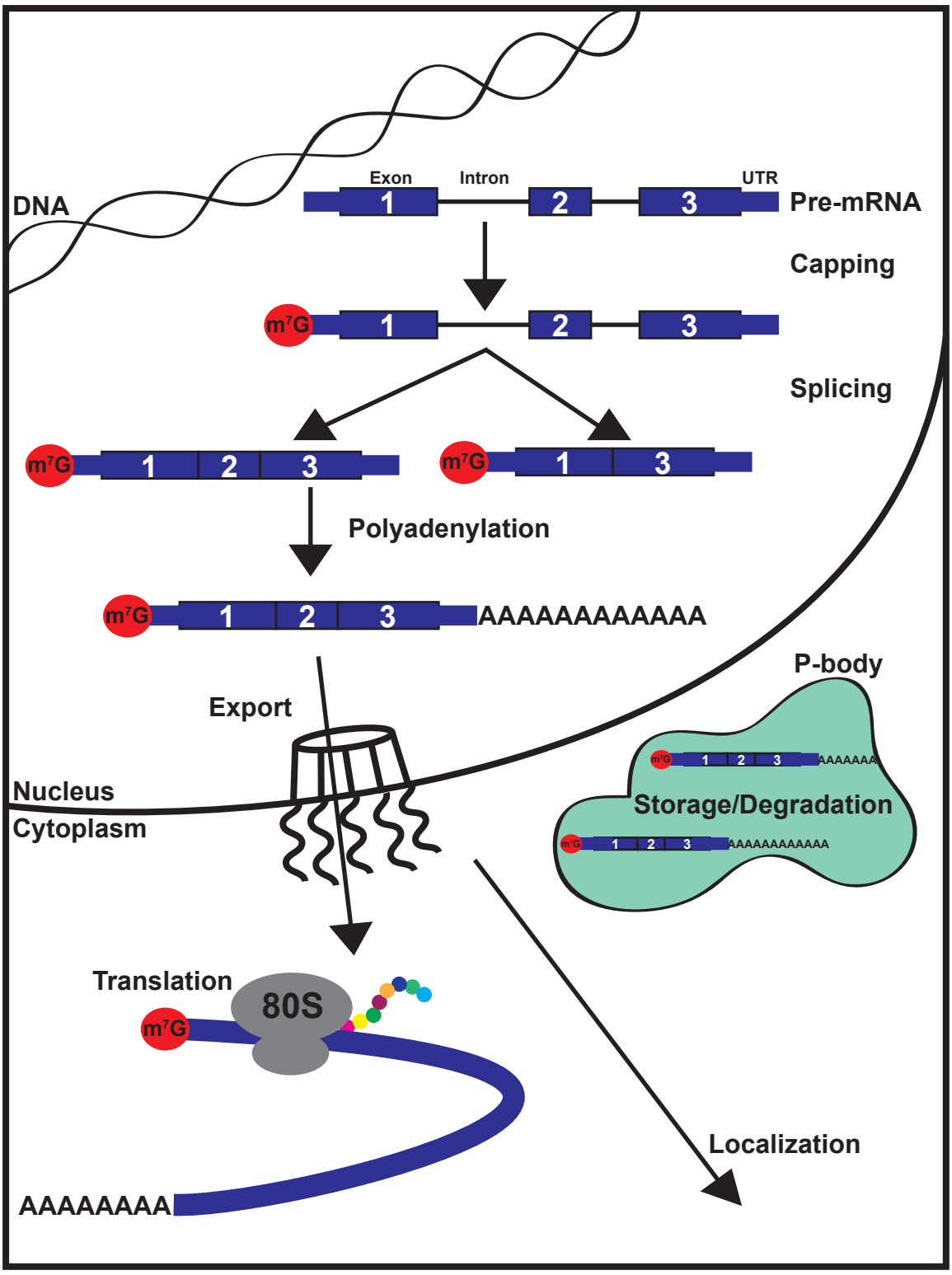

DNA
Exon
Intron
UTR
1
2
3
Pre-mRNA
Capping
m7G
Splicing
Polyadenylation
AAAAAAAAAAAA
P-body
Export
Nucleus
Cytoplasm
Storage/Degradation
AAAAAAA
AAAAAAAAAAAA
Translation
80S
AAAAAAAA
Localization

