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Wei-Hsuan Lee April 9, 2019

Investigating Genetic Interactions Between the RNA Binding Protein *dNab2* and Planar Cell Polarity (PCP) Components in *Drosophila melanogaster*

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a thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

Investigating Genetic Interactions Between the RNA Binding Protein *dNab2* and Planar Cell Polarity (PCP) Components in *Drosophila melanogaster*

By Wei-Hsuan Lee

Mutations in the ubiquitously expressed polyadenosine RNA binding protein (RBP) ZC3H14 are linked to a monogenic, non-syndromic autosomal recessive intellectual disability. ZC3H14 has a functionally conserved ortholog, dNab2, in *Drosophila melanogaster*. Previous research in the Moberg and Corbett Labs has shown that dNab2 is autonomously required within neurons to pattern axon projection in the mushroom body (MB). MB axons lacking dNab2 project aberrantly across the brain midline and show evidence of defective branching. Similarly, mutations in components of the well-conserved planar cell polarity (PCP) pathway, which controls the planar orientation of static and motile cells, also cause MB axon mis-projection. Previous research in the Moberg Lab also shows that flies lacking *dNab2* display kink sensory bristles (Pak et al., 2011), which is similar to the randomized orientation of sensory bristles observed in PCP mutants (Seifert & Mlodzik 2007). To assess if *dNab2* controls axon projection and sensory bristle orientation in conjunction with the PCP pathway, and to identify genes that interact with dNab2 in neurodevelopment and other cellular contexts, we utilized a dNab2 overexpression model as the basis of a modifier screen. A preliminary test in the Moberg lab suggested that an allele of the Wnt receptor *frizzled* (*fz*) could suppress a rough eye phenotype caused by dNab2 overexpression, which provided foundation and motivation for broader examination of PCP candidates. This approach found evidence that alleles of several PCP pathway components could dominantly rescue a rough-eye phenotype caused by excess dNab2. These PCP mutations could also rescue the near-lethal phenotype of a *dNab2* null allele and

partially rescue an associated kinked thoracic bristle phenotype. Surprisingly, the rescue of *dNab2* rough-eye phenotype could be observed in progeny of PCP allele 'carriers' that did not inherit the allele, suggesting an indirect modification effect through the germline. In sum, these findings reveal a consistent pattern of genetic interactions between PCP components and the *dNab2* RBP that seem to reflect complex regulatory interactions between PCP signaling and *dNab2*. Future experiments could focus on molecular mechanism(s) that underlie these effects in neurons and the germline.

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My thanks to Dr. Ken Moberg, my advisor, who ideated the project and directly supervised me on the project. This project is built upon Chris Rounds and Zenyth Shepherd's preliminary findings on the connections between *dNab2* and PCP. In addition to my other committee members, Binta Jalloh, Chris Rounds, and Edwin Corgiat served as graduate mentors throughout my time conducting research in the Moberg Lab and contributed data and experiments to this project, for which I am grateful.

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CHAPTER 1: BACKGROUND

Intellectual disability (ID) consists of a cluster of developmental disorders characterized by defects in adaptive functioning and an IQ score under 70 (Ropers et al., 2008), and has caused an enormous socioeconomic burden on public health. Autosomal forms of genetic ID are often more common than X-linked ones, and as recently shown, autosomal recessive ID (ARID) is extremely heterogeneous (Jamra 2018). Mutations in genes that encode RNA binding proteins (RBPs) often lead to tissue-specific disease pathology, particularly within the brain and nervous system (Castello et al., 2013).

The *ZC3H14* gene encodes a ubiquitously expressed zinc-finger polyadenosine RNA-binding protein that plays a key role in post-transcriptional regulation of gene expression, including regulating mRNA stability, nuclear transport and localization, and mRNA translation, thus controlling neuronal RNA metabolism. (Kelly et al., 2012 & Wigington et al., 2014) and see **Figure 1**. *ZC3H14* is evolutionarily and functionally conserved among yeast, mice, flies, and humans. A nonsense mutation of the *ZC3H14* gene has been linked to a form of monogenic, non-syndromic autosomal recessive intellectual disability (NS-ARID). Patients of this NS-ARID are first identified in two independent Iranian families and are the offspring of consanguineous marriage between cousins (Kelly et al., 2014 & Chang Hui et al, 2011). To further study the effects of mutations in *ZC3H14* in neurodevelopment, the *Drosophila* model system is exploited. *Drosophila* nuclear polyadenosine binding (dNab2) is a functionally conserved ortholog of the human ZC3H14. Previous biochemical and genetic studies indicate that dNab2 restricts bulk RNA poly(A) tail length, and that loss of dNab2 leads to developmental and behavioral defects such as reduced adult viability, kink thoracic bristles, locomotor dysfunction, and brain morphological difference (Pak et al., 2011). These defects are fully rescued by neuronal dNab2 re-expression, and partially rescued by human ZC3H14 expression in fly neurons (Kelly et al., 2016; Kelly et al., 2014; Pak et al., 2011), showing the conserved role for human ZC3H14 and fly dNab2. Utilizing a monogenic fly model, an organism with a complex nervous system and a

short gestation period, provides a genetically tractable system to investigate the post-transcriptional roles of this RBP in neurons and other cell types. Identification of commonalities between the two orthologs opens the possibility to uncover disease mechanisms underlying ZC3H14 mutations, and could lead to development of broadly applicable diagnostic and therapeutic approaches to treating human ID. Since mutations linked to ID converge on common pathways, the study of the ZC3H14 fly model can also help elucidate common cell biological defects underlying IDs as a whole.

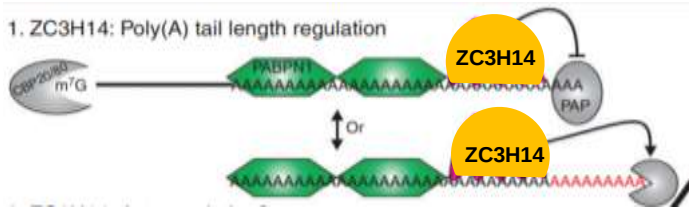


Figure 1. Model for the function of ZC3H14. ZC3H14 plays a role in poly(A) tail length regulation of other mRNA transcripts.

Figure adapted from Wigington et al., 2014

Evidence of links between *dNab2* and planar polarity: Unpublished data from the Corbett and Ping Chen Lab (Emory University) reveals that *ZC3H14* knockout mice displayed abnormal orientation of stereocilia in the cochlea, a structure in the inner ear (**see Figure 2**). As stereocilia orientation is controlled by the planar cell polarity (PCP) pathway (Kelly & Chen 2014), these data suggest a potential requirement for *ZC3H14* in normal PCP signaling. In addition, previous research in the Moberg lab has shown that dNab2 mutant flies show kink thoracic bristles (**see Figure 3**). This is a mutant phenotype linked to the PCP pathway, since research has shown that flies with mutations in core PCP genes *Frizzled* (Fz) and *Dishevelled* (Dsh) show randomized orientation of actin hairs and sensory bristles on the surface of the wing and thorax (Seifert & Mlodzik 2007). Together, these data

show potential connections between dNab2 and multiple components of the PCP pathway that will be further explored in this thesis.

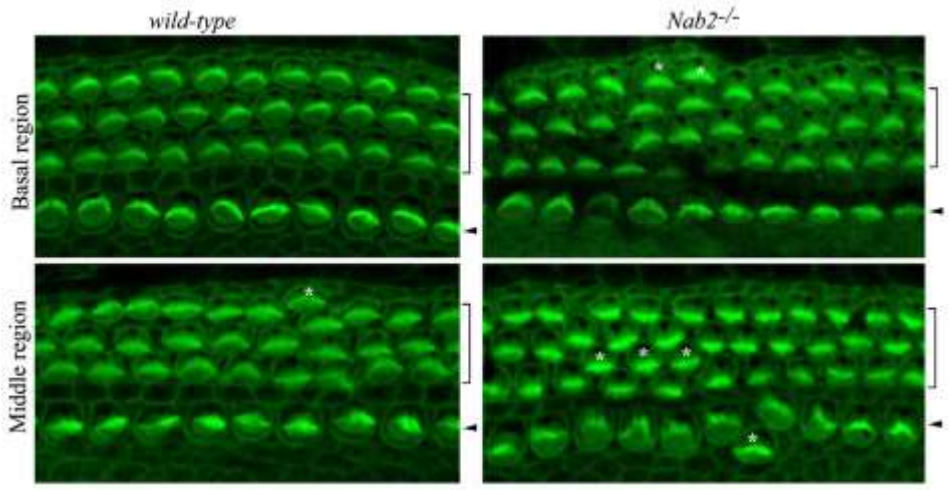


Figure 2. Images of stereocilia (green) in cochlear inner ear hair cells in *wildtype* (left) and *ZC3H14* knockout (right; labeled as "*Nab2-/-*") mice. *Wild type* shows the normal organization of 4 rows of hair cells (3 top and 1 bottom). Asterisks denote cells in an abnormal "5th row" position, which is rare in *wt* but common in *ZC3H14-/-* cochlea.

Images gathered by P. Chen lab

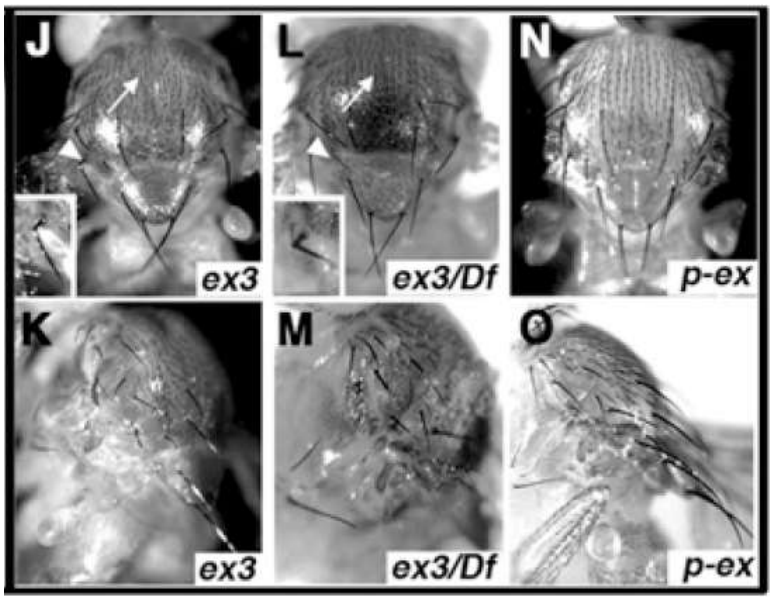


Figure 3. Front and side views of the thorax showing thoracic bristles. dNab2^{ex3} (J and K) and dNab2^{ex3}/Df (L and M) mutants show kink major thoracic bristles and disorganized

minor thoracic bristles (arrows in J and L) compared with p-ex controls (N and O).

