cells. Therefore, we will be taking advantage of the readily accessible *Drosophila* larval neural stem cells, located in the central brain region (**Figure 2.7A**; (Homem and Knoblich 2012; Li, Wang, and Groth 2014)).

Mammalian and *Drosophila* neural stem cells both perform asymmetric cell division by separating conserved cell fate determinants through intrinsic mechanisms (Knoblich 2008). Additionally, asymmetric cell division requires asymmetric centrosome activities in both humans and *Drosophila,* as loss of this asymmetry results in tumorigenesis (Neumuller and Knoblich 2009; Jana et al. 2016). Additionally, the orthologous CPEB proteins of humans and *Drosophila* are similarly expressed in neuronal tissues. Therefore, *Drosophila* neural stem cells are an excellent model for investigating if one conserved mechanism of spatial and temporal regulation of centrosome activity is mediated by CPEB proteins.

There exists a vast genetic toolbox for manipulating the *Drosophila* genome, permitting detailed study of cellular and molecular mechanisms (Hales et al. 2015). For our experiments, we utilized *Drosophila* lines in which expression of the neuronal CPEB protein, Orb2, was genetically ablated (**Figure 2.7B**). Predominately, we utilize a previously characterized null mutation called $orb2^{\Delta 36}$. This mutation, generated through FLP-FRT, genetically removes the *orb2* coding sequence from the third chromosome (Xu et al. 2012). Therefore, no RNA or protein product is produced in homozygous $orb2^{\Delta 36}$ animals. (Xu et al. 2012). Additionally, we take advantage the *Drosophila* GAL4-*UAS* system for targeted genetic knockdown of *orb2*. In this system, expression of the *Saccharomyces cerevisiae* transcription activation factor GAL4 is driven by a *Drosophila* cell-specific promotor. To achieve knockdown specifically in *Drosophila* neural stem cells, we utilized the promoter *worniu* (Lai et al. 2012; Albertson et al. 2004). In cells with GAL4 expression, GAL4 binds a DNA enhancer element called the upstream activating sequence (*UAS*) which regulates expression of a target gene. For genetic knockdown of *orb2*, the gene under control of the UAS is an $orb2^{RNAi}$ sequence, which results in the degradation of endogenous *orb2* mRNA such that little to no Orb2 protein product is capable of being translated in the cell (Duffy 2002).

Using this arsenal of genetically modified animals, we next investigated the effects of *orb2* perturbation on asymmetric centrosome maturation within the *Drosophila* neural stem cell. To do this, we dissected third larval instar central brains and chemically fixed the intact tissue to preserve the spatial localization of proteins and cells within the tissue (Lerit, Plevock, and Rusan 2014). The tissue was then subjected to immunofluorescent labeling. Immunofluorescence utilizes primary antibodies to detect the protein of interest, followed by a fluorescently tagged secondary antibody that recognizes the primary antibody allowing for amplification of signal. Stained larval brains were then visualized using a spinning disk confocal microscope, which excites the

fluorophores of the secondary antibodies using lasers of specified wavelengths. After excitation, the fluorophores emit a detectable photon, which is captured by a complementary metal oxide semiconductor (CMOS) camera. The camera converts the number of photons it detects into an image, in which the value of each pixel corresponds to the number of photons measured at that location. Therefore, areas with high concentrations of the protein of interest will have many primary and secondary antibodies bound, resulting in a high quantity of excited fluorophores and comparably higher pixel values (also called intensity) compared to areas of relatively lower protein concentration. These images can then be quantified to determine statistical differences in localized concentration of the probed targets (**Figure 2.7C**).

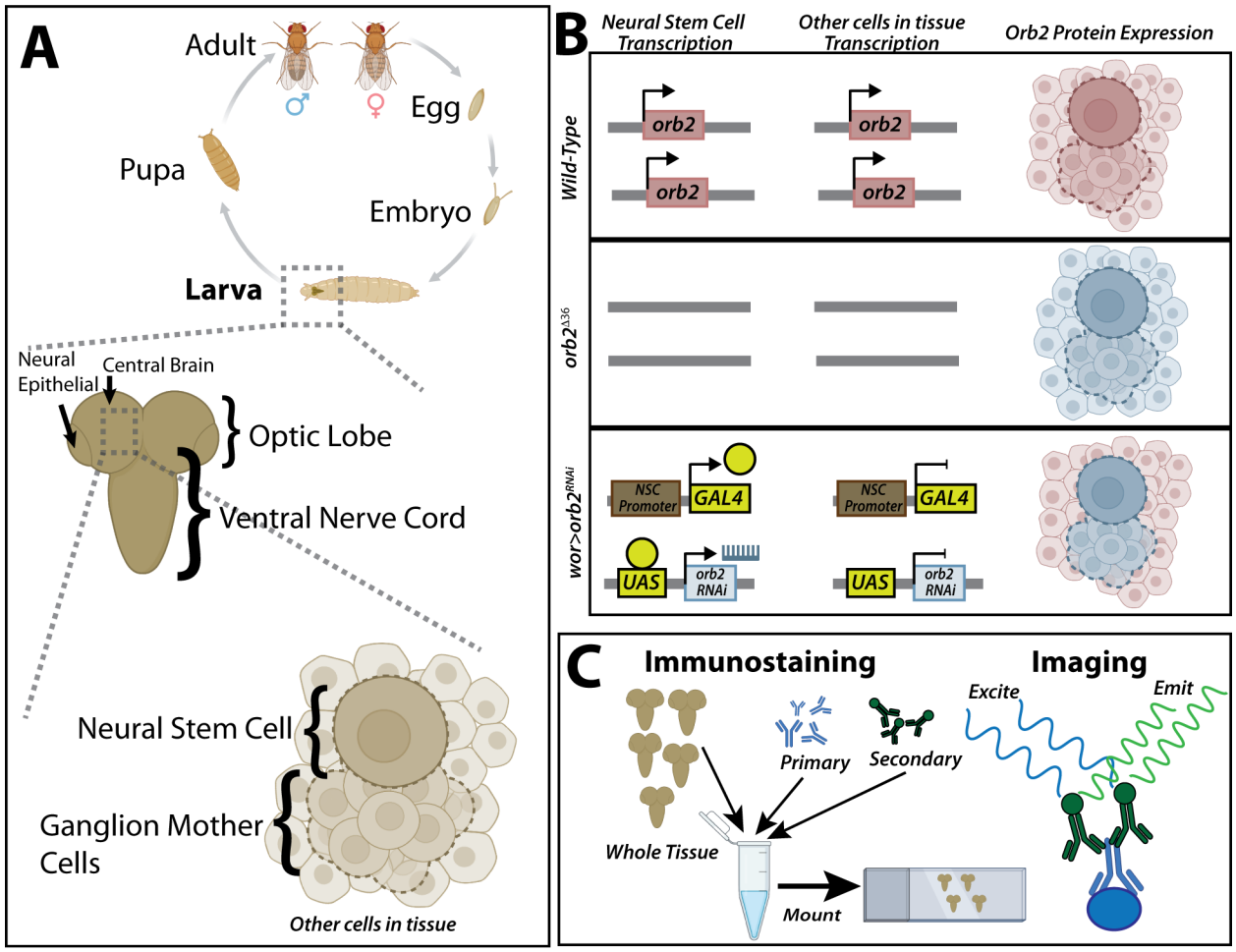


Figure 2.7 - Methodology of investigation

(A) The *Drosophila* life cycle has many distinct developmental stages. The larva stage is a late developmental stage. The developing central nervous system (CNS) located in the most anterior portion of the larva (dotted box), can be dissected out for further examination. The CNS (enlarged) is composed of two distinct morphological structures; two spherical optic lobes and a ventral nerve cord. The optic lobes can be morphologically defined further with the neural epithelium located peripheral to the central brain region (arrows). In the central brain region, hundreds of self-renewing neural stem cells (NSC) can be found amongst other neuronally derived cells. NSCs (enlarged) are distinct as they are physically larger and rounder than most other cells. Repeated rounds of asymmetric cell division generate a cluster of differentiating cells, the ganglion mother cells (GMCs). (B) *Drosophila* genetic lines relevant to this study. In wild-type control animals (top) endogenous expression of *orb2* (pink rectangle) is unaffected in all cell types. Endogenous levels of Orb2 protein (pink cells) are produced in all cells. In $orb2^{\Delta 36}$ animals (middle) the endogenous *orb2* genetic locus is completely removed from all cells (grey DNA lacking *orb2* gene). No Orb2 protein product is produced in any cells (blue). In $wor>orb2^{RNAi}$ animals (bottom) knockdown of Orb2 protein is cell-type specific. In NSCs, the NSC-promoter *worniu (*brown rectangle*)* drives expression of the yeast *GAL4* (top yellow rectangle). GAL4 protein (yellow circle) then drives $orb2^{RNAi}$ by binding to the upstream activation sequence (UAS; bottom yellow rectangle) which promotes transcription of the $orb2^{RNAi}$ locus (blue rectangle). The resulting siRNA (blue lines) binds to endogenous *orb2* and promotes its degradation, preventing Orb2 protein translation (blue neural stem cell). In other cell types, the *worniu* promoter is not activated, and no GAL4 protein is produced. Without GAL4 protein expression, the *orb2* siRNAi is not produced and Orb2 protein levels are unaffected (pink cells). (C) Immunofluorescence of whole larval brain tissue. Whole larval brains (brown) are dissected from live animals and fixed prior to immunostaining. Primary antibodies (blue) recognize and bind the target protein (blue circle) and are added to the brains. Secondary antibodies (green), which are conjugated to a fluorophore (green sphere) and recognize the specific primary antibodies, are then added. The brains are then mounted on a slide and imaged using a laser confocal microscope. A specific light wavelength (blue squiggle lines) excites the fluorophore of the secondary antibodies. After excitation, the fluorophore emits a different light wavelength (green squiggle lines), which can be detected by a fluorescent camera and converted into imaging data. Icons are from biorender.com.

2.3.3. Summary of Chapter Two

Centrosomes are microtubule-organizing centers that contribute to numerous cellular processes. Disruption of cellular function results in devastating, multisystem human syndromes. Centrosomes modulate their microtubule-nucleating activity through compositional and structural changes of the pericentriolar material which surrounds the centrioles. Although many centrosome genes are identified, the mechanisms that centrosomes utilize to transition between different pericentriolar material states is not well understood. Recent studies from our laboratory and others suggests RNA localized to the centrosome may play a functional role in modulating centrosome activity. Through bioinformatic analysis of centrosome genes, we implicate the CPEB protein as

a potential binding partner of centrosome RNAs. We also suggest a phenotypic parallel between CPEB proteins and the 3' UTR of centrosome genes in human cancers. Using quantitative imaging techniques of *Drosophila* larval neural stem cells, we investigate a previously undefined role for Orb2 in regulating centrosome activities.

2.4. Supplemental Information

Supplemental Table S2.1 – 3' UTR RNA sequences and analysis of *Drosophila* centrosome asymmetry genes and homologous human genes.
Excel file available as supplement file. Data used for 3' UTR analysis of *Drosophila* centrosome asymmetry genes and homologous human genes. (Tab 1; *Fly)* All *Drosophila* centrosome asymmetry genes along with relevant identifiers, chromosome location, RNA and protein isoforms, and 3'UTR sequence with CPE sites indicated (canonical $UUUU_{(n)}AU$ (light green), $UUUU_{(n)}A$ (dark green), $UUUU_{(n)}C$ (yellow), $UUUU_{(n)}G$ (red). Sequences displayed in **Figure 2.4** are highlighted grey. (Tab 2; *fly.to.human.homology*) DRSC Integrative Ortholog Prediction Tool (DIOPT) raw data indicating human homologous genes for *Drosophila* centrosome asymmetry genes. (Tab 3; *Human*) All human genes identified as a homologous *Drosophila* centrosome asymmetry gene along with relevant identifiers, RNA and protein isoforms, and 3'UTR sequence with CPE sites indicated (canonical $UUUU_{(n)}AU$ (light green), $UUUU_{(n)}A$ (dark green), $UUUU_{(n)}C$ (yellow), $UUUU_{(n)}G$ (red). Sequences displayed in **Figure 2.4** are highlighted grey.

Supplemental Table S2.2 – Data and analysis of primary site frequency across cancer cases.
Excel file available as supplement file. First three tabs display raw data downloaded from GDC. Tabs 4 and 5 are enrichment analysis. (Tab 1; *All Cases*) Data from all cases from the included studies. (Tab 2; *CPEB cases*) Data from cases which contained a mutation in a CPEB2-4 gene. (Tab 3; 3' UTR centrosome cases) Data from cases which contained a mutation in the 3' UTR of a centrosome asymmetry gene. (*Tab 4; Enrichment analysis*) Chi-squared goodness of fit analysis to determine if distribution of primary sites between either CPEB cases or 3' UTR cases differed from the expected primary site distribution of all cases included in the studies. *** P-value <0.001. (*Tab 5; Graph*) Sums for primary sites with the biggest fold-changes. Data was used in **Figure 2.6**)

Chapter 3. RNA-binding protein Orb2 causes microcephaly and supports centrosome asymmetry in neural stem cells

A version of this manuscript was submitted to
Molecular Biology of the Cell and currently under revision

Beverly V. Robinson, Junnan Fang, Dipen S. Mehta, Joseph Buehler, and Dorothy A. Lerit

Pre-Print Available
bioRxiv 2021.11.23.469707; doi: https://doi.org/10.1101/2021.11.23.469707

Experimentation

B.V.R– Conducted the experimentation and analysis performed in Figure 3.2, Figure 3.3, and Figure 3.4; aided in the troubleshooting of the western blot in Figure 3.5A; performed the analysis for the data in Figure 3.5C-E; conceptualized the model proposed in Figure 3.5F; conducted the experimentation and analysis for Supplemental Figure S3.1; aided in the mining and analysis of the data used in Supplemental Figure S3.2 and Supplement Table S3.1; performed analysis of data requested for revisions.

J.F - Aided in the troubleshooting and conducted the experimentation and analysis performed in Figure 3.5A,B.

D.S.M– Aided in the troubleshooting and conducted the experimentation and analysis performed in Figure 3.5A,B; performed analysis of data requested for revisions.

J.D.B– Aided in the mining and analysis of the data used in Supplemental Figure S3.1 and Supplement Table S3.1.

D.A.L– Conducted the experimentation and analysis performed in Figure 3.1; performed the experimentation for the data presented in Figure 3.5C-E; conducted experimentation and analysis for requested revisions.

Writing

B.V.R – Original draft, review & editing

J.F - Review & editing

J.D.B - Review & editing

D.A.L - Original draft, review & editing

3.1. Abstract

To maintain a balance of self-renewal versus neurogenesis, neural stem cells (NSCs) undergo asymmetric cell division along an invariant polarity axis instructed by centrosomes. During interphase, the NSC centrosomes are defined by marked asymmetries in protein composition and functional activity as microtubule-organizing centers. Here, we show a conserved RNA-binding protein, Orb2, supports centrosome asymmetry in interphase NSCs. While Orb2 localizes to the active apical centrosome, it promotes the transient inactivation of the basal centrosome required for centrosome segregation and spindle morphogenesis. Orb2 is required cell autonomously within NSCs to support centrosome asymmetry and maintenance of the stem cell pool. We suggest Orb2 plays opposing roles in centrosome activation and inactivation, possibly through the translational regulation of multiple mRNA substrates. Conversely, loss of *orb2* manifests in microcephaly independent of Orb2 function in NSCs. Bioinformatics uncovers a significant overlap among RNA targets between *Drosophila* Orb2 and human CPEB4, consistent with a conserved role for CPEB proteins in centrosome regulation and neurodevelopment.

3.2. Introduction

Neural stem cells (NSCs) undergo asymmetric cell division (ACD) along an invariant apical-basal polarity axis to segregate cell fate determinants, giving rise to two differentially fated progeny: a self-renewing stem cell and a ganglion mother cell (GMC) destined for neural differentiation (Doe et al. 1991; Knoblich, Jan, and Jan 1995; Kraut et al. 1996; Broadus and Doe 1997). This balance in NSC self-renewal is critical for neurogenesis, as its deregulation can lead to brain tumors or neurodevelopmental disorders, such as microcephaly (Bond et al. 2002; Cabernard and Doe 2009). Key to NSC homeostasis are centrosomes, which instruct the division axis and organize the bipolar mitotic spindle required to segregate the pro-stem and pro-

differentiation cell fate determinants (Cabernard and Doe 2009; Januschke and Gonzalez 2010; Wang et al. 2011).

Centrosomes are microtubule (MT)-organizing centers (MTOC) consisting of a central pair of centrioles surrounded by pericentriolar material (PCM), which recruits the γ-Tubulin (γTub) ring complex required for MT nucleation (Conduit, Wainman, and Raff 2015). Normally, centrosomes recruit the robust levels of PCM necessary for microtubule-nucleating activity just before mitotic onset, a process called centrosome maturation (Gould and Borisy 1977; Khodjakov and Rieder 1999). Following mitotic exit, centrosomes shed PCM.

In NSCs, however, centrosomes are subject to an asymmetric centrosome maturation cycle, wherein the apical (daughter) centrosome recruits PCM and organizes MTs, while the basal-fated (mother) centrosome is transiently inactivated until mitotic onset (Rusan and Peifer 2007; Rebollo et al. 2007; Conduit and Raff 2010; Januschke et al. 2011). NSC centrosome asymmetry is implicated in apical-basal spindle pole alignment and centrosome segregation (Januschke and Gonzalez 2010; Januschke et al. 2013; Lerit and Rusan 2013; Ramdas Nair et al. 2016). A basic molecular framework required for NSC centrosome asymmetry involves asymmetric localization of Centrobin (Cnb) and Polo kinase to the daughter centrosome in a mechanism also requiring Wdr62 to promote centrosome maturation (Ramdas Nair et al. 2016; Januschke et al. 2013; Gallaud et al. 2020). Conversely, transient inactivation of the basal centrosome requires Bld10/Cep135, Pericentrin-like protein (PLP), and Polo-like kinase 4 (PLK4/SAK) (Lerit and Rusan 2013; Singh, Ramdas Nair, and Cabernard 2014; Gambarotto et al. 2019). Nevertheless, how centrosome asymmetry is regulated remains incompletely understood.

Intriguingly, *Cnb*, *Cep135*, *plp*, *polo*, and *Wdr62* mRNAs were identified as putative mRNA targets for the RNA-binding protein (RBP) Orb2 through an unbiased transcriptomics

study, raising the possibility that Orb2 might regulate centrosome asymmetry in NSCs (Stepien et al. 2016). Orb2 is a member of the cytoplasmic polyadenylation element binding (CPEB) proteins orthologous to mammalian CPEB2–4 and implicated in mRNA localization and translational control (Huang et al. 2006; Keleman et al. 2007; Hafer et al. 2011). Although prior work supports a role for *orb2* in NSC spindle orientation and neuronal specification, whether Orb2 contributes to centrosome regulation is unknown (Hafer et al. 2011).

Here, we identify an NSC-autonomous role for Orb2 in establishing centrosome asymmetry associated with misaligned spindles and NSC loss. We also identify an NSC-independent role for Orb2 in regulating brain size, as *orb2* loss leads to microcephaly. Finally, we examine potential targets of Orb2 and propose a revised model of asymmetric centrosome maturation.

3.3. Results and Discussion

3.3.1. Orb2 localizes to centrosomes within cycling NSCs

To investigate whether Orb2 functions within larval brain NSCs to support centrosome asymmetry, we first examined endogenous Orb2 localization using monoclonal antibodies (Hafer et al. 2011). Although Orb2 localizes to neuronal lineages, subcellular localization of Orb2 is not well defined (Hafer et al. 2011; Keleman et al. 2007). We visualized Orb2 relative to the centriole marker Asterless (Asl; (Varmark et al. 2007)) and the PCM marker Centrosomin (Cnn; (Megraw et al. 1999; Vaizel-Ohayon and Schejter 1999)). Orb2 appeared dispersed throughout the cytoplasm with a notable enrichment on the apical centrosome (**Figure 3.1A**, *interphase*). To directionally measure protein localization to centrosomes, we calculated an asymmetry index (AI; *Methods*). Apical centrosome enrichment of Orb2 was observed in N=21/24 interphase NSCs, indicated by positive Orb2 AI values (**Figure 3.1B**). On average, interphase apical centrosomes

contained 1.7-fold more Orb2 than basal centrosomes (**Figure 3.1C**; $p<0.01$ by t-test). Upon mitotic entry, Orb2 localization increased at both centrosomes, demonstrating Orb2 is recruited to active centrosomes during centrosome maturation (**Figure 3.1A**, *prophase* and *metaphase*). Previous work indicates CPEB proteins localize to centrosomes in *Xenopus* oocytes, embryos, and cultured mammalian cells (Groisman et al. 2000; Pascual et al. 2020; Eliscovich et al. 2008). Our localization analysis similarly reveals Orb2 enrichments at active NSC centrosomes, raising the possibility that Orb2 may normally function to regulate centrosome activity locally at centrosomes.

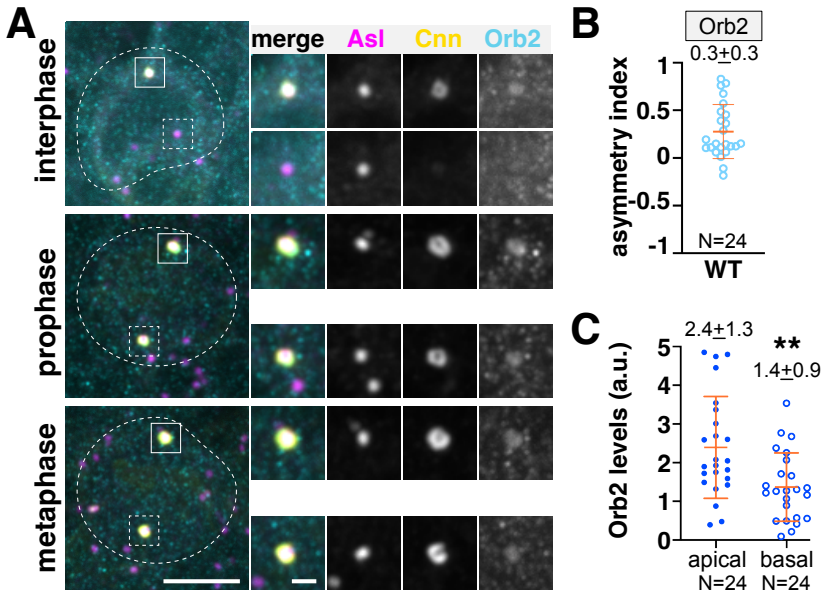


Figure 3.1 - Orb2 localizes to active NSC centrosomes.
(A) Maximum-intensity projections of WT NSCs (dashed circles) stained for Asl (magenta), Cnn (PCM; yellow), and Orb2 (cyan). Solid and dashed boxes note apical vs. basal centrosomes; enlarged at right. (B) Orb2 AI and (C) Orb2 levels on apical vs. basal centrosomes quantified from N=24 late interphase NSCs. Mean + SD displayed; **, p=0.003 by Welch's t-test. Bars: 5 µm, 1 µm (inset).

3.3.2. Orb2 disrupts centrosome activity in interphase NSCs

To determine if Orb2 contributes to centrosome asymmetry, we examined γTub distributions at apical and basal centrosomes in wild-type (WT) vs. *orb2* null mutant NSCs during late interphase, when centrosomes are normally asymmetric (Rusan and Peifer 2007; Rebollo et

al. 2007). As anticipated, Asl displayed symmetric distributions among apical and basal centrosomes in both genotypes (mean AI ± S.D.= 0.0±0.2 for WT and 0.1±0.3 for *orb2*; **Figure 3.2A–C**). In comparison, γTub was significantly enriched on the apical centrosome in WT NSCs (**Figure 3.2A, C**). In contrast to WT, *orb2* NSCs showed impaired centrosome asymmetry, evident by increased γTub localization to the basal centrosome and decreased AI values (**Figure 3.2B, C**). γTub AI was reduced by over 20% within *orb2* NSCs, as compared to WT (**Figure 3.2C**; p=0.05 by Kolmogorov-Smirnov test). Consistently, ~30% of *orb2* NSCs (N= 12/40) had γTub AI values >2 S.D. from the WT mean (**Figure 3.2D**; $p< 0.0001$ by chi-square test). Measuring the levels of γTub localized at the apical and basal centrosomes revealed a 2.7-fold increase in γTub recruitment to the basal centrosomes of symmetrized *orb2* NSCs relative to WT (**Figure 3.2E**; $p<0.0001$ by Mann-Whitney test). In parallel, γTub recruitment to the apical centrosomes trended lower in *orb2* NSCs. We conclude Orb2 primarily promotes centrosome asymmetry during interphase by blocking the precocious activation of the basal centrosome.

Considering Orb2 is more enriched at the apical centrosome, Orb2-dependent repression of the basal centrosome during interphase likely occurs from a distance, perhaps from within the cytoplasm. In contrast, Orb2 localized to active centrosomes is permissive for PCM recruitment. These data showcase opposing functions of Orb2 at the apical versus basal centrosomes.

