

REVIEW

Links between mRNA splicing, mRNA quality control, and intellectual disability

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In recent years, the impairment of RNA binding proteins that play key roles in the post-transcriptional regulation of gene expression has been linked to numerous neurological diseases. These RNA binding proteins perform critical mRNA processing steps in the nucleus, including splicing, polyadenylation, and export. In many cases, these RNA binding proteins are ubiquitously expressed raising key questions about why only brain function is impaired. Recently, mutations in the *ZC3H14* gene, encoding an evolutionarily conserved, polyadenosine RNA binding protein, have been linked to a nonsyndromic form of autosomal recessive intellectual disability. Thus far, research on *ZC3H14* and its Nab2 orthologs in budding yeast and *Drosophila* reveals that *ZC3H14*/Nab2 is important for mRNA processing and neuronal patterning. Two recent studies now provide evidence that *ZC3H14*/Nab2 may function in the quality control of mRNA splicing and export and could help to explain the molecular defects that cause neuronal dysfunction and lead to an inherited form of intellectual disability. These studies on *ZC3H14*/Nab2 reveal new clues to the puzzle of why loss of the ubiquitously expressed *ZC3H14* protein specifically affects neurons.

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Intellectual disability is a neurodevelopmental disorder characterized by reduced intellectual functioning (I.Q. ≤ 70) and deficits in adaptive behavior diagnosed by the age of 18 years^[1-3]. Intellectual disability has been estimated to affect 1-3% of the population worldwide^[4, 5]. Amongst the growing list of genes linked to intellectual disability (> 700)^[2], many of these genes encode RNA binding proteins that play critical roles in post-transcriptional regulation of gene expression. Recently, mutations in the *ZC3H14* gene, encoding an evolutionarily conserved, zinc finger polyadenosine RNA binding protein, have been linked to a severe nonsyndromic form of autosomal recessive intellectual disability (I.Q. ~30-50)^[6]. Here, we highlight

two recent studies^[7, 8] on the role of the *ZC3H14* protein and its budding yeast ortholog, Nab2, in the quality control of mRNA splicing and export that could provide insights into the function of *ZC3H14* in the brain and how impairment of *ZC3H14* could give rise to intellectual disability.

The human *ZC3H14* (zinc finger CCCH domain-containing #14) protein, also known as MSUT2^[9], belongs to an evolutionarily conserved family of nuclear zinc finger polyadenosine RNA binding (Pab) proteins that include *S. cerevisiae* Nab2 and *Drosophila* dNab2^[6, 10, 11]. Splice variants of *ZC3H14* give rise to at least four human *ZC3H14* protein isoforms - Isoform 1-4; *ZC3H14* Isoforms