Figure Source: Pak et al., 2011

The Planar Cell Polarity Pathway (PCP)

The PCP pathway polarizes cells into well-organized structures in the planar, two-dimensional axis of the epithelial surface. It establishes polarity of cells in a given tissue to coordinate growth and extension of their cellular structures (e.g. cilia and hair follicles), and it is thus very important for normal tissue development and function. This pathway was first discovered in genetic studies in *Drosophila,* when mutations in certain PCP alleles (*Wnt* and *Fz*) resulted in disoriented wing hairs. In *Drosophila*, this polarization governs orientation of wing hairs, sensory bristles on the external cuticle, and the hexagonal array of ommatidia in the adult eye, while in vertebrates, it governs orientation of stereocilia in the sensory epithelium of the inner ear and hair follicles (Seifert & Mlodzik 2007). Importantly, evidence suggests that PCP also controls the direction of neuronal growth cone extension in the nervous system of flies and mice (Shimizu, K., Sato, M. & Tabata 2011; Lyuksyutova et al., 2003). Mutations of genes in the PCP pathway has also been shown to be strongly linked to human diseases such as neural tube closure defects and adolescent idiopathic scoliosis (AIS), due to uncoordinated growth of cilia and improper formation of the spine (Andersen et al., 2017). The PCP pathway has since become a target for research in diseases related to ciliogenesis and neurogenesis.

The PCP pathway is composed of a two opposing adhesion complexes that polarize static and migrating cells across their planar (rather than apicobasal) axis (**see Figure 4).** There are two evolutionarily conserved patterning mechanisms that underlie planar polarity in *Drosophila*: the Frizzled (Fz)/Flamingo (Fmi) core complex and the Fat (Ft)/ Dachsous (Ds) system. The two signaling complexes act in parallel and reinforce correct PCP establishment through their independent inputs. PCP is activated by binding of a Wnt ligand to a Fz receptor, which then induces membrane clustering

of one of the PCP protein complexes that in turn triggers cytoplasmic actin polymerization. This pathway is thus regarded as a noncanonical, ß-catenin independent pathway that diverges from the canonical Wnt signaling pathway at the *Dishevelled* (*Dsh*) gene, and its functioning is known to be independent of transcription. (Roque & Torban 2015). Overall, PCP is critical for the generation of both global and local directional information for tissue growth and patterning in multicellular organisms, and the associated cellular responses are diverse, ranging from the regulation of actin cytoskeletal organization, cell adhesion and movement, (Simons & Marek 2018), as well as the development of the nervous system (Tissir & Goffinet 2013).

The Frizzled and Fat PCP Pathways

Frizzled: The core proteins in the *Frizzled* (*Fz*) planar polarity pathway (aka 'core' PCP), *Van Gogh (Vang), Prickle (Pk), Starry Night (Fmi or Stan), Frizzled (Fz), Dishevelled (Dsh), and Diego (Dgo),* align the polarity of each cell with that of its neighbors through an intercellular feedback loop, enabling polarity to propagate from cell to cell (Ma et al., 2003). Proteins of the core PCP become asymmetrically localized at the junctions between cells to form intercellular complexes that coordinate planar polarity between neighboring cells. Intracellular inhibitory interactions lead to the formation of a Fz-Fmi-Dsh-Dgo complex on one side and a Vang-Fmi-Pk complex on the opposite pole, creating a planar orientation axis (Yang et al., 2016). The proximal localization of *Vang* and *Pk* and the distal localization of *Dsh, Fz, and Dgo* in the cellular domain lead to an asymmetrical development of the wing hair on the distal end of the wing cell (**see Figure 5)**.

In contrast to their intracellular antagonism, the Fz-Fmi-Dsh-Dgo and Vang-Fmi-Pk complexes stabilize each other across cell to cell junctions and thus engage in positive-feedback. The antagonizing and stabilizing interactions between the two complexes lead to asymmetric activation of downstream effectors, specifically protein GTPases and the Jun N-terminal kinase (JNK) and see **Figure 4**. These molecules in turn direct asymmetric actin polymerization, cytoskeletal rearrangements, and neuronal projection. For instance, due to the mechanistic balance of two opposing systems in the PCP pathway, loss or overexpression of any core PCP genes can lead to disturbance of the localization and activation of other core PCP and downstream components. The amount of PCP factors needs to be in perfect balance as well, as too much and too little of these components results in similar defects (Yang et al., 2016).

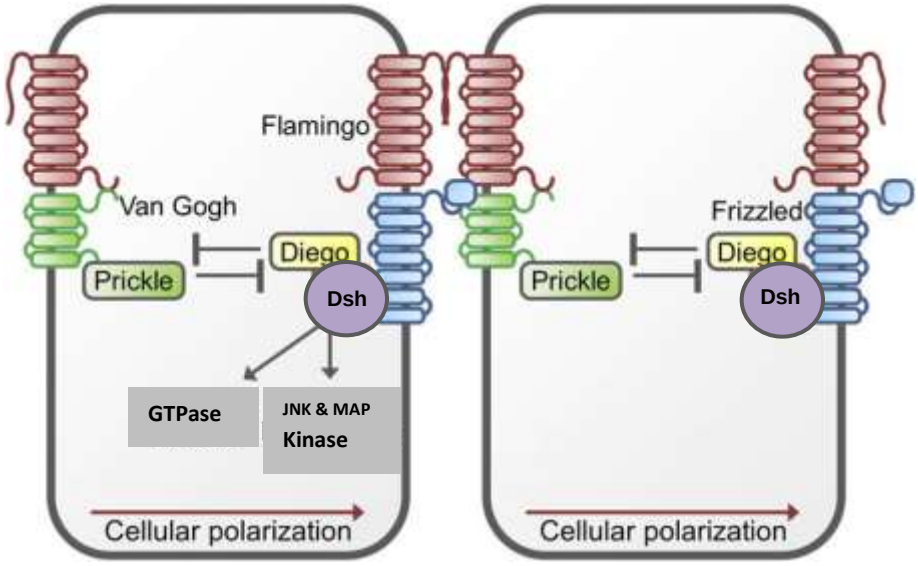


Figure 4. A model for organization of the core PCP pathway in *Drosophila* and their downstream targets. Flamingo and Frizzled are receptor proteins that Dsh, shown above, binds to, and elicits downstream pathways such as the JNK and MAP kinase pathways that direct actin polymerization. The proximal Van Gogh and Prickle complex antagonizes the distal Diego and Dsh complex, creating unidirectional polarity.

Figure adapted from Foulquier et al., 2017

Fat/Dachsous: The Fat/Dachsous (Fj/Ds) system dictates global orientation by transducing a directional signal to individual cells (Goodrich & Strutt, 2011). This pathway acts in parallel with the core PCP pathway to control bristle orientation in the *Drosophila* abdomen (**see Figure 6**). The Ft/Ds pathway can act independently of the core pathway to mediate effects on growth control, cell division orientation in the wing and eye discs, and the polarity of larval denticles (Goodrich & Strutt, 2011). This pathway has also been shown to increase cell proliferation, increase size of non-dividing cells, aid cell recruitment, and many other processes that are required for the regenerative response to injury (Montez & Morata 2017).

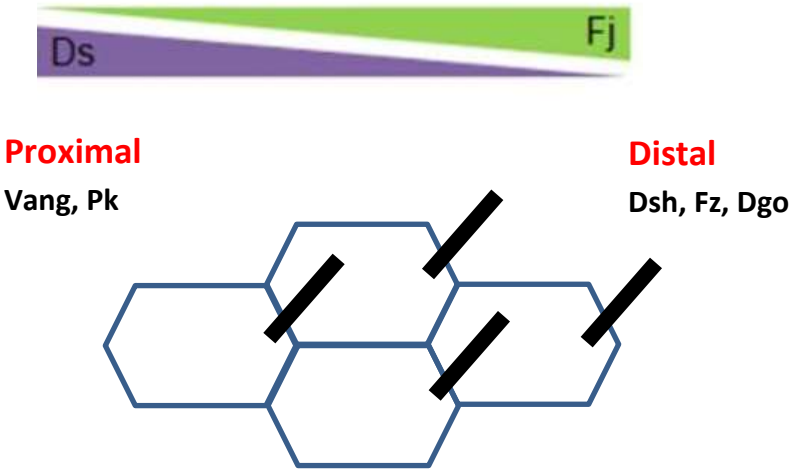


Figure 5. PCP signaling in the *Drosophila* wing. Epithelial cells in the wing blade generate an actin hair (black rods) pointing distally in wildtype flies. The asymmetries of global and core proteins generate tissue polarity. Global PCP components Ds and Fj are expressed in opposing gradients across the wing, while the core PCP proteins are asymmetrically localized at the cell junctions between neighboring cells. Vang and Pk are proximally located and Dsh, Fz, and Dgo are located on the distal cellular domain.

Figure adapted from Schnell & Carroll 2014.

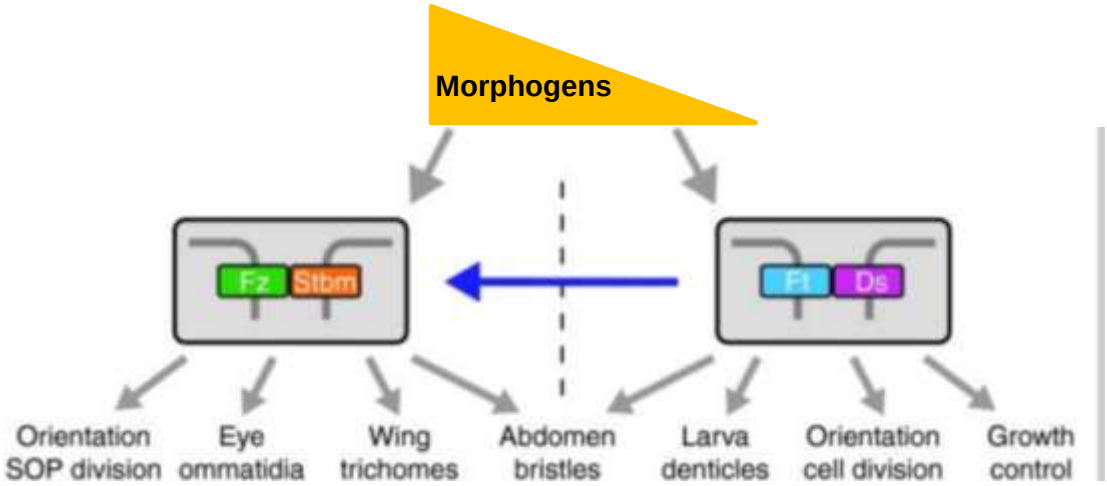


Figure 6. Relationships between the core and Ft/Ds pathways in *Drosophila*. Both the Wnt/Fz (aka Fz/Stbm) and Ft/Ds pathways can respond independently to upstream morphogenetic patterning information (gradient in orange). The core pathway interacts directly with effectors to control polarity of trichomes in the wing, the orientation of sensory organ precursor (SOP) divisions, and the rotation of ommatidia in the eye. The Ft/Ds pathway can alter polarity via the core pathway (blue arrow) by an unknown mechanism.