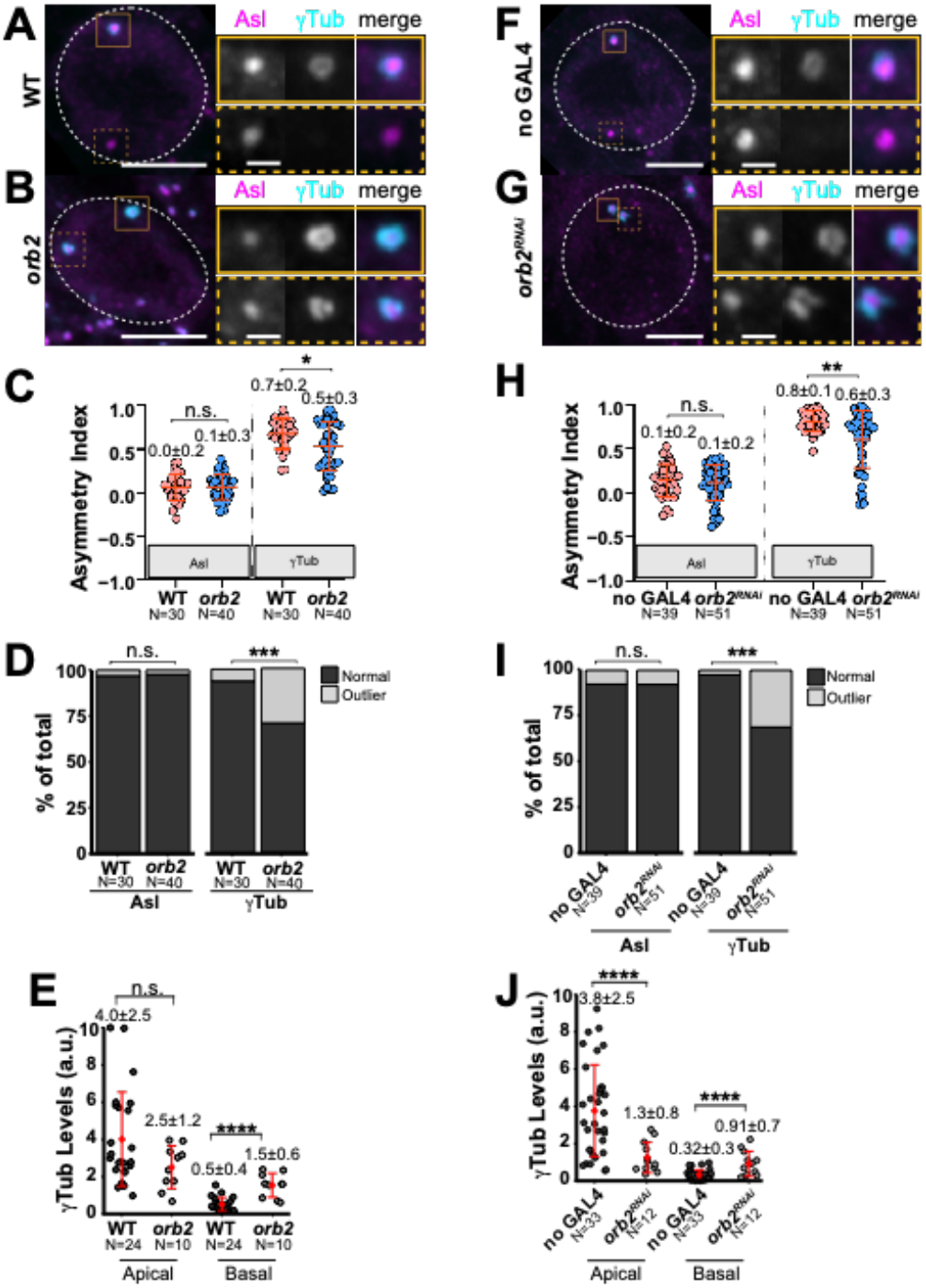


Figure 3.2 - Orb2 contributes to centrosome asymmetry.
Maximum intensity projections of interphase NSCs (dashed circles) stained for Asl (centrioles, magenta) and γTub (PCM, cyan). Solid and dashed boxes mark apical vs. basal centrosomes; enlarged at right. (A) WT NSC with γTub enriched at the apical centrosome. (B) *orb2* NSC with γTub at both centrosomes. (C) AIs of Asl and γTub in N=30 NSCs from n=8 WT brains and N=40 NSCs from n=10 *orb2* brains. Each dot is a measurement from one cell. (D) Frequency distribution of Asl and γTub AIs in WT vs. *orb2* NSCs. Light grey (outlier) values are > 2 S.D. from the control mean. (E) Scatter plot of γTub levels at apical vs. basal centrosomes in N=24 NSCs from n=8 WT brains and N=10 symmetrized NSCs from n=7 *orb2* brains

(defined as AI >2 S.D. from WT mean). (F) no GAL4 control NSCs resemble WT. (G) *orb2*^{RNAi} NSC with Tub at both centrosomes. (H) AIs from N=39 NSCs from n=6 no GAL4 brains and N=51 NSC from n=6 *orb2*^{RNAi} brains. (I) Frequency distribution of Asl and γTub AIs in control vs. *orb2*^{RNAi} brains. (J) Scatter plot of γTub at centrosomes from N=39 NSCs from n=6 control brains and N=16 NSCs from n=6 worGAL4>*orb2*^{RNAi} brains. Mean ± SD displayed. The experiments were repeated >3 independent replicates and significance determined by (C, H) Kolmogorov-Smirnov test, (D, I) chi-square test, (E, J) Mann-Whitney test: n.s., not significant; *, p<0.05; **p<0.01 ***, p<0.001; and ****, p<0.0001. Bars: 5 μm, 1 μm (insets).

3.3.3. Orb2-dependent centrosome regulation is cell autonomous

Asymmetric cell division is regulated through intrinsic and extrinsic cellular pathways (Siegrist and Doe 2006; Doe 2008). To elucidate if the reduction of centrosome asymmetry observed in *orb2* mutants arose from a requirement for Orb2 within NSCs, we depleted *orb2* specifically in NSCs using an *orb2* dsRNA transgene (*UAS-orb2*^{RNAi}) driven by the NSC-specific *worniu* (*wor*)-*GAL4* (Albertson et al. 2004). Both the no *GAL4* control and the *wor-GAL4>orb2*^{RNAi} (hereafter, *orb2*^{RNAi}) interphase NSCs showed equal distributions of Asl at apical and basal centrosomes (**Figure 3.2F–H**). While controls appeared WT with an enrichment of γTub on the apical centrosome, a subset of *orb2*^{RNAi} NSCs (~30%; N=16/51) recruited γTub to the basal centrosome precociously and showed reduced γTub at the apical centrosome (**Figure 3.2G–J**). These data indicate Orb2 promotes centrosome asymmetry cell autonomously within NSCs and further implicate differential regulation of apical versus basal centrosomes by Orb2. The basal centrosome is more sensitive to Orb2 loss, as suggested by the ~3-fold γTub enrichment observed in *orb2* mutant or *orb2*^{RNAi} NSCs. Orb2 may repress basal centrosome activity either by repressing pro-maturation factors (e.g., *Cnb*, *polo*, or *Wdr62* mRNAs), and/or by promoting the expression of factors required for basal centrosome inactivation, such as Cep135 or PLP.

3.3.4. Loss of *orb2* is associated with supernumerary centrosomes

The precocious activation of the basal centrosome is associated with errant centrosome segregation during asymmetric cell division, resulting in both centrosomes retained within the self-renewing stem cell (Lerit and Rusan 2013). To assay whether *orb2* loss similarly impairs centrosome segregation, we quantified the frequency of supernumerary centrosomes in WT and *orb2* NSCs. While >90% WT NSCs (N=25/30) had the expected 2 centrosomes, ~35% *orb2* mutant NSCs (N=15/40) had extra centrosomes (**Figure 3.3A–C**; $p<0.05$ by chi-square test). A similar frequency of supernumerary centrosomes was observed in $orb2^{RNAi}$ NSCs (~25%; N=13/51; $p<0.05$ by chi-square test), demonstrating Orb2 functions within NSC to regulate centrosome number (**Figure 3.3D–F**). Because the two active interphase centrosomes observed upon loss of *orb2* are confined to the apical half of the NSC (**Figure 3.2B, G**), these are likely retained within the stem cell and amplified in the next cell cycle.

3.3.5. Orb2 is required for mitotic spindle morphogenesis

Given that Orb2 helps regulate centrosome activity and segregation, we next examined its role in spindle orientation. During asymmetric cell division, the NSC mitotic spindle normally orients along an invariant apical-basal polarity axis entrained by the concerted action of multiple protein complexes (Siegrist and Doe 2005; Siller, Cabernard, and Doe 2006; Cabernard and Doe 2009). Localization of Bazooka (Baz)/Par-3 to the apical cortex in late interphase initiates NSC polarization (Wodarz and Huttner 2003; Knoblich 2008), while localization of the adapter protein Miranda (Mira) to the basal cortex during mitosis represents a late polarization step (Shen et al. 1998; Rolls et al. 2003; Atwood and Prehoda 2009). WT and *orb2* mutant NSCs showed similar distributions of Baz and Mira to the apical and basal cortices, respectively, indicating that

polarization is not significantly disrupted in *orb2* larval NSCs (**Figure 3.3G–I**; n.s. by chi-square test).

Prior work identified *αpkc* mRNA as a target for Orb2 translational repression in adult brains and testes (Mastushita-Sakai et al. 2010; Xu, Tyagi, and Schedl 2014). Further, loss of *orb2* impairs αPKC localization in the testes and embryonic NSCs, the later also showing disrupted spindle orientation (Hafer et al. 2011; Xu, Tyagi, and Schedl 2014). However, our findings indicate larval NSCs polarize correctly in the absence of Orb2, arguing Orb2 may have distinct functions and mRNA targets within the larval brain.

We next used multiple coordinate analysis to define spindle orientation in mitotic WT and *orb2* NSCs stained for β-Tubulin (βTub) to label MTs, Baz, and Asl (see *Methods*; **Figure 3.3J–L**). First, we measured spindle orientation (θ_1), the angle between the apical cortical polarity and spindle alignment axes (**Figure 3.3J, M–N**). In WT, most spindles aligned within 30° of the polarity axis, yet ~20% of *orb2* NSC spindles were misoriented >30°, indicating *orb2* spindles are more randomized than controls (**Figure 3.3M, N**; $p<0.0001$ by chi-square test).

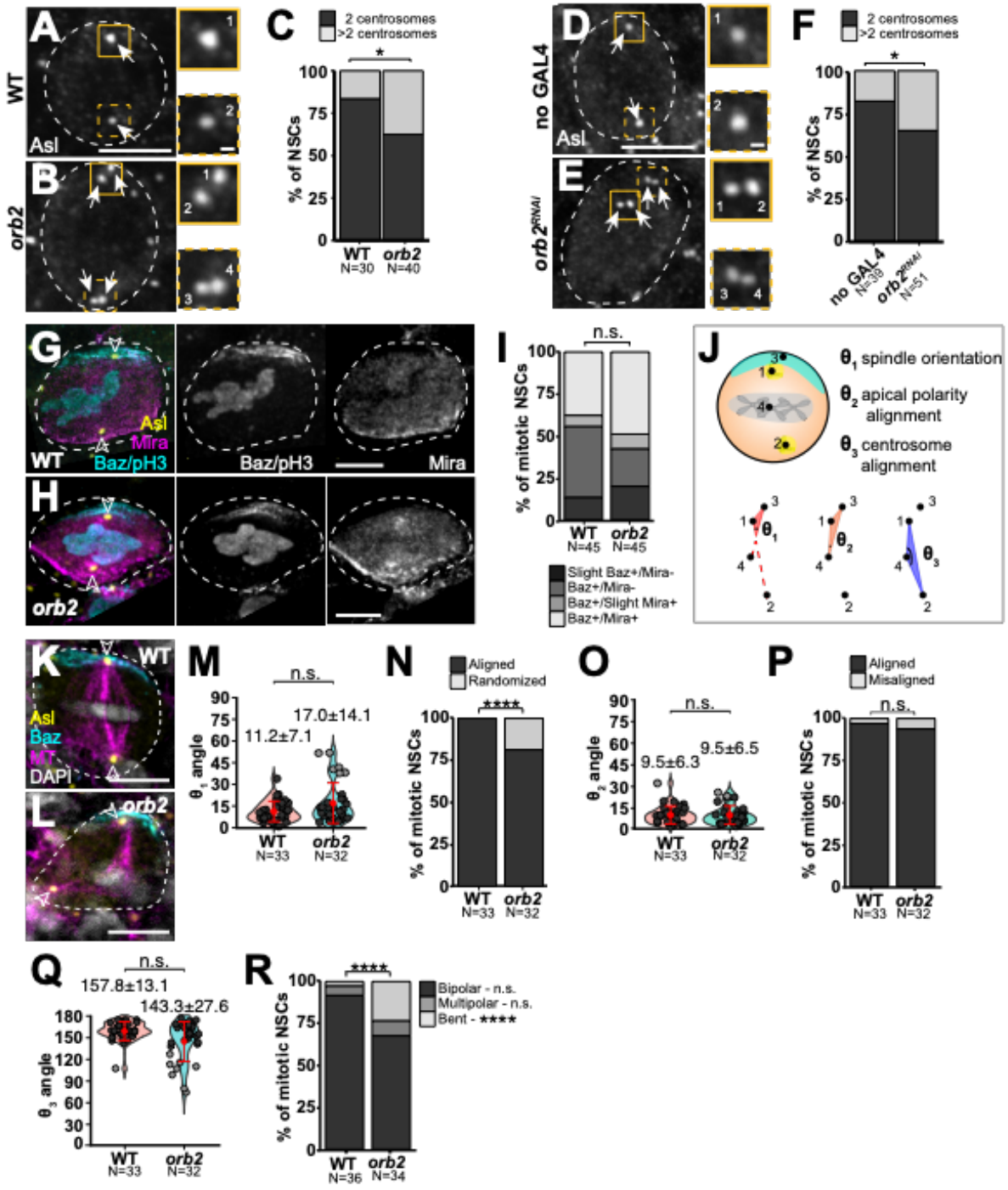


Figure 3.3 - Orb2 is required for centrosome segregation and spindle morphogenesis.
Maximum intensity projections of interphase NSCs (dashed circles) from (A) WT, (B) *orb2*, (D) no GAL4 control, and (E) *orb2*^{RNAi} stained for Asl (arrows). Solid and dashed boxes mark apical vs. basal centrosomes; enlarged at right. (C and F) Frequency distributions of supernumerary centrosomes in interphase NSCs. (G) WT and (H) *orb2* NSCs stained for Baz (apical cortex, cyan), pH3 (mitotic, cyan), Mira (basal cortex, magenta), and Asl (yellow; arrowheads). (I) Frequency distribution of apical Baz and/or basal Mira crescents in mitotic NSCs. (J) Cartoon depicts points used to measure within mitotic NSCs: θ_1 spindle orientation, θ_2 apical polarity alignment, and θ_3 centrosome alignment (Methods). (K) WT and (L) *orb2* NSCs stained for MTs (βTub, magenta), Asl (yellow), Baz (cyan), and DAPI (DNA, grey). (M) Plot

and (N) frequency distribution of θ_1 spindle orientation. (O) Plot and (P) frequency distribution of θ_2 apical polarity alignment. (Q) Plot and (R) frequency distribution of θ_3 centrosome alignment from N=33 WT and N=32 *orb2* mitotic NSCs from a single experiment across two replicates. Light grey datapoints are >2 SD from the WT mean. Mean ± SD indicated; significance determined by (C, F, I, N, P, R) Chi-squared test or (M, O, Q) Two-tailed t-test. n.s., not significant; *, $p<0.05$; and ****, $p<0.0001$. Bars: 5 µm; 1 µm (insets).

To ascertain how defects in spindle orientation arise, we next quantified apical polarity alignment, θ_2, the angle between the apical centrosome and polarity axes (**Figure 3.3J, O–P**). The anchoring of the apical centrosome to the apical cortex happens during early interphase and influences polarization in NSCs and other stem cells (Yu et al. 2003; Januschke and Gonzalez 2010; Inaba, Venkei, and Yamashita 2015). Both WT and *orb2* NSCs showed alignment of the apical centrosome to the apical polarity axis, consistent with our observations that apical polarity is unaffected in *orb2* mutants (**Figure 3.3G-I, O-P**).

Finally, we examined centrosome alignment, θ_3, the angle between the apical and basal centrosome axes (**Figure 3.3J, Q-R**). About 90% of WT mitotic NSCs aligned the basal centrosome ~180° away from the apical centrosome (**Figure 3.3K, Q-R**). In contrast, θ_3 is reduced in *orb2* NSCs (**Figure 3.3L, Q**). While the average reduction in θ_3 is not statistically significant, a subpopulation of *orb2* NSCs (~20%; N=7/32 cells) show defective θ_3 <120° (**Figure 3.3L, Q**). Consequently, spindle morphogenesis is significantly impaired, resulting in a higher frequency (~25%; N=9/34 cells; $p<0.0001$ by chi-square test) of bent spindles in *orb2* NSCs relative to WT (**Figure 3.3R**). Taken together, these data argue the precocious activation of the basal centrosome in *orb2* NSCs during interphase impairs centrosome migration to the basal cortex, resulting in aberrant spindle alignment and morphology. However, it remains formally possible that *orb2* may have distinct functions in interphase centrosome asymmetry and mitotic spindle orientation.

3.3.6. Loss of *orb2* results in microcephaly

Defects in centrosome regulation contribute to neurodevelopmental disorders, including microcephaly and intellectual disability (Robinson, Faundez, and Lerit 2020). Previous work illustrates *orb2* is crucial for learning and memory in the adult *Drosophila* brain (Keleman et al. 2007; Mastushita-Sakai et al. 2010; Kruttner et al. 2012; Kacsoh et al. 2015; Hervas et al. 2016; Sanguanini and Cattaneo 2018). Moreover, reduced brain volumes were noted from serial sectioning adult *orb2* brains (Kruttner et al. 2012). Thus, to assess larval neurodevelopment in *orb2* mutants, we measured the volume of single optic lobes from age-matched third instar larva (Link et al. 2019). Compared to WT brains, *orb2* brains were significantly smaller (**Figure 3.4A,B,E**; $p<0.0001$ by t-test). About 75% (N=10/13 brains) of *orb2* brains had volumes < 2-S.D. from WT, consistent with microcephaly.

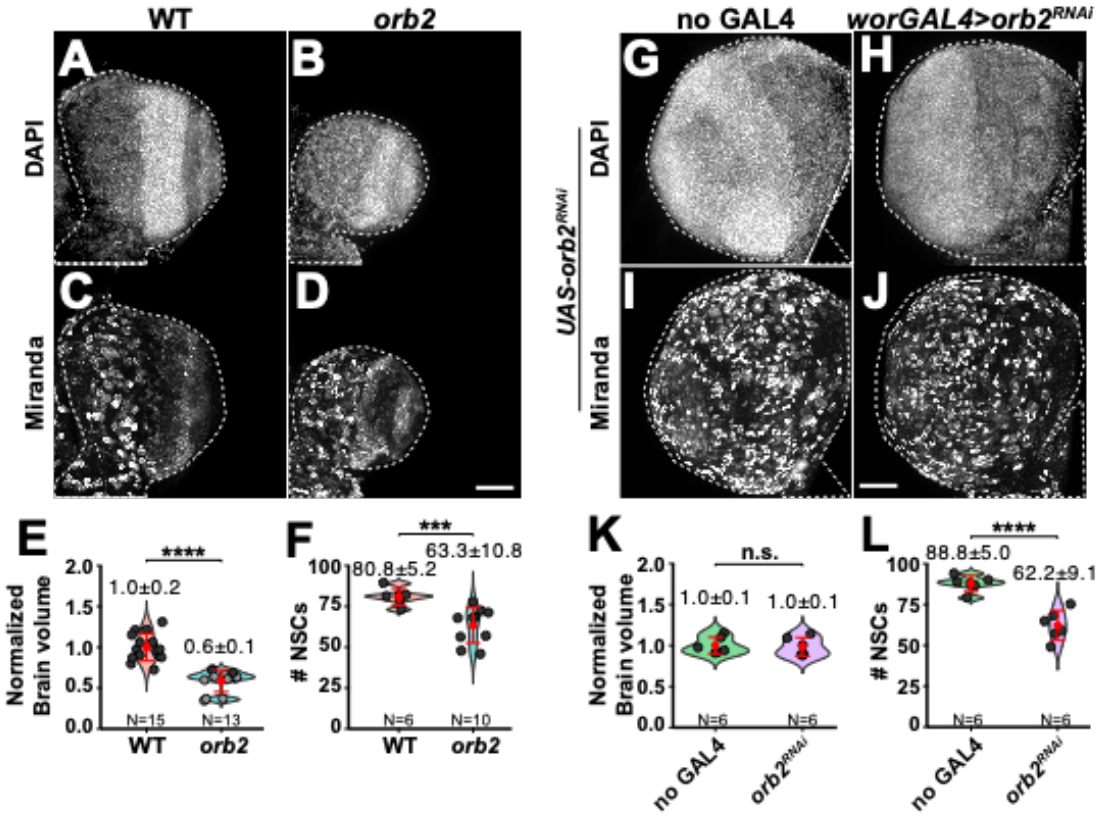


Figure 3.4 - NSC-autonomous and non-autonomous Orb2 activities support neurodevelopment.

Projected third instar larval optic lobes (dashed lines) stained for DAPI or Mira. In age-matched brains, (A) WT are larger than (B) *orb2* and (C) WT have more NSCs than (D) *orb2*. (E) Volume quantified from N=15 WT and 13 *orb2* brains. (F) Quantification of NSCs in N=6 WT and 10 *orb2* brains. In age-matched brains, (G) no GAL4 controls are sized as (H) $orb2^{RNAi}$ brains. Yet, (I) no GAL4 controls have more NSCs than (J) $orb2^{RNAi}$. (K) Volume and (L) NSC counts quantified from N=6 brains per genotype. Brain volumes were measured from one lobe per brain and normalized to the control mean (Link et al., 2019). Mean ± SD displayed. Significance determined by Student's t-test: n.s., not significant; *, $p<0.05$***; and $p<0.001$. Bars: 40 μm.

To determine if NSC loss contributes to *orb2*-dependent microcephaly, we counted the number of Mira+ NSCs in control and *orb2* brains. While WT had ~80 NSCs, *orb2* mutants showed a ~20% reduction with ~60 NSCs per lobe (**Figure 3.4C,D,F**; $p<0.001$ by t-test). These data indicate Orb2 is required to maintain the NSC pool.

Are the centrosome and spindle defects observed in *orb2* mutants correlated with NSC loss and/or microcephaly? To begin to address this question, we depleted *orb2* specifically in NSCs and compared the brain volume of age matched $orb2^{RNAi}$ brains relative to no *GAL4* controls and noted no difference (**Figure 3.4G,H,K**), indicating *orb2*-dependent microcephaly is nonautonomous to NSCs – Orb2 is required in other cell types to affect brain size. In contrast, NSC loss is a cell autonomous response to *orb2* depletion. Similar to *orb2* mutants, we detected ~30% fewer NSC per optic lobe in $orb2^{RNAi}$ brains relative to no *GAL4* controls (**Figure 3.4I,J,L**; $p<0.0001$ by t-test). That the frequency of NSC loss in *orb2* mutants is similar to the incidence of centrosome defects argues these responses are correlated. Moreover, NSC loss is separable from *orb2*-dependent microcephaly. Consistent with the idea that Orb2 supports neurodevelopment in multiple cellular lineages, high levels of Orb2 were detected in the cytoplasm of numerous (non-NSC) cells within the larval brain (Hafer et al. 2011).

To elucidate mechanisms underlying NSC loss in *orb2* mutants, we first tested the hypothesis that NSCs are eliminated by cell death. We quantified the coincidence of pro-apoptotic

cleaved Caspase 3 (CC3; (Fan and Bergmann 2010)) in cells marked with the NSC-specific Deadpan (Dpn) antibody (Bier et al. 1992). Similar rates of apoptosis were observed in WT and *orb2* NSCs, indicating NSC loss occurs by other mechanisms (**Supplemental Figure S3.1A–C**; n.s. by t-test). Another mechanism whereby NSCs may be depleted is via premature differentiation (Cabernard and Doe 2009; Lai and Doe 2014; Abdel-Salam et al. 2020). Normally, the pro-differentiation marker Prospero (Pros) is confined to the nucleus of the differentiating GMCs and absent from NSCs (Vaessin et al. 1991; Doe et al. 1991), while retention of Pros in the NSC nucleus promotes premature differentiation (Lai and Doe 2014). We quantified the coincidence of Pros with Dpn and detected no significant difference relative to WT, indicating premature differentiation does not precipitate NSC loss in *orb2* mutants (**Supplemental Figure S3.1**D–F; n.s. by t-test).

Finally, we examined whether *orb2* affects mitotic progression, reasoning impaired NSC self-renewal might contribute to NSC loss. However, the mitotic indices in WT and *orb2* brains were not significantly different (33.6$\pm$4.9% per lobe in N=6 WT vs. 36.3$\pm$10.1% in N=10 *orb2* brains; **Supplemental Figure S3.1**G–I; n.s. by t-test). These data suggest NSC loss is not due to increased quiescence. Loss of NSCs observed in *orb2* mutants may be actuated by non-apoptotic cell death pathways. Alternatively, the altered neuronal specification observed in *orb2* embryos may impinge upon larval neurodevelopment, resulting in fewer NSCs (Hafer et al. 2011).

3.3.7. Orb2 regulates PLP protein levels in larval brains

Orb2 is an RNA-binding protein known to promote or repress translation of its target mRNAs (Mastushita-Sakai et al. 2010; Xu et al. 2012; Khan et al. 2015). Aspects of the *orb2* null phenotype in NSCs resemble *plp* loss, as both mutants display precocious activation of the basal

centrosome in interphase NSCs coincident with supernumerary centrosome and spindle defects (Lerit and Rusan 2013). Given these similarities, we tested if Orb2 regulates PLP protein expression by comparing PLP levels in WT vs. *orb2* larval brain extracts. Semi-quantitative western blotting uncovered an average 30% reduction in PLP in *orb2* relative to WT, suggesting Orb2 promotes PLP translation (**Figure 3.5A, B**). To assay a requirement for Orb2 in regulating PLP in NSCs, we examined PLP localization to the apical versus basal centrosomes in interphase NSCs. In WT NSCs, PLP was enriched on the inactive, basal centrosome, consistent with prior work (**Figure 3.5C**; (Lerit and Rusan 2013; Singh, Ramdas Nair, and Cabernard 2014). Despite a reduction in PLP levels in whole brain extracts, robust localization of PLP to centrosomes was detected in *orb2* NSCs, comparable to WT (**Figure 3.5C, D**; n.s. by Kolmogorov-Smirnov test). Further, levels of PLP localized at apical vs. basal centrosomes were not significantly different from WT (**Figure 3.5E**). Taken together, these data imply Orb2-dependent regulation of PLP may occur outside of NSCs. While the consequences of PLP regulation by Orb2 remain unclear, they are unlikely to impinge upon microcephaly, as *plp* null animals have normal sized brains (Martinez-Campos et al. 2004). We conclude Orb2 likely regulates other mRNA targets to affect centrosome asymmetry and brain volume.