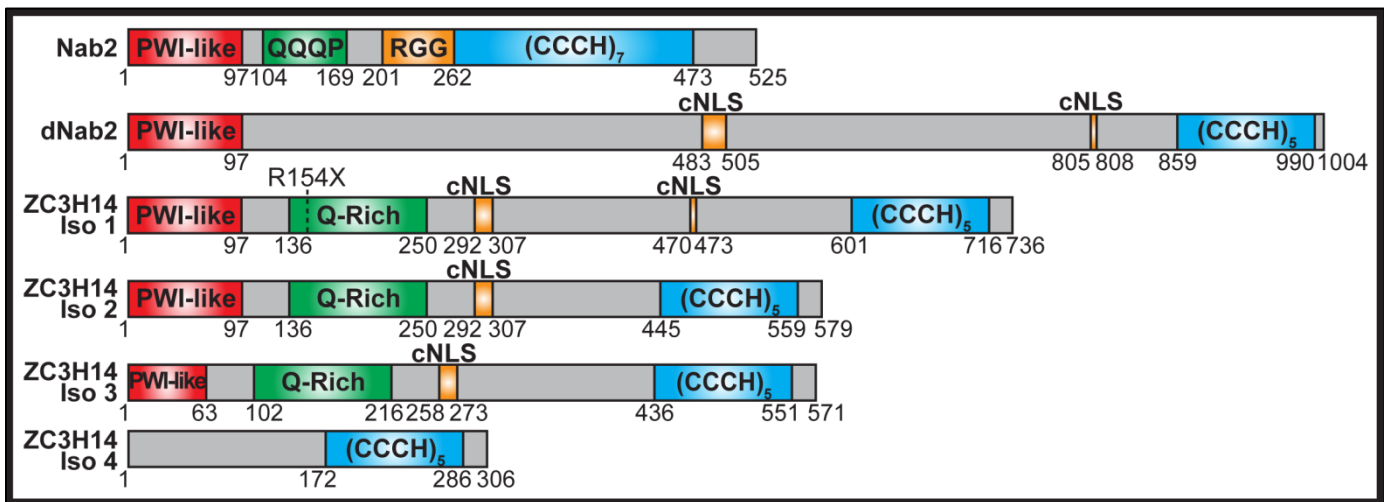


Figure 1. Domain structure of *S. cerevisiae* Nab2, *Drosophila melanogaster* dNab2, human ZC3H14 Isoform 1-4 polyadenosine RNA binding protein. The ZC3H14/Nab2 proteins contain the following domains: an N-terminal Pro-Trp-Ile (PWI)-like fold domain (red), important for protein-protein interaction and mRNA export in Nab2^[13, 14, 18, 19, 31], a Glu (Q)-rich domain (green), an RGG motif/predicted classical nuclear localization signal (cNLS) (orange), important for localization to the nucleus in Nab2^[13], and a C-terminal tandem Cys₃His (CCCH) zinc finger domain (blue), critical for specific binding to polyadenosine RNA^[10, 11, 20]. The Arg154Stop (R154X) premature termination codon mutation in ZC3H14 linked to intellectual disability that eliminates ZC3H14 Isoform 1-3^[6] is depicted above ZC3H14 Isoform 1. Amino acid positions of domains are shown below each protein.

1-3 are ubiquitously expressed in all tissues of the body while Isoform 4 is primarily expressed in the testes^[12]. The best-characterized ZC3H14 mutation linked to intellectual disability creates a premature termination codon - R154X - that causes loss of expression of ZC3H14 Isoform 1-3^[6]. Human ZC3H14 Isoform 1-3, fly dNab2, and budding yeast Nab2 each contain an N-terminal PWI-like domain, a nuclear targeting signal (cNLS or RGG domain) and a C-terminal tandem CCCH zinc finger domain (Figure 1)^[6, 10-14]. Studies on *S. cerevisiae* Nab2 have shown that Nab2 is an essential, nuclear protein that shuttles between the nucleus and cytoplasm and plays key roles in mRNA export, mRNA stability, and regulation of poly(A) tail length on bulk RNA^[11, 13, 15-17]. The Nab2 PWI-like domain serves as a protein-protein interaction domain that binds nuclear pore-associated proteins, such as Mlp1, and other factors and is important for nuclear mRNA export^[13, 14, 18, 19]. The Nab2 tandem zinc finger domain, containing seven CCCH zinc fingers, binds specifically to polyadenosine RNA with high affinity^[10, 20]. Notably, *nab2* N-terminal domain yeast mutants exhibit nuclear accumulation of poly(A) RNA and *nab2* zinc finger mutants show extended bulk poly(A) tails^[13, 15, 18, 20]. Like Nab2, the tandem zinc finger domains of ZC3H14 and dNab2, containing five zinc fingers, specifically bind to polyadenosine RNA^[6, 10]. Importantly, cells depleted for ZC3H14 or dNab2 show extended bulk poly(A) tails, indicating that ZC3H14 and dNab2 also regulate poly(A) tail length^[6, 21].

Studies on dNab2 using *dNab2* mutant fly models have provided insight into the critical function of dNab2 in

neurons. Mutant flies that lack dNab2 have reduced viability and locomotor activity, impaired short-term memory, and defects in the neuronal patterning in the learning and memory center (mushroom body) of the fly brain^[6, 22]. Critically, expression of dNab2 only in the neurons of *dNab2* zygotic mutant flies rescues the viability, locomotor activity and neuronal patterning in the flies, demonstrating that dNab2 is essential for proper neuronal function and also that expression of dNab2 only in neurons is sufficient to support proper neuronal function^[6, 22]. In addition, neuronal expression of human ZC3H14 Isoform 1 in *dNab2* mutant flies rescues function, indicating that ZC3H14 is a functional ortholog of dNab2^[21].

Work on ZC3H14 has shown that mouse ZC3H14 is enriched in murine hippocampal neurons that are critical for memory in the brain, supporting a role for ZC3H14 in neuronal function^[6]. Moreover, mouse ZC3H14 colocalizes with poly(A) RNA in nuclear speckles, which are known centers of pre-mRNA processing, in rodent hippocampal neurons^[6], suggesting ZC3H14 could function in neuronal RNA processing. Human ZC3H14 Isoform 1, but not Isoform 4, also localizes to nuclear speckles in mammalian cells^[12]. Combined, these data suggest that ZC3H14 could regulate specific RNA processing steps to coordinate neuronal function and that ZC3H14 loss could lead to neuronal dysfunction and intellectual disability via dysregulation of neuronal RNA processing. Further studies on the molecular functions of ZC3H14 and its orthologs are therefore warranted to elucidate the critical role(s) of ZC3H14 in proper neuronal function.

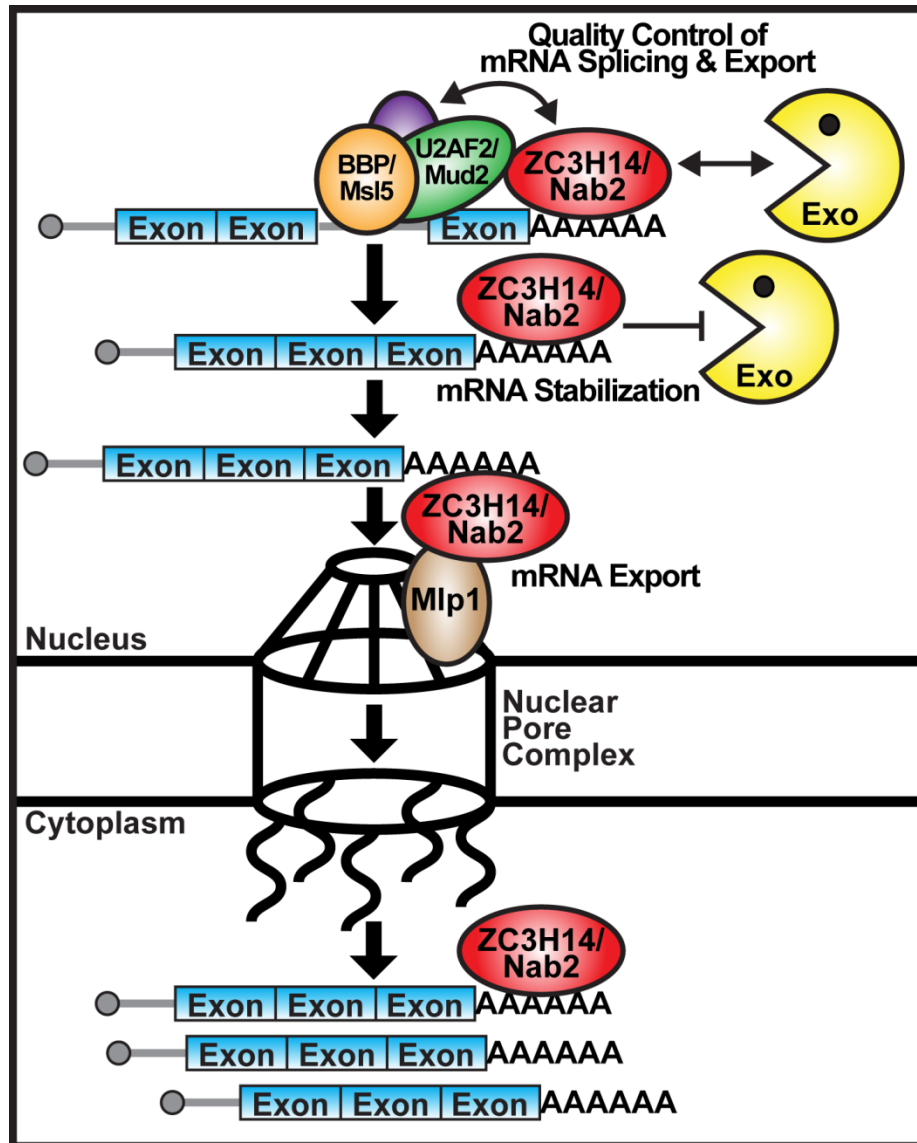


Figure 2. Model for ZC3H14/Nab2 function in quality control of mRNA splicing and export. ZC3H14/Nab2 binds to the poly(A) tail of a transcript and checks for interaction with early splicing factors, such as branchpoint recognition factors, U2AF2/Mud2 and BBP/Msl5, to detect improperly spliced and unspliced pre-mRNA. If ZC3H14/Nab2 binds to splicing factors on the pre-mRNA, ZC3H14/Nab2 could mark the transcript as unspliced, retain the transcript in the nucleus, and trigger recruitment of the RNA exosome (Exo) to the transcript for degradation. If ZC3H14/Nab2 does not bind to splicing factors, the mRNA transcript could be marked as spliced and ZC3H14/Nab2, together with other export factors, could stabilize and remodel the