Figure adapted from Goodrich and Strutt, 2011

Planar Polarity in the Nervous System

Although the role of PCP is most evident in epithelial cells, it is also responsible for shaping the development and function of the nervous system in *Drosophila* and mammals. PCP components in *Drosophila* and vertebrates are involved in axon guidance, target recognition, dendritic growth and maintenance, and neuronal migration (Tissir & Goffinet 2013). Several core PCP genes, including *frizzled* (*frz*), *strabismus* (*stbm*), *flamingo* (*fmi*), and *dishevelled* (*dsh*), are cooperatively required for axonal targeting and branching of the fly mushroom bodies (MBs), a pair of structures in the *Drosophila* brain that contain neurons required for olfactory learning and memory (Shimizu, K., Sato, M. & Tabata 2011). Previous research in the Moberg and Corbett Labs has shown that *dNab2* loss also alters axon projection in the MBs (Kelly et al., 2016 also **see Figure 7**), suggesting that *dNab2* may regulate an mRNA encoding a PCP component. The mutant MB phenotypes caused by defective PCP signaling pathway including loss of MB alpha lobes and fusion of MB beta lobes (Shimizu, K., Sato, M. & Tabata 2011), are similar to those of MB phenotypes identified in *dNab2* mutants. Therefore, the primary aim of this thesis is to elucidate the strength and breadth of interactions between *dNab2* and alleles in the *Drosophila* planar cell polarity pathway.

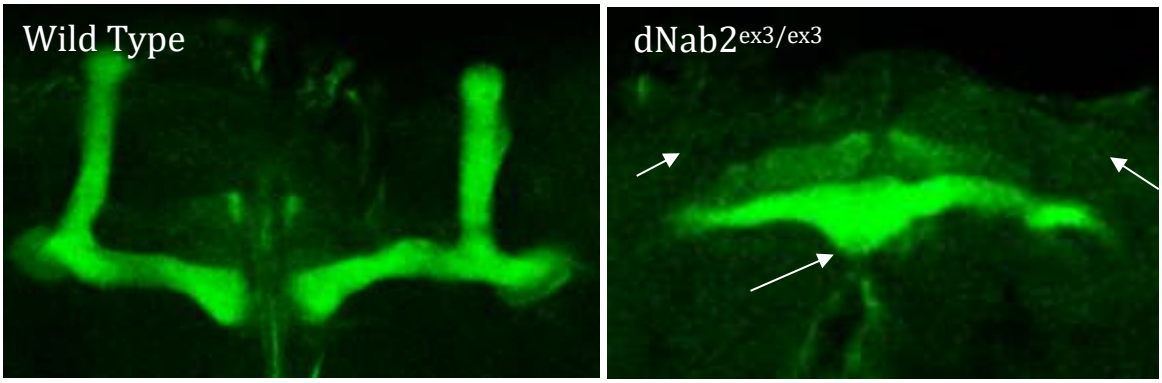


Figure 7. MB phenotypes of dNab2$^{ex3/ex3}$ mutants. Wild Type flies show normal MB structure with alpha-lobes intact and beta-lobes unfused (left image). dNab2 $^{ex3/ex3}$ flies show missing alpha-lobes and fused beta lobes (white arrows). Fly brains were dissected and stained with a mouse Fas II antibody and imaged with a confocal microscope.

B. Jalloh assisted with brain dissections and E. Corgiat with Imaging

CHAPTER 2: PCP ALLELES INTERACT WITH A *dNAB2* TRANSGENE IN THE EYE

Introduction

The *Gal4/UAS* system is used to drive tissue-specific expression of a transgene (*UAS-gene*) of interest in *Drosophila* (Brand & Perrimon 1993) and see **Figure 8**. The use of the *GMR-Gal4* driver (Glass Multimerized Reporter) to induce *UAS* transgene expression in the developing eye is a well validated system to a generate a gain-of-function phenotype that can be used for a dominant modifier screen. Previous experiments in the Moberg and Corbett Labs exploited the finding that dNab2 overexpression in *Drosophila* retinal cells (‘*GMR>dNab2’*) produces a rough-eye phenotype that is readily modified by interacting genes such as *PABP2, hiragii* (Poly A polymerase) (Pak et al., 2011). Bienkowski et al. (2017) used this approach to identify the *Drosophila* Fragile-X homolog, *dFmr1*, as a dNab2 interacting candidate.

Here we utilized a similar *GMR>dNab2* modification approach to test modification of the rough eye phenotype to gain insight into the breadth and scope of interactions between dNab2 and PCP core and downstream components. In this study, I assessed 13 genes in the core and downstream PCP pathway, and 1 gene in the canonical Wnt signaling pathway for dominant modification of the *GMR>dNab2* phenotype. The alleles selected play various roles in axon guidance, MB development, and neuronal projections that are defective in dNab2 mutant flies (Kelly et al., 2016 **See Summary Chart**). Assessing whether reduced PCP gene dosage (heterozygosity) for different mutations of these alleles that are critical for neuronal development affected dNab2 phenotypes in retinal neurons can elucidate the differential interactions dNab2 has on different PCP candidates and give direction to further research in other cell types.

Methods

Drosophila Stocks and Genetics

Crosses were maintained in 25°C humidified incubators with 12hr light-dark cycles. Stocks were obtained from Bloomington *Drosophila* Stock Center at Indiana University (BDSC). The *ex3* (null) and *pex41* (*precise excision 41;* a wild type control) *dNab2* alleles have been described previously (Pak et al., 2011). Drivers: *GMR* (BDSC# 1350). Alleles: $dNab2^{EP}$, dsh^{a3}, dsh^{6}, dsh^{1}, $appl^{d}$, $puc^{MI11060}$, $pk^{sple-13}$, $pk^{sple-14}$, pk^{30}, $wnt4^{C1}$, $tap^{MI10541}$, fz^{Jb}, $vang^{6}$, fmi^{frz3} , and $pygo^{s123}$(non PCP).

Dominant modifier screen

A stock overexpressing *UAS-dNab2* under *the GMR-Gal4* eye specific promoter (*GMR>Gal4*) was crossed to 13 loss-of-function PCP alleles and 1 Wnt signaling pathway allele (listed above). Progeny overexpressing dNab2 in the eye in the background of heterozygosity for loss-of-function PCP candidate alleles, were assessed for modification (i.e. enhancement or suppression) of the *GMR>Gal4* rough eye phenotype. Interactions were categorized as ‘enhancing’, ‘suppressing’, or ‘neutral’ (based on pigmentation loss and disorganization of honey comb structure) under a white-light dissecting microscope (**see Figure 9**). Adult eyes were imaged with a Canon Rebel T3 Camera. Images were processed with Image J.

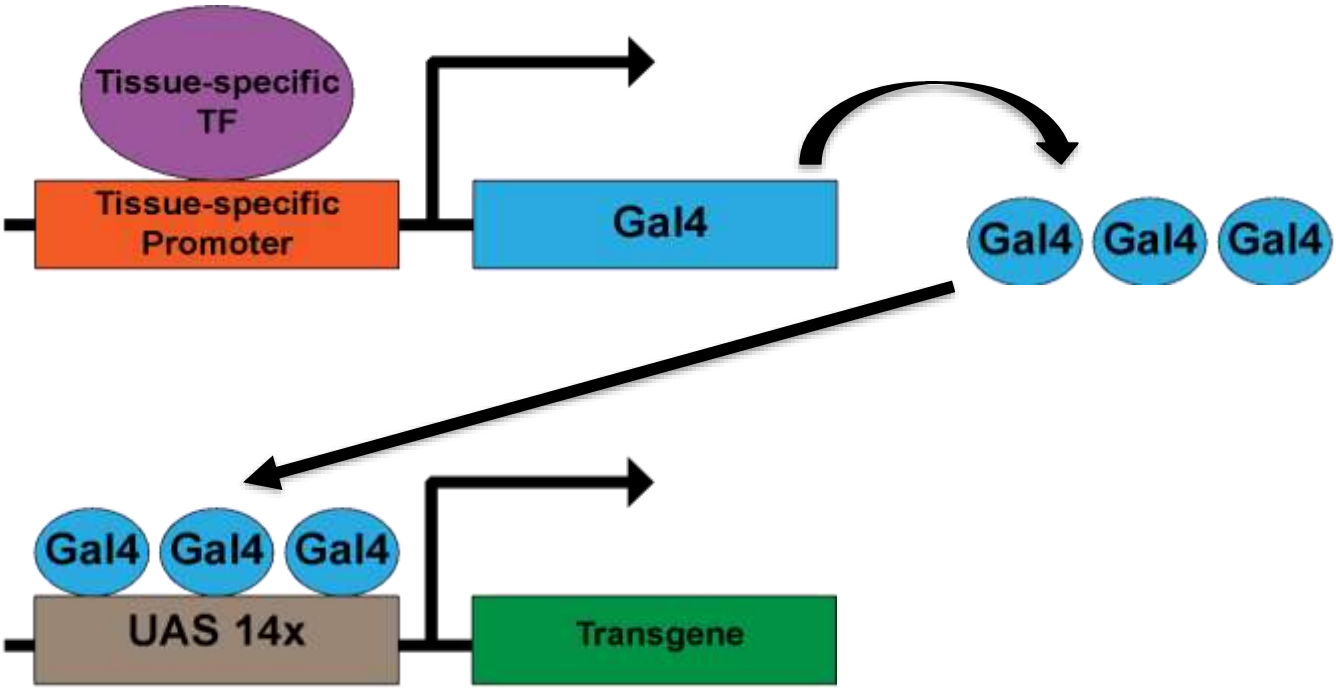


Figure 8. Schematic of the UAS-Gal 4 System in driving tissue specific gene expression
A tissue-specific transcription factor binds to the promoter to drive expression of the yeast *Gal4* gene. The production of *Gal4* transcription binds to the upstream activating sequence (UAS) of the transgene and drives expression. In the construct utilized in this thesis, an eye-specific promoter and transcription factor is used, and the targeted transgene is *dNab2*.