FIGURE 1.1. An overview of eukaryotic post-transcriptional processing. DNA is transcribed by RNA PolII to produce a premature mRNA transcript, or pre-mRNA. Pre-mRNAs undergo extensive processing in the nucleus to produce an export-competent mature mRNA transcript. These processing events include the addition of a 7-methylguanosine cap structure to the 5' end of the message, the removal, or splicing, out of intronic regions, as well as the addition of a 3' poly(A) tail. Fully processed mRNAs are exported through the nuclear pore complex where they can interact with translation machinery to produce proteins, be transported to specific cellular locations such as axonal synapses, or stored/degraded in distinct cytoplasmic foci such as p-bodies.

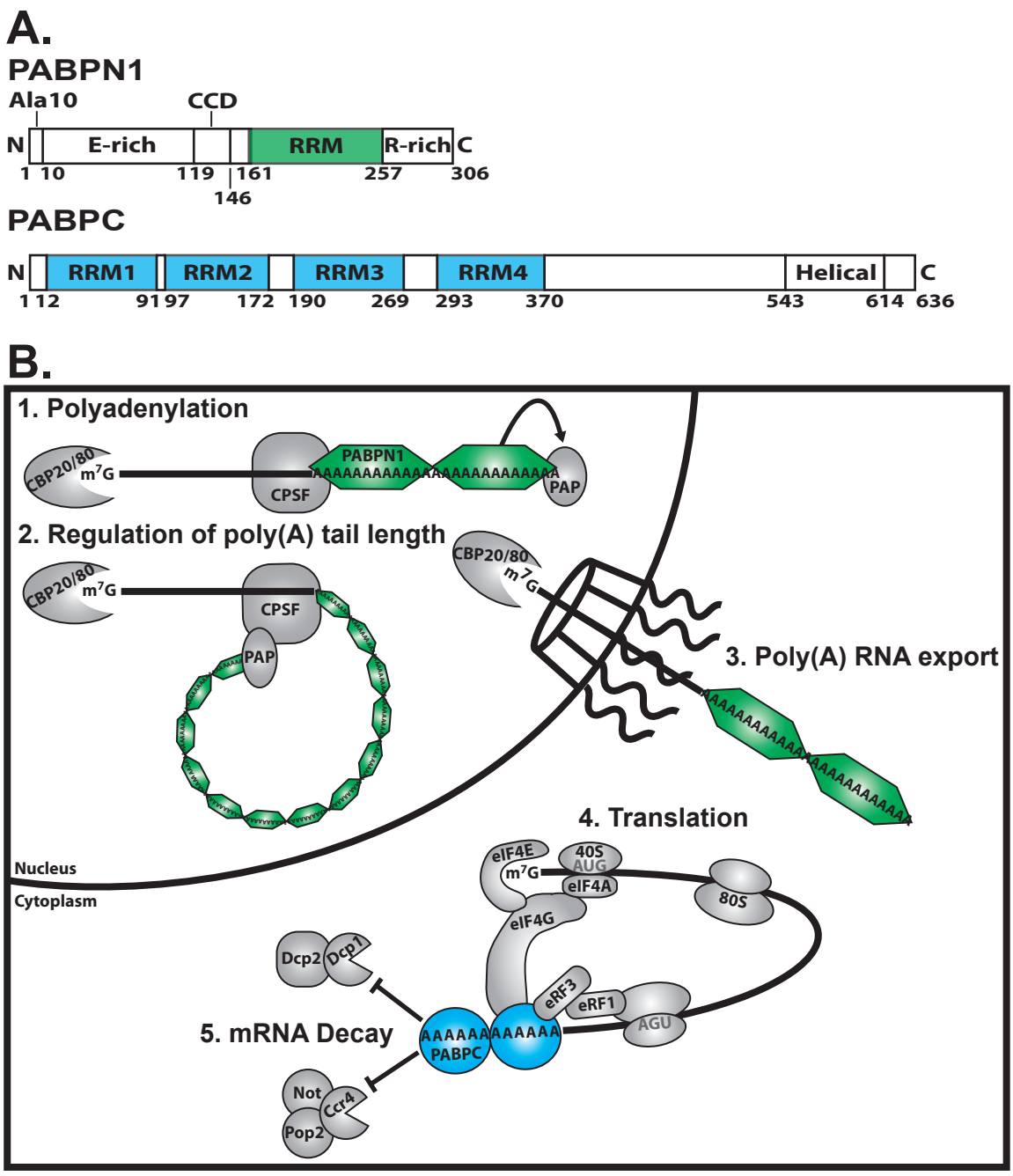


FIGURE 1.2. The canonical poly(A) binding proteins, PABPN1 and PABPC, play multiple roles in post-transcriptional processing. *A*) The domain structures for the canonical Pabs PABPN1 and PABPC are schematized here. PABPN1 is a nuclear Pab that contains a stretch of 10 alanines (Ala10) that are expanded in OPMD, a glutamic acid-rich domain (E-rich), a coiled-coil domain (CCD), an RNA recognition motif (RRM) that is responsible for high affinity polyadenosine RNA binding, as well as an arginine-rich (R-rich) domain at the C-terminus. PABPC is a cytoplasmic Pab that interacts with polyadenosine RNA via RRMs (RRM1-4) and contains a C-terminal Helical domain. *B*) The role of PABPN1 (green hexagon) in modulating 3'end processing of mRNA transcripts is well established and consists of the following molecular functions:

1. Polyadenylation: PABPN1 interacts with poly(A) polymerase to stimulate processive polyadenylation; 2. Regulation of poly(A) tail length: PABPN1 interacts with the cleavage and polyadenylation specificity factor (CPSF) to modulate and ensure proper poly(A) tail length; and 3. Poly(A) RNA export: Although whether the role is direct or indirect is unknown, defects in PABPN1 function can lead to nuclear accumulation of poly(A) RNA. This observation together with the fact that PABPN1 shuttles between the nucleus and the cytoplasm have led to the suggestion that PABPN1 function is required for efficient poly(A) RNA export from the nucleus. PABPC (blue circle) plays a well-defined role in modulating gene expression including: 4. Translation: PABPC binds to eukaryotic translation initiation factor 4G (eIF4G), which bridges interactions between the 5'- and 3'-ends of the mRNA and facilitates efficient translation initiation and 5. mRNA decay: PABPC binds to eukaryotic release factor 3 (eRF3), which facilitates ribosome recycling and thus inhibits mRNA decay by protecting the poly(A) tail from decapping enzymes (Dcp1 and Dcp2) as well as exonucleases such as the Ccr4-Pop2-Not complex. The following factors are also incorporated into the model shown: Cap binding proteins 20 and 80 (CBP20/80); 7 methylguanosine cap (m7G); eukaryotic translation initiation factor 4E (eIF4E); eukaryotic small ribosome (40S); eukaryotic translation initiation factor 4A (eIF4A); eukaryotic ribosome (80S); and eukaryotic release factor 1 (eRF1).