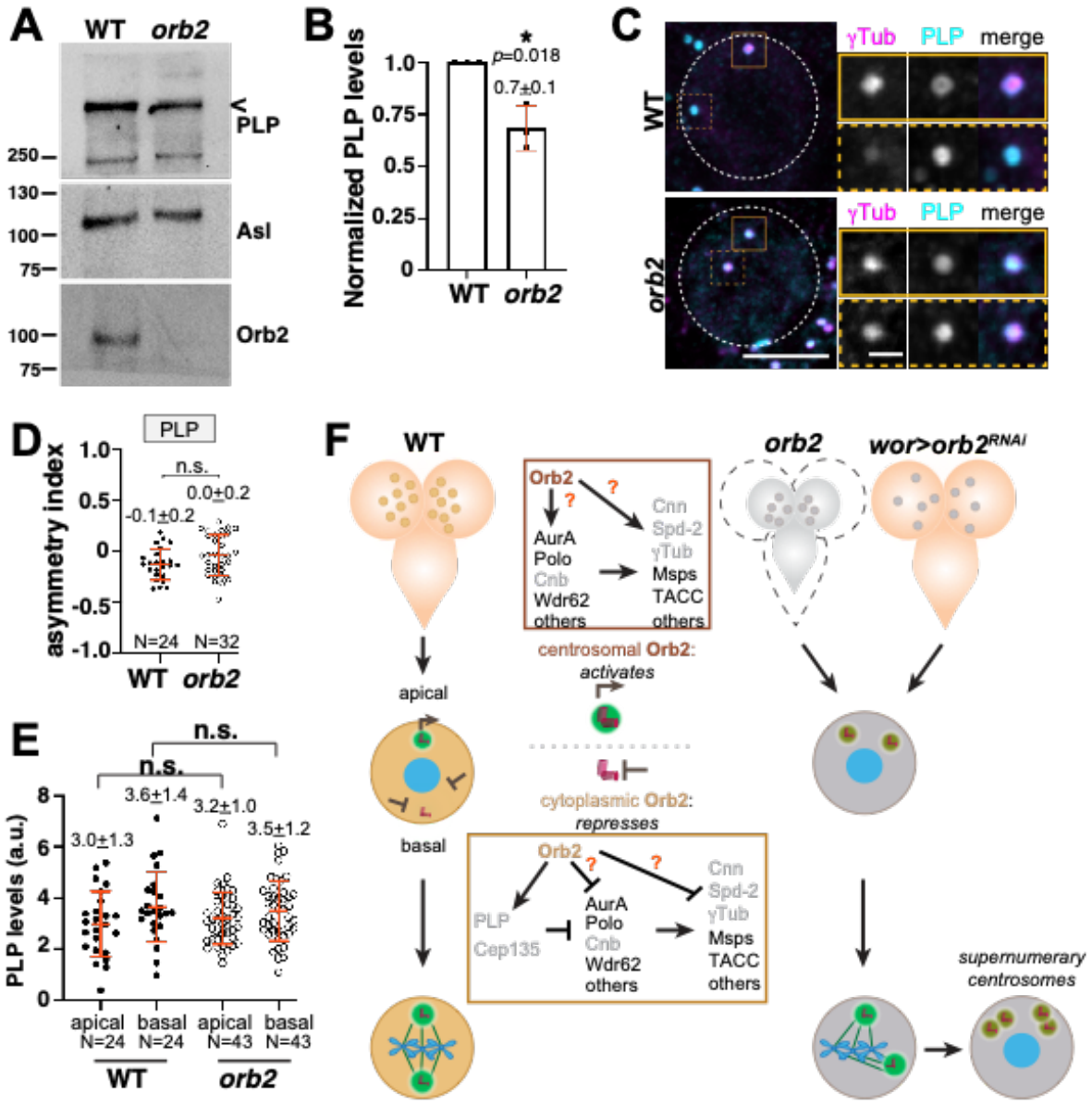


Figure 3.5 - Orb2 regulates PLP protein levels.
(A) Immunoblot of PLP, Asl, and Orb2 proteins from WT and *orb2* third instar larval brain extracts. (B) Normalized (to Asl) relative PLP levels in WT and *orb2* larval brains from 3 biological replicates. (C) Max projected WT and *orb2* NSCs (dashed circles) stained for γTub (magenta) and PLP (cyan). Solid and dashed boxes note apical vs. basal centrosomes; enlarged at right. (D) PLP AI calculated from N=24 WT and 32 *orb2* interphase NSCs. (E) PLP localization to apical and basal centrosomes in N=24 WT vs. N=43 *orb2* interphase NSCs. Data representative from a single experiment across 2 replicates. (F) Model depicts NSC-autonomous function of Orb2 to support interphase centrosome asymmetry and NSC-independent requirement for normal brain volume. Loss of Orb2 activity (grey cells) impairs basal centrosome inactivation (PCM, green). Centrosome-localized Orb2 (WT apical; top box) enhances centrosome activation, perhaps by translational activation of one or more targets (black, Orb2 target BC>3; see **Supplement Table S3.1**). Conversely, cytoplasmic Orb2 supports basal centrosome inactivation (bottom box), required for centrosome separation, spindle orientation, and centrosome segregation to the daughter

cells. Mean + SD displayed; *, $p<0.05$ by unpaired, one-tailed t-test; n.s., not significant. Uncropped, replicated blots are available to view: https://doi.org/10.6084/m9.figshare.17052722 Bars: 5 μm; insets 1 μm.

To refine the list of targets that may be involved in centrosome regulation and neurodevelopment, we compared the Orb2 mRNA targets identified in *Drosophila* S2 cells (Stepien et al. 2016) to a recent list of mRNA targets bound by CPEB4 in HeLa cells (Pascual et al. 2020) and identified 1083 overlapping genes (**Supplemental Figure S3.2A**; **Supplement Table S3.1**). Gene ontology (GO) analysis for cellular components uncovered a significant enrichment of organelle terms (**Supplemental Figure S3.2B**). In contrast, centrosome-related ontologies were not significantly enriched among the overlapping genes (**Supplemental Figure S3.2C**). However, because Orb2 has multiple orthologs, CPEB2-4, other putative Orb2 targets absent from the CPEB4 dataset are omitted from these pairwise analyses. We examined centrosome-related ontologies of Orb2 targets with a biologic complexity (BC) $\geq$ 3, a metric of reproducibility across biological replicates (Licatalosi et al. 2008). This analysis identified 2150 mRNAs, including 53 mRNAs annotated with centrosome ontologies, which may be subject to Orb2 regulation (**Supplemental Figure S3.2D**; **Supplement Table S3.1**).

3.3.8. Model Summary

Our data indicate Orb2 is required for robust inactivation of the basal centrosome and may also enhance apical centrosome activity in interphase NSCs. Orb2 also functions in other cell types for normal brain size (**Figure 3.5F**). Given its local enrichment, we propose Orb2 promotes apical centrosome maturation (**Figure 3.5F**, *top box*), while cytoplasmic Orb2 inactivates the basal centrosome (**Figure 3.5F**, *bottom box*). Consistent with opposing activities at the apical vs. basal

centrosomes, Orb2 activates or represses the translation of its targets depending on its oligomerization status (Khan et al. 2015). Whether on-site translation occurs at the apical centrosome is an intriguing hypothesis that remains to be tested.

Microcephaly is quite heterogenous; however, a significant number of genes associated with heritable microcephaly and intellectual disability are also associated with centrosome biogenesis and regulation (Thornton and Woods 2009; Jayaraman, Bae, and Walsh 2018; Robinson, Faundez, and Lerit 2020). Our study implicates Orb2 at the intersection of these pathways.

3.4. Materials and Methods

3.4.1. Fly Stocks

The following strains and transgenic lines were used: y^1w^{1118} (Bloomington *Drosophila* Stock Center (BDSC) #1495) was used as the WT control unless otherwise noted. *orb2* brains were isolated from the homozygous null allele, $orb2^{\Delta 36}$ (gift from P. Schedl, Princeton University (Xu et al. 2012)). NSC-specific depletion of *orb2* by $orb2^{RNAi}$ ($P\{TRiP.HMJ22715\}^{attP40}$; BDSC #60424) was driven by $(P\{wor.GAL4.A\}^2$; BDSC #56555). All strains were maintained on Bloomington formula cornmeal-agar media (Lab-Express, Inc.; Ann Arbor, MI) at 25°C in a light and temperature-controlled incubator.

3.4.2. Immunofluorescence

Crawling third instar larva were used for dissections. Larval brains were prepared for immunofluorescence as previously described (Lerit, Plevock, and Rusan 2014). Briefly, brains were dissected in Schneider's *Drosophila* Medium (ThermoFisher Scientific, #21720024), fixed

in 9% paraformaldehyde for 15 min, blocked in PBT buffer (Phosphate Buffered Saline (PBS) supplemented with 1% BSA and 0.1% Tween-20) for one hour at room temperature prior to overnight incubation in primary antibodies in PBT with nutation at 4°C. Samples were further blocked with modified PBT (2% BSA, 0.1% Tween-20, and 4% normal goat serum in PBS) for 1 hour before incubation for two hours at room temperature with secondary antibody and DAPI. Brains were oriented and mounted in Aqua-Poly/Mount (Polysciences, Inc) prior to imaging.

The following primary antibodies were used: guinea pig anti-Asl (1:4000, gift from G. Rogers, University of Arizona), mouse anti-GTU88 (γTub; 1:250–350, Sigma T6557), rabbit anti-Cnn (1:4000 gift from Tim Megraw, Florida State University), guinea pig anti-PLP (1:4000, gift from Nasser Rusan, NIH), rabbit anti-phospho-Histone H3 Ser10 (pH3; 1:2000, Sigma-Millipore, 05-570), mouse anti-βTub (clone E7, 1:250; Developmental Studies Hybridoma Bank (DSHB)), mouse anti-Orb2 (undiluted 1:1 mix of clones 2D11 and 4G8 (DSHB); (Xu et al. 2012)), rat anti-Mira (1:500, Abcam, ab197788), rabbit anti-Baz (1:2000, gift from A. Harris, University of Toronto), rat anti-Dpn (1:500, Abcam, ab195173), rabbit anti-cleaved Caspase 3 (CC3, 1:75; Cell Signaling Technology, 9661s), and mouse anti-Pros (clone MR1A, 1:500; DSHB). Secondary antibodies: Alexa Fluor 488, 568, and 647 (1:500, Molecular Probes). DAPI (ThermoFisher Scientific) was used at 10 ng/mL.

3.4.3. Microscopy

Images were acquired on a Nikon Ti-E inverted microscope fitted with a Yokogawa CSU-X1 spinning disk head (Yokogawa Corp. of America), Orca Flash 4.0 v2 CMOS camera (Hamamatsu Corp.), Perfect Focus system (Nikon), and a Nikon LU-N4 solid-state laser launch (15 mW; 405, 488, 561, and 647 nm) using the following Nikon objectives: 100x 1.49-NA Apo

Total Internal Reflection Fluorescence oil immersion, 40x 1.3-NA Plan Fluor oil immersion, and 2x× 0.75-NA Plan Apo. Images were acquired at 25°C through Nikon Elements AR software on a 64-bit HP Z440 workstation (Hewlett-Packard).

3.4.4. Image analysis

Images were assembled using Fiji (National Institutes of Health;(Schindelin et al. 2012)), Adobe Photoshop, and Adobe Illustrator software to separate or merge channels, crop regions of interest, generate maximum-intensity projections, and adjust brightness and contrast.

Centrosome asymmetry

Interphase NSCs were identified by the absence of pH3, round nuclear morphology, and presence of duplicated centrosomes in large (≥10 μm) cells. To blind the experimenter to genotype, maximum projected images were generated, randomized, and used to measure background-subtracted integrated densities from regions of interest (ROIs) centered at the apical or basal centrosomes. Apical centrosome integrated density (A) and basal centrosome integrated density (B) were used to calculate the asymmetry index, $(A-B)/(A+B)$ (Lerit and Rusan 2013). The data were then unblinded and mean ± S.D. were calculated per genotype.

Polarity, NSC number, and mitotic index

Mitotic NSCs were identified by the presence of pH3 in large, Mira+ cells. To score polarity, maximum projected images were anonymized to blind the experimenter to genotype, and each NSC was scored for the absence or presence of Baz or Mira crescents at the apical or basal cortices, respectively. All Mira+ NSCs were counted to calculate the number of NSCs per optic lobe. Mitotic index is defined as the number of Mira+, pH3+ NSCs per total Mira+ NSCs.

Spindle morphology

Z-stack images of mitotic NSCs labeled with anti-βTub to label the mitotic spindle, Asl to mark the centrioles, and Baz to label the apical polarity axis were randomized to blind the experimenter to genotype. The point tool in Fiji was used to record the X,Y, and Z coordinates of four ROIs per cell: 1) center of the apical centrosome, 2) center of the basal centrosome, 3) center of the Baz apical crescent, and 4) center of the DAPI+ condensed chromosomes. These points were used to calculate four different vectors, and vector analysis was used to calculate the angles between specified vectors. The following angles (θ) were calculated: θ_1= *spindle orientation*, the angle between the vectors defined by points 1 and 2 (division axis) relative to points 3 and 4 (polarity axis); θ_2= *apical polarity alignment* is the angle between the vectors defined by points 3 and 4 (apical polarity axis) relative to points 1 and 4 (apical centrosome axis); and θ_3= *centrosome alignment* is the angle between the vectors defined by points 1 and 4 (apical centrosome axis) relative to points 2 and 4 (basal centrosome axis). Angles that fell outside $\pm$ 2 S.D. from the control mean were defined as defective. For spindle alignment, NSCs with spindle angles >75^{o} were classified as orthogonal.

Age-matched brain volume

To age match larva, 20 female virgins and 10 males of the appropriate genotype were allowed to seed a vial for 24 hrs. After removal of the adults, vials were incubated at 25 ^{o}C until crawling third instar larva emerged. *orb2* null progeny showed a developmental delay of 24-48 hrs as compared to controls; null larva took ~7-8 days to emerge. Age-matched crawling third instar larva were dissected and prepared for immunofluorescence. The entire volume of the DAPI-labeled brain was imaged. Imaris software (Oxford Instruments) was used to select an ROI of the optic lobe and measure the volume using the 3D surfaces tool (Link et al. 2019).

NSC differentiation and death rate

NSCs were identified by the presence of Dpn. To score for premature differentiation, maximum projected images of Dpn and Pros stained brains were randomized to blind the experimenter to genotype, and an ROI of the central brain region was used to calculate the Pearson's Correlation Coefficient on background-subtracted and automatic threshold–masked images using the Coloc 2 plugin for Fiji (Schindelin et al. 2012). To score cell death, a similar analysis was run on Dpn and CC3 stained brains. To assess specificity of overlapping signals for both experiments, the red channel (Pros or CC3) was rotated 90° clockwise, and colocalization was remeasured.

3.4.5. Immunoblotting

Larval brain extracts were prepared from 20 crawling third instar larva dissected in Schneider's medium, removed of imaginal discs, transferred to fresh media, then rinsed once in cold PBST. Samples were homogenized on ice in 30 μL of fresh PBST using a cordless motor and plastic, disposable homogenizer, supplemented with 20 μL 5x SDS loading buffer, boiled for 10 min at 95 °C, then stored at -20 °C or resolved on a commercial 7.5% polyacrylamide gel (Bio-Rad, #4568023. Proteins were transferred to a 0.2 μm nitrocellulose membrane (GE Healthcare) by wet transfer in a buffer containing 25 mM Tris-HCl, pH 7.6, 192 mM glycine, 10% methanol, and 0.02% SDS at 4 °C. Membranes were blocked in 5% dry milk in TBST (Tris-buffered saline, 0.05% Tween-20), washed well with TBST, and incubated overnight at 4 °C with primary antibodies. After washing with TBST, membranes were incubated for 1.5 hr in secondary antibodies diluted 1:5000 in TBST. Bands were visualized with Clarity ECL substrate (Bio-Rad, 1705061) on a Bio-Rad ChemiDoc imaging system.

The following primary antibodies were used: rabbit anti-PLP (1:4000, gift from Nasser Rusan, NIH), guinea pig anti-Asl (1:10,000, gift from Greg Rogers, University of Arizona), and mouse anti-Orb2 (1:25 dilution each of 2D11 and 4G8; DSHB, Paul Schedl, Princeton University (Hafer et al. 2011)). Secondary antibodies: goat anti-mouse HRP (1:5000, ThermoFisher #31430), goat anti-rabbit HRP (1:5000, ThermoFisher #31460), and goat anti-guinea pig HRP (1:5000, ThermoFisher #A18769). Densitometry was measured in Adobe Photoshop and protein levels are normalized to the Asl loading control. Full-size, replicated blots are available to view on FigShare: https://doi.org/10.6084/m9.figshare.17052722 . The experiment was repeated with three biological replicates per genotype.

3.4.6. Bioinformatics

Gene names were converted to FlyBase identifiers using the Flybase.org tool 'Query by symbols'(Larkin et al. 2021). Overlapping genes were identified by the 'COUNT IF' function in Excel and Venn diagrams were plotted in R-Studio. GO cellular component analysis was done using the Panther statistical overrepresentation test (http://www.pantherdb.org/), and Fisher's exact test was used to generate an adjusted p-value, i.e., false discovery rate (FDR) (Mi et al. 2021).

3.4.7. Statistical analysis

Data were plotted and statistical analysis performed using Microsoft Excel, GraphPad Prism, and RStudio software. Normality distributions were determined using a D'Agnostino-Pearson or Shapiro-Wilk normality test. Goodness-of-Fit chi-squared analysis were performed using control distributions as the expected distributions. Data were further analyzed using parametric two-tailed t-test, non-parametric Mann-Whitney test, or Kolmogorov-Smirnov (KS)

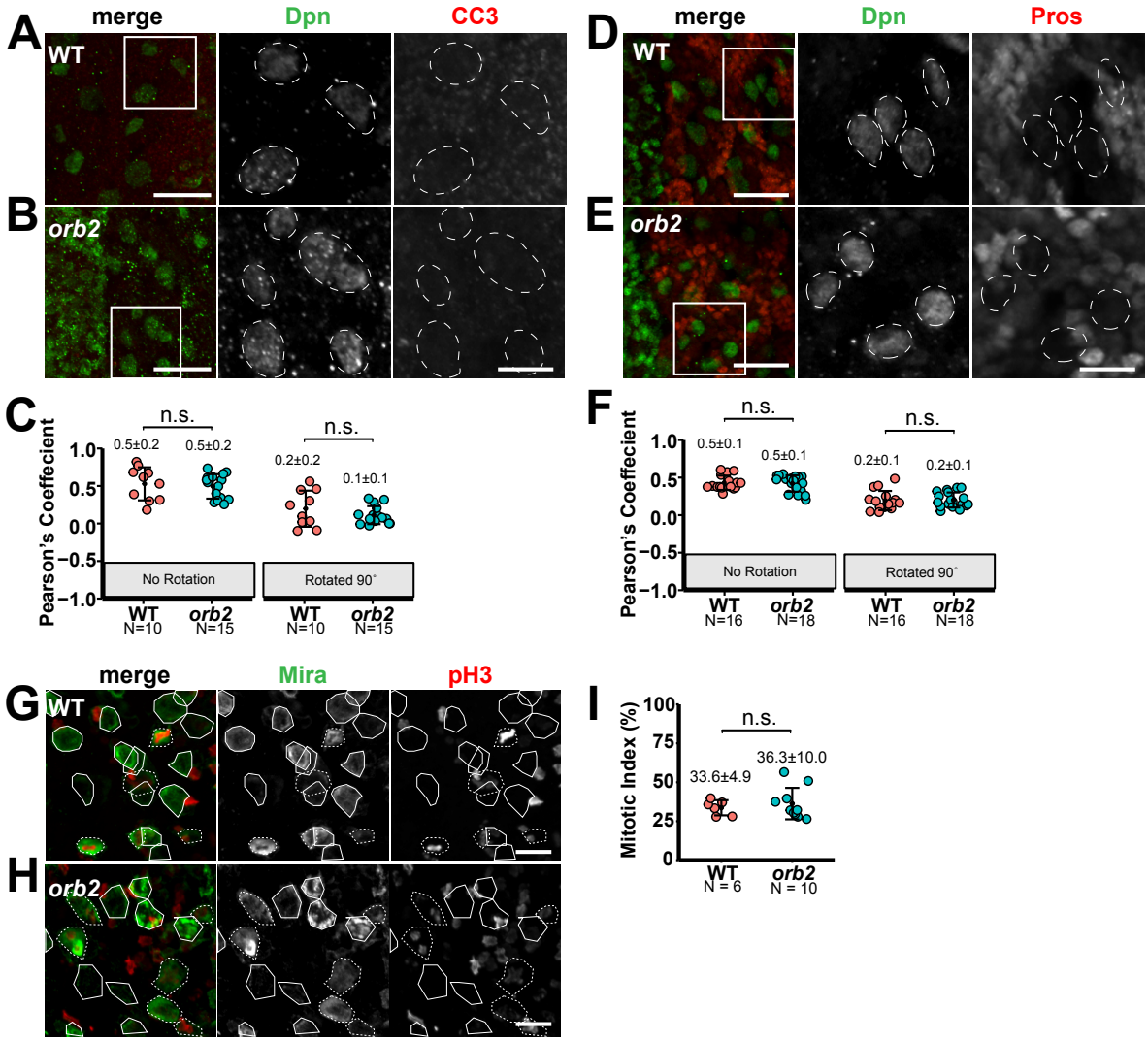
test, or an appropriate post-hoc test as noted in the figure legends. Data are plotted as mean ± S.D. and are representative results from at 2 or more independent experiments.

3.5. Acknowledgements

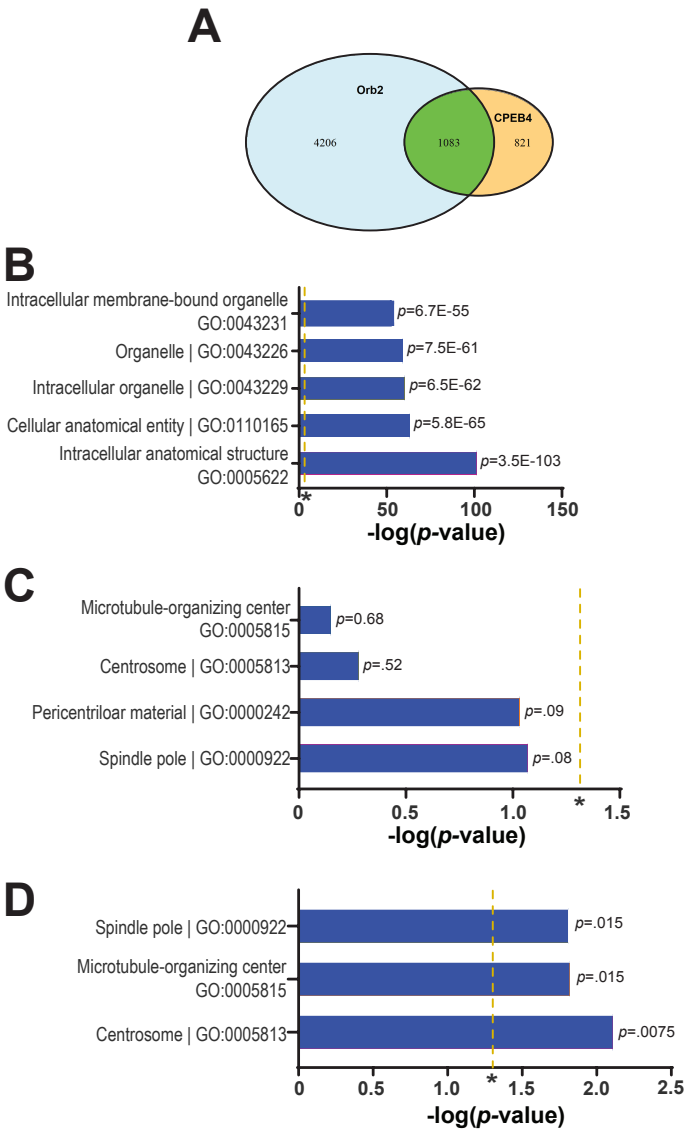
We thank Drs. Timothy Megraw, Nasser Rusan, Greg Rogers, and Paul Schedl for gifts of reagents. We are grateful to members of the Lerit lab and Drs. Victor Faundez and Nasser Rusan for constructive feedback on the manuscript.

This work was supported by NIH grant T32GM008490 (BVR), American Heart Association grant 20POST35210023 (to JF), and R01GM138544 (to DAL).