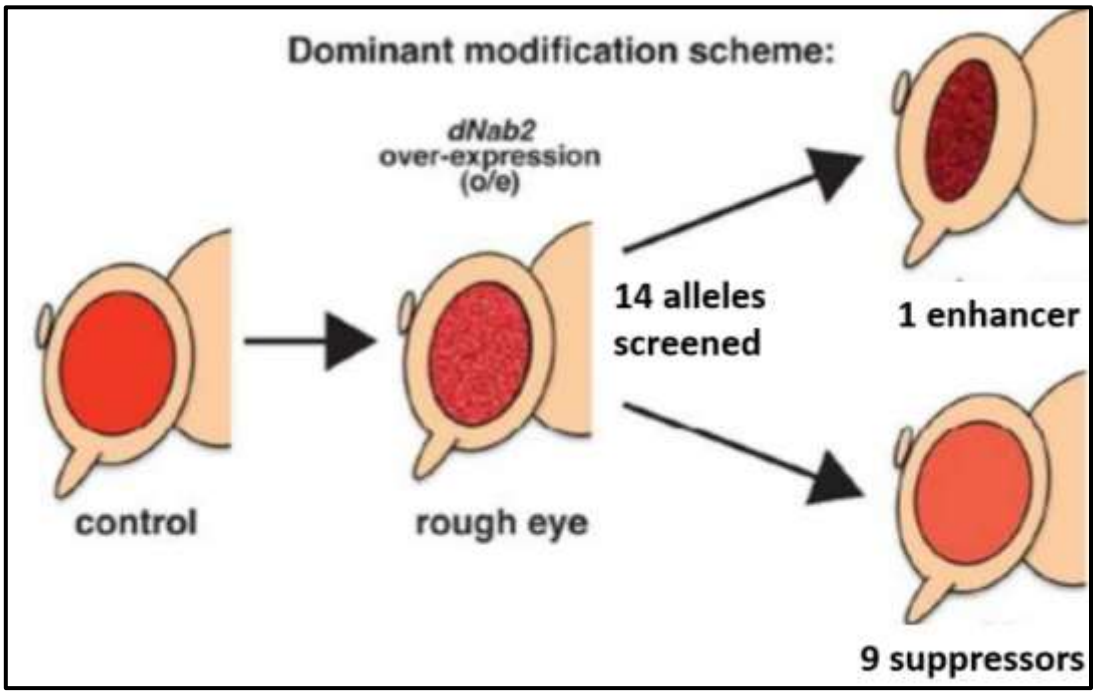


Figure adapted from Edwin Corgiat & Chris Rounds

Figure 9. Schematic of the GMR>dNab2 dominant modification eye screen. GMR-Gal4 overexpression (o/e) of dNab2 from the dNab2 ^{EP3716} allele leads to a rough eye phenotype that was enhanced by 1 and suppressed by 9 of the 14 candidate alleles.

Figure adapted from Bienkowski et al.,2017

Results and Discussion

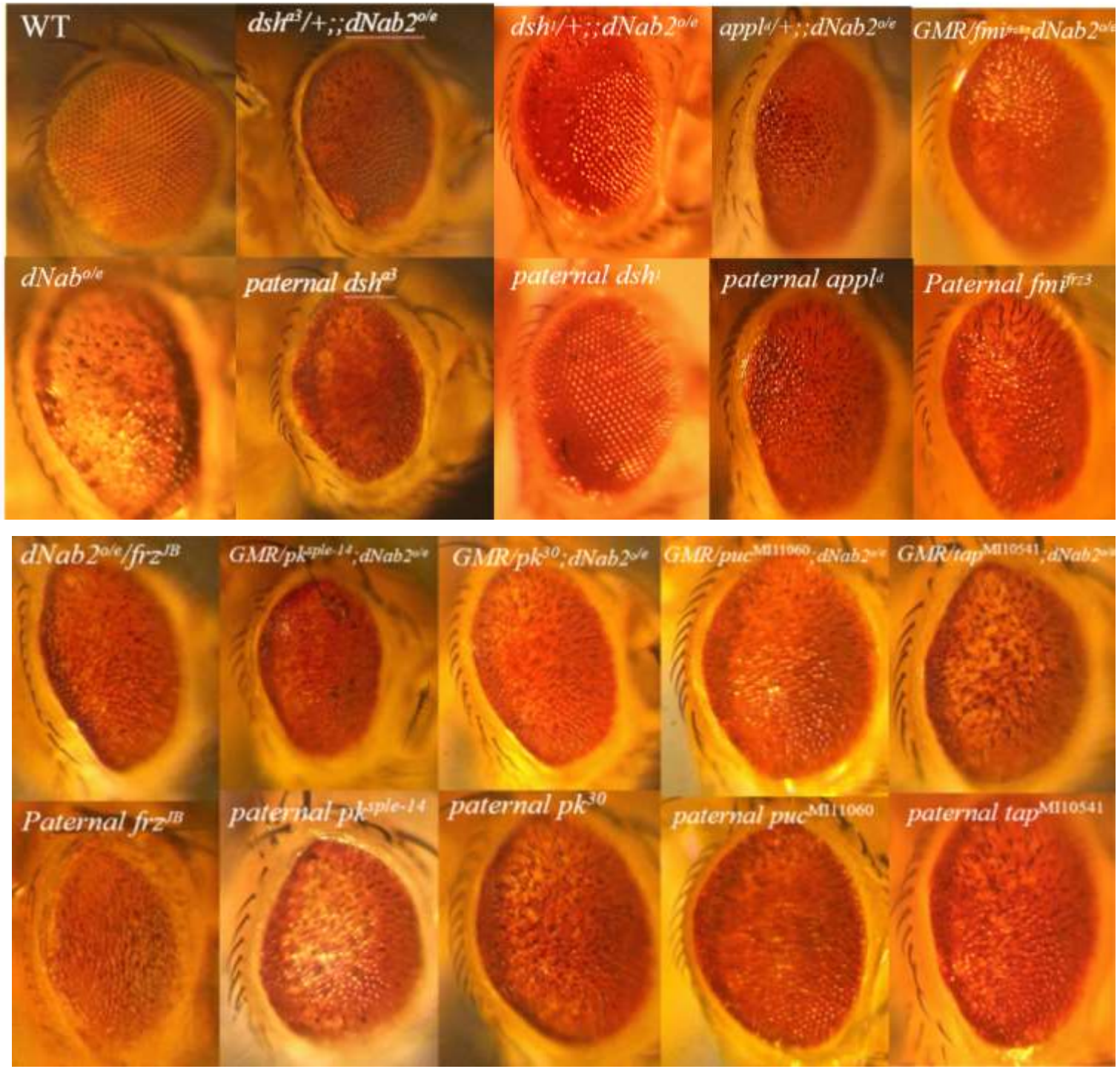
Here we report the results of a candidate-based screen of PCP alleles for dominant genetic interactions with the *Drosophila dNab2* gene, which encodes an RBP whose human ortholog, *ZC3H14*, is lost in an inherited intellectual disability. As previously described, *GMR>dNab2* eyes ("*dNab2$^{o/e}$*" in **Figure 10A**) lack full pigmentation and have disorganized ommatidia, likely due to effects of excess dNab2 on endogenous retinal mRNAs. Through a candidate based genetic screen of 13 different core and downstream PCP alleles and 1 allele outside of the PCP pathway, we found that heterozygosity for loss-of-function alleles of a number of genes strongly modified the *dNab2* overexpression rough-eye phenotype: the nine (9) alleles *dsh^{a3}*, *dsh^{1}*, *appl^{d}*, *puc^{MI11060}*,*pk$^{sple-14}$*, *pk^{30}*, *tap^{MI10541}*, *fz^{Jb}*, *and fmi^{frz3}* dominantly suppressed; 1 allele, *wnt4^{C1}*, dominantly enhanced; the last four alleles, *pygo^{s123}*, *dsh^{6}*, *vang^{6}* and *pk$^{sple-13}$*(4), show no dominant effects. (**Figures 9**). Significantly, the *dsh^{1}* allele results is a Lysine-to-Methionine change (K417M) that selectively impairs Dsh protein function in the non-canonical Wnt/PCP signaling but remains wildtype with respect to canonical Wnt/armadillo signaling (Axelrod et al., 1998). These results argue strongly that dNab2 interacts functionally only with the non-canonical Wnt/PCP pathway in the fly eye. The fact that *pygo^{s123}*, an allele in the canonical Wnt signaling pathway, shows no modification of the phenotype, also supports the proposed specificity of *dNab2* interaction with the PCP pathway.

These results show that reducing the genetic dosage of certain PCP candidates by approximately one-half suppresses effects of dNab2 overexpression in retinal neurons; the simplest

explanation of these data is that overexpressed dNab2 enhances PCP 'activity' which is then reduced to near wildtype levels by mutant PCP alleles. However, as the PCP pathway is comprised of two antagonizing complexes in balance (**see Figure 4**), too much or too little of a given PCP protein could cause a change in the overall equilibrium that mimics 'gain' of the opposing complex. Thus, the genetic effects of PCP alleles may not mean that PCP activity is elevated in *GMR>dNab2* flies. The mechanism by which PCP alleles modify activating effects remain unknown. Based on research done in Bienkowski et al, 2017, we speculate that dNab2, an RNA-binding protein, could bind to the poly (A) tail of PCP mRNAs, leading to translational modification that influences PCP mRNA levels and protein function in normal development of the *Drosophila* eye.

To rule out the possibility of a background modifier carrying out the phenotypic rescue, we tested multiple *dsh* alleles, including dsh^{a3}, and dsh^{1}, and found consistent dominant suppression, indicating that these candidates are required for excess dNab2 to disrupt eye morphology. We observed differential rescue among the *dsh* and *pk* alleles tested, which can be explained by the different functions of the PCP proteins that these mutations disrupt. In addition, we observe differential modification results among alleles of the same gene, such as dsh^{1}, dsh^{6}, and dsh^{a3}. Since these different alleles could result in a null mutation or a hypomorphic mutation, influencing normal function of *Dsh* differently, we speculate that the differential allele strength plays a role in the modification of eye phenotype. There could be a "threshold requirement" of the PCP protein level required to carry out normal function causing a few *Dsh* alleles to show modification (dsh^{1}, $dsh^{a3)}$, whereas the other $(dsh^{6)}$ doesn't. Identified interacting genes include both core and downstream PCP targets, suggesting that dNab2 interactions are consistent throughout the PCP pathway. This pattern of genetic links suggests that dNab2 may interact with the PCP pathway in retinal neurons.