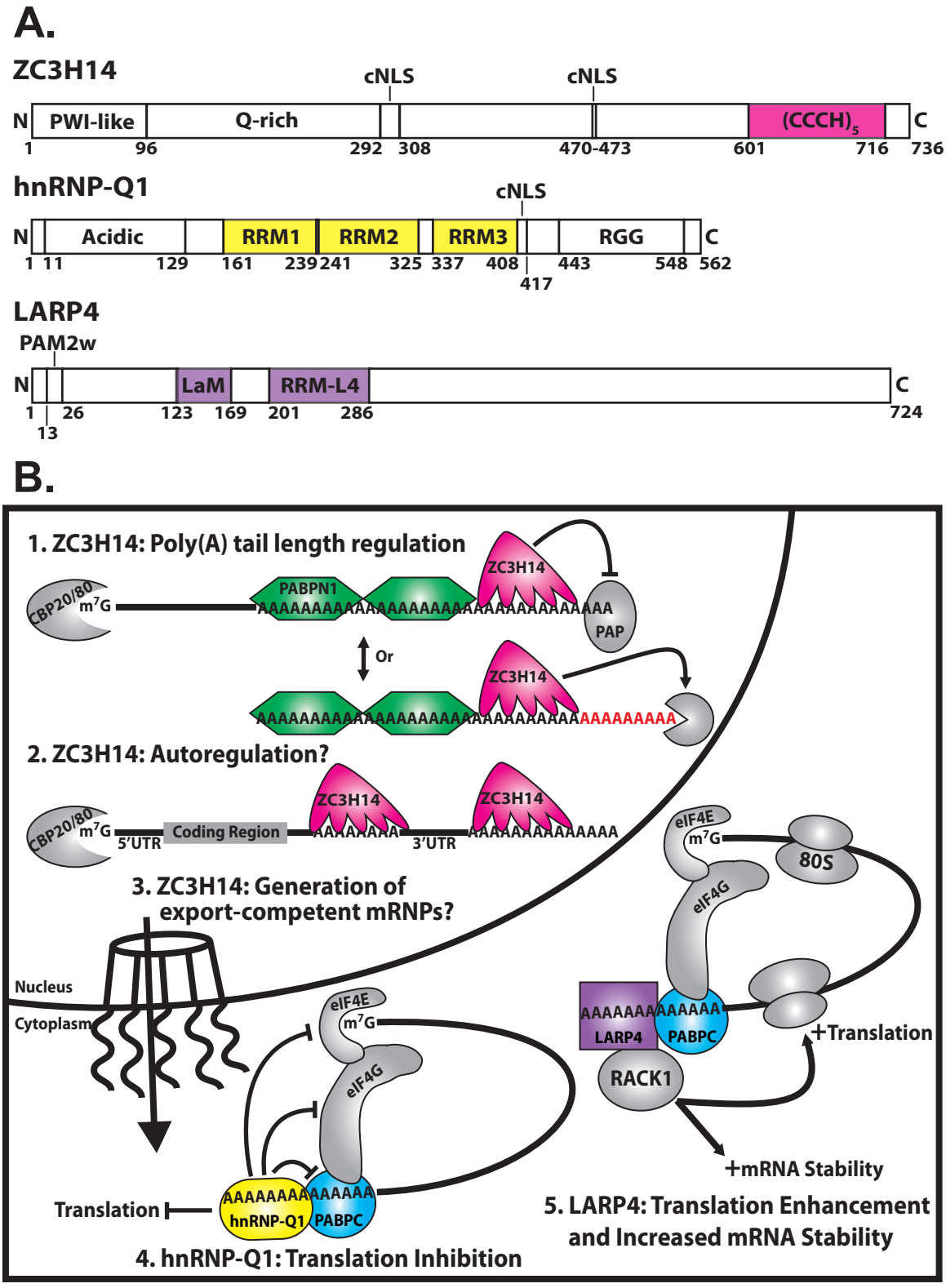
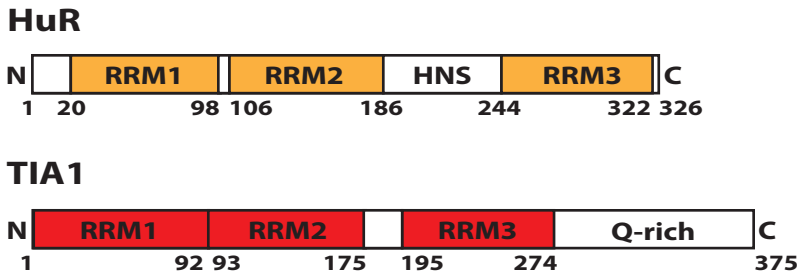