3.6. Supplemental Information



Supplemental Figure S3.1 - NSC differentiation in WT *vs orb2* mutants.
Maximum intensity projections of (A) WT and (B) *orb2* brains stained with Dpn (green; NSC nuclei) and CC3 (red; pro-apoptotic. Insets (boxes) are enlarged to the right to highlight NSCs (dashed ovals). (C) Quantification of the Pearson's correlation coefficient between Dpn and CC3 from N=10 WT and 15 *orb2* optic lobes pooled from two replicates. The CC3 channel was rotated clockwise (90°) to test for specificity of overlapping signals. (D) Maximum intensity projections of WT and (E) orb2 brains stained with Dpn (green) and Pros (red; differentiated nuclei). (F) Quantification of the Pearson's correlation coefficient between Dpn and Pros from N=16 WT and 18 *orb2* optic lobes pooled from two replicates. The Pros channel was rotated clockwise (90°) to test for specificity of overlapping signals. In (D) and (F), each data point is from one ROI per optic lobe. (G) Projected images of WT and (H) *orb2* NSCs (dashed ovals) stained for Mira (green; NSCs) and the mitotic marker pH3 (red). (I) Quantification of mitotic index from N=6 WT and 10 *orb2* brains. n.s., not significant by Student's t-test. Bars: (A–E) 20 µm and 8 µm (insets); (G and H) 10 µm.



Supplemental Figure S3.2 - Ontological analysis of Orb2 and human CPEB4.
A) Venn diagram showing relative transcript pool sizes and overlap detected from the Stepien et al. Orb2 CLIP and Pascual et al. CPEB4 datasets (Stepien et al. 2016; Pascual et al. 2020). B) Top 5 gene ontologies overrepresented in the overlapping transcripts by lowest raw p value. C) Overrepresentation tests for gene ontologies implicated in the centrosome were not significant. D) Overrepresentation test of genes in the Orb2 CLIPseq with BC >3 yields significant centrosome ontologies. -log *p*-values are plotted with a significance cut-off of p=0.05, noted by the dashed line and asterisk. Genes are listed in **Supplement Table S3.1**.

Supplement Table S3.1 - Orb2 and CPEB4 common RNA targets.
Sheet 1 lists FlyBase IDs for all transcripts detected in (Stepien et al. 2016; Pascual et al. 2020). Sheet 2 lists overlapping genes; column E is sortable to list genes with centrosome ontologies. Sheet 3 lists GO terms overrepresented in the overlapping genes, column J is sortable to list genes with centrosome ontologies. Sheet 4 lists Orb2 targets from (Stepien et al. 2016) with BC $\geq$ 3; column F is sortable to list genes with centrosome ontologies. Sheet 5 shows an overrepresentation test for centrosome ontologies from the Orb2 BC $\geq$ 3 gene list. Sheet 6 lists each gene included in the indicated centrosome ontologies.

Chapter 4. General Discussion

4.1. Summary of Results

Our understanding of centrosome activity, structure, and regulation has made significant advancements since the initial discovery of the membrane-less organelle in the late 1870s (Qi and Zhou 2021). Genetic and proteomic studies in multiple model systems confirm a high level of conservation within the centrosome structural and composition across species (Bettencourt-Dias 2013; Bettencourt-Dias and Glover 2007; Azimzadeh and Bornens 2005). Functional studies defined the role of the centrosome as the primary microtubule-organizing center required for spindle formation, intracellular transport, polarization, cell motility, and ciliogenesis (Badano, Teslovich, and Katsanis 2005). Consistent with multifunctionality, the centrosome proteome contains hundreds of proteins with new ones routinely being discovered (Danielsson et al. 2020). Despite this, how centrosome gene expression is regulated still represents an unknown in the field. Elucidating answers to this unknown will expand our understanding of how centrosome activity is regulated.

Detailed imaging of the centrosome within the last ten years revealed dynamic, specific, and local enrichments of mRNA, protein, and translation occurring within the pericentriolar material, the electron-dense matrix that surrounds the two inner centrioles of a centrosome (Vasquez-Limeta and Loncarek 2021; Bowler et al. 2019; Sahabandu et al. 2019; Gartenmann et al. 2017; Fu and Glover 2012; Lau et al. 2012; Sonnen et al. 2012; Sepulveda et al. 2018). It is now understood the pericentriolar material exists in multiple structural conformations. The regulation thereof allowing the centrosome to modulate its activity level in response to cellular cues (Fry et al. 2017; Mennella et al. 2014). Still, little is understood about the mechanisms required to rapidly transition the pericentriolar material between different conformational states (e.g., interphase versus mitosis). By defining the pathways that regulate the accumulation and

shedding of pericentriolar material, we may discover novel therapeutics aimed at ameliorating the wide range of conditions that occur when centrosome activity is dysregulated.

Chapter One implicates centrosome genes in human neurodevelopment. Using data mined from the Human Phenotype Ontology public database (Kohler et al. 2019), 12% of genes annotated with the microcephaly phenotype also have centrosome-related annotations, indicating a role in structural brain development. Similarly, 11% of genes annotated with the intellectual disability phenotype also have centrosome-related annotations, indicating a role in brain function (**Figure 1.1**). In support of multiple, diverse roles within the human brain, Chapter One also reviews previously published data to illustrate three different cellular mechanisms through which centrosomes regulate *Drosophila* neurogenesis. Disruption of centrosome activity can differentially affect cell fate following asymmetric cell division based on how and when centrosome activity is altered, which provides one explanation for how loss of centrosome activity contributes to different phenotypic observations in human pathologies ((Robinson, Faundez, and Lerit 2020); **Figure 1.2**). Key to asymmetric cell division in neural stem cells is the ability to intrinsically and asymmetrically regulate centrosome maturation (Chen and Yamashita 2021). This is done in part by generating asymmetric protein enrichments at each centrosome (Lerit and Rusan 2013; Januschke et al. 2013; Ramdas Nair et al. 2016; Singh, Ramdas Nair, and Cabernard 2014; Gallaud et al. 2020). Protein enrichments and genes contributing to asymmetric centrosome maturation are summarized in Chapter One ((Robinson, Faundez, and Lerit 2020); **Figure 1.3**). Lastly, Chapter One conceptualizes a novel paradigm in which RNA-binding proteins are required to generate these distinct protein enrichments. In summary, Chapter One leverages bioinformatics to highlight the role of centrosome function in human brain development and reviews how insights into centrosome regulation gained from *Drosophila* neural stem cells may be used to define the

cellular mechanisms in which centrosomes function during neurogenesis and how such mechanisms may contribute to human pathologies.

Chapter Two proposes the biological question of this thesis: *How do centrosomes modulate pericentriolar material levels rapidly in time and space?* An in-depth literature review of centrosome structure and biogenesis illustrates that how pericentriolar material is shed and recruited to the centrosome during the cell cycle is incompletely understood and reveal centrosomes as recently identified sites of active translation (Lin, Xie, and Chan 2022; Zein-Sabatto and Lerit 2021). These findings further support the plausibility that post-transcriptional regulation of centrosome RNAs by one or more RNA-binding proteins modulate centrosome regulation. In further support of post-transcriptional regulation of centrosome genes as a mechanism of centrosome control, results from bioinformatic analysis show that variations of the CPE sequence ($UUUU_{(n)}AU$), the sequence specific motif recognized and bound by CPEB family proteins, are repeated within the 3' UTR of *Drosophila* centrosome asymmetry genes ((Stepien et al. 2016); **Figure 2.4**). Additionally, the CPE sites are conserved in the human orthologs, suggesting CPE sites play a functional role in both *Drosophila* and humans (Pascual et al. 2020). Furthermore, analysis using data mined from NCI's GDC harmonized database (Heath et al. 2021) suggests human cancers with mutations in the 3' UTR of centrosome genes phenocopy human cancers with mutations in CPEB genes (**Figure 2.6**). Taken together, these previously published results and exploratory analyses provide rationale for the hypothesis of this thesis: *CPEB proteins regulate Drosophila centrosome activities through post-transcriptional mechanisms, and this likely represents a conserved mechanism of centrosome regulation*. To address this biological question and hypothesis, we utilized asymmetrically dividing *Drosophila* larval neural stem cells

depleted for the *orb2* gene coupled with quantitative image analysis to identify the consequences of *orb2* loss on centrosome activities (**Figure 2.7**).

The results discussed in Chapter Three indicate Orb2 has two distinct localization patterns within neural stem cells. Firstly, Orb2 localizes diffusely to the cytoplasm. Secondly, Orb2 concentrates as dense puncta at active centrosomes (**Figure 3.1**). More recent, unpublished data, suggest this localization may be non-specific; however, those experiments are still ongoing within the laboratory. Orb2 was reported to exist in two forms, a diffuse monomeric form and an oligomerized amyloid form (Cervantes et al. 2016; Hervas et al. 2016; Keleman et al. 2007; Kruttner et al. 2012; Khan et al. 2015). Therefore, we postulate Orb2 forms two distinct populations of Orb2, a monomeric Orb2 that localizes diffusely to the cytoplasm and an oligomeric Orb2 that localizes specifically to active centrosomes. Additionally, Orb2 functions intrinsically in the regulation of centrosome activity as loss of neural stem cell *orb2* results in aberrant centrosome activities. Immunofluorescence for the γ-TuRC component, γ-Tubulin, reveals ~20% of mutant neural stem cells exhibit two microtubule nucleating centrosomes in interphase compared to controls which have <10%. Additionally, *orb2* mutants display a slight reduction in overall centrosome microtubule nucleating capacity as active *orb2* centrosomes exhibit slightly lower florescent intensity compared to controls. Together, these data indicate a reduction in the normally asymmetric pericentriolar material distribution (**Figure 1.3**). In contrast, immunofluorescence of a centriole component, Asl, remains consistent between *orb2* mutants and controls, indicating the centriole structure is unaffected (**Figure 3.2**). Monomeric Orb2 frequently represses translation of its mRNA target, whereas oligomeric Orb2 frequently promotes translation (Cervantes et al. 2016; Khan et al. 2015). Therefore, this dual responsibility in centrosome activation and repression could be explained by opposing activities of the two Orb2 populations.

Whether Orb2 exists in different conformations within neural stem cells is an interesting area for future study.

orb2 mutant neural stem cells exhibit other centrosome-related phenotypes, including centrosome amplification and mitotic spindle morphology defects (**Figure 3.3D-R**). These data suggest Orb2 regulates multiple centrosome regulatory pathways throughout the cell cycle. An overall reduction of the neural stem cell population in *orb2* brains suggests the observed centrosome defects impair neural stem cell homeostasis (**Figure 3.4C,D,I,J**). Lastly, the results discussed in Chapter Three indicate that Orb2 activities are cell type-specific, and *orb2* loss causes cell-specific phenotypes. Orb2 regulates PLP expression levels in cellularly heterogenous *Drosophila* brain lysates; however, in neural stem cells, PLP levels and localization appear unaffected (**Figure 3.5A-E**). Similarly, *orb2* is a novel microcephaly gene, however this phenotype is not dependent on *orb2* expression in neural stem cells (**Figure 3.4A,B,G,H**). Taken together, these data show that even 150 years after the initial discovery of the centrosome, there is much still to learn regarding its regulation, and previously undefined mechanisms, such as contributions of RNA-binding proteins and potential substrates must be explored to elucidate the nuances of centrosome regulation.

4.2. Implications of Findings

The findings provided in Chapter Three demonstrate for the first time the requirement of an RNA-binding protein as a regulator of centrosome activities. Additionally, these findings suggest RNA-binding proteins are a component of a still-to-be defined cellular mechanism of centrosome regulation conserved across species. Taken together, the results described in this dissertation allow us to further speculate on the biological purpose of centrosome-localized RNA

and hint at a novel mechanism in which centrosomes dynamically change the local pericentriolar material protein composition allowing for rapid transitions in centrosome microtubule nucleating ability through localization and local translation of centrosome genes. Further testing is needed to parse apart the specific cellular interactions that allow an RNA-binding protein to regulate centrosome activities and this work lays the foundation for those mechanistic studies.

In addition to mechanisms that regulate centrosome activities, the findings described herein allow us to speculate how numerous centrosome-related conditions frequently exhibit diverse and multi-system phenotypes (Wu et al. 2021; Qi and Zhou 2021). To fine-tune gene expression, RNA-binding proteins form differential regulatory complexes with a range of different affinities and specificities, exist in different conformational states from post-translational modifications, and respond to differential cellular signals (Gebauer et al. 2021). Therefore, genetic diseases that involve RNA-binding proteins frequently have pleiotropic phenotypic consequences. For example, Fragile X Mental Retardation Protein (FMRP) is a ubiquitously expressed RNA-binding protein encoded by the *FMR1* gene (Verheij et al. 1993). In Fragile X Syndrome, the *FMR1* gene undergoes repeat expansion in its 5'UTR (Verkerk et al. 1991). Fragile X Syndrome is the most common hereditary intellectual disability (de Vries et al. 1993). A longitudinal study which mapped brain regions sensitive to *FMR1* loss reveals that regions such as the caudate and fusiform gyri exhibit aberrant early (i.e. prenatal) neurodevelopment in patients with Fragile X Syndrome compared to age and developmental-matched controls. Conversely, regions such as the orbital gyri, basal forebrain, and large segments of the thalamus appear anatomically similar in both Fragile X Syndromic patients and controls at early time points, but experience region specific sensitivities postnatally resulting in anatomical alterations at later time points (Hoeft et al. 2010). Therefore, Fragile X Syndrome highlights how RNA-binding proteins can exhibit both regional and temporal-

specific regulatory functions (O'Donnell and Warren 2002; Raj et al. 2021). Furthermore, a mechanism in which centrosome activities are regulated by the fine tuning of RNA-binding proteins explains how the pleiotropic effects observed in centrosome diseases may arise as tissue-specific RNA-binding protein interactions may differentially regulate centrosomes. In support of tissue-specific interactions, Orb2 localizes and promotes the translation of *αpkc* mRNA during spermatogenesis, ultimately influencing polarity of the developing spermatid, and promotes polarized localization of αPKC protein in embryonic neural stem cells (Xu et al. 2012; Hafer et al. 2011). However, in *Drosophila* larval neural stem cells, Orb2 does not appear to significantly contribute to polarity (**Figure 3.3A-C**). Similarly, our results indicate Orb2 regulates PLP levels in *Drosophila* brain tissue lysates (**Figure 3.5A, B**); however, loss of *orb2* in *Drosophila* embryos does not significantly alter PLP levels (Fang and Lerit 2022). Furthermore, Orb2 functions within an undefined cell-type/s to regulate brain size and PLP expression (**Figure 3.4**; **Figure 3.5A-E**). A decrease in PLP levels is not sufficient to account for microcephaly in *orb2* mutants, as *plp* null animals show normal brain size (Martinez-Campos et al. 2004). We speculate cell type-specific interactions between Orb2 and other regulatory proteins influence these varied phenotypes. An intriguing hypothesis conceptualized from this work is the fine tuning of centrosome activities by tissue-specific RNA-binding protein interactions promote the pleiotropic phenotypes observed in human centrosome diseases.

Lastly, this work implicates *orb2* as a novel neural stem cell-independent microcephaly gene in *Drosophila* (**Figure 3.4A, B, G, H**). The role of Orb2 in regulating brain size was previously only briefly noted in adult brains (Keleman et al. 2007). Microcephaly was not previously associated with mammalian CPEB proteins. However, as there are three mammalian orthologs of Orb2 (CPEB2-4; (Mendez and Richter 2001; Richter 2007); **Figure 2.5**), a role for

CPEB proteins in regulating mammalian brain size may be obscured due to redundancy within the CPEB family. This work, therefore, introduces a previously undefined pathology of microcephaly independent of neural stem cell division and dependent of CPEB proteins.

4.3. Limitations and Future Directions

The studies described in Chapter Three reveal an Orb2-dependent pathway of centrosome regulation contributing to asymmetric cell division. However, these studies were limited to *Drosophila* larval neural stem cells. Therefore, the scope of this study is limited in both species and cell-type. Although the basic structure of the centrosome is conserved (**Figure 2.1**), variations exist both in structure and biogenesis between *Drosophila* and mammalian centrosomes as well between centrosomes in different cell types (Loncarek and Bettencourt-Dias 2018; Bornens 2012). Additional testing is required to confirm the requirement of CPEB proteins in modulating centrosome activities in both other cell types and species. Concurrent work exploring CPEB function in other *Drosophila* cell types illustrates Orb, but not Orb2, influences PLP expression and consequent incorporation into the pericentriolar material of early embryonic centrosomes. Importantly, this work highlights Orb differentially contributes to *plp* mRNA expression in different cell types. In *orb* deficient ovarian lysates, *plp* mRNA levels are increased, suggesting Orb normally functions to attenuate *plp* transcript expression and/or stability in this tissue. Consistent with its maternal contribution, similar levels of *plp* mRNA are detected in *orb* depleted embryos. Conversely, PLP protein levels are depleted in *orb* deficient embryos, likely due to a shortened *plp* poly(A) tail, suggesting Orb normally functions to promote *plp* translation. In contrast, PLP levels are unchanged in *orb* deficient ovaries. These data hint that the CPEB protein Orb differential regulates the same target transcript (*plp*) in different tissue types (embryo versus ovary) (Fang and Lerit 2022).

Although PLP protein levels were reduced in *orb2* whole brain lysates (**Figure 3.5A**), we did not specifically examine protein content within neural stem cells. As RNA-binding proteins fine-tune gene regulation through post-translational mechanisms regulated by the local subcellular environment, whole tissue analyses may miss neural stem cell specific alterations. Therefore, future analysis to expand our knowledge of CPEB protein activities must both explore additional cell types and elucidate a neural stem cell specific Orb2 mechanism, perhaps using isolated neural stem cells for biochemical analysis.

Furthermore, divergence between the human and *Drosophila* neurodevelopmental pathways limits the conclusions drawn from the bioinformatic studies comparing *Drosophila* Orb2 targets/activities to human CPEB2-4 targets/activities (**Figure 2.4**; **Figure 2.6**; **Supplemental Figure S3.2**). For example, loss of *plp*, the *Drosophila* ortholog of human microcephaly gene *PCNT,* does not cause microcephaly in *Drosophila* suggesting either an additional function of the *PCNT* gene in human development not present in *Drosophila* (e.g., supporting neocortical expansion and stratification), or a parallel, redundant pathway present in *Drosophila* (Lerit and Rusan 2013; Martinez-Campos et al. 2004). Additionally, in both *Drosophila* and *Aplysia*, CPEB oligomerization is required for long term memory formation (Majumdar et al. 2012; Si et al. 2010). Of the mammalian CPEB proteins, only CPEB3 functions in long-term memory and also forms amyloid-like oligomers (Stephan et al. 2015). Thus some, but not all, mammalian CPEB proteins may function in homologous functional pathways as Orb2. Regardless, identifying *orb2* as a novel *Drosophila* microcephaly gene and centrosome regulator within larval neural stem cells allows us to speculate on the pathogenesis of human neurological conditions, but further studies must be conducted to determine which, if any, mammalian CPEB proteins function in similar pathological pathways.

The results reported herein indicate the RNA-binding protein Orb2 regulates centrosome activities in *Drosophila* neural stem cells. An important next step of this work is to elucidate the mechanism/s by which Orb2 directs these activities. As outlined in Chapter Three, we hypothesize two distinct Orb2 populations function antagonistically on centrosome maturation (**Figure 3.1**; **Figure 3.5F**). Following phosphorylation, Orb2A promotes Orb2 oligomerization (White-Grindley et al. 2014). Oligomerization of Orb2B switches Orb2 from a translational repressor to a translational activator (Khan et al. 2015). Therefore, we hypothesize centrosomal Orb2 will differ from cytoplasmic Orb2 in either composition (Orb2A to Orb2B ratio) and/or conformation (phosphorylation and/or oligomerization status). These differences would ultimately result in different Orb2 functions (promotion versus inhibition of translation). Confirmation of two functionally distinct Orb2 populations within neural stem cells will allow us to untangle Orb2-dependent pathways of centrosome regulation. Numerous methodologies exist that would allow for specific characterization of the centrosomal versus cytoplasmic Orb2. First, isolation of centrosomes or proximity labeling of Orb2 at the centrosome vs Orb2 at other organelles could reveal specific centrosomal versus cytoplasmic Orb2 compositions and conformations (Meigs and Kaplan 2008; Branon et al. 2018; Rhee et al. 2013; Trinkle-Mulcahy 2019). Following identification of specific centrosome vs cytoplasmic Orb2 features, it may be feasible to disrupt one subpopulation versus the other to probe the specific spatial Orb2 functions. Therefore, characterizing both the cytoplasmic and centrosomal Orb2 populations will be an important next step in defining the mechanisms of Orb2-mediated centrosome regulation.

The centrosome phenotypes that arise in *orb2* mutants could result directly from loss of Orb2-dependent regulation of centrosomal RNAs. However, it is presently unknown if mRNAs localize to neural stem cell centrosomes. Similarly, it is unknown if Orb2 functions as a

translational regulator to modulate centrosome activity and/or brain size. Conversely, *orb2* phenotypes could arise as indirect consequences of dysregulated gene expression of a non-centrosome gene disrupting centrosome protein functions. Therefore, another important next step toward understanding mechanisms of Orb2-dependent centrosome regulation is identification of direct downstream targets of Orb2. Putative Orb2 targets were recently identified by PAR-CLIP in *Drosophila* S2 cells, and putative CPE sites were identified in centrosome genes; however, a direct interaction between Orb2 and these CPE sites has not been validated, nor is it known if such interactions persist in neural stem cells ((Stepien et al. 2016); **Figure 2.4**). Although we examined *plp* as a putative Orb2 target given both *orb2* and *plp* mutant interphase neural stem cells exhibit two inactive centrosomes (**Figure 3.2**; (Lerit and Rusan 2013)), our results suggest PLP localization is not regulated at neural stem cell centrosomes by an Orb2-dependent mechanism (**Figure 3.5C-E**). Intriguingly, the centrosome asymmetry gene *wdr62* was recently identified to cause neural stem cell-independent microcephaly ((Ramdas Nair et al. 2016; Lim et al. 2017)). We showed *orb2* mutant animals also exhibit centrosome asymmetry defects and neural stem cell-independent microcephaly (**Figure 3.2; Figure 3.4**). Furthermore, the previously mentioned PAR-CLIP experiment performed in *Drosophila* S2 cells identified *wdr62* as a frequent target (BC>3) of Orb2B. Direct binding of Orb2 to the CPE sites of *wdr62* is strongly supported by the parallel finding that *wdr62* is not pulled down by Orb2B with mutated RNA-binding domains (Stepien et al. 2016). A speculative model derived from these previous studies and this dissertation suggests Orb2 binds the CPE sites in *wdr62* RNA and regulates Wdr62 protein expression in neuronal tissues to direct multiple *Drosophila* neurodevelopmental pathways. In neural stem cells, Orb2 would represses translation of *wdr62* at the basal centrosome to direct the transient inactivation of the basal centrosome during interphase. In glial and astrocyte cells, Orb2 would promote *wdr62*

expression necessary for timely mitotic progression and *Drosophila* brain growth (Chen et al. 2014; Lim et al. 2017). Loss of Orb2 would thusly result in an overexpression of Wdr62 at the basal centrosome in neural stem cells, promoting the aberrant neural stem cell phenotypes we observed in this study. Concurrently, *orb2* loss would deplete Wdr62 in glial and astrocyte cells, ultimately leading to *wdr62* -dependent microcephaly. This model is highly speculative, and although *wdr62* should be prioritized when investigating Orb2 mechanisms in *Drosophila* larval brain tissue, future studies that elucidate whether Orb2 directly interacts with CPE-containing RNAs in *Drosophila* brain lysates will be a critical next step in defining mechanisms underlying both Orb2-dependent centrosome regulation and Orb2-dependent microcephaly. Towards this end, it is essential to determine if the Orb2 RNA-binding domain is required for Orb2 function in regulating centrosomes and/or brain size. While a requirement for the RNA-binding domain would suggest Orb2 regulates RNA targets to control neurodevelopment, an absence of this requirement would alternatively suggest Orb2 may rely upon protein-protein interactions for its functions.

Elucidating the targets and mechanisms of Orb2-dependent centrosome regulation in *Drosophila* neural stem cells is the most immediate future direction that requires pursuit, but the findings presented herein suggest a functionally conserved mechanism in which RNA-binding proteins regulate centrosome activities. Therefore, another future direction is to define the degree of this functional conservation. Firstly, can these centrosome phenotypes be recapitulated in CPEB-mutant mammalian cells and can mammalian CPEB proteins rescue *Drosophila orb2* mutants? Phenotypic similarities and successful functional rescue between *Drosophila* and mammalian cells would suggest a conserved role of CPEB proteins in centrosome regulation. Secondly, are CPEB proteins the only family of RNA-binding proteins responsible for regulating centrosome activities, or do the findings in this work represent a more global cellular mechanism

of centrosome regulation? We investigated the nucleotide sequences of *Drosophila* neural stem cell centrosome asymmetry gene 3' UTRs (**Figure 2.4**). Future studies should probe both the secondary structures of centrosome 3' UTRs as well as expand the target pool to include centrosome genes not asymmetrically regulated. Identification of another consensus RNA-binding protein motif would suggest a global mechanism of centrosome gene regulation directed by RNA-binding proteins. Therefore, future directions of this work may include characterizing the functional conservation of CPEB proteins specifically and other RNA-binding proteins in centrosome regulation. Once a molecular pathway of Orb2-dependent centrosome regulation is identified, it may represent a pathway of interest for identification of new therapeutics in the treatment of human centrosome-related diseases.