A


WT
dsh3/+;;dNab2o/e
dsh1/+;;dNab2o/e
appld/+;;dNab2o/e
GMR/fmiRNAi;dNab2o/e
dNabo/e
paternal dsh3
paternal dsh1
paternal appld
Paternal fmifrz3
dNab2o/e/frzJB
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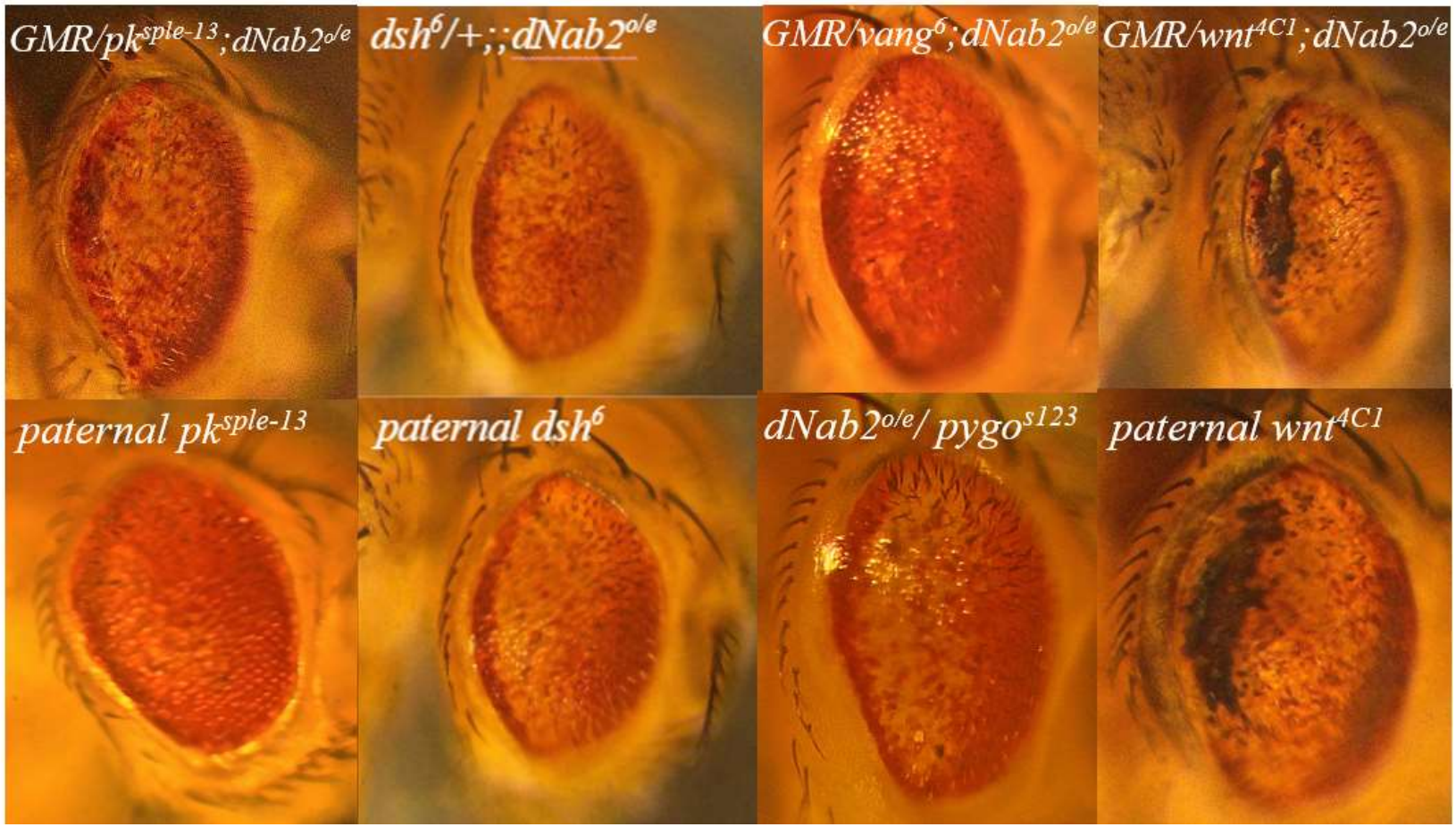

GMR/pk^sple-13;dNab2^o/e
dsh^6/+;;dNab2^o/e
GMR/vang^6;dNab2^o/e
GMR/wnt^4C1;dNab2^o/e
paternal pk^sple-13
paternal dsh^6
dNab2^o/e/ pygo^s123
paternal wnt^4C1

C

Allele	Effect			
	With allele		Transgenerational	
	Pigment	Structure	Pigment	Structure
dsh^{a3}	Suppressed	Suppressed	Suppressed	Suppressed
dsh^{1}	Suppressed	Suppressed	Suppressed	Suppressed
dsh^{6}	Neutral	Neutral	Neutral	Neutral
$appl^{d}$	Suppressed	Suppressed	Suppressed	Suppressed
fz^{Jb}	Suppressed	Suppressed	Suppressed	Suppressed
$wnt4^{c1}$	Enhanced	Neutral	Enhanced	Neutral
$pk^{sple-13}$	Neutral	Neutral	Suppressed	Suppressed
$pk^{sple-14}$	Suppressed	Suppressed	Neutral	Suppressed
pk^{30}	Suppressed	Suppressed	Suppressed	Neutral
$puc^{MI11060}$	Suppressed	Suppressed	Suppressed	Neutral
fmi^{frz3}	Suppressed	Suppressed	Suppressed	Suppressed
$tap^{MI10541}$	Suppressed	Suppressed	Neutral	Neutral
$vang^{6}$	Neutral	Neutral	N/A	N/A
$pygo^{s123}$	Neutral	Neutral	N/A	N/A

Figure 10. PCP alleles dominantly modify the overexpressed *dNab2$^{o/e}$* eye phenotype by eye pigment and structure. Visualization of modified *dNab2$^{o/e}$* phenotype in progeny of 13 PCP alleles and 1 non-PCP allele. A wild type eye (top, far left) shows honeycomb structure and no loss in pigmentation. A *dNab2$^{o/e}$* eye (bottom, far left) shows significant loss in pigmentation and disorganized, rough ommatidia. Top rows of both panels show alleles tested for dominant modification of *dNab2$^{o/e}$* eye phenotype. Images on bottom row of both panels, denoted 'paternal PCP', show indirect, dominant modification of the eye phenotype without inheritance of the corresponding PCP allele. Eye effect scored by change in pigment and ommatidia. (A) Candidate alleles that show dominant suppression. (B) Candidate alleles that shows dominant enhancement or no effect on the rough-eye phenotype. (C) Table summary of all candidate alleles tested.

E. Corgiat assisted with image processing

CHAPTER 3: INDIRECT MODIFICATION INTERACTIONS BETWEEN PCP AND *dNAB2*

Introduction

In the course of the experiments described above, we noticed an unexpected and potentially significant "indirect modification effect" in which PCP alleles present in the parental germline could dominantly rescue *dNab2* overexpression eye phenotypes in the progeny. This rescue occurred even through the *GMR-dNab2* modified F1 progeny inherited the wild-type copy of the PCP gene. This "indirect modification", also described as a "transgenerational" effect, is independent of genotype and presumably operates through the parental germline, perhaps via an epigenetic mechanism. Such speculation is corroborated by Honnen et al., 2012, a paper that shows some evidence of a role for PCP in controlling longevity in the germline of C. elegans through translocating transcription factors into the nucleus. Through a dominant modifier eye screen, survival assay, and examination of the dNab2 kink bristle phenotype, we probed the generality of this unexpected dNab2-PCP transgenerational interaction in different developmental and tissue contexts. Although mechanistically unclear, we speculate that the dNab2-PCP "transgenerational interaction" operates through a different mechanism than the dNab2-PCP direct interaction presented in **Chapter 2.**

Methods

Drosophila Stocks and Genetics

Crosses were maintained in the same conditions, and stocks were obtained from the same institution (BDSC) as described in Chapter 2.

Results & Discussion

Through the course of the experiments, we observed an indirect modification effect in the dominant suppression of dNab2 phenotypes. In the dominant modifier eyes screen, progeny without inheriting certain PCP mutant alleles, including dsh^{a3}, dsh^{1}, $appl^{d}$, fz^{Jb}, and pk^{30}, $puc^{MI11060}$, fmi^{frz3} exhibit the same suppression as those that carried one copy of the allele **(Figure 11 A)**. $Wnt4^{C1}$ shows indirect dominant enhancement of the phenotype, while dsh^{6} and $pk^{sple\text{-}14}$show no modification **(Figure 11 B)**. Surprisingly, $pk^{sple\text{-}13}$, a PCP allele that did not show direct, dominant suppression in progeny that carried the allele, instead showed an indirect rescue without inheriting the allele. This finding suggests that the modification of the germline by PCP components could balance of the amount of PCP proteins required for normal function. This also implies that in the $pk^{sple\text{-}13}$ allele, the indirect modification mechanism, potentially operating through the germline, occurs, but the direct modification with the allele, through another mechanism, does not. Together, these results suggest that dNab2 interacts with PCP components across generations through an unknown mechanism that is different from that of the direct heterozygous modification.

Our discovery that PCP alleles can rescue eye defects caused by overexpression of dNab2 without actually being inherited by the progeny was unexpected but could be important. There are two commonly proposed mechanisms that could explain this observed phenomenon: the maternal loading hypothesis and the epigenetic inheritance hypothesis. Previous research has established that maternal contribution of mRNAs and proteins may be a major determinant of developmental robustness and survival of progeny (Schüpbach & Wieschaus, 1986). However, our results do not align with this maternal provisioning hypothesis, since the observed suppression effect could be passed through the male germline. Therefore, we speculate that alleles in the PCP pathway may modify chromatin in the germline that could be passed on to progeny, which modulates dNab2 expression or regulates dNab2

interactions with other genes crucial to development later in morphogenesis. One study in *C. elegans* provides some evidence of a role for PCP in controlling longevity in the germline (Honnen et al., 2012). This work identifies the action *Vang-1* has in translocating the DAF-16/FoxO transcription factor into the nucleus where it activates gene expression for distinct processes in the germline, outlining the nuclear role of a key player in the PCP pathway. Vang-1 in *C. elegans* shows sequence similarities and conservation of overall domain architecture compared to Van Gogh in *Drosophila*. This previous work provides a foundation for our speculation that PCP components could act upstream of *dNab2*, playing a nuclear role in modifying the heritable state of chromatin, thereby effecting a change in gene expression in the germline.