A.
ZC3H14
cNLS
cNLS
N
PWI-like
Q-rich
(CCCH)5
C
1
96
292
308
470-473
601
716
736
hnRNP-Q1
cNLS
N
Acidic
RRM1
RRM2
RRM3
RGG
C
1
11
129
161
239
241
325
337
408
417
443
548
562
LARP4
PAM2w
N
LaM
RRM-L4
C
1
13
26
123
169
201
286
724
B.
1. ZC3H14: Poly(A) tail length regulation
CBP20/80
m7G
PABPN1
ZC3H14
PAP
Or
ZC3H14
2. ZC3H14: Autoregulation?
CBP20/80
m7G
5'UTR
Coding Region
ZC3H14
ZC3H14
3'UTR
3. ZC3H14: Generation of
export-competent mRNPs?
Nucleus
Cytoplasm
eIF4E
m7G
eIF4G
AAAAAAA
LARP4
PABPC
RACK1
80S
+Translation
+mRNA Stability
Translation
AAAAAAA
hnRNP-Q1
PABPC
4. hnRNP-Q1: Translation Inhibition
5. LARP4: Translation Enhancement
and Increased mRNA Stability

FIGURE 1.3. Novel Pab family members. *A*) The domain structures for three novel Pab family members (ZC3H14, hnRNP-Q1, and LARP4) are schematized here. ZC3H14 is a novel nuclear Pab that interacts with polyadenosine RNA via tandem CysCysCysHis (CCCH) zinc fingers and also contains an N-terminal Proline Tryptophan Isoleucine-like (PWI-like) fold that mediates interactions with the nuclear pore, a glutamine-rich (Q-rich) domain of unknown function, and two putative classical nuclear localization signals (cNLS). hnRNP-Q1 is a novel cytoplasmic Pab that is presumed to bind polyadenosine RNA via RRMs (RRM1-3) and contains an Acidic N-terminal domain as well as an Arginine Glycine Glycine (RGG) domain, both of which mediate protein-protein interactions. A putative weak cNLS is also present in hnRNP-Q1. LARP4 is a novel cytoplasmic Pab that interacts with polyadenosine RNA via a La Motif (LaM) in conjunction with an RRM-like 4 domain (RRM-L4). LARP4 interacts with PABPC via a poly(A) binding protein interacting motif (PAM2) domain that contains an atypical tryptophan in the consensus sequence (PAM2w). *B*) The functions proposed for the new members of the Pab family described here (ZC3H14, hnRNP-Q1, and LARP4) are illustrated. The novel nuclear zinc finger Pab, ZC3H14 (pink five-fingered shape) plays a role in 1. poly(A) tail length regulation: ZC3H14 could limit poly(A) tail length either by inhibiting Poly(A) Polymerase (PAP) or by recruiting a 3'-5' exonuclease (grey Pac-man); 2. Autoregulation: Like its S. cerevisiae counterpart, Nab2, ZC3H14 may bind to and autoregulate its own mRNA transcript via an A15 stretch present in the 3'UTR; and 3. Generation of export-competent mRNPs: ZC3H14 could play a direct role in the generation of properly packaged mRNPs that are poised for export but most data to support this function comes from studies of S. cerevisiae Nab2. Alternatively, proper polyadenylation could be required to assemble export-competent mRNPs and the role for Nab2/ZC3H14 could be indirect. The novel cytoplasmic Pab, hnRNP-Q1 (yellow ellipse), plays a role in 4. Translation inhibition: hnRNP-Q1 competes with PABPC for binding to poly(A) tails and consequently preventing the formation of the translation initiation complex. The other novel cytoplasmic Pab, LARP4 (purple rectangle), is implicated in 5. Translation enhancement and increased mRNA stability: LARP4 interacts with PABPC as well as the ribosome-associated protein, RACK1, to positively modulate mRNA translation and decay. The following factors are also incorporated into the model shown: Cap binding proteins 20 and 80 (CBP20/80); 7 methylguanosine cap (m7G); eukaryotic translation initiation factor 4E (eIF4E); eukaryotic translation initiation factor 4G (eIF4G); and eukaryotic ribosome (80S).