This work also demonstrates Orb2 functions in neuronal tissue homeostasis. Firstly, *orb2* is implicated as a novel microcephaly gene independent of its expression in neural stem cells (**Figure 3.4**). An important future direction is to identify the cell type *orb2* expression is required in for proper brain formation. Orb2 was previously identified as strongly expressed in a subset of cells in the *Drosophila* brain (Hafer et al. 2011). Could *orb2* expression in these cells be required for brain size in *Drosophila*? *wdr62*-dependent microcephaly is linked to proper function of glial and astrocyte cells (Lim et al. 2017). Could these cells similarly play a role in Orb2-dependent microcephaly? Future studies exploring *orb2* function in other cell types within the *Drosophila* brain are required to determine the pathology of Orb2-dependent microcephaly. Secondly, *orb2* is required for homeostasis of stem cell number, as loss of *orb2* leads to fewer neural stem cells (**Figure 3.4**). *Drosophila* neurogenesis occurs in two developmental waves. The first wave, which occurs during embryogenesis, involves symmetric divisions of neural stem cells that allow for the expansion of the stem cell pool. The second wave, which occurs during larval development,

involves asymmetric cell divisions, which allow for the stem cell population to maintain its cell numbers while also providing cells for differentiation (Ramon-Canellas, Peterson, and Morante 2019). We found depletion of neural stem cells is cell autonomous. Although we assayed cell apoptosis, premature differentiation, and mitotic stalling, we were unable to identify the cellular mechanism responsible for the observed neural stem cell depletion (**Supplemental Figure S3.1**). Future studies which investigate the initial wave of embryonic neurogenesis and other cell death pathways may reveal the developmental pathways disrupted in *orb2 Drosophila* brains resulting in reduced neural stem cell numbers. Live imaging would also be a key future direction to examine whether the perturbed mitotic spindles evident in *orb2* mutant neural stem cells are subject to mitotic stalling or other cell cycle errors.

4.4. Conclusion

In summary, we present a body of work identifying the RNA-binding protein Orb2 as a regulator of centrosome activities, asymmetric cell division, and brain development in *Drosophila* larval neural stem cells. We identified and described several centrosome-related phenotypes that arise from loss of *orb2,* including aberrant centrosome maturation, centrosome amplification, defective spindle morphogenesis, and reduction of the centrosome protein PLP. Taken together, these data suggest *orb2* regulates centrosomes and spindles throughout the cell cycle. Additionally, our data are consistent with the hypothesis post-transcriptional regulation of centrosome RNAs may represent a conserved mechanism to regulate centrosome activity. Consistent with this idea, our laboratory recently directly tested the requirement of centrosome-localized *centrocortin* (*cen*) mRNA in *Drosophila* embryos and found mislocalization of *cen* results in mitotic errors (Ryder, Fang, and Lerit 2020). We also identified *orb2* as a novel *Drosophila* microcephaly gene, independent of *orb2* function in neural stem cells. This finding illustrates the diversity of pathways

regulated by Orb2 during neurogenesis is likely still underestimated. The work presented in this dissertation introduces a new player in the centrosome field, which may provide novel mechanistic insights which will advance our understanding of how centrosome activities are regulated. It is imperative that centrosome regulation is defined in depth, as identification of the cellular players and mechanisms tasked with centrosome regulation may lead to the development of therapeutics for human diseases resulting from centrosome dysfunction. Therefore, these foundational studies provide invaluable data for the fields of centrosome, RNA, and developmental biology.

Chapter 5. References

Abdel-Salam, G. M. H., I. S. M. Sayed, H. H. Afifi, S. F. Abdel-Ghafar, M. R. Abouzaid, S. I. Ismail, M. S. Aglan, M. Y. Issa, H. T. El-Bassyouni, G. El-Kamah, L. K. Effat, M. Eid, M. S. Zaki, S. A. Temtamy, and M. S. Abdel-Hamid. 2020. 'Microcephalic osteodysplastic primordial dwarfism type II: Additional nine patients with implications on phenotype and genotype correlation', *American Journal of Medical Genetics. Part A*, 182: 1407-20.

Albertson, R., C. Chabu, A. Sheehan, and C. Q. Doe. 2004. 'Scribble protein domain mapping reveals a multistep localization mechanism and domains necessary for establishing cortical polarity', *Journal of Cell Science*, 117: 6061-70.

Albertson, R., and C. Q. Doe. 2003. 'Dlg, Scrib and Lgl regulate neuroblast cell size and mitotic spindle asymmetry', *Nature Cell Biology*, 5: 166-70.

Aldaz, H., L. M. Rice, T. Stearns, and D. A. Agard. 2005. 'Insights into microtubule nucleation from the crystal structure of human gamma-tubulin', *Nature*, 435: 523-7.

Allanson, J. E., C. Cunniff, H. E. Hoyme, J. McGaughran, M. Muenke, and G. Neri. 2009. 'Elements of morphology: standard terminology for the head and face', *American Journal of Medical Genetics. Part A*, 149A: 6-28.

Alliegro, M. C., M. A. Alliegro, and R. E. Palazzo. 2006. 'Centrosome-associated RNA in surf clam oocytes', *Proceedings of the National Academy of Sciences of the United States of America*, 103: 9034-8.

Alvarez-Rodrigo, I., T. L. Steinacker, S. Saurya, P. T. Conduit, J. Baumbach, Z. A. Novak, M. G. Aydogan, A. Wainman, and J. W. Raff. 2019. 'Evidence that a positive feedback loop drives centrosome maturation in fly embryos', *Elife*, 8.

Alvey, P. L. 1985. 'An investigation of the centriole cycle using 3T3 and CHO cells', *Journal of Cell Science*, 78: 147-62.

Andersen, J. S., C. J. Wilkinson, T. Mayor, P. Mortensen, E. A. Nigg, and M. Mann. 2003. 'Proteomic characterization of the human centrosome by protein correlation profiling', *Nature*, 426: 570-4.

Ashburner, M., C. A. Ball, J. A. Blake, D. Botstein, H. Butler, J. M. Cherry, A. P. Davis, K. Dolinski, S. S. Dwight, J. T. Eppig, M. A. Harris, D. P. Hill, L. Issel-Tarver, A. Kasarskis, S. Lewis, J. C. Matese, J. E. Richardson, M. Ringwald, G. M. Rubin, and G. Sherlock. 2000. 'Gene ontology: tool for the unification of biology. The Gene Ontology Consortium', *Nature Genetics*, 25: 25-9.

Atwood, S. X., and K. E. Prehoda. 2009. 'aPKC phosphorylates Miranda to polarize fate determinants during neuroblast asymmetric cell division', *Current Biology*, 19: 723-9.

Avidor-Reiss, T., and J. Gopalakrishnan. 2013. 'Building a centriole', *Current Opinion in Cell Biology*, 25: 72-7.

Aydogan, M. G., A. Wainman, S. Saurya, T. L. Steinacker, A. Caballe, Z. A. Novak, J. Baumbach, N. Muschalik, and J. W. Raff. 2018. 'A homeostatic clock sets daughter centriole size in flies', *J Cell Biol*, 217: 1233-48.

Azimzadeh, J., and W. F. Marshall. 2010. 'Building the centriole', *Current Biology*, 20: R816-25.

Azimzadeh, Juliette, and Michel Bornens. 2005. 'The Centrosome in Evolution.' in, *Centrosomes in Development and Disease*.

Bachmann-Gagescu, R., J. C. Dempsey, I. G. Phelps, B. J. O'Roak, D. M. Knutzen, T. C. Rue, G. E. Ishak, C. R. Isabella, N. Gorden, J. Adkins, E. A. Boyle, N. de Lacy, D. O'Day, A. Alswaid, A. R. Ramadevi, L. Lingappa, C. Lourenco, L. Martorell, A. Garcia-Cazorla, H. Ozyurek, G. Haliloglu, B. Tuysuz, M. Topcu, Genomics University of Washington Center for Mendelian, P. Chance, M. A. Parisi, I. A. Glass, J. Shendure, and D. Doherty. 2015. 'Joubert syndrome: a model for untangling recessive disorders with extreme genetic heterogeneity', *Journal of Medical Genetics*, 52: 514-22.

Badano, J. L., T. M. Teslovich, and N. Katsanis. 2005. 'The centrosome in human genetic disease', *Nat Rev Genet*, 6: 194-205.

Bahe, S., Y. D. Stierhof, C. J. Wilkinson, F. Leiss, and E. A. Nigg. 2005. 'Rootletin forms centriole-associated filaments and functions in centrosome cohesion', *J Cell Biol*, 171: 27-33.

Balestra, F. R., P. Strnad, I. Fluckiger, and P. Gonczy. 2013. 'Discovering regulators of centriole biogenesis through siRNA-based functional genomics in human cells', *Developmental Cell*, 25: 555-71.

Bally-Cuif, L., W. J. Schatz, and R. K. Ho. 1998. 'Characterization of the zebrafish Orb/CPEB-related RNA binding protein and localization of maternal components in the zebrafish oocyte', *Mechanisms of Development*, 77: 31-47.

Barkovich, A. J., R. Guerrini, R. I. Kuzniecky, G. D. Jackson, and W. B. Dobyns. 2012. 'A developmental and genetic classification for malformations of cortical development: update 2012', *Brain*, 135: 1348-69.

Barr, J., S. Charania, R. Gilmutdinov, K. Yakovlev, Y. Shidlovskii, and P. Schedl. 2019. 'The CPEB translational regulator, Orb, functions together with Par proteins to polarize the Drosophila oocyte', *PLoS Genet*, 15: e1008012.

Basto, R., K. Brunk, T. Vinadogrova, N. Peel, A. Franz, A. Khodjakov, and J. W. Raff. 2008. 'Centrosome amplification can initiate tumorigenesis in flies', *Cell*, 133: 1032-42.

Basto, R., J. Lau, T. Vinogradova, A. Gardiol, C. G. Woods, A. Khodjakov, and J. W. Raff. 2006. 'Flies without centrioles', *Cell*, 125: 1375-86.

Bergalet, J., D. Patel, F. Legendre, C. Lapointe, L. P. Benoit Bouvrette, A. Chin, M. Blanchette, E. Kwon, and E. Lecuyer. 2020. 'Inter-dependent Centrosomal Co-localization of the cen and ik2 cis-Natural Antisense mRNAs in Drosophila', *Cell Reports*, 30: 3339-52 e6.

Berger, C., H. Harzer, T. R. Burkard, J. Steinmann, S. van der Horst, A. S. Laurenson, M. Novatchkova, H. Reichert, and J. A. Knoblich. 2012. 'FACS purification and transcriptome analysis of drosophila neural stem cells reveals a role for Klumpfuss in self-renewal', *Cell Reports*, 2: 407-18.

Betschinger, J., and J. A. Knoblich. 2004. 'Dare to be different: asymmetric cell division in Drosophila, C. elegans and vertebrates', *Current Biology*, 14: R674-85.

Betschinger, J., K. Mechtler, and J. A. Knoblich. 2003. 'The Par complex directs asymmetric cell division by phosphorylating the cytoskeletal protein Lgl', *Nature*, 422: 326-30.

Bettencourt-Dias, M. 2013. 'Q&A: Who needs a centrosome?', *BMC Biology*, 11: 28.

Bettencourt-Dias, M., and D. M. Glover. 2007. 'Centrosome biogenesis and function: centrosomics brings new understanding', *Nature Reviews: Molecular Cell Biology*, 8: 451-63.

Bettencourt-Dias, M., F. Hildebrandt, D. Pellman, G. Woods, and S. A. Godinho. 2011. 'Centrosomes and cilia in human disease', *Trends in Genetics*, 27: 307-15.

Bier, E., H. Vaessin, S. Younger-Shepherd, L. Y. Jan, and Y. N. Jan. 1992. 'deadpan, an essential pan-neural gene in Drosophila, encodes a helix-loop-helix protein similar to the hairy gene product', *Genes and Development*, 6: 2137-51.

Blachon, S., X. Cai, K. A. Roberts, K. Yang, A. Polyanovsky, A. Church, and T. Avidor-Reiss. 2009. 'A proximal centriole-like structure is present in Drosophila spermatids and can serve as a model to study centriole duplication', *Genetics*, 182: 133-44.

Blacque, O. E., and M. R. Leroux. 2006. 'Bardet-Biedl syndrome: an emerging pathomechanism of intracellular transport', *Cellular and Molecular Life Sciences*, 63: 2145-61.

Blencowe, B., S. Brenner, T. Hughes, and Q. Morris. 2009. 'Post-transcriptional gene regulation: RNA-protein interactions, RNA processing, mRNA stability and localization', *Pacific Symposium on Biocomputing*: 545-8.

Bloodgood, Robert A. 2009. 'From Central to Rudimentary to Primary: The History of an Underappreciated Organelle Whose Time Has Come.The Primary Cilium.' in Roger D. Sloboda (ed.), *Primary Cilia* (Academic Press).

Blower, M. D., E. Feric, K. Weis, and R. Heald. 2007. 'Genome-wide analysis demonstrates conserved localization of messenger RNAs to mitotic microtubules', *J Cell Biol*, 179: 1365-73.

Boehm, V., and N. H. Gehring. 2016. 'Exon Junction Complexes: Supervising the Gene Expression Assembly Line', *Trends in Genetics*, 32: 724-35.

Bond, J., E. Roberts, G. H. Mochida, D. J. Hampshire, S. Scott, J. M. Askham, K. Springell, M. Mahadevan, Y. J. Crow, A. F. Markham, C. A. Walsh, and C. G. Woods. 2002. 'ASPM is a major determinant of cerebral cortical size', *Nature Genetics*, 32: 316-20.

Bond, J., E. Roberts, K. Springell, S. B. Lizarraga, S. Scott, J. Higgins, D. J. Hampshire, E. E. Morrison, G. F. Leal, E. O. Silva, S. M. Costa, D. Baralle, M. Raponi, G. Karbani, Y. Rashid, H. Jafri, C. Bennett, P. Corry, C. A. Walsh, and C. G. Woods. 2005. 'A centrosomal mechanism involving CDK5RAP2 and CENPJ controls brain size', *Nature Genetics*, 37: 353-5.

Bond, J., and C. G. Woods. 2006. 'Cytoskeletal genes regulating brain size', *Current Opinion in Cell Biology*, 18: 95-101.

Bornens, M. 2012. 'The centrosome in cells and organisms', *Science*, 335: 422-6.

Bornens, M., M. Paintrand, J. Berges, M. C. Marty, and E. Karsenti. 1987. 'Structural and chemical characterization of isolated centrosomes', *Cell Motility and the Cytoskeleton*, 8: 238-49.

Bornens, Michel, and Juliette Azimzadeh. 2007. 'Origin and Evolution of the Centrosome.' in, *Eukaryotic Membranes and Cytoskeleton: Origins and Evolution* (Springer New York: New York, NY).

Boveri, T. 2008. 'Concerning the origin of malignant tumours by Theodor Boveri. Translated and annotated by Henry Harris', *Journal of Cell Science*, 121 Suppl 1: 1-84.

Boveri, Theodor. 1887. *Ueber den Antheil des Spermatozoon an der Theilung des Eies* (Gesellschaft f??r Morphologie und Physiologie zu M??nchen: M??nchen).

———. 1888. *Zellen-Studien: Die Befruchtung und Teilung des Eies von Ascaris megalocephala* (G. Fischer).

———. 1895. 'Über die Befruchtungs-und Entwickelungsfähigkeit kernloser Seeigel-Eier und über die Möglichkeit ihrer Bastardirung', *Archiv für Entwicklungsmechanik der Organismen*, 2: 394-443.

Bowler, M., D. Kong, S. Sun, R. Nanjundappa, L. Evans, V. Farmer, A. Holland, M. R. Mahjoub, H. Sui, and J. Loncarek. 2019. 'High-resolution characterization of centriole distal appendage morphology and dynamics by correlative STORM and electron microscopy', *Nat Commun*, 10: 993.

Bowman, S. K., R. A. Neumuller, M. Novatchkova, Q. Du, and J. A. Knoblich. 2006. 'The Drosophila NuMA Homolog Mud regulates spindle orientation in asymmetric cell division', *Developmental Cell*, 10: 731-42.

Branon, T. C., J. A. Bosch, A. D. Sanchez, N. D. Udeshi, T. Svinkina, S. A. Carr, J. L. Feldman, N. Perrimon, and A. Y. Ting. 2018. 'Efficient proximity labeling in living cells and organisms with TurboID', *Nature Biotechnology*, 36: 880-87.

Braun, D. A., and F. Hildebrandt. 2017. 'Ciliopathies', *Cold Spring Harbor Perspectives in Biology*, 9.

Breslow, D. K., and A. J. Holland. 2019. 'Mechanism and Regulation of Centriole and Cilium Biogenesis', *Annual Review of Biochemistry*, 88: 691-724.

Brinkley, B. R. 1985. 'Microtubule organizing centers', *Annual Review of Cell Biology*, 1: 145-72.

Brinkley, B. R., and J. Cartwright, Jr. 1971. 'Ultrastructural analysis of mitotic spindle elongation in mammalian cells in vitro. Direct microtubule counts', *J Cell Biol*, 50: 416-31.

Brinkley, B. R., and T. M. Goepfert. 1998. 'Supernumerary centrosomes and cancer: Boveri's hypothesis resurrected', *Cell Motility and the Cytoskeleton*, 41: 281-8.

Broadus, J., and C. Q. Doe. 1997. 'Extrinsic cues, intrinsic cues and microfilaments regulate asymmetric protein localization in Drosophila neuroblasts', *Current Biology*, 7: 827-35.

Broadus, J., S. Fuerstenberg, and C. Q. Doe. 1998. 'Staufen-dependent localization of prospero mRNA contributes to neuroblast daughter-cell fate', *Nature*, 391: 792-5.

Buffin, E., D. Emre, and R. E. Karess. 2007. 'Flies without a spindle checkpoint', *Nature Cell Biology*, 9: 565-72.

Buxbaum, A. R., G. Haimovich, and R. H. Singer. 2015. 'In the right place at the right time: visualizing and understanding mRNA localization', *Nature Reviews: Molecular Cell Biology*, 16: 95-109.

Cabernard, C., and C. Q. Doe. 2009. 'Apical/basal spindle orientation is required for neuroblast homeostasis and neuronal differentiation in Drosophila', *Developmental Cell*, 17: 134-41.

Carvalho-Santos, Z., J. Azimzadeh, J. B. Pereira-Leal, and M. Bettencourt-Dias. 2011. 'Evolution: Tracing the origins of centrioles, cilia, and flagella', *J Cell Biol*, 194: 165-75.

Caussinus, E., and F. Hirth. 2007. 'Asymmetric stem cell division in development and cancer', *Progress in Molecular and Subcellular Biology*, 45: 205-25.

Cervantes, S. A., T. H. Bajakian, M. A. Soria, A. S. Falk, R. J. Service, R. Langen, and A. B. Siemer. 2016. 'Identification and Structural Characterization of the N-terminal Amyloid Core of Orb2 isoform A', *Scientific Reports*, 6: 38265.

Chan, J. Y. 2011. 'A clinical overview of centrosome amplification in human cancers', *International Journal of Biological Sciences*, 7: 1122-44.

Chang, J. S., L. Tan, and P. Schedl. 1999. 'The Drosophila CPEB homolog, orb, is required for oskar protein expression in oocytes', *Developmental Biology*, 215: 91-106.

Chavali, P. L., M. Putz, and F. Gergely. 2014. 'Small organelle, big responsibility: the role of centrosomes in development and disease', *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 369.

Chen, C., and Y. M. Yamashita. 2021. 'Centrosome-centric view of asymmetric stem cell division', *Open Biol*, 11: 200314.

Chen, E. Y., C. M. Tan, Y. Kou, Q. Duan, Z. Wang, G. V. Meirelles, N. R. Clark, and A. Ma'ayan. 2013. 'Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool', *BMC Bioinformatics*, 14: 128.

Chen, J. F., Y. Zhang, J. Wilde, K. C. Hansen, F. Lai, and L. Niswander. 2014. 'Microcephaly disease gene Wdr62 regulates mitotic progression of embryonic neural stem cells and brain size', *Nat Commun*, 5: 3885.

Chen, Y., Y. H. Tsai, and S. H. Tseng. 2016. 'Regulation of the Expression of Cytoplasmic Polyadenylation Element Binding Proteins for the Treatment of Cancer', *Anticancer Research*, 36: 5673-80.

Chouaib, R., A. Safieddine, X. Pichon, A. Imbert, O. S. Kwon, A. Samacoits, A. M. Traboulsi, M. C. Robert, N. Tsanov, E. Coleno, I. Poser, C. Zimmer, A. Hyman, H. Le Hir, K. Zibara, M. Peter, F. Mueller, T. Walter, and E. Bertrand. 2020. 'A Dual Protein-mRNA Localization Screen Reveals Compartmentalized Translation and Widespread Co-translational RNA Targeting', *Developmental Cell*, 54: 773-91 e5.

Conduit, P. T., Z. Feng, J. H. Richens, J. Baumbach, A. Wainman, S. D. Bakshi, J. Dobbelaere, S. Johnson, S. M. Lea, and J. W. Raff. 2014. 'The centrosome-specific phosphorylation of Cnn by Polo/Plk1 drives Cnn scaffold assembly and centrosome maturation', *Developmental Cell*, 28: 659-69.

Conduit, P. T., and J. W. Raff. 2010. 'Cnn dynamics drive centrosome size asymmetry to ensure daughter centriole retention in Drosophila neuroblasts', *Current Biology*, 20: 2187-92.

Conduit, P. T., A. Wainman, and J. W. Raff. 2015. 'Centrosome function and assembly in animal cells', *Nature Reviews: Molecular Cell Biology*, 16: 611-24.

Coutinho, A. M., G. Oliveira, C. Katz, J. Feng, J. Yan, C. Yang, C. Marques, A. Ataide, T. S. Miguel, L. Borges, J. Almeida, C. Correia, A. Currais, C. Bento, L. Mota-Vieira, T. Temudo, M. Santos, P. Maciel, S. S. Sommer, and A. M. Vicente. 2007. 'MECP2 coding sequence and 3'UTR variation in 172 unrelated autistic patients', *American Journal of Medical Genetics. Part B: Neuropsychiatric Genetics*, 144B: 475-83.

D'Ambrogio, A., K. Nagaoka, and J. D. Richter. 2013. 'Translational control of cell growth and malignancy by the CPEBs', *Nature Reviews: Cancer*, 13: 283-90.

Danielsson, F., D. Mahdessian, U. Axelsson, D. Sullivan, M. Uhlen, J. S. Andersen, P. J. Thul, and E. Lundberg. 2020. 'Spatial Characterization of the Human Centrosome Proteome Opens Up New Horizons for a Small but Versatile Organelle', *Proteomics*, 20: e1900361.

Darling, S., A. B. Fielding, D. Sabat-Pospiech, I. A. Prior, and J. M. Coulson. 2017. 'Regulation of the cell cycle and centrosome biology by deubiquitylases', *Biochemical Society Transactions*, 45: 1125-36.

Darnell, J. C., and J. D. Richter. 2012. 'Cytoplasmic RNA-binding proteins and the control of complex brain function', *Cold Spring Harbor Perspectives in Biology*, 4: a012344.

David, R. 2011. 'Cell cycle: building the centriole', *Nature Reviews: Molecular Cell Biology*, 12: 342.

de Vries, B. B., A. M. Wiegers, E. de Graaff, A. J. Verkerk, J. O. Van Hemel, D. J. Halley, J. P. Fryns, L. M. Curfs, M. F. Niermeijer, and B. A. Oostra. 1993. 'Mental status and fragile X expression in relation to FMR-1 gene mutation', *European Journal of Human Genetics*, 1: 72-9.

Debec, A. 1978. 'Haploid cell cultures of Drosophila melanogaster', *Nature*, 274: 255-6.

Degrassi, F., M. Damizia, and P. Lavia. 2019. 'The Mitotic Apparatus and Kinetochores in Microcephaly and Neurodevelopmental Diseases', *Cells*, 9.

Delgehyr, N., J. Sillibourne, and M. Bornens. 2005. 'Microtubule nucleation and anchoring at the centrosome are independent processes linked by ninein function', *Journal of Cell Science*, 118: 1565-75.

Devakumar, D., A. Bamford, M. U. Ferreira, J. Broad, R. E. Rosch, N. Groce, J. Breuer, M. A. Cardoso, A. J. Copp, P. Alexandre, L. C. Rodrigues, and I. Abubakar. 2018. 'Infectious causes of microcephaly: epidemiology, pathogenesis, diagnosis, and management', *Lancet Infectious Diseases*, 18: e1-e13.

Dieks, J. K., A. Baumer, E. Wilichowski, A. Rauch, and M. Sigler. 2014. 'Microcephalic osteodysplastic primordial dwarfism type II (MOPD II) with multiple vascular complications misdiagnosed as Dubowitz syndrome', *European Journal of Pediatrics*, 173: 1253-6.

Dippell, R. V. 1976. 'Effects of nuclease and protease digestion on the ultrastructure of Paramecium basal bodies', *J Cell Biol*, 69: 622-37.

Dix, C. I., and J. W. Raff. 2007. 'Drosophila Spd-2 recruits PCM to the sperm centriole, but is dispensable for centriole duplication', *Current Biology*, 17: 1759-64.

Dobbelaere, J., F. Josue, S. Suijkerbuijk, B. Baum, N. Tapon, and J. Raff. 2008. 'A genome-wide RNAi screen to dissect centriole duplication and centrosome maturation in Drosophila', *PLoS Biology*, 6: e224.

Doe, C. Q. 2008. 'Neural stem cells: balancing self-renewal with differentiation', *Development*, 135: 1575-87.

Doe, C. Q., Q. Chu-LaGraff, D. M. Wright, and M. P. Scott. 1991. 'The prospero gene specifies cell fates in the Drosophila central nervous system', *Cell*, 65: 451-64.

Dolk, H. 1991. 'The predictive value of microcephaly during the first year of life for mental retardation at seven years', *Developmental Medicine and Child Neurology*, 33: 974-83.

Dong, G. 2015. 'Building a ninefold symmetrical barrel: structural dissections of centriole assembly', *Open Biol*, 5.

Doxsey, S. J., P. Stein, L. Evans, P. D. Calarco, and M. Kirschner. 1994. 'Pericentrin, a highly conserved centrosome protein involved in microtubule organization', *Cell*, 76: 639-50.