Research has also shown potential cross-talk between the PCP and Wnt signaling pathway in shaping the nervous system. There is a well-known function of Wnts as ligands for Fz in the PCP pathway for the axonal development of the mushroom body of the *Drosophila* brain. Having a canonical Wnt protein, Wnt5, as a candidate ligand for the PCP pathway (Shimizu, K., Sato, M. & Tabata) shows the interconnectedness of the canonical Wnt and PCP pathway in neurodevelopment, thus providing foundation for our speculation of the nuclear role PCP candidates may play.

CHAPTER 4: POTENTIAL MECHANISM OF THE INDIRECT dNAB2-PCP INNTERACTION

Introduction

To test different hypotheses for the cause of this indirect modification interaction observed between dNab2 and PCP components, which include maternal loading and epigenetic inheritance through the germline, we performed a *dsh*^{1} RNAi germline knockdown on male flies. RNA interference (RNAi) is an endogenous cellular mechanism triggered by double-stranded RNA (dsRNA) which leads to the degradation of homologous RNAs (Ameres & Zamore 2013), thus silencing targeted genes. Through introduction of exogenous double-stranded RNA into cells, targeted RNAi knockdown is frequently exploited by scientists as a useful complement to traditional genetic mutants for identifying and characterizing gene function. In *Drosophila*, RNAi has been applied in cultured cells or in vivo to perturb the function of single genes or to systematically probe gene function on a genome-wide scale (Heigwer et al., 2018). Here, through a cross with females overexpressing dNab2 in retinal neurons and males with the *dsh*^{1} RNAi knockdown, we aim to examine whether this indirect modification effect observed previously, where transmittance of the modified phenotype is independent of genotype in the progeny, remains. This experiment provides evidence for our speculation that PCP plays a role in modifying the heritable state of chromatin in the *Drosophila* germline.

Methods

Drosophila Stocks and Genetics

Crosses were maintained in the same conditions, and stocks were obtained from the same institution (BDSC) as described in Chapter 2. The *ex3* (null) *dNab2* alleles have been described previously (Pak et al., 2011). Drivers: *GMR* (BDSC# 1350), *nanos-Gal4* (*nos>dsh*^{RNAi})

Germline RNA-Mediated Interference (RNAi)

RNA-mediated interference is carried out by an introduction of exogenous double-stranded RNA into cells to silence targeted genes. Here we use a *nanos-Gal4* driver to knock down expression of *dsh* in the male germline.

Dominant Modifier Eye Screen

A stock overexpressing *UAS-dNab2* under *the GMR-Gal4* eye specific promoter (*GMR>Gal4*) was crossed to a male fly with germline specific dsh^{RNAi} knockdown. Progeny overexpressing dNab2 in the eye in the background without paternal inheritance of the dsh^{RNAi} were assessed for modification (i.e. enhancement or suppression) of the *GMR>Gal4* rough eye phenotype. Interactions were categorized as 'enhancing', 'suppressing', or 'neutral' (based on pigmentation loss and disorganization of honey comb eye structure) under a white-light dissecting microscope. Adult eyes were imaged with a Leica microscope.

Results/Discussion

With a *dsh* RNAi germline knockdown on male flies crossed to female flies overexpressing dNab2, we generated progeny that either inherited the dsh^{RNAi} allele or not. Here we observed the same dominant suppression effect in the progeny with (not shown in figure) and without the dsh^{RNAi} allele, showing that the copy of dsh^{RNAi} allele in the paternal genotype exerted its effect on the overexpression eye phenotype in the progeny (**Figure 11**). This result rejects the maternal loading hypothesis, showing that the suppression affect could be passed down through the male germline, and validates our speculation that certain PCP components could modify the epigenetic markers on chromatin that can be transmitted through the germline.

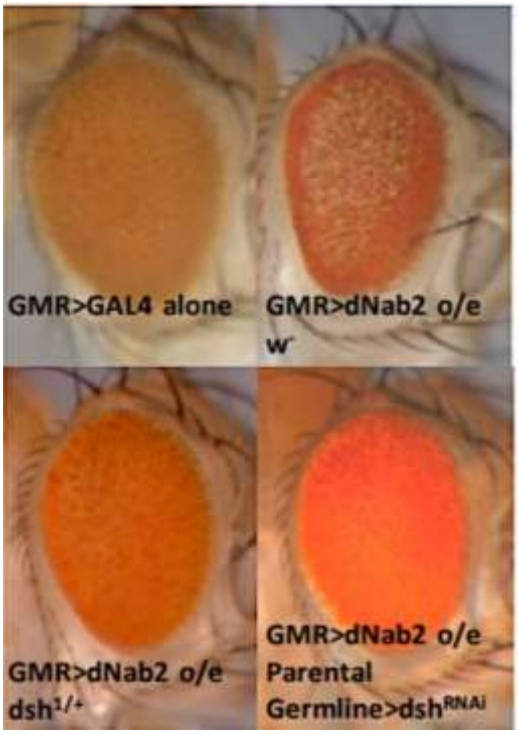


Figure 11. *dsh*^{RNAi} in the paternal germline modifies the *GMR>dNab2* eye phenotype in the subsequent generation. Top left: A control adult female eye with only the *GMR-Gal4* driver. Top right: A adult female *GMR>dNab2* eye phenotype shows loss of pigmentation and rough eye structure. Bottom left: *dsh*^{1} heterozygosity suppresses *GMR>dNab2*$^{o/e}$ phenotype. Bottom right: Knockdown of *dsh* in the paternal germline with the driver *nanos-Gal4* (*nos>dsh*^{RNAi}) also suppresses the *GMR>dNab2* phenotype in F1 progeny.

Figure Source: Christopher Rounds

CHAPTER 5: PCP ALLELES RESCUE EFFECTS OF dNab2 GENOMIC LOSS

Introduction

Utilizing the dNab2 overexpression model as the basis of a modifier screen is a preliminary, artificial gene-screening platform to identify interacting candidates. Observing consistent interactions between dNab2 and PCP in the fly eye, we want to probe the breadth of these interactions in different cellular contexts. To examine whether PCP candidates interact with endogenous dNab2, we selected four (4) different PCP candidate alleles that showed strong dominant modification in the *GMR>dNab2* eye screen: dsh^{1}, $appl^{d}$, $pk^{sple\text{-}14}$ (suppression), and $wnt4^{C1}$ (enhancement) for further experimentation. We then crossed these alleles into the endogenous *dNab2* null model ($dNab2^{ex3}/dNab2^{ex3}$) and examined whether dNab2-PCP interactions also occur with genomic alleles. Through this experiment, we can understand whether these interactions occur in different cellular contexts beyond retinal neurons, and the relative strengths of *dNab2* interactions with the selected *PCP* candidates.

Methods

Drosophila Stocks and Genetics

Male flies carrying one copy of dsh^{1} (on the X chromosome) and one copy of the $dNab2^{ex3}$ mutant allele were crossed to heterozygous *dNab2*/TM6B females, generating female progeny carrying one copy of dsh^{1} mutant allele that were *dNab2* null and male progeny without the dsh^{1} allele that were also dNab2 null. The $appl^{d}$ allele was crossed in the same way. Stocks: $sp/cyo;dNab2^{ex3}/TM6B$, sco/cyo;;mkrs/TM6B, $dNab2^{ex3}$/TM6B. Alleles: dsh^{1}, $appl^{d}$, $pk^{sple\text{-}14}$, and $wnt4^{C1}$. The *ex3* and *pex41* (precise excision 41) and alleles have been described previously (Pak et al., 2011).

Survival & Kink Bristle Assay

Crosses were maintained in 25°C humidified incubators with 12hr light-dark cycles. Number of surviving progeny were counted the day of eclosion and up to 3 days after to assess survival. Both sides of the *Drosophila* shoulder were examined for the kink thoracic bristle phenotype associated with dNab2 loss (Pak et al., 2011). Those with one kinked bristle were scored as 'positive' for the phenotype.

Results and Discussion

Wild type *pex41* flies exhibit a 97% survival while *dNab2* null flies show a ~8% survival. dsh^{1} heterozygosity, or a 'direct modification' of the *dNab2* null phenotype, rescues survival to ~56%. The $appl^{d}$ heterozygosity rescues to 34% survival. Thus, adult viability appears to be partially rescued by dsh^{1} and $appl^{d}$ heterozygosity. On the other hand, $wnt4^{C1}$ heterozygosity showed enhancement of *dNab2* lethality, decreasing viability to 2%, which is consistent with the enhancement effect of *GMR>dNab2* by $wnt4^{C1}$ observed in the dominant modifier eye screen. $pk^{sple-14}$ showed no effect on lethality. These results suggest that *dNab2* differentially interacts with multiple components in the PCP pathway, and that the cellular requirement for PCP components for normal retinal tissue development and adult viability may be distinct, as some alleles that modifies *GMR>dNab2* overexpression do not affect $dNab2^{ex3}$ null phenotypes. Based on the PCP interactions with the $dNab2^{ex3}$ allele, we hypothesize that *dNab2* loss over-activates the PCP pathway, perhaps because dNab2 acts as a translational repressor of a PCP mRNA, corroborated by previous data on *dNab2* and *Fmr1* target mRNAs (Bienkowski et al., 2017). Loss of dNab2 thus enhances PCP signaling, allowing translation of PCP proteins to reach the threshold requirement, which may influence downstream biological processes that are crucial to *Drosophila* survival to adulthood.

In addition to rescue of lethality of *dNab2* null flies through "direct" modification with PCP heterozygosity, we observed the same indirect modification, as observed in the *GMR>dNab2* dominant modifier eye screen in the endogenous *dNab2* model. The survival rescue observed occurred in a dose-dependent manner in dsh^{1}, showing lowest survival for *dNab2* null flies (~8%), and ~27% survival in flies without the dsh^{1} mutant allele (genomic wild type for *dsh*), and ~47% percent rescue in flies with dsh^{1} heterozygosity. However, the other two (2) PCP candidates alleles examined, $appl^{d}$ and $wnt4^{C1}$, and did not show transgenerational rescue of $dNab2^{ex3}$ survival (**Table 1**). These results suggest that interactions with dsh^{1} are fairly specific, and persist through the germline, whereas other PCP candidates may not. The potential mechanism of this effect is not known. However, in *C. elegan*, the PCP pathway controls activity of the insulin-responsive transcription factor DAF-16/FoxO in the germline (Honnen et al., 2012), which establishes one way that PCP alleles could cause heritable chromatin changes in the germline that could be passed on to progeny.