A.


HuR
N
RRM1
RRM2
HNS
RRM3
C
1
20
98
106
186
244
322
326
TIA1
N
RRM1
RRM2
RRM3
Q-rich
C
1
92
93
175
195
274
375

B.

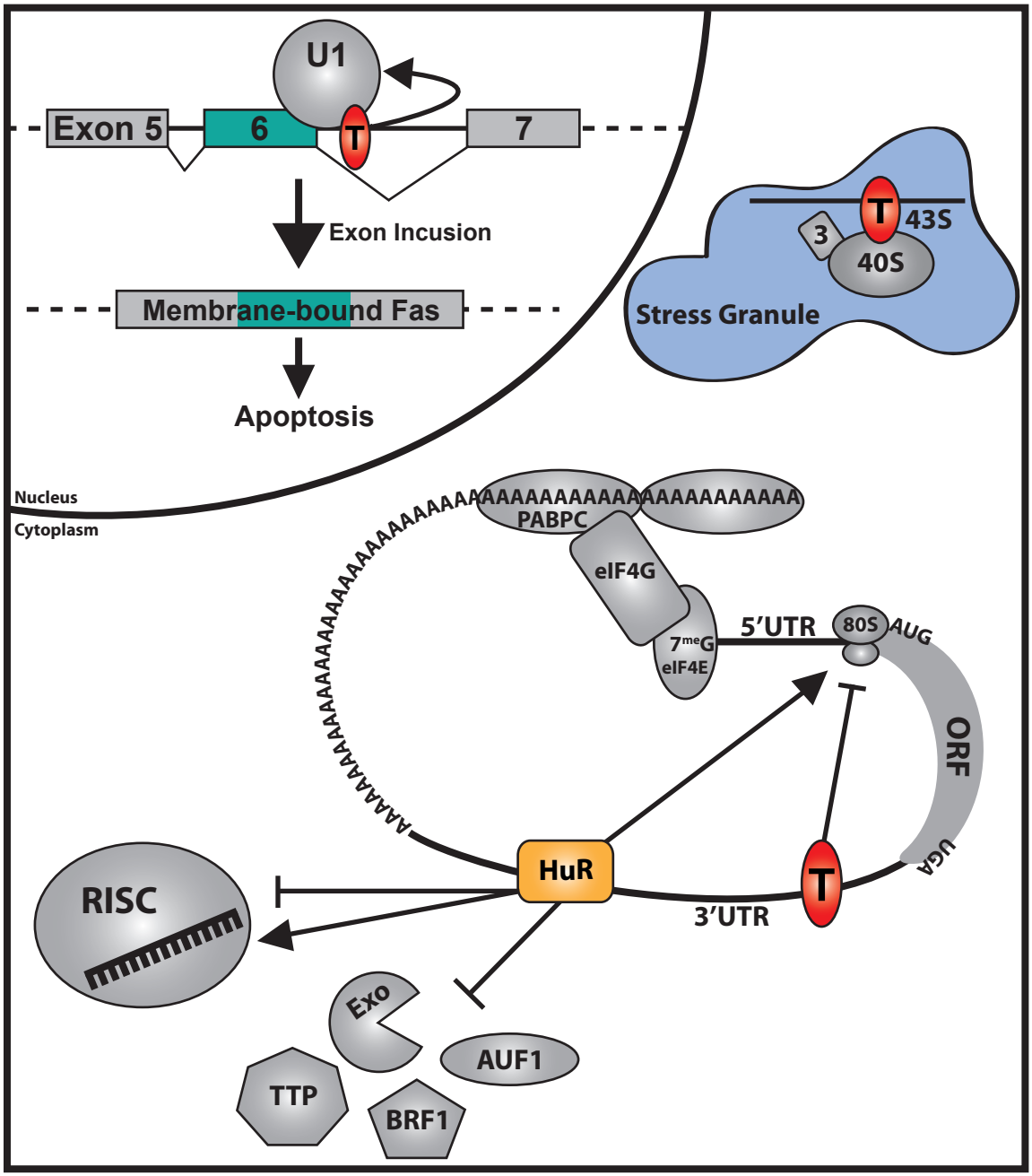

U1
Exon 5
6
7
T
Exon Incusion
Membrane-bound Fas
Apoptosis
Nucleus
Cytoplasm
T
43S
3
40S
Stress Granule
PABPC
eIF4G
7meG
eIF4E
5'UTR
80S
AUG
ORF
UGA
HuR
3'UTR
RISC
Exo
AUF1
TTP
BRF1