Duesberg, P. 1999. 'Are centrosomes or aneuploidy the key to cancer?', *Science*, 284: 2091-2.

Duffy, J. B. 2002. 'GAL4 system in Drosophila: a fly geneticist's Swiss army knife', *Genesis*, 34: 1-15.

Dynes, J. L., and O. Steward. 2012. 'Arc mRNA docks precisely at the base of individual dendritic spines indicating the existence of a specialized microdomain for synapse-specific mRNA translation', *Journal of Comparative Neurology*, 520: 3105-19.

Dzhindzhev, N. S., G. Tzolovsky, Z. Lipinszki, M. Abdelaziz, J. Debski, M. Dadlez, and D. M. Glover. 2017. 'Two-step phosphorylation of Ana2 by Plk4 is required for the sequential loading of Ana2 and Sas6 to initiate procentriole formation', *Open Biol*, 7.

Dzhindzhev, N. S., G. Tzolovsky, Z. Lipinszki, S. Schneider, R. Lattao, J. Fu, J. Debski, M. Dadlez, and D. M. Glover. 2014. 'Plk4 phosphorylates Ana2 to trigger Sas6 recruitment and procentriole formation', *Current Biology*, 24: 2526-32.

Egger, B., J. Q. Boone, N. R. Stevens, A. H. Brand, and C. Q. Doe. 2007. 'Regulation of spindle orientation and neural stem cell fate in the Drosophila optic lobe', *Neural Dev*, 2: 1.

Eliscovich, C., I. Peset, I. Vernos, and R. Mendez. 2008. 'Spindle-localized CPE-mediated translation controls meiotic chromosome segregation', *Nature Cell Biology*, 10: 858-65.

Enos, S. J., M. Dressler, B. F. Gomes, A. A. Hyman, and J. B. Woodruff. 2018. 'Phosphatase PP2A and microtubule-mediated pulling forces disassemble centrosomes during mitotic exit', *Biol Open*, 7.

Fan, Y., and A. Bergmann. 2010. 'The cleaved-Caspase-3 antibody is a marker of Caspase-9-like DRONC activity in Drosophila', *Cell Death and Differentiation*, 17: 534-9.

Fang, J., and D. A. Lerit. 2022. 'Orb-dependent polyadenylation contributes to PLP expression and centrosome scaffold assembly', *Development*, 149.

Farache, D., L. Emorine, L. Haren, and A. Merdes. 2018. 'Assembly and regulation of gamma-tubulin complexes', *Open Biol*, 8.

Feng, Z., A. Caballe, A. Wainman, S. Johnson, A. F. M. Haensele, M. A. Cottee, P. T. Conduit, S. M. Lea, and J. W. Raff. 2017. 'Structural Basis for Mitotic Centrosome Assembly in Flies', *Cell*, 169: 1078-89 e13.

Ferenz, N. P., R. Paul, C. Fagerstrom, A. Mogilner, and P. Wadsworth. 2009. 'Dynein antagonizes eg5 by crosslinking and sliding antiparallel microtubules', *Current Biology*, 19: 1833-8.

Ferguson, R. L., and J. L. Maller. 2010. 'Centrosomal localization of cyclin E-Cdk2 is required for initiation of DNA synthesis', *Current Biology*, 20: 856-60.

Fernandez-Miranda, G., and R. Mendez. 2012. 'The CPEB-family of proteins, translational control in senescence and cancer', *Ageing Res Rev*, 11: 460-72.

Firat-Karalar, E. N., and T. Stearns. 2015. 'Probing mammalian centrosome structure using BioID proximity-dependent biotinylation', *Methods in Cell Biology*, 129: 153-70.

Flemming, Walther. 1875. *Studien in der Entwicklungsgeschichte der Najaden* (KK Staatsdruckerei).

Fong, C. S., G. Mazo, T. Das, J. Goodman, M. Kim, B. P. O'Rourke, D. Izquierdo, and M. F. Tsou. 2016. '53BP1 and USP28 mediate p53-dependent cell cycle arrest in response to centrosome loss and prolonged mitosis', *Elife*, 5.

Fox, C. A., M. D. Sheets, and M. P. Wickens. 1989. 'Poly(A) addition during maturation of frog oocytes: distinct nuclear and cytoplasmic activities and regulation by the sequence UUUUUAU', *Genes and Development*, 3: 2151-62.

Frank, V., A. I. den Hollander, N. O. Bruchle, M. N. Zonneveld, G. Nurnberg, C. Becker, G. Du Bois, H. Kendziorra, S. Roosing, J. Senderek, P. Nurnberg, F. P. Cremers, K. Zerres, and C. Bergmann. 2008. 'Mutations of the CEP290 gene encoding a centrosomal protein cause Meckel-Gruber syndrome', *Human Mutation*, 29: 45-52.

Freeman, M. R., and C. Q. Doe. 2001. 'Asymmetric Prospero localization is required to generate mixed neuronal/glial lineages in the Drosophila CNS', *Development*, 128: 4103-12.

Fry, A. M., J. Sampson, C. Shak, and S. Shackleton. 2017. 'Recent advances in pericentriolar material organization: ordered layers and scaffolding gels', *F1000Res*, 6: 1622.

Fu, J., and D. M. Glover. 2012. 'Structured illumination of the interface between centriole and peri-centriolar material', *Open Biol*, 2: 120104.

Fu, J., I. M. Hagan, and D. M. Glover. 2015. 'The centrosome and its duplication cycle', *Cold Spring Harbor Perspectives in Biology*, 7: a015800.

Gall, Joseph G. 2005. 'Early Studies on Centrioles and Centrosomes.' in, *Centrosomes in Development and Disease*.

Gallaud, E., A. Ramdas Nair, N. Horsley, A. Monnard, P. Singh, T. T. Pham, D. Salvador Garcia, A. Ferrand, and C. Cabernard. 2020. 'Dynamic centriolar localization of Polo and Centrobin in early mitosis primes centrosome asymmetry', *PLoS Biology*, 18: e3000762.

Galletta, B. J., C. J. Fagerstrom, T. A. Schoborg, T. A. McLamarrah, J. M. Ryniawec, D. W. Buster, K. C. Slep, G. C. Rogers, and N. M. Rusan. 2016. 'A centrosome interactome provides insight into organelle assembly and reveals a non-duplication role for Plk4', *Nat Commun*, 7: 12476.

Gambarotto, D., C. Pennetier, J. M. Ryniawec, D. W. Buster, D. Gogendeau, A. Goupil, M. Nano, A. Simon, D. Blanc, V. Racine, Y. Kimata, G. C. Rogers, and R. Basto. 2019. 'Plk4 Regulates Centriole Asymmetry and Spindle Orientation in Neural Stem Cells', *Developmental Cell*, 50: 11-24 e10.

Ganem, N. J., S. A. Godinho, and D. Pellman. 2009. 'A mechanism linking extra centrosomes to chromosomal instability', *Nature*, 460: 278-82.

Gartenmann, L., A. Wainman, M. Qurashi, R. Kaufmann, S. Schubert, J. W. Raff, and I. M. Dobbie. 2017. 'A combined 3D-SIM/SMLM approach allows centriole proteins to be localized with a precision of approximately 4-5 nm', *Current Biology*, 27: R1054-R55.

Gebauer, F., T. Schwarzl, J. Valcarcel, and M. W. Hentze. 2021. 'RNA-binding proteins in human genetic disease', *Nat Rev Genet*, 22: 185-98.

Gemble, S., R. Wardenaar, K. Keuper, N. Srivastava, M. Nano, A. S. Mace, A. E. Tijhuis, S. V. Bernhard, D. C. J. Spierings, A. Simon, O. Goundiam, H. Hochegger, M. Piel, F. Foijer, Z. Storchova, and R. Basto. 2022. 'Genetic instability from a single S phase after whole-genome duplication', *Nature*, 604: 146-51.

Gerstberger, S., M. Hafner, M. Ascano, and T. Tuschl. 2014. 'Evolutionary conservation and expression of human RNA-binding proteins and their role in human genetic disease', *Advances in Experimental Medicine and Biology*, 825: 1-55.

Ghadimi, B Michael, Dan L Sackett, Michael J Difilippantonio, Evelin Schröck, Thomas Neumann, Annukka Jauho, Gert Auer, and Thomas Ried. 2000. 'Centrosome amplification and instability occurs exclusively in aneuploid, but not in diploid colorectal cancer cell lines, and correlates with numerical chromosomal aberrations', *Genes, Chromosomes and Cancer*, 27: 183-90.

Giansanti, M. G., E. Bucciarelli, S. Bonaccorsi, and M. Gatti. 2008. 'Drosophila SPD-2 is an essential centriole component required for PCM recruitment and astral-microtubule nucleation', *Current Biology*, 18: 303-9.

Glisovic, T., J. L. Bachorik, J. Yong, and G. Dreyfuss. 2008. 'RNA-binding proteins and post-transcriptional gene regulation', *FEBS Letters*, 582: 1977-86.

Glover, D. M., C. Gonzalez, and J. W. Raff. 1993. 'The centrosome', *Scientific American*, 268: 62-8.

Godinho, S. A., M. Kwon, and D. Pellman. 2009. 'Centrosomes and cancer: how cancer cells divide with too many centrosomes', *Cancer and Metastasis Reviews*, 28: 85-98.

Godinho, S. A., and D. Pellman. 2014. 'Causes and consequences of centrosome abnormalities in cancer', *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 369.

Gogendeau, D., K. Siudeja, D. Gambarotto, C. Pennetier, A. J. Bardin, and R. Basto. 2015. 'Aneuploidy causes premature differentiation of neural and intestinal stem cells', *Nat Commun*, 6: 8894.

Gomes, E., and J. Shorter. 2019. 'The molecular language of membraneless organelles', *Journal of Biological Chemistry*, 294: 7115-27.

Gonzalez, C. 2007. 'Spindle orientation, asymmetric division and tumour suppression in Drosophila stem cells', *Nat Rev Genet*, 8: 462-72.

Goshima, G., R. Wollman, S. S. Goodwin, N. Zhang, J. M. Scholey, R. D. Vale, and N. Stuurman. 2007. 'Genes required for mitotic spindle assembly in Drosophila S2 cells', *Science*, 316: 417-21.

Gould, R. R., and G. G. Borisy. 1977. 'The pericentriolar material in Chinese hamster ovary cells nucleates microtubule formation', *J Cell Biol*, 73: 601-15.

Groisman, I., Y. S. Huang, R. Mendez, Q. Cao, W. Theurkauf, and J. D. Richter. 2000. 'CPEB, maskin, and cyclin B1 mRNA at the mitotic apparatus: implications for local translational control of cell division', *Cell*, 103: 435-47.

Habedanck, R., Y. D. Stierhof, C. J. Wilkinson, and E. A. Nigg. 2005. 'The Polo kinase Plk4 functions in centriole duplication', *Nature Cell Biology*, 7: 1140-6.

Hafer, N., S. Xu, K. M. Bhat, and P. Schedl. 2011. 'The Drosophila CPEB protein Orb2 has a novel expression pattern and is important for asymmetric cell division and nervous system function', *Genetics*, 189: 907-21.

Hafner, A. S., P. G. Donlin-Asp, B. Leitch, E. Herzog, and E. M. Schuman. 2019. 'Local protein synthesis is a ubiquitous feature of neuronal pre- and postsynaptic compartments', *Science*, 364.

Hake, L. E., R. Mendez, and J. D. Richter. 1998. 'Specificity of RNA binding by CPEB: requirement for RNA recognition motifs and a novel zinc finger', *Molecular and Cellular Biology*, 18: 685-93.

Hake, L. E., and J. D. Richter. 1994. 'CPEB is a specificity factor that mediates cytoplasmic polyadenylation during Xenopus oocyte maturation', *Cell*, 79: 617-27.

Hales, K. G., C. A. Korey, A. M. Larracuente, and D. M. Roberts. 2015. 'Genetics on the Fly: A Primer on the Drosophila Model System', *Genetics*, 201: 815-42.

Harripaul, R., N. Vasli, A. Mikhailov, M. A. Rafiq, K. Mittal, C. Windpassinger, T. I. Sheikh, A. Noor, H. Mahmood, S. Downey, M. Johnson, K. Vleuten, L. Bell, M. Ilyas, F. S. Khan, V. Khan, M. Moradi, M. Ayaz, F. Naeem, A. Heidari, I. Ahmed, S. Ghadami, Z. Agha, S. Zeinali, R. Qamar, H. Mozhdehipanah, P. John, A. Mir, M. Ansar, L. French, M. Ayub, and J. B. Vincent. 2018. 'Mapping autosomal recessive intellectual disability: combined microarray and exome sequencing identifies 26 novel candidate genes in 192 consanguineous families', *Molecular Psychiatry*, 23: 973-84.

Hartman, H., J. P. Puma, and T. Gruney, Jr. 1974. 'Evidence for the association of RNA with the ciliary basal bodies of Tetrahymena', *Journal of Cell Science*, 16: 241-59.

Hayashi, Y., and A. K. Majewska. 2005. 'Dendritic spine geometry: functional implication and regulation', *Neuron*, 46: 529-32.

Heath, A. P., V. Ferretti, S. Agrawal, M. An, J. C. Angelakos, R. Arya, R. Bajari, B. Baqar, J. H. B. Barnowski, J. Burt, A. Catton, B. F. Chan, F. Chu, K. Cullion, T. Davidsen, P. M. Do, C. Dompierre, M. L. Ferguson, M. S. Fitzsimons, M. Ford, M. Fukuma, S. Gaheen, G. L. Ganji, T. I. Garcia, S. S. George, D. S. Gerhard, F. Gerthoffert, F. Gomez, K. Han, K. M. Hernandez, B. Issac, R. Jackson, M. A. Jensen, S. Joshi, A. Kadam, A. Khurana, K. M. J. Kim, V. E. Kraft, S. Li, T. M. Lichtenberg, J. Lodato, L. Lolla, P. Martinov, J. A. Mazzone, D. P. Miller, I. Miller, J. S. Miller, K. Miyauchi, M. W. Murphy, T. Nullet, R. O. Ogwara, F. M. Ortuno, J. Pedrosa, P. L. Pham, M. Y. Popov, J. J. Porter, R. Powell, K. Rademacher, C. P. Reid, S. Rich, B. Rogel, H. Sahni, J. H. Savage, K. A. Schmitt, T. J. Simmons, J. Sislow, J. Spring, L. Stein, S. Sullivan, Y. Tang, M. Thiagarajan, H. D. Troyer, C. Wang, Z. Wang, B. L. West, A. Wilmer, S. Wilson, K. Wu, W. P. Wysocki, L. Xiang, J. T. Yamada, L. Yang, C. Yu, C. K. Yung, J. C. Zenklusen, J. Zhang, Z. Zhang, Y. Zhao, A. Zubair, L. M. Staudt, and R. L. Grossman. 2021. 'The NCI Genomic Data Commons', *Nature Genetics*, 53: 257-62.

Heidemann, S. R., G. Sander, and M. W. Kirschner. 1977. 'Evidence for a functional role of RNA in centrioles', *Cell*, 10: 337-50.

Hervas, R., M. Del Carmen Fernandez-Ramirez, A. Galera-Prat, M. Suzuki, Y. Nagai, M. Bruix, M. Menendez, D. V. Laurents, and M. Carrion-Vazquez. 2021. 'Divergent CPEB prion-like domains reveal different assembly mechanisms for a generic amyloid-like fold', *BMC Biology*, 19: 43.

Hervas, R., L. Li, A. Majumdar, C. Fernandez-Ramirez Mdel, J. R. Unruh, B. D. Slaughter, A. Galera-Prat, E. Santana, M. Suzuki, Y. Nagai, M. Bruix, S. Casas-Tinto, M. Menendez, D. V. Laurents, K. Si, and M. Carrion-Vazquez. 2016. 'Molecular Basis of Orb2 Amyloidogenesis and Blockade of Memory Consolidation', *PLoS Biology*, 14: e1002361.

Hieronymus, H., and P. A. Silver. 2004. 'A systems view of mRNP biology', *Genes and Development*, 18: 2845-60.

Hinchcliffe, Edward H., Frederick J. Miller, Matthew Cham, Alexey Khodjakov, and Greenfield Sluder. 2001. 'Requirement of a Centrosomal Activity for Cell Cycle Progression through G₁ into S Phase', *Science*, 291: 1547-50.

Hoeft, F., J. C. Carter, A. A. Lightbody, H. Cody Hazlett, J. Piven, and A. L. Reiss. 2010. 'Region-specific alterations in brain development in one- to three-year-old boys with fragile X syndrome', *Proceedings of the National Academy of Sciences of the United States of America*, 107: 9335-9.

Homem, C. C., and J. A. Knoblich. 2012. 'Drosophila neuroblasts: a model for stem cell biology', *Development*, 139: 4297-310.

Homem, C. C., M. Repic, and J. A. Knoblich. 2015. 'Proliferation control in neural stem and progenitor cells', *Nature Reviews: Neuroscience*, 16: 647-59.

Hu, H., K. Kahrizi, L. Musante, Z. Fattahi, R. Herwig, M. Hosseini, C. Oppitz, S. S. Abedini, V. Suckow, F. Larti, M. Beheshtian, B. Lipkowitz, T. Akhtarkhavari, S. Mehvari, S. Otto, M. Mohseni, S. Arzhangi, P. Jamali, F. Mojahedi, M. Taghdiri, E. Papari, M. J. Soltani Banavandi, S. Akbari, S. H. Tonekaboni, H. Dehghani, M. R. Ebrahimpour, I. Bader, B. Davarnia, M. Cohen, H. Khodaei, B. Albrecht, S. Azimi, B. Zirn, M. Bastami, D. Wieczorek, G. Bahrami, K. Keleman, L. N. Vahid, A. Tzschach, J. Gartner, G. Gillessen-Kaesbach, J. R. Varaghchi, B. Timmermann, F. Pourfatemi, A. Jankhah, W. Chen, P. Nikuei, V. M. Kalscheuer, M. Oladnabi, T. F. Wienker, H. H. Ropers, and H. Najmabadi. 2019. 'Genetics of intellectual disability in consanguineous families', *Molecular Psychiatry*, 24: 1027-39.

Huang, Y. S., M. C. Kan, C. L. Lin, and J. D. Richter. 2006. 'CPEB3 and CPEB4 in neurons: analysis of RNA-binding specificity and translational control of AMPA receptor GluR2 mRNA', *EMBO Journal*, 25: 4865-76.

Inaba, M., Z. G. Venkei, and Y. M. Yamashita. 2015. 'The polarity protein Baz forms a platform for the centrosome orientation during asymmetric stem cell division in the Drosophila male germline', *Elife*, 4.

Ivshina, M., P. Lasko, and J. D. Richter. 2014. 'Cytoplasmic polyadenylation element binding proteins in development, health, and disease', *Annual Review of Cell and Developmental Biology*, 30: 393-415.

Izumi, H., and Y. Kaneko. 2012. 'Evidence of asymmetric cell division and centrosome inheritance in human neuroblastoma cells', *Proceedings of the National Academy of Sciences of the United States of America*, 109: 18048-53.

Izumi, Y., N. Ohta, K. Hisata, T. Raabe, and F. Matsuzaki. 2006. 'Drosophila Pins-binding protein Mud regulates spindle-polarity coupling and centrosome organization', *Nature Cell Biology*, 8: 586-93.

Jakobsen, L., K. Vanselow, M. Skogs, Y. Toyoda, E. Lundberg, I. Poser, L. G. Falkenby, M. Bennetzen, J. Westendorf, E. A. Nigg, M. Uhlen, A. A. Hyman, and J. S. Andersen. 2011. 'Novel asymmetrically localizing components of human centrosomes identified by complementary proteomics methods', *EMBO Journal*, 30: 1520-35.

Jana, S. C., M. Bettencourt-Dias, B. Durand, and T. L. Megraw. 2016. 'Drosophila melanogaster as a model for basal body research', *Cilia*, 5: 22.

Januschke, J., and C. Gonzalez. 2010. 'The interphase microtubule aster is a determinant of asymmetric division orientation in Drosophila neuroblasts', *J Cell Biol*, 188: 693-706.

Januschke, J., S. Llamazares, J. Reina, and C. Gonzalez. 2011. 'Drosophila neuroblasts retain the daughter centrosome', *Nat Commun*, 2: 243.

Januschke, J., J. Reina, S. Llamazares, T. Bertran, F. Rossi, J. Roig, and C. Gonzalez. 2013. 'Centrobin controls mother-daughter centriole asymmetry in Drosophila neuroblasts', *Nature Cell Biology*, 15: 241-8.

Jayaraman, D., B. I. Bae, and C. A. Walsh. 2018. 'The Genetics of Primary Microcephaly', *Annu Rev Genomics Hum Genet*, 19: 177-200.

Jeong, Y., J. Lee, K. Kim, J. C. Yoo, and K. Rhee. 2007. 'Characterization of NIP2/centrobin, a novel substrate of Nek2, and its potential role in microtubule stabilization', *Journal of Cell Science*, 120: 2106-16.

Jung, H., C. G. Gkogkas, N. Sonenberg, and C. E. Holt. 2014. 'Remote control of gene function by local translation', *Cell*, 157: 26-40.

Kacsoh, B. Z., J. Bozler, S. Hodge, M. Ramaswami, and G. Bosco. 2015. 'A novel paradigm for nonassociative long-term memory in Drosophila: predator-induced changes in oviposition behavior', *Genetics*, 199: 1143-57.

Keck, J. M., M. H. Jones, C. C. Wong, J. Binkley, D. Chen, S. L. Jaspersen, E. P. Holinger, T. Xu, M. Niepel, M. P. Rout, J. Vogel, A. Sidow, J. R. Yates, 3rd, and M. Winey. 2011. 'A cell cycle phosphoproteome of the yeast centrosome', *Science*, 332: 1557-61.

Kedde, M., M. van Kouwenhove, W. Zwart, J. A. Oude Vrielink, R. Elkon, and R. Agami. 2010. 'A Pumilio-induced RNA structure switch in p27-3' UTR controls miR-221 and miR-222 accessibility', *Nature Cell Biology*, 12: 1014-20.

Keleman, K., S. Kruttner, M. Alenius, and B. J. Dickson. 2007. 'Function of the Drosophila CPEB protein Orb2 in long-term courtship memory', *Nature Neuroscience*, 10: 1587-93.

Keller, L. C., and W. F. Marshall. 2008. 'Isolation and proteomic analysis of Chlamydomonas centrioles', *Methods in Molecular Biology*, 432: 289-300.

Khan, M. R., L. Li, C. Perez-Sanchez, A. Saraf, L. Florens, B. D. Slaughter, J. R. Unruh, and K. Si. 2015. 'Amyloidogenic Oligomerization Transforms Drosophila Orb2 from a Translation Repressor to an Activator', *Cell*, 163: 1468-83.

Khodjakov, A., and C. L. Rieder. 1999. 'The sudden recruitment of gamma-tubulin to the centrosome at the onset of mitosis and its dynamic exchange throughout the cell cycle, do not require microtubules', *J Cell Biol*, 146: 585-96.

Kim, J. H., and J. D. Richter. 2006. 'Opposing polymerase-deadenylase activities regulate cytoplasmic polyadenylation', *Molecular Cell*, 24: 173-83.

Kleylein-Sohn, J., J. Westendorf, M. Le Clech, R. Habedanck, Y. D. Stierhof, and E. A. Nigg. 2007. 'Plk4-induced centriole biogenesis in human cells', *Developmental Cell*, 13: 190-202.

Knoblich, J. A. 2008. 'Mechanisms of asymmetric stem cell division', *Cell*, 132: 583-97.

———. 2010. 'Asymmetric cell division: recent developments and their implications for tumour biology', *Nature Reviews: Molecular Cell Biology*, 11: 849-60.

Knoblich, J. A., L. Y. Jan, and Y. N. Jan. 1995. 'Asymmetric segregation of Numb and Prospero during cell division', *Nature*, 377: 624-7.

Knockleby, J., and H. Lee. 2010. 'Same partners, different dance: involvement of DNA replication proteins in centrosome regulation', *Cell Cycle*, 9: 4487-91.

Kohler, S., L. Carmody, N. Vasilevsky, J. O. B. Jacobsen, D. Danis, J. P. Gourdine, M. Gargano, N. L. Harris, N. Matentzoglu, J. A. McMurry, D. Osumi-Sutherland, V. Cipriani, J. P. Balhoff, T. Conlin, H. Blau, G. Baynam, R. Palmer, D. Gratian, H. Dawkins, M. Segal, A. C. Jansen, A. Muaz, W. H. Chang, J. Bergerson, S. J. F. Laulederkind, Z. Yuksel, S. Beltran, A. F. Freeman, P. I. Sergouniotis, D. Durkin, A. L. Storm, M. Hanauer, M. Brudno, S. M. Bello, M. Sincan, K. Rageth, M. T. Wheeler, R. Oegema, H. Lourghi, M. G. Della Rocca, R. Thompson, F. Castellanos, J. Priest, C. Cunningham-Rundles, A. Hegde, R. C. Lovering, C. Hajek, A. Olry, L. Notarangelo, M. Similuk, X. A. Zhang, D. Gomez-Andres, H. Lochmuller, H. Dollfus, S. Rosenzweig, S. Marwaha, A. Rath, K. Sullivan, C. Smith, J. D. Milner, D. Leroux, C. F. Boerkoel, A. Klion, M. C. Carter, T. Groza, D. Smedley, M. A. Haendel, C. Mungall, and P. N. Robinson. 2019. 'Expansion of the Human Phenotype Ontology (HPO) knowledge base and resources', *Nucleic Acids Research*, 47: D1018-D27.

Kollman, J. M., J. K. Polka, A. Zelter, T. N. Davis, and D. A. Agard. 2010. 'Microtubule nucleating gamma-TuSC assembles structures with 13-fold microtubule-like symmetry', *Nature*, 466: 879-82.