Genotype	**% viability (of expected)**
$dnab2^{ex3}/dnab2^{ex3}$	~8%
$dsh^{1}/+;;dnab2^{ex3}/dnab2^{ex3}$	47%
$y/+;;dnab2^{ex3}/dnab2^{ex3}$(paternal dsh^{1})	26.5%
$appl^{d}/+;;dnab2^{ex3}/dnab2^{ex3}$	34%
$y/+;;dnab2^{ex3}/dnab2^{ex3}$(paternal $appl^{d}$)	6%
$wnt4^{C1};;dnab2^{ex3}/dnab2^{ex3}$	2.4%
cyo/+;;$dnab2^{ex3}/dnab2^{ex3}$(paternal $wnt4^{C1}$)	2.4%
pex41	97%

Table 1. Summary of the percentage (%) viability of the indicated genotypes (shown as a % of expected). *dsh*^{1} and *appl*^{d} heterozygosity increased *dNab2*$^{ex3/ex3}$ viability, whereas *wnt4*^{c1} heterozygosity decreased viability.

Data Contribution: Binta Jalloh, dNab2$^{ex3/ex3}$ *Survival*

We observed a rescue of the *dNab2*^{ex3} null kinked-bristle phenotype by PCP heterozygosity as well. While almost all (>90%) *dNab2*^{ex3} null flies show bristle kinking, heterozygotes of *dsh*^{1}, *appl*^{d}, and *wnt4*^{C1} reduce this to ~50% (**see Figure 12**). However, due to the poor viability of *pk*$^{sple-14}$ heterozygotes, the effect of the *pk*$^{sple-14}$ allele may not be conclusive. Overall, these results are consistent with previous findings on the tissue-specific requirement of PCP proteins in normal development, the tissue-specific nature of dNab2-PCP interactions, and confirm the existence of *dNab2* and PCP interactions beyond retinal neurons. Moreover, we also observe an indirect modification of the *dNab2*^{ex3} kinked-bristle phenotype among F1 progeny that did not inherit the given PCP allele, which is consistent with the rescue effect observed in flies with PCP heterozygosity (**Figure 12**).

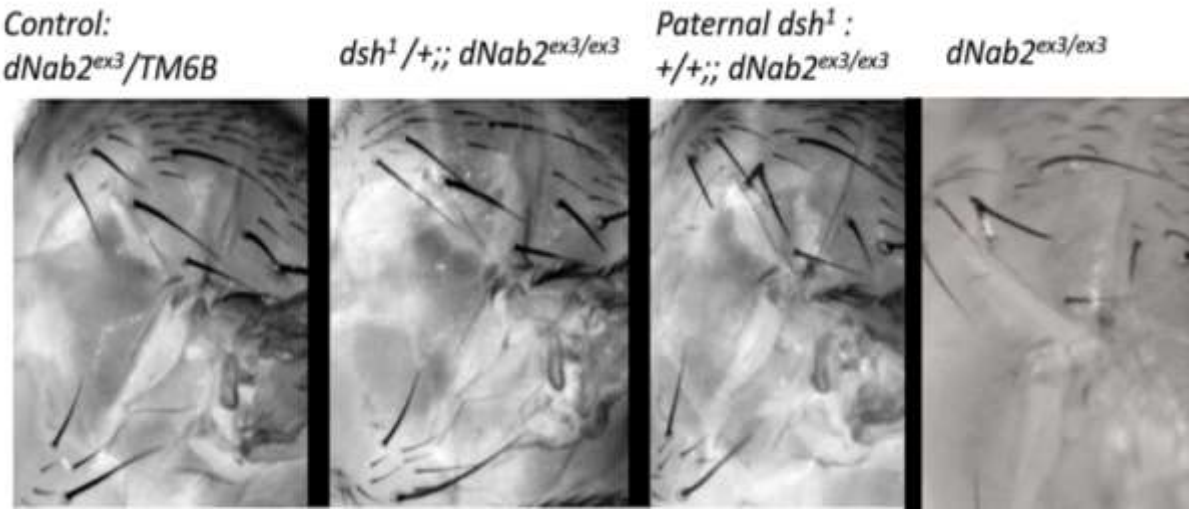


Figure 12. ~50% of the flies examined showed rescue of the kinked-bristle phenotype with PCP heterozygosity. Wild type flies (far left) show straight thoracic bristles, while *dNab2* mutants (far right) show kinked thoracic bristles. *dsh*^{1} heterozygosity partially rescues this phenotype directly (*dsh*^{1}*/+;;dNab2*$^{ex3/ex3}$) and through the paternal germline (*Paternal dsh*^{1}*:+/+;;dNab2*$^{ex3/ex3}$). *Appl*^{d}, and *wnt4*^{C1}alleles also show similar rescue effects.

Figure collaboration: E. Corgiat

CHAPTER 6: CONCLUSIONS AND FUTURE DIRECTIONS

Conclusions

The primary aim of this thesis is to elucidate connections between dNab2 and candidates in the planar cell polarity (PCP) pathway, which were originally noted by Zenyth Shepherd, a summer undergraduate researcher who identified an allele of the *Wnt* receptor *frizzled* (*fz*) that could suppress the *GMR-dNab2* rough eye phenotype (Moberg Lab, unpublished). Using the overexpression of *dNab2* in retinal neurons as a genetic screening platform, we examined thirteen different alleles in the PCP pathway and one (1) in the canonical Wnt signaling pathway and identified ten (10) candidates that interact with *dNab2*. Through various experiments we discovered differential effects of dNab2 on PCP in different genetic configurations. We found that of the eleven PCP modifiers in the *GMR-dNab2* eye screen, three (dsh^{1}, $appl^{d}$, and $wnt4^{C1}$) also dominantly modified survival rates of *dNab2* null flies, whereas one ($pk^{sple\text{-}14}$) did not. A number of these PCP alleles also dominantly modified a kinked thoracic bristle phenotype that is highly penetrant in *dNab2* null flies. As PCP controls polymerization of actin and assembly of bristles in the adult thorax (Singh & Mlodzik 2012), we presume that defective PCP signaling may contribute to thoracic bristle defects in *dNab2* mutants. Specifically, the allele dsh^{1}, which we observe to show consistent suppression effects across the three phenotypes, is selectively deficient only in the noncanonical Wnt signaling pathway. Therefore, the downstream targets that dsh^{1} might impact would be intact in the canonical Wnt signaling pathway, showing that the dominant modification of dNab2 mutant phenotypes that we observe are likely the result from a disrupted PCP signaling pathway. Unpublished data in the Moberg lab also show a partial MB rescue effect on mutant alpha-lobe thinning and beta-lobe fusion by heterozygosity of PCP allele ($appl^{d}$), suggesting connections between *dNab2* and PCP in neuronal development (Rounds, Moberg

unpublished, and **see Appendix 1**). Overall, we speculate that there are different cellular requirements of PCP proteins for normal retinal and actin development, and survival to adulthood in *dNab2* mutants.

The differences in suppression by different PCP alleles may be due to the relative dosage of each PCP factor, which I will refer to as the 'hourglass' model. This model proposes that there are different endogenous amounts of each PCP protein in different cell types, and that the least abundant of these proteins acts as a rate-limiting protein for PCP activity, similar to the narrowest point in an hourglass. Alleles of these limiting factors will therefore have greater effect on PCP signaling in each tissue, resulting in a different pattern of PCP allele modification of *dNab2* phenotypes depending on the tissue. For instance, dsh^{1} and $appl^{d}$ may be more rate limiting in retinal neurons, whereas $pk^{sple-14}$ and $wnt4^{C1}$ are not, and consequently alleles of these factors have weaker suppressive effects on *dNab2* phenotypes.

Although the transgenic overexpression of *dNab2* and the *dNab2* null model discussed above **(CHAPTERS 2 and 5)** yield different speculations of the function of dNab2 on PCP components, the results in the dNab2 overexpression model could be interpreted as a dominant negative effect that mimics a loss-of-function phenotype as shown in the *dNab2* null model. From these results, *dNab2* appears to interact with different components in the linear PCP pathway, controlling their cellular abundance in a way that is crucial to different aspects of development. This speculation is based on Bienkowski et al., 2017, and his work shows that CAMKII mRNA steady state levels are altered upon interaction with *dNab2*.

We speculate that the genetic interaction between *dNab2* and PCP alleles occurs in large part because the dNab2 protein binds and regulates the splicing, translation and/or stability by binding to the poly (A) tail of one or more mRNAs that encode PCP proteins (**see Summary Model**). This model is supported by data from the Corbett Lab showing that mice lacking the dNab2 homolog ZC3H14

show elevated levels of the *Vang* related PCP protein $Vang^{l2}$ in the brain (Rha et al., 2017; Morris & Corbett 2018). The Moberg and Corbett labs have also identified genetic interactions between dNab2 and non-PCP components, suggesting that *dNab2* is not a specific regulator of the PCP pathway. For instance, dNab2 interacts with 26 genes in the translational pathway centered around *dfmr1* (Bienkowski et al., 2017). These results imply that the *dNab2* function may be diverse, tissue-specific, and developmental stage-specific resulting in the variety of observed phenotypes in brain morphology, locomotion, and behavior (Pak et al., 2011; Kelly et al., 2016; Rha et al., 2017). It seems likely that no sole interaction with one specific pathway is responsible for all of the *dNab2* mutant phenotypes. This also explains the discrepancy between survival rescue and kink bristle rescue. *dNab2* may function to control a large group of mRNAs in cells, so that its apparent role within the PCP pathway is not responsible for all the different dNab2 mutant phenotypes.

$Wnt4^{C1}$ is an allele that shows consistent dominant enhancement of the *dNab2* rough-eye phenotype, which is important to note. The $Wnt4^{C1}$ is a mutation caused by a 3 base pair deletion of Glutamine 299, an in-frame deletion, and the loss of this one amino acid could lead to a change in structure of the $Wnt4^{C1}$ protein. Since the interactions between dNab2 and $Wnt4^{C1}$ show effects opposite to the other alleles screened in the *dNab2* overexpression model and the endogenous *dNab2* null model, we suspect that $Wnt4^{C1}$ could be a neomorphic allele, gaining a new function that causes an enhanced phenotype. This new function could potentially be over-activating the PCP pathway, leading to an intracellular imbalance of PCP proteins that act on downstream targets that give rise to dysfunctional signaling.