FIGURE 1.4. HuR and TIA1, two U-rich RNA binding proteins, play multifunctional roles in post-transcriptional processing. *A*) The domain structures for HuR and TIA1 are schematized here. HuR is a U-rich element RNA binding protein that binds primarily to the 3'UTRs of target transcripts via two N-terminal RNA Recognition Motifs (RRMs). A third C-terminal RRM has affinity for polyadenosine RNA and is thought to interact with the poly(A) tail. HuR localizes to the nucleus at steady-state but has been shown to shuttle between the nucleus and cytoplasm in response to various stimuli. The shuttling of HuR is mediated by the HuR Nucleocytoplasmic Shuttling sequence (HNS). TIA1 is another RNA binding protein with affinity for U-rich sequences. TIA1 contains three tandem RRMs followed by a prion-related Glutamine-rich (Q-rich) domain. Extensive binding studies have established that RRM 2 is responsible for binding to U-rich RNA while RRM3 enhances this binding event. RRM1 and the Q-rich domain mediate interactions with the U1 snRNP, which influences splice site selection. TIA1 also shuttles between the nucleus and cytoplasm, but displays a steady-state nuclear localization. The nucleocytoplasmic shuttling of TIA1 is mediated by sequence elements in RRMs 2 and 3. *B*) The well-studied functions of HuR and TIA1 are schematized here. HuR and TIA1 are represented as an orange rectangle and red oval, respectively. All other proteins are in grey. TIA1 has a well-studied role in splicing. In the nucleus, TIA1 interacts with U-rich intronic sequences located downstream of 5' weak splice sites (shown here binding downstream of exon 6 of the *Fas* pre-mRNA). Binding of TIA1 to these intronic sequences results in recruitment of the U1 snRNP (U1), which is one of the initial factors present at 5' splice sites and assists in the commitment to pre-mRNA splicing at that location. Therefore, TIA1 promotes the inclusion of otherwise "cryptic" exons. The *Fas* pre-mRNA is alternatively spliced to form two divergent protein products. The exon-included variant (shown here) encodes a membrane-bound Fas protein that transduces apoptotic signaling. TIA1 also has a well-studied role in translational repression in the cytoplasm. TIA1 interacts with U-rich elements in the 3'UTR of target mRNAs and promotes the formation of a noncanonical preinitiation complex (composed of the 40S ribosomal subunit (40S), eIF3 (3) and other components, resulting an a 43S complex), which is a stress granule core component, thus resulting in stress granule recruitment and translational repression. HuR also binds to U-rich elements primarily within the 3'UTRs of target transcripts and in doing so, increases mRNA stability. The observed increase in stability is thought to be the result of HuR blocking the access of other destabilizing U-rich binding proteins, such as TTP, AUF1 and BRF1, as well as exoribonucleases (Exo). HuR has also been shown to positively regulate the translation of target mRNAs via interactions with the 3'UTR. Finally, recent work has described a complex relationship between HuR and the miRNA machinery (RISC). These independent studies have observed both coordinate and competitive relationsips between HuR and miRNAs, suggesting that the role of HuR in interfacing with miRNAs is context- and transcript-dependent. The following factors are also incorporated into the model shown: poly(A) binding protein C1 (PABPC); open reading frame (ORF); 7 methylguanosine cap (m7G); eukaryotic translation initiation factor 4E (eIF4E); eukaryotic translation initiation factor 4G (eIF4G); and eukaryotic ribosome