Komori, H., K. L. Golden, T. Kobayashi, R. Kageyama, and C. Y. Lee. 2018. 'Multilayered gene control drives timely exit from the stem cell state in uncommitted progenitors during Drosophila asymmetric neural stem cell division', *Genes and Development*, 32: 1550-61.

Kraut, R., W. Chia, L. Y. Jan, Y. N. Jan, and J. A. Knoblich. 1996. 'Role of inscuteable in orienting asymmetric cell divisions in Drosophila', *Nature*, 383: 50-5.

Kruttner, S., B. Stepien, J. N. Noordermeer, M. A. Mommaas, K. Mechtler, B. J. Dickson, and K. Keleman. 2012. 'Drosophila CPEB Orb2A mediates memory independent of Its RNA-binding domain', *Neuron*, 76: 383-95.

Kuleshov, M. V., M. R. Jones, A. D. Rouillard, N. F. Fernandez, Q. Duan, Z. Wang, S. Koplev, S. L. Jenkins, K. M. Jagodnik, A. Lachmann, M. G. McDermott, C. D. Monteiro, G. W. Gundersen, and A. Ma'ayan. 2016. 'Enrichr: a comprehensive gene set enrichment analysis web server 2016 update', *Nucleic Acids Research*, 44: W90-7.

Kwon, O. S., R. Mishra, A. Safieddine, E. Coleno, Q. Alasseur, M. Faucourt, I. Barbosa, E. Bertrand, N. Spassky, and H. Le Hir. 2021. 'Exon junction complex dependent mRNA localization is linked to centrosome organization during ciliogenesis', *Nat Commun*, 12: 1351.

Lai, S. L., and C. Q. Doe. 2014. 'Transient nuclear Prospero induces neural progenitor quiescence', *Elife*, 3.

Lai, S. L., M. R. Miller, K. J. Robinson, and C. Q. Doe. 2012. 'The Snail family member Worniu is continuously required in neuroblasts to prevent Elav-induced premature differentiation', *Developmental Cell*, 23: 849-57.

Lambert, J. D., and L. M. Nagy. 2002. 'Asymmetric inheritance of centrosomally localized mRNAs during embryonic cleavages', *Nature*, 420: 682-6.

Lara-Gonzalez, P., F. G. Westhorpe, and S. S. Taylor. 2012. 'The spindle assembly checkpoint', *Current Biology*, 22: R966-80.

Larkin, A., S. J. Marygold, G. Antonazzo, H. Attrill, G. Dos Santos, P. V. Garapati, J. L. Goodman, L. S. Gramates, G. Millburn, V. B. Strelets, C. J. Tabone, J. Thurmond, and Consortium FlyBase. 2021. 'FlyBase: updates to the Drosophila melanogaster knowledge base', *Nucleic Acids Research*, 49: D899-D907.

Lau, L., Y. L. Lee, S. J. Sahl, T. Stearns, and W. E. Moerner. 2012. 'STED microscopy with optimized labeling density reveals 9-fold arrangement of a centriole protein', *Biophysical Journal*, 102: 2926-35.

Lawo, S., M. Hasegan, G. D. Gupta, and L. Pelletier. 2012. 'Subdiffraction imaging of centrosomes reveals higher-order organizational features of pericentriolar material', *Nature Cell Biology*, 14: 1148-58.

Leal, G., D. Comprido, and C. B. Duarte. 2014. 'BDNF-induced local protein synthesis and synaptic plasticity', *Neuropharmacology*, 76 Pt C: 639-56.

Lecuyer, E., H. Yoshida, N. Parthasarathy, C. Alm, T. Babak, T. Cerovina, T. R. Hughes, P. Tomancak, and H. M. Krause. 2007. 'Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function', *Cell*, 131: 174-87.

Lee, C. E., K. S. Singleton, M. Wallin, and V. Faundez. 2020. 'Rare Genetic Diseases: Nature's Experiments on Human Development', *iScience*, 23: 101123.

Lee, K., and K. Rhee. 2011. 'PLK1 phosphorylation of pericentrin initiates centrosome maturation at the onset of mitosis', *J Cell Biol*, 195: 1093-101.

Leitch, C. C., N. A. Zaghloul, E. E. Davis, C. Stoetzel, A. Diaz-Font, S. Rix, M. Alfadhel, R. A. Lewis, W. Eyaid, E. Banin, H. Dollfus, P. L. Beales, J. L. Badano, and N. Katsanis. 2008. 'Hypomorphic mutations in syndromic encephalocele genes are associated with Bardet-Biedl syndrome', *Nature Genetics*, 40: 443-8.

Lennox, A. L., H. Mao, and D. L. Silver. 2018. 'RNA on the brain: emerging layers of post-transcriptional regulation in cerebral cortex development', *Wiley Interdiscip Rev Dev Biol*, 7.

Lerit, D. A. 2022. 'Signed, sealed, and delivered: RNA localization and translation at centrosomes', *Molecular Biology of the Cell*, 33: pe3.

Lerit, D. A., K. M. Plevock, and N. M. Rusan. 2014. 'Live imaging of Drosophila larval neuroblasts', *J Vis Exp*.

Lerit, D. A., and J. S. Poulton. 2016. 'Centrosomes are multifunctional regulators of genome stability', *Chromosome Research*, 24: 5-17.

Lerit, D. A., and N. M. Rusan. 2013. 'PLP inhibits the activity of interphase centrosomes to ensure their proper segregation in stem cells', *J Cell Biol*, 202: 1013-22.

Lesage, B., I. Gutierrez, E. Marti, and C. Gonzalez. 2010. 'Neural stem cells: the need for a proper orientation', *Current Opinion in Genetics and Development*, 20: 438-42.

Li, K., and T. C. Kaufman. 1996. 'The homeotic target gene centrosomin encodes an essential centrosomal component', *Cell*, 85: 585-96.

Li, S., H. Wang, and C. Groth. 2014. 'Drosophila neuroblasts as a new model for the study of stem cell self-renewal and tumour formation', *Bioscience Reports*, 34.

Licatalosi, D. D., A. Mele, J. J. Fak, J. Ule, M. Kayikci, S. W. Chi, T. A. Clark, A. C. Schweitzer, J. E. Blume, X. Wang, J. C. Darnell, and R. B. Darnell. 2008. 'HITS-CLIP yields genome-wide insights into brain alternative RNA processing', *Nature*, 456: 464-9.

Lim, N. R., B. Shohayeb, O. Zaytseva, N. Mitchell, S. S. Millard, D. C. H. Ng, and L. M. Quinn. 2017. 'Glial-Specific Functions of Microcephaly Protein WDR62 and Interaction with the Mitotic Kinase AURKA Are Essential for Drosophila Brain Growth', *Stem Cell Reports*, 9: 32-41.

Lin, M., S. S. Xie, and K. Y. Chan. 2022. 'An updated view on the centrosome as a cell cycle regulator', *Cell Division*, 17: 1.

Lingle, W. L., and J. L. Salisbury. 1999. 'Altered centrosome structure is associated with abnormal mitoses in human breast tumors', *American Journal of Pathology*, 155: 1941-51.

Link, N., H. Chung, A. Jolly, M. Withers, B. Tepe, B. R. Arenkiel, P. S. Shah, N. J. Krogan, H. Aydin, B. B. Geckinli, T. Tos, S. Isikay, B. Tuysuz, G. H. Mochida, A. X. Thomas, R. D. Clark, G. M. Mirzaa, J. R. Lupski, and H. J. Bellen. 2019. 'Mutations in ANKLE2, a ZIKA Virus Target, Disrupt an Asymmetric Cell Division Pathway in Drosophila Neuroblasts to Cause Microcephaly', *Developmental Cell*, 51: 713-29 e6.

Loncarek, J., and M. Bettencourt-Dias. 2018. 'Building the right centriole for each cell type', *J Cell Biol*, 217: 823-35.

Lorenzo-Betancor, O., P. R. Blackburn, E. Edwards, R. Vazquez-do-Campo, E. W. Klee, C. Labbe, K. Hodges, P. Glover, A. N. Sigafoos, A. I. Soto, R. L. Walton, S. Doxsey, M. B. Bober, S. Jennings, K. J. Clark, Y. Asmann, D. Miller, W. D. Freeman, J. Meschia, and O. A. Ross. 2018. 'PCNT point mutations and familial intracranial aneurysms', *Neurology*, 91: e2170-e81.

Loyer, N., and J. Januschke. 2020. 'Where does asymmetry come from? Illustrating principles of polarity and asymmetry establishment in Drosophila neuroblasts', *Current Opinion in Cell Biology*, 62: 70-77.

Lu, F., R. Lan, H. Zhang, Q. Jiang, and C. Zhang. 2009. 'Geminin is partially localized to the centrosome and plays a role in proper centrosome duplication', *Biologie Cellulaire*, 101: 273-85.

Magescas, J., J. C. Zonka, and J. L. Feldman. 2019. 'A two-step mechanism for the inactivation of microtubule organizing center function at the centrosome', *Elife*, 8.

Mahen, R., and A. R. Venkitaraman. 2012. 'Pattern formation in centrosome assembly', *Current Opinion in Cell Biology*, 24: 14-23.

Majumdar, A., W. C. Cesario, E. White-Grindley, H. Jiang, F. Ren, M. R. Khan, L. Li, E. M. Choi, K. Kannan, F. Guo, J. Unruh, B. Slaughter, and K. Si. 2012. 'Critical role of amyloid-like oligomers of Drosophila Orb2 in the persistence of memory', *Cell*, 148: 515-29.

Malerod, L., R. Le Borgne, A. Lie-Jensen, A. H. Eikenes, A. Brech, K. Liestol, H. Stenmark, and K. Haglund. 2018. 'Centrosomal ALIX regulates mitotic spindle orientation by modulating astral microtubule dynamics', *EMBO Journal*, 37.

Malumbres, M., and M. Barbacid. 2007. 'Cell cycle kinases in cancer', *Current Opinion in Genetics and Development*, 17: 60-5.

Mao, H., J. J. McMahon, Y. H. Tsai, Z. Wang, and D. L. Silver. 2016. 'Haploinsufficiency for Core Exon Junction Complex Components Disrupts Embryonic Neurogenesis and Causes p53-Mediated Microcephaly', *PLoS Genet*, 12: e1006282.

Mao, H., L. J. Pilaz, J. J. McMahon, C. Golzio, D. Wu, L. Shi, N. Katsanis, and D. L. Silver. 2015. 'Rbm8a haploinsufficiency disrupts embryonic cortical development resulting in microcephaly', *Journal of Neuroscience*, 35: 7003-18.

Mardin, B. R., F. G. Agircan, C. Lange, and E. Schiebel. 2011. 'Plk1 controls the Nek2A-PP1gamma antagonism in centrosome disjunction', *Current Biology*, 21: 1145-51.

Marthiens, V., and R. Basto. 2020. 'Centrosomes: The good and the bad for brain development', *Biologie Cellulaire*, 112: 153-72.

Marthiens, V., M. Piel, and R. Basto. 2012. 'Never tear us apart--the importance of centrosome clustering', *Journal of Cell Science*, 125: 3281-92.

Martinez-Campos, M., R. Basto, J. Baker, M. Kernan, and J. W. Raff. 2004. 'The Drosophila pericentrin-like protein is essential for cilia/flagella function, but appears to be dispensable for mitosis', *J Cell Biol*, 165: 673-83.

Mastushita-Sakai, T., E. White-Grindley, J. Samuelson, C. Seidel, and K. Si. 2010. 'Drosophila Orb2 targets genes involved in neuronal growth, synapse formation, and protein turnover', *Proceedings of the National Academy of Sciences of the United States of America*, 107: 11987-92.

Matsumoto, Y., and J. L. Maller. 2004. 'A centrosomal localization signal in cyclin E required for Cdk2-independent S phase entry', *Science*, 306: 885-8.

Matsuo, K., K. Ohsumi, M. Iwabuchi, T. Kawamata, Y. Ono, and M. Takahashi. 2012. 'Kendrin is a novel substrate for separase involved in the licensing of centriole duplication', *Current Biology*, 22: 915-21.

McClintock, M. A., C. I. Dix, C. M. Johnson, S. H. McLaughlin, R. J. Maizels, H. T. Hoang, and S. L. Bullock. 2018. 'RNA-directed activation of cytoplasmic dynein-1 in reconstituted transport RNPs', *Elife*, 7.

McGrew, L. L., E. Dworkin-Rastl, M. B. Dworkin, and J. D. Richter. 1989. 'Poly(A) elongation during Xenopus oocyte maturation is required for translational recruitment and is mediated by a short sequence element', *Genes and Development*, 3: 803-15.

McLamarrah, T. A., D. W. Buster, B. J. Galletta, C. J. Boese, J. M. Ryniawec, N. A. Hollingsworth, A. E. Byrnes, C. W. Brownlee, K. C. Slep, N. M. Rusan, and G. C. Rogers. 2018. 'An ordered pattern of Ana2 phosphorylation by Plk4 is required for centriole assembly', *J Cell Biol*, 217: 1217-31.

Meghini, F., T. Martins, X. Tait, K. Fujimitsu, H. Yamano, D. M. Glover, and Y. Kimata. 2016. 'Targeting of Fzr/Cdh1 for timely activation of the APC/C at the centrosome during mitotic exit', *Nat Commun*, 7: 12607.

Megraw, T. L., K. Li, L. R. Kao, and T. C. Kaufman. 1999. 'The centrosomin protein is required for centrosome assembly and function during cleavage in Drosophila', *Development*, 126: 2829-39.

Meigs, T. E., and D. D. Kaplan. 2008. 'Isolation of centrosomes from cultured Mammalian cells', *CSH Protoc*, 2008: pdb prot5039.

Mendez, R., and J. D. Richter. 2001. 'Translational control by CPEB: a means to the end', *Nature Reviews: Molecular Cell Biology*, 2: 521-9.

Mennella, V., D. A. Agard, B. Huang, and L. Pelletier. 2014. 'Amorphous no more: subdiffraction view of the pericentriolar material architecture', *Trends in Cell Biology*, 24: 188-97.

Mennella, V., B. Keszthelyi, K. L. McDonald, B. Chhun, F. Kan, G. C. Rogers, B. Huang, and D. A. Agard. 2012. 'Subdiffraction-resolution fluorescence microscopy reveals a domain of the centrosome critical for pericentriolar material organization', *Nature Cell Biology*, 14: 1159-68.

Mi, H., D. Ebert, A. Muruganujan, C. Mills, L. P. Albou, T. Mushayamaha, and P. D. Thomas. 2021. 'PANTHER version 16: a revised family classification, tree-based classification tool, enhancer regions and extensive API', *Nucleic Acids Research*, 49: D394-D403.

Mi, H., A. Muruganujan, D. Ebert, X. Huang, and P. D. Thomas. 2019. 'PANTHER version 14: more genomes, a new PANTHER GO-slim and improvements in enrichment analysis tools', *Nucleic Acids Research*, 47: D419-D26.

Mierke, Claudia Tanja. 2020. 'Cell Cycle, DNA Replication, Centrosomes, Centrioles and Cell Division.' in, *Cellular Mechanics and Biophysics*.

Mikule, K., B. Delaval, P. Kaldis, A. Jurcyzk, P. Hergert, and S. Doxsey. 2007. 'Loss of centrosome integrity induces p38-p53-p21-dependent G1-S arrest', *Nature Cell Biology*, 9: 160-70.

Miller, S., M. Yasuda, J. K. Coats, Y. Jones, M. E. Martone, and M. Mayford. 2002. 'Disruption of dendritic translation of CaMKIIalpha impairs stabilization of synaptic plasticity and memory consolidation', *Neuron*, 36: 507-19.

Mitchell-Dick, A., A. Chalem, L. J. Pilaz, and D. L. Silver. 2019. 'Acute Lengthening of Progenitor Mitosis Influences Progeny Fate during Cortical Development in vivo', *Developmental Neuroscience*, 41: 300-17.

Mittasch, M., V. M. Tran, M. U. Rios, A. W. Fritsch, S. J. Enos, B. Ferreira Gomes, A. Bond, M. Kreysing, and J. B. Woodruff. 2020. 'Regulated changes in material properties underlie centrosome disassembly during mitotic exit', *J Cell Biol*, 219.

Mochida, G. H. 2009. 'Genetics and biology of microcephaly and lissencephaly', *Seminars in Pediatric Neurology*, 16: 120-6.

Moritz, M., M. B. Braunfeld, V. Guenebaut, J. Heuser, and D. A. Agard. 2000. 'Structure of the gamma-tubulin ring complex: a template for microtubule nucleation', *Nature Cell Biology*, 2: 365-70.

Moroy, T., and C. Geisen. 2004. 'Cyclin E', *International Journal of Biochemistry and Cell Biology*, 36: 1424-39.

Mottier-Pavie, V., and T. L. Megraw. 2009. 'Drosophila bld10 is a centriolar protein that regulates centriole, basal body, and motile cilium assembly', *Molecular Biology of the Cell*, 20: 2605-14.

Muller, H., D. Schmidt, S. Steinbrink, E. Mirgorodskaya, V. Lehmann, K. Habermann, F. Dreher, N. Gustavsson, T. Kessler, H. Lehrach, R. Herwig, J. Gobom, A. Ploubidou, M. Boutros, and B. M. Lange. 2010. 'Proteomic and functional analysis of the mitotic Drosophila centrosome', *EMBO Journal*, 29: 3344-57.

Nakahata, Y., and R. Yasuda. 2018. 'Plasticity of Spine Structure: Local Signaling, Translation and Cytoskeletal Reorganization', *Frontiers in Synaptic Neuroscience*, 10: 29.

Nazarov, S., A. Bezler, G. N. Hatzopoulos, V. Nemcikova Villimova, D. Demurtas, M. Le Guennec, P. Guichard, and P. Gonczy. 2020. 'Novel features of centriole polarity and cartwheel stacking revealed by cryo-tomography', *EMBO Journal*, 39: e106249.

Neumuller, R. A., and J. A. Knoblich. 2009. 'Dividing cellular asymmetry: asymmetric cell division and its implications for stem cells and cancer', *Genes and Development*, 23: 2675-99.

'Neurodevelopmental Disorders.' in. 2013. *Diagnostic and Statistical Manual of Mental Disorders*.

Ng, D. C. H., D. K. Richards, R. J. Mills, U. Y. Ho, H. L. Perks, C. R. Tucker, H. K. Voges, J. K. Pagan, and J. E. Hudson. 2020. 'Centrosome Reduction Promotes Terminal Differentiation of Human Cardiomyocytes', *Stem Cell Reports*, 15: 817-26.

Nicholas, A. K., E. A. Swanson, J. J. Cox, G. Karbani, S. Malik, K. Springell, D. Hampshire, M. Ahmed, J. Bond, D. Di Benedetto, M. Fichera, C. Romano, W. B. Dobyns, and C. G. Woods. 2009. 'The molecular landscape of ASPM mutations in primary microcephaly', *Journal of Medical Genetics*, 46: 249-53.

Nigg, E. A., and A. J. Holland. 2018. 'Once and only once: mechanisms of centriole duplication and their deregulation in disease', *Nature Reviews: Molecular Cell Biology*, 19: 297-312.

Nigg, E. A., and J. W. Raff. 2009. 'Centrioles, centrosomes, and cilia in health and disease', *Cell*, 139: 663-78.

Nigg, E. A., and T. Stearns. 2011. 'The centrosome cycle: Centriole biogenesis, duplication and inherent asymmetries', *Nature Cell Biology*, 13: 1154-60.

Nishiyama, J. 2019. 'Plasticity of dendritic spines: Molecular function and dysfunction in neurodevelopmental disorders', *Psychiatry and Clinical Neurosciences*, 73: 541-50.

O'Donnell, W. T., and S. T. Warren. 2002. 'A decade of molecular studies of fragile X syndrome', *Annual Review of Neuroscience*, 25: 315-38.

O'Neill, R. S., T. A. Schoborg, and N. M. Rusan. 2018. 'Same but different: pleiotropy in centrosome-related microcephaly', *Molecular Biology of the Cell*, 29: 241-46.

Oegema, K., C. Wiese, O. C. Martin, R. A. Milligan, A. Iwamatsu, T. J. Mitchison, and Y. Zheng. 1999. 'Characterization of two related Drosophila gamma-tubulin complexes that differ in their ability to nucleate microtubules', *J Cell Biol*, 144: 721-33.

Ohashi, R., and N. Shiina. 2020. 'Cataloguing and Selection of mRNAs Localized to Dendrites in Neurons and Regulated by RNA-Binding Proteins in RNA Granules', *Biomolecules*, 10.

Ohshiro, T., T. Yagami, C. Zhang, and F. Matsuzaki. 2000. 'Role of cortical tumour-suppressor proteins in asymmetric division of Drosophila neuroblast', *Nature*, 408: 593-6.

Pagan, J. K., A. Marzio, M. J. Jones, A. Saraf, P. V. Jallepalli, L. Florens, M. P. Washburn, and M. Pagano. 2015. 'Degradation of Cep68 and PCNT cleavage mediate Cep215 removal from the PCM to allow centriole separation, disengagement and licensing', *Nature Cell Biology*, 17: 31-43.

Palazzo, R. E., J. M. Vogel, B. J. Schnackenberg, D. R. Hull, and X. Wu. 2000. 'Centrosome maturation', *Current Topics in Developmental Biology*, 49: 449-70.

Paridaen, J. T., M. Wilsch-Brauninger, and W. B. Huttner. 2013. 'Asymmetric inheritance of centrosome-associated primary cilium membrane directs ciliogenesis after cell division', *Cell*, 155: 333-44.

Pascreau, G., M. E. Churchill, and J. L. Maller. 2011. 'Centrosomal localization of cyclins E and A: structural similarities and functional differences', *Cell Cycle*, 10: 199-205.

Pascual, R., C. Segura-Morales, M. Omerzu, N. Bellora, E. Belloc, C. L. Castellazzi, O. Reina, E. Eyras, M. M. Maurice, A. Millanes-Romero, and R. Mendez. 2020. 'mRNA spindle localization and mitotic translational regulation by CPEB1 and CPEB4', *RNA*.

Passemard, S., A. M. Kaindl, and A. Verloes. 2013. 'Microcephaly.' in, *Handbook of Clinical Neurology*.

Peng, C. Y., L. Manning, R. Albertson, and C. Q. Doe. 2000. 'The tumour-suppressor genes lgl and dlg regulate basal protein targeting in Drosophila neuroblasts', *Nature*, 408: 596-600.

Pepper, D. A., and B. R. Brinkley. 1977. 'Localization of tubulin in the mitotic apparatus of mammalian cells by immunofluorescence and immunoelectron microscopy', *Chromosoma*, 60: 223-35.

———. 1980. 'Tubulin nucleation and assembly in mitotic cells: evidence for nucleic acids in kinetochores and centrosomes', *Cell Motility*, 1: 1-15.

Pereira, G., T. U. Tanaka, K. Nasmyth, and E. Schiebel. 2001. 'Modes of spindle pole body inheritance and segregation of the Bfa1p-Bub2p checkpoint protein complex', *EMBO Journal*, 20: 6359-70.

Pihan, G. A., A. Purohit, J. Wallace, H. Knecht, B. Woda, P. Quesenberry, and S. J. Doxsey. 1998. 'Centrosome defects and genetic instability in malignant tumors', *Cancer Research*, 58: 3974-85.

Pilaz, L. J., J. J. McMahon, E. E. Miller, A. L. Lennox, A. Suzuki, E. Salmon, and D. L. Silver. 2016. 'Prolonged Mitosis of Neural Progenitors Alters Cell Fate in the Developing Brain', *Neuron*, 89: 83-99.

Pique, M., J. M. Lopez, S. Foissac, R. Guigo, and R. Mendez. 2008. 'A combinatorial code for CPE-mediated translational control', *Cell*, 132: 434-48.

Pirozzi, F., B. Nelson, and G. Mirzaa. 2018. 'From microcephaly to megalencephaly: determinants of brain size', *Dialogues in Clinical Neuroscience*, 20: 267-82.

Poulton, J. S., J. C. Cuningham, and M. Peifer. 2017. 'Centrosome and spindle assembly checkpoint loss leads to neural apoptosis and reduced brain size', *J Cell Biol*, 216: 1255-65.

Qi, F., and J. Zhou. 2021. 'Multifaceted roles of centrosomes in development, health, and disease', *Journal of Molecular Cell Biology*, 13: 611-21.

Qu, W. R., Q. H. Sun, Q. Q. Liu, H. J. Jin, R. J. Cui, W. Yang, B. Song, and B. J. Li. 2020. 'Role of CPEB3 protein in learning and memory: new insights from synaptic plasticity', *Aging*, 12: 15169-82.

Raff, J. W., D. R. Kellogg, and B. M. Alberts. 1993. 'Drosophila gamma-tubulin is part of a complex containing two previously identified centrosomal MAPs', *J Cell Biol*, 121: 823-35.

Raj, N., Z. T. McEachin, W. Harousseau, Y. Zhou, F. Zhang, M. E. Merritt-Garza, J. M. Taliaferro, M. Kalinowska, S. G. Marro, C. M. Hales, E. Berry-Kravis, M. W. Wolf-Ochoa, V. Martinez-Cerdeno, M. Wernig, L. Chen, E. Klann, S. T. Warren, P. Jin, Z. Wen, and G. J. Bassell. 2021. 'Cell-type-specific profiling of human cellular models of fragile X syndrome reveal PI3K-dependent defects in translation and neurogenesis', *Cell Reports*, 35: 108991.

Ramdas Nair, A., P. Singh, D. Salvador Garcia, D. Rodriguez-Crespo, B. Egger, and C. Cabernard. 2016. 'The Microcephaly-Associated Protein Wdr62/CG7337 Is Required to Maintain Centrosome Asymmetry in Drosophila Neuroblasts', *Cell Reports*, 14: 1100-13.