Our serendipitous discovery of a transgenerational, ‘indirect’ effect of PCP alleles on *dNab2* phenotypes was unexpected and difficult to explain. We speculate that this effect may occur because PCP is involved in chromatin regulation or histone modifications in *Drosophila* germline that are

passed on to progeny, which eventually affects expression of genes that contribute to the observed *dNab2* overexpression and *dNab2* null phenotypes. There is one published study in *C. elegans* that provides some evidence of a role for PCP in controlling longevity via effects in the germline (Honnen et al., 2012). This work identifies the action *Vang-1* (the worm PCP component *Vang*) has in translocating the DAF-16/FoxO transcription factor into the nucleus where it activates gene expression for distinct processes in the germline, highlighting the nuclear role of a core candidate gene in the PCP pathway.

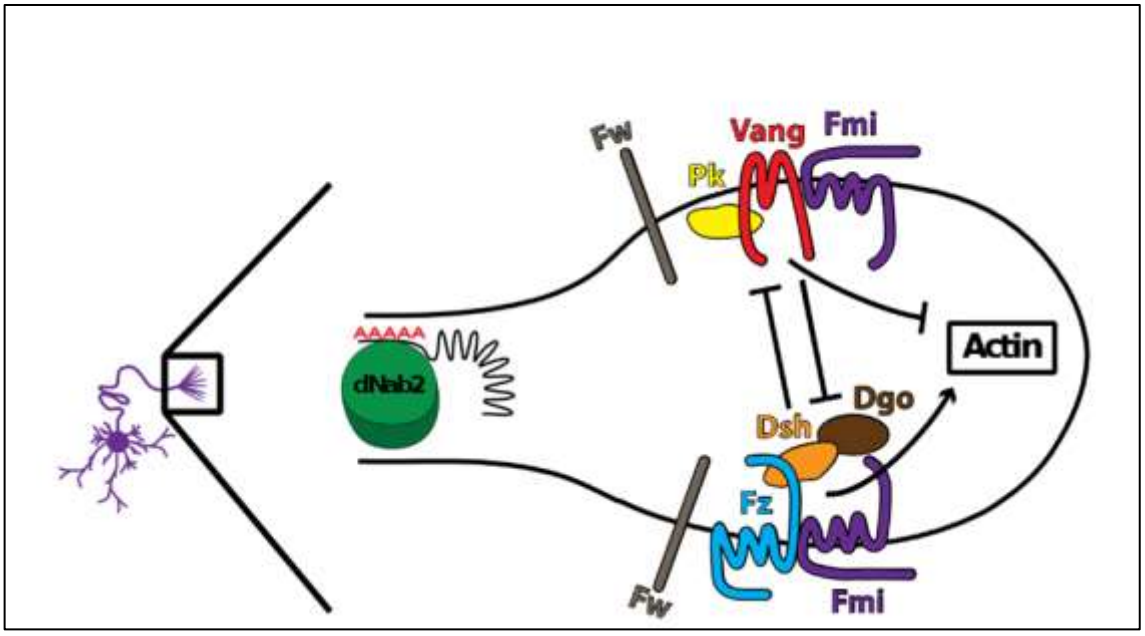
Altogether, the data presented in this thesis provide evidence that there are robust and consistent interactions between *dNab2* and planar cell polarity components, and that they may co-regulate mRNAs encoding tissue-specific developmental factors. However, the functional hierarchy of *dNab2* and PCP and the interacting mechanisms remain unclear. The results also suggest a potential nuclear role of PCP components in the *Drosophila* germline, raising the possibility that dysregulation of PCP components may be crucial to biological processes that contribute to defects in *Drosophila* lacking *dNab2* or in humans lacking *ZC3H14*.

Future Directions

Further studies in the Moberg and Corbett labs could examine how PCP signaling pathways could be altered in *dNab2* mutants to understand molecular mechanism(s) that underlie the observed effects in neurons and the germline presented in this thesis. We could perform a qPCR experiment to examine steady state levels of different PCP mRNAs to confirm our speculation of the role *dNab2* has on regulating PCP transcription levels. We could further perform immunoblotting to compare the relative protein levels of flies heterozygous to the candidate PCP mutant alleles and those without inheriting those alleles to understand whether the suppression effect is dosage dependent. Examining the persistence of the indirect modification effect through multiple generations would confirm the

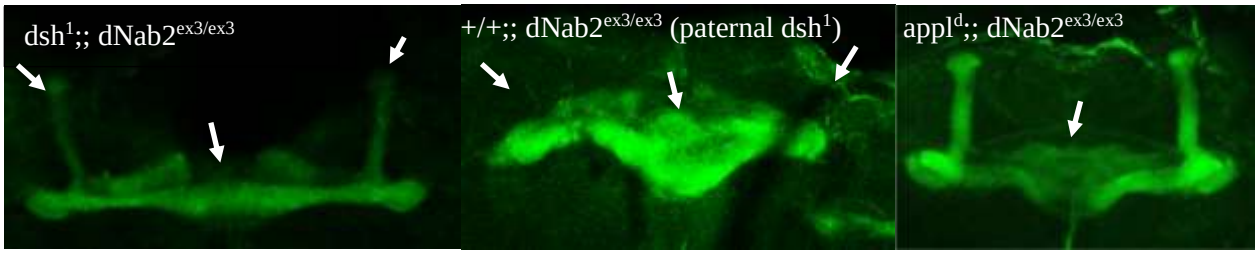
"transgenerational" nature of this mechanism. Although MB phenotypes in PCP heterozygosity in *dNab2* null flies have been preliminarily examined, further quantification of data could confirm dNab2-PCP interactions in neurons (**Appendix 1**). We could also perform an RNAi knockdown of *dsh* in the germline and look for PCP specific phenotypes, such as swirling of wing hairs, in the progeny. We could also examine whether the absence of *dNab2* would lead to mis-localization of PCP components within neurons or other affected cell types.

As PCP signaling is a ubiquitous mechanism of tissue patterning in developmental processes, defects in PCP are linked to human diseases. Several studies have shown that PCP gene mutations lead to failure of convergent extension, misoriented cell division, uncoordinated growth of cilia, and other defects in tissue development and function (Butler & Wallingford 2017). Neural tube closure defects and adolescent idiopathic scoliosis (AIS), diseases which proper formation of the spine is inhibited, are both caused by mutations in Vang1, a core PCP allele (Andersen et al., 2017). As more studies identify novel candidates in the PCP pathway that are linked to human diseases, further research could investigate the molecular interactions between PCP components and *ZC3H14* in the development of other human diseases.



Summary Model. Model of dNab2 interaction with PCP components in actin polymerization and assembly in neurons. dNab2 regulates the splicing, translation and/or stability by binding to the poly A tail of one or more mRNAs that encode PCP proteins. This regulation could lead to positive or negative modulation of actin polymerization, depending on the interacting PCP mRNA.

Diagram source: Edwin Corgiat



Appendix 1. PCP heterozygosity could potentially rescue dNab2$^{ex3/ex3}$ phenotype. Flies with *dsh^{1}* heterozygosity display the same MB phenotypes as dNab2$^{ex3/ex3}$ flies (thinned alpha-lobes and fused beta-lobes, far left image). Flies that contain *dsh^{1}* in the paternal germline shows MB defects as well (missing alpha-lobes and fused beta-lobes, center image). Flies with *appl^{d}* heterozygosity shows partial rescue of the MB phenotype (normal alpha-lobes, beta lobes still fused, far right image).

Proteins	Alleles Examined	Signaling Pathway	Biological Function	Molecular Function	Genetic Lesion
Dishevelled (Dsh)	*dsh*^{A3}, *dsh*^{6}, *dsh*^{1}	Core Frz-dependent PCP, Core Canonical Wnt	Positive regulation of axon guidance and extension Establishment of epithelial and ommatidial PCP	Frz-binding	*dsh*^{a3} : R413H amino acid change *dsh*^{6} :hypomorphic allele *dsh*^{1} : K417M amino acid change
Frizzled (Fz)	*fz*^{Jb}			Wnt-binding transmembrane G protein-coupled receptor	Nonsense mutation: W355term amino acid change
Starry Night (Fmi/Stan)	*fmi*^{frz3}	Core Frz-dependent PCP		Transmembrane G protein-coupled receptor	Hypomorphic allele
Van Gogh (Vang)	*vang*^{6}		Negative regulation of Wnt Signaling and actin polymerization Positive regulation of axon extension and guidance	Frz-binding transmembrane protein	2bp deletion at nucleotide 243→frameshift mutation at amino acid 81
Prickle (Pk)	*pk*$^{sple-13}$, *pk*$^{sple-14}$, *pk*^{30}			Direct interactions with transmembrane proteins (Vang and Fmi)	*pk*$^{sple-13}$: Lesion present between 40.2 and 66.2kb downstream of zero (zero being 973bp proximal to the *pk* transcription start site.) *pk*$^{sple-14}$: Lesion present between 41.3 and 48.6kb downstream of zero *pk*^{30} : Deletion of 1306bp
β amyloid protein precursor-like (Appl)	*appl*^{d}	Downstream PCP	MB development	Interact with Vang	Deletion of central portion of *Appl.* Exon containing translational start site and stop codon retained
Wnt oncogene analog 4 (Wnt4)	*wnt4*^{C1}		Motor, retinal neuron axon guidance	Frz binding	3 bp deletion of Glu 299

Puckered (Puc)	*puc*^{MI11060}		Actin cytoskeleton organization, establishment of epithelial PCP	Protein phosphatase (Jun-N-terminal kinase pathway)	Transposable element insertion at 3R
Target of Poxn (Tap)	*tap*^{MI10541}		Axon guidance, MB development	DNA binding, protein dimerization	Transposable element insertion at 3L
Pygopus (Pygo)	*pygo*^{s123}	Canonical Wnt	Embryonic pattern specification	Methylated histone binding transcription coactivator	Loss of function allele: Small deletion close to *pygo* translation initiation site→ truncation that retains only 51 amino acids.

Summary Chart. Table of pathways, molecular, and biological function of alleles examined.
Many of the candidate alleles examined for interactions with dNab2 are involved in normal MB development and axon guidance. *Information Source: Flybase*

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