Ramon-Canellas, P., H. P. Peterson, and J. Morante. 2019. 'From Early to Late Neurogenesis: Neural Progenitors and the Glial Niche from a Fly's Point of View', *Neuroscience*, 399: 39-52.

Rao, C. V., Y. M. Yang, M. V. Swamy, T. Liu, Y. Fang, R. Mahmood, M. Jhanwar-Uniyal, and W. Dai. 2005. 'Colonic tumorigenesis in BubR1+/-ApcMin/+ compound mutant mice is linked to

premature separation of sister chromatids and enhanced genomic instability', *Proceedings of the National Academy of Sciences of the United States of America*, 102: 4365-70.
Rao, M.S., M. Carpenter, and M.C. Vemuri. 2012. *Neural Development and Stem Cells* (Springer New York).
Rattner, J. B., and S. G. Phillips. 1973. 'Independence of centriole formation and DNA synthesis', *J Cell Biol*, 57: 359-72.
Rebollo, E., P. Sampaio, J. Januschke, S. Llamazares, H. Varmark, and C. Gonzalez. 2007. 'Functionally unequal centrosomes drive spindle orientation in asymmetrically dividing Drosophila neural stem cells', *Developmental Cell*, 12: 467-74.
Reina, J., and C. Gonzalez. 2014. 'When fate follows age: unequal centrosomes in asymmetric cell division', *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 369.
Rhee, H. W., P. Zou, N. D. Udeshi, J. D. Martell, V. K. Mootha, S. A. Carr, and A. Y. Ting. 2013. 'Proteomic mapping of mitochondria in living cells via spatially restricted enzymatic tagging', *Science*, 339: 1328-31.
Richter, J. D. 2007. 'CPEB: a life in translation', *Trends in Biochemical Sciences*, 32: 279-85.
Rieder, C. L. 1979. 'Ribonucleoprotein staining of centrioles and kinetochores in newt lung cell spindles', *J Cell Biol*, 80: 1-9.
Rieder, C. L., and H. Maiato. 2004. 'Stuck in division or passing through: what happens when cells cannot satisfy the spindle assembly checkpoint', *Developmental Cell*, 7: 637-51.
Robinson, B. V., V. Faundez, and D. A. Lerit. 2020. 'Understanding microcephaly through the study of centrosome regulation in Drosophila neural stem cells', *Biochemical Society Transactions*, 48: 2101-15.
Roghi, C., R. Giet, R. Uzbekov, N. Morin, I. Chartrain, R. Le Guellec, A. Couturier, M. Doree, M. Philippe, and C. Prigent. 1998. 'The Xenopus protein kinase pEg2 associates with the centrosome in a cell cycle-dependent manner, binds to the spindle microtubules and is involved in bipolar mitotic spindle assembly', *Journal of Cell Science*, 111 (Pt 5): 557-72.
Rolls, M. M., R. Albertson, H. P. Shih, C. Y. Lee, and C. Q. Doe. 2003. 'Drosophila aPKC regulates cell polarity and cell proliferation in neuroblasts and epithelia', *J Cell Biol*, 163: 1089-98.
Rusan, N. M., and M. Peifer. 2007. 'A role for a novel centrosome cycle in asymmetric cell division', *J Cell Biol*, 177: 13-20.
Ryder, P. V., J. Fang, and D. A. Lerit. 2020. 'centrocortin RNA localization to centrosomes is regulated by FMRP and facilitates error-free mitosis', *J Cell Biol*, 219: e202004101.
Ryder, P. V., and D. A. Lerit. 2018. 'RNA localization regulates diverse and dynamic cellular processes', *Traffic*, 19: 496-502.
Safieddine, A., E. Coleno, S. Salloum, A. Imbert, A. M. Traboulsi, O. S. Kwon, F. Lionneton, V. Georget, M. C. Robert, T. Gostan, C. H. Lecellier, R. Chouaib, X. Pichon, H. Le Hir, K. Zibara, F. Mueller, T. Walter, M. Peter, and E. Bertrand. 2021. 'A choreography of centrosomal mRNAs reveals a conserved localization mechanism involving active polysome transport', *Nat Commun*, 12: 1352.
Sahabandu, N., D. Kong, V. Magidson, R. Nanjundappa, C. Sullenberger, M. R. Mahjoub, and J. Loncarek. 2019. 'Expansion microscopy for the analysis of centrioles and cilia', *Journal of Microscopy*, 276: 145-59.
Samuels, T. J., Y. Arava, A. I. Jarvelin, F. Robertson, J. Y. Lee, L. Yang, C. P. Yang, T. Lee, D. Ish-Horowicz, and I. Davis. 2020. 'Neuronal upregulation of Prospero protein is driven by alternative mRNA polyadenylation and Syncrip-mediated mRNA stabilisation', *Biol Open*, 9.
Samuels, T. J., A. I. Jarvelin, D. Ish-Horowicz, and I. Davis. 2020. 'Imp/IGF2BP levels modulate individual neural stem cell growth and division through myc mRNA stability', *Elife*, 9.
Sanguanini, M., and A. Cattaneo. 2018. 'A continuous model of physiological prion aggregation suggests a role for Orb2 in gating long-term synaptic information', *R Soc Open Sci*, 5: 180336.

Santos-Cortez, R. L. P., V. Khan, F. S. Khan, Z. U. Mughal, I. Chakchouk, K. Lee, M. Rasheed, R. Hamza, A. Acharya, E. Ullah, M. A. N. Saqib, I. Abbe, G. Ali, M. J. Hassan, S. Khan, Z. Azeem, I. Ullah, M. J. Bamshad, D. A. Nickerson, I. Schrauwen, W. Ahmad, M. Ansar, and S. M. Leal. 2018. 'Novel candidate genes and variants underlying autosomal recessive neurodevelopmental disorders with intellectual disability', *Human Genetics*, 137: 735-52.

Sayer, J. A., E. A. Otto, J. F. O'Toole, G. Nurnberg, M. A. Kennedy, C. Becker, H. C. Hennies, J. Helou, M. Attanasio, B. V. Fausett, B. Utsch, H. Khanna, Y. Liu, I. Drummond, I. Kawakami, T. Kusakabe, M. Tsuda, L. Ma, H. Lee, R. G. Larson, S. J. Allen, C. J. Wilkinson, E. A. Nigg, C. Shou, C. Lillo, D. S. Williams, B. Hoppe, M. J. Kemper, T. Neuhaus, M. A. Parisi, I. A. Glass, M. Petry, A. Kispert, J. Gloy, A. Ganner, G. Walz, X. Zhu, D. Goldman, P. Nurnberg, A. Swaroop, M. R. Leroux, and F. Hildebrandt. 2006. 'The centrosomal protein nephrocystin-6 is mutated in Joubert syndrome and activates transcription factor ATF4', *Nature Genetics*, 38: 674-81.

Schaefer, M., M. Petronczki, D. Dorner, M. Forte, and J. A. Knoblich. 2001. 'Heterotrimeric G proteins direct two modes of asymmetric cell division in the Drosophila nervous system', *Cell*, 107: 183-94.

Schieweck, R., T. Riedemann, I. Forne, M. Harner, K. E. Bauer, D. Rieger, F. Y. Ang, S. Hutten, A. F. Demleitner, B. Popper, S. Derdak, B. Sutor, M. Bilban, A. Imhof, and M. A. Kiebler. 2021. 'Pumilio2 and Staufen2 selectively balance the synaptic proteome', *Cell Reports*, 35: 109279.

Schindelin, J., I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J. Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak, and A. Cardona. 2012. 'Fiji: an open-source platform for biological-image analysis', *Nat Methods*, 9: 676-82.

Schoborg, T., A. L. Zajac, C. J. Fagerstrom, R. X. Guillen, and N. M. Rusan. 2015. 'An Asp-CaM complex is required for centrosome-pole cohesion and centrosome inheritance in neural stem cells', *J Cell Biol*, 211: 987-98.

Schockel, L., M. Mockel, B. Mayer, D. Boos, and O. Stemmann. 2011. 'Cleavage of cohesin rings coordinates the separation of centrioles and chromatids', *Nature Cell Biology*, 13: 966-72.

Semichov, S. B. 1984. '[Theoretical basis of the newest American classification of mental disorders (Diagnostic and Statistical Manual of Mental Disorders, 3d Edition--DSM-III, Washington, D.C., APA, 1980, 494 p.)].' in, *Zhurnal Nevropatologii i Psikhiatrii Imeni S. S. Korsakova*.

Sepulveda, G., M. Antkowiak, I. Brust-Mascher, K. Mahe, T. Ou, N. M. Castro, L. N. Christensen, L. Cheung, X. Jiang, D. Yoon, B. Huang, and L. E. Jao. 2018. 'Co-translational protein targeting facilitates centrosomal recruitment of PCNT during centrosome maturation in vertebrates', *Elife*, 7: e34959.

Shah, P. S., N. Link, G. M. Jang, P. P. Sharp, T. Zhu, D. L. Swaney, J. R. Johnson, J. Von Dollen, H. R. Ramage, L. Satkamp, B. Newton, R. Huttenhain, M. J. Petit, T. Baum, A. Everitt, O. Laufman, M. Tassetto, M. Shales, E. Stevenson, G. N. Iglesias, L. Shokat, S. Tripathi, V. Balasubramaniam, L. G. Webb, S. Aguirre, A. J. Willsey, A. Garcia-Sastre, K. S. Pollard, S. Cherry, A. V. Gamarnik, I. Marazzi, J. Taunton, A. Fernandez-Sesma, H. J. Bellen, R. Andino, and N. J. Krogan. 2018. 'Comparative Flavivirus-Host Protein Interaction Mapping Reveals Mechanisms of Dengue and Zika Virus Pathogenesis', *Cell*, 175: 1931-45 e18.

Sharp, J. A., J. J. Plant, T. K. Ohsumi, M. Borowsky, and M. D. Blower. 2011. 'Functional analysis of the microtubule-interacting transcriptome', *Molecular Biology of the Cell*, 22: 4312-23.

Shen, C. P., J. A. Knoblich, Y. M. Chan, M. M. Jiang, L. Y. Jan, and Y. N. Jan. 1998. 'Miranda as a multidomain adapter linking apically localized Inscuteable and basally localized Staufen and Prospero during asymmetric cell division in Drosophila', *Genes and Development*, 12: 1837-46.

Shinohara, H., N. Sakayori, M. Takahashi, and N. Osumi. 2013. 'Ninein is essential for the maintenance of the cortical progenitor character by anchoring the centrosome to microtubules', *Biol Open*, 2: 739-49.

Si, K., Y. B. Choi, E. White-Grindley, A. Majumdar, and E. R. Kandel. 2010. 'Aplysia CPEB can form prion-like multimers in sensory neurons that contribute to long-term facilitation', *Cell*, 140: 421-35.

Si, K., S. Lindquist, and E. R. Kandel. 2003. 'A neuronal isoform of the aplysia CPEB has prion-like properties', *Cell*, 115: 879-91.

Siegrist, S. E., and C. Q. Doe. 2005. 'Microtubule-induced Pins/Galphai cortical polarity in Drosophila neuroblasts', *Cell*, 123: 1323-35.

———. 2006. 'Extrinsic cues orient the cell division axis in Drosophila embryonic neuroblasts', *Development*, 133: 529-36.

———. 2007. 'Microtubule-induced cortical cell polarity', *Genes and Development*, 21: 483-96.

Siller, K. H., C. Cabernard, and C. Q. Doe. 2006. 'The NuMA-related Mud protein binds Pins and regulates spindle orientation in Drosophila neuroblasts', *Nature Cell Biology*, 8: 594-600.

Silver, D. L., D. E. Watkins-Chow, K. C. Schreck, T. J. Pierfelice, D. M. Larson, A. J. Burnetti, H. J. Liaw, K. Myung, C. A. Walsh, N. Gaiano, and W. J. Pavan. 2010. 'The exon junction complex component Magoh controls brain size by regulating neural stem cell division', *Nature Neuroscience*, 13: 551-8.

Singh, P., A. Ramdas Nair, and C. Cabernard. 2014. 'The centriolar protein Bld10/Cep135 is required to establish centrosome asymmetry in Drosophila neuroblasts', *Current Biology*, 24: 1548-55.

Sir, J. H., M. Putz, O. Daly, C. G. Morrison, M. Dunning, J. V. Kilmartin, and F. Gergely. 2013. 'Loss of centrioles causes chromosomal instability in vertebrate somatic cells', *J Cell Biol*, 203: 747-56.

Sluder, G. 2005. 'Two-way traffic: centrosomes and the cell cycle', *Nature Reviews: Molecular Cell Biology*, 6: 743-8.

Sonnen, K. F., L. Schermelleh, H. Leonhardt, and E. A. Nigg. 2012. '3D-structured illumination microscopy provides novel insight into architecture of human centrosomes', *Biol Open*, 1: 965-76.

Srsen, V., N. Gnadt, A. Dammermann, and A. Merdes. 2006. 'Inhibition of centrosome protein assembly leads to p53-dependent exit from the cell cycle', *J Cell Biol*, 174: 625-30.

Stearns, T. 2015. 'Cell biology. Centrioles, in absentia', *Science*, 348: 1091-2.

Stephan, J. S., L. Fioriti, N. Lamba, L. Colnaghi, K. Karl, I. L. Derkatch, and E. R. Kandel. 2015. 'The CPEB3 Protein Is a Functional Prion that Interacts with the Actin Cytoskeleton', *Cell Reports*, 11: 1772-85.

Stepien, B. K., C. Oppitz, D. Gerlach, U. Dag, M. Novatchkova, S. Kruttner, A. Stark, and K. Keleman. 2016. 'RNA-binding profiles of Drosophila CPEB proteins Orb and Orb2', *Proceedings of the National Academy of Sciences of the United States of America*, 113: E7030-E38.

Sullenberger, C., A. Vasquez-Limeta, D. Kong, and J. Loncarek. 2020. 'With Age Comes Maturity: Biochemical and Structural Transformation of a Human Centriole in the Making', *Cells*, 9.

Sunkel, C. E., and D. M. Glover. 1988. 'polo, a mitotic mutant of Drosophila displaying abnormal spindle poles', *Journal of Cell Science*, 89 (Pt 1): 25-38.

Sutton, M. A., and E. M. Schuman. 2006. 'Dendritic protein synthesis, synaptic plasticity, and memory', *Cell*, 127: 49-58.

Tachibana, K. E., M. A. Gonzalez, G. Guarguaglini, E. A. Nigg, and R. A. Laskey. 2005. 'Depletion of licensing inhibitor geminin causes centrosome overduplication and mitotic defects', *EMBO Rep*, 6: 1052-7.

Tan, L., J. S. Chang, A. Costa, and P. Schedl. 2001. 'An autoregulatory feedback loop directs the localized expression of the Drosophila CPEB protein Orb in the developing oocyte', *Development*, 128: 1159-69.

The Gene Ontology, Consortium. 2019. 'The Gene Ontology Resource: 20 years and still GOing strong', *Nucleic Acids Research*, 47: D330-D38.

Theis, M., K. Si, and E. R. Kandel. 2003. 'Two previously undescribed members of the mouse CPEB family of genes and their inducible expression in the principal cell layers of the hippocampus', *Proceedings of the National Academy of Sciences of the United States of America*, 100: 9602-7.

Thornton, G. K., and C. G. Woods. 2009. 'Primary microcephaly: do all roads lead to Rome?', *Trends in Genetics*, 25: 501-10.

Tiruchinapalli, D. M., Y. Oleynikov, S. Kelic, S. M. Shenoy, A. Hartley, P. K. Stanton, R. H. Singer, and G. J. Bassell. 2003. 'Activity-dependent trafficking and dynamic localization of zipcode binding protein 1 and beta-actin mRNA in dendrites and spines of hippocampal neurons', *Journal of Neuroscience*, 23: 3251-61.

Tischer, J., S. Carden, and F. Gergely. 2021. 'Accessorizing the centrosome: new insights into centriolar appendages and satellites', *Current Opinion in Structural Biology*, 66: 148-55.

Tongiorgi, E., M. Righi, and A. Cattaneo. 1997. 'Activity-dependent dendritic targeting of BDNF and TrkB mRNAs in hippocampal neurons', *Journal of Neuroscience*, 17: 9492-505.

Trinkle-Mulcahy, L. 2019. 'Recent advances in proximity-based labeling methods for interactome mapping', *F1000Res*, 8.

Tsou, M. F., W. J. Wang, K. A. George, K. Uryu, T. Stearns, and P. V. Jallepalli. 2009. 'Polo kinase and separase regulate the mitotic licensing of centriole duplication in human cells', *Developmental Cell*, 17: 344-54.

Vaessin, H., E. Grell, E. Wolff, E. Bier, L. Y. Jan, and Y. N. Jan. 1991. 'prospero is expressed in neuronal precursors and encodes a nuclear protein that is involved in the control of axonal outgrowth in Drosophila', *Cell*, 67: 941-53.

Vaizel-Ohayon, D., and E. D. Schejter. 1999. 'Mutations in centrosomin reveal requirements for centrosomal function during early Drosophila embryogenesis', *Current Biology*, 9: 889-98.

Van Beneden, E. 1876. 'Contribution al'histoire de la vesiculaire germinative et du premier embryonnaire', *Bull Acad R Belg*, 42: 35-97.

Varmark, H., S. Llamazares, E. Rebollo, B. Lange, J. Reina, H. Schwarz, and C. Gonzalez. 2007. 'Asterless is a centriolar protein required for centrosome function and embryo development in Drosophila', *Current Biology*, 17: 1735-45.

Vasquez-Limeta, A., and J. Loncarek. 2021. 'Human centrosome organization and function in interphase and mitosis', *Seminars in Cell and Developmental Biology*, 117: 30-41.

Verheij, C., C. E. Bakker, E. de Graaff, J. Keulemans, R. Willemsen, A. J. Verkerk, H. Galjaard, A. J. Reuser, A. T. Hoogeveen, and B. A. Oostra. 1993. 'Characterization and localization of the FMR-1 gene product associated with fragile X syndrome', *Nature*, 363: 722-4.

Verkerk, A. J., M. Pieretti, J. S. Sutcliffe, Y. H. Fu, D. P. Kuhl, A. Pizzuti, O. Reiner, S. Richards, M. F. Victoria, F. P. Zhang, and et al. 1991. 'Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome', *Cell*, 65: 905-14.

Vessey, J. P., G. Amadei, S. E. Burns, M. A. Kiebler, D. R. Kaplan, and F. D. Miller. 2012. 'An asymmetrically localized Staufen2-dependent RNA complex regulates maintenance of mammalian neural stem cells', *Cell Stem Cell*, 11: 517-28.

Vorobjev, I. A., and S. Chentsov Yu. 1982. 'Centrioles in the cell cycle. I. Epithelial cells', *J Cell Biol*, 93: 938-49.

Wang, C., S. Li, J. Januschke, F. Rossi, Y. Izumi, G. Garcia-Alvarez, S. S. Gwee, S. B. Soon, H. K. Sidhu, F. Yu, F. Matsuzaki, C. Gonzalez, and H. Wang. 2011. 'An ana2/ctp/mud complex regulates spindle orientation in Drosophila neuroblasts', *Developmental Cell*, 21: 520-33.

Wang, X., J. W. Tsai, J. H. Imai, W. N. Lian, R. B. Vallee, and S. H. Shi. 2009. 'Asymmetric centrosome inheritance maintains neural progenitors in the neocortex', *Nature*, 461: 947-55.

Watemberg, N., S. Silver, S. Harel, and T. Lerman-Sagie. 2002. 'Significance of microcephaly among children with developmental disabilities', *Journal of Child Neurology*, 17: 117-22.

Weaver, B. A., A. D. Silk, C. Montagna, P. Verdier-Pinard, and D. W. Cleveland. 2007. 'Aneuploidy acts both oncogenically and as a tumor suppressor', *Cancer Cell*, 11: 25-36.

White-Grindley, E., L. Li, R. Mohammad Khan, F. Ren, A. Saraf, L. Florens, and K. Si. 2014. 'Contribution of Orb2A stability in regulated amyloid-like oligomerization of Drosophila Orb2', *PLoS Biology*, 12: e1001786.

Williams, B. R., V. R. Prabhu, K. E. Hunter, C. M. Glazier, C. A. Whittaker, D. E. Housman, and A. Amon. 2008. 'Aneuploidy affects proliferation and spontaneous immortalization in mammalian cells', *Science*, 322: 703-9.

Wodarz, A., and W. B. Huttner. 2003. 'Asymmetric cell division during neurogenesis in Drosophila and vertebrates', *Mechanisms of Development*, 120: 1297-309.

Wohlschlegel, J. A., B. T. Dwyer, S. K. Dhar, C. Cvetic, J. C. Walter, and A. Dutta. 2000. 'Inhibition of eukaryotic DNA replication by geminin binding to Cdt1', *Science*, 290: 2309-12.

Wong, Y. L., J. V. Anzola, R. L. Davis, M. Yoon, A. Motamedi, A. Kroll, C. P. Seo, J. E. Hsia, S. K. Kim, J. W. Mitchell, B. J. Mitchell, A. Desai, T. C. Gahman, A. K. Shiau, and K. Oegema. 2015. 'Cell biology. Reversible centriole depletion with an inhibitor of Polo-like kinase 4', *Science*, 348: 1155-60.

Wu, J., and A. Akhmanova. 2017. 'Microtubule-Organizing Centers', *Annual Review of Cell and Developmental Biology*, 33: 51-75.

Wu, Q., X. Yu, L. Liu, S. Sun, and S. Sun. 2021. 'Centrosome-phagy: implications for human diseases', *Cell & Bioscience*, 11: 49.

Xu, S., N. Hafer, B. Agunwamba, and P. Schedl. 2012. 'The CPEB protein Orb2 has multiple functions during spermatogenesis in Drosophila melanogaster', *PLoS Genet*, 8: e1003079.

Xu, S., S. Tyagi, and P. Schedl. 2014. 'Spermatid cyst polarization in Drosophila depends upon apkc and the CPEB family translational regulator orb2', *PLoS Genet*, 10: e1004380.

Yamamoto, S., M. Jaiswal, W. L. Charng, T. Gambin, E. Karaca, G. Mirzaa, W. Wiszniewski, H. Sandoval, N. A. Haelterman, B. Xiong, K. Zhang, V. Bayat, G. David, T. Li, K. Chen, U. Gala, T. Harel, D. Pehlivan, S. Penney, Lelm Vissers, J. de Ligt, S. N. Jhangiani, Y. Xie, S. H. Tsang, Y. Parman, M. Sivaci, E. Battaloglu, D. Muzny, Y. W. Wan, Z. Liu, A. T. Lin-Moore, R. D. Clark, C. J. Curry, N. Link, K. L. Schulze, E. Boerwinkle, W. B. Dobyns, R. Allikmets, R. A. Gibbs, R. Chen, J. R. Lupski, M. F. Wangler, and H. J. Bellen. 2014. 'A drosophila genetic resource of mutants to study mechanisms underlying human genetic diseases', *Cell*, 159: 200-14.

Yamashita, Y. M., A. P. Mahowald, J. R. Perlin, and M. T. Fuller. 2007. 'Asymmetric inheritance of mother versus daughter centrosome in stem cell division', *Science*, 315: 518-21.

Yoshihara, Y., M. De Roo, and D. Muller. 2009. 'Dendritic spine formation and stabilization', *Current Opinion in Neurobiology*, 19: 146-53.

Yu, F., Y. Cai, R. Kaushik, X. Yang, and W. Chia. 2003. 'Distinct roles of Galphai and Gbeta13F subunits of the heterotrimeric G protein complex in the mediation of Drosophila neuroblast asymmetric divisions', *J Cell Biol*, 162: 623-33.

Yu, F., X. Morin, Y. Cai, X. Yang, and W. Chia. 2000. 'Analysis of partner of inscuteable, a novel player of Drosophila asymmetric divisions, reveals two distinct steps in inscuteable apical localization', *Cell*, 100: 399-409.

Yuste, R., and T. Bonhoeffer. 2004. 'Genesis of dendritic spines: insights from ultrastructural and imaging studies', *Nature Reviews: Neuroscience*, 5: 24-34.

Zebrowski, D. C., S. Vergarajauregui, C. C. Wu, T. Piatkowski, R. Becker, M. Leone, S. Hirth, F. Ricciardi, N. Falk, A. Giessl, S. Just, T. Braun, G. Weidinger, and F. B. Engel. 2015. 'Developmental alterations in centrosome integrity contribute to the post-mitotic state of mammalian cardiomyocytes', *Elife*, 4.

Zein-Sabatto, H., and D. A. Lerit. 2021. 'The Identification and Functional Analysis of mRNA Localizing to Centrosomes', *Front Cell Dev Biol*, 9: 782802.

Zhang, X., M. H. Chen, X. Wu, A. Kodani, J. Fan, R. Doan, M. Ozawa, J. Ma, N. Yoshida, J. F. Reiter, D. L. Black, P. V. Kharchenko, P. A. Sharp, and C. A. Walsh. 2016. 'Cell-Type-Specific Alternative Splicing Governs Cell Fate in the Developing Cerebral Cortex', *Cell*, 166: 1147-62 e15.

Zheng, Y., V. Mennella, S. Marks, J. Wildonger, E. Elnagdi, D. Agard, and T. L. Megraw. 2016. 'The Seckel syndrome and centrosomal protein Ninein localizes asymmetrically to stem cell

centrosomes but is not required for normal development, behavior, or DNA damage response in Drosophila', *Molecular Biology of the Cell*, 27: 1740-52.

Zou, C., J. Li, Y. Bai, W. T. Gunning, D. E. Wazer, V. Band, and Q. Gao. 2005. 'Centrobin: a novel daughter centriole-associated protein that is required for centriole duplication', *J Cell Biol*, 171: 437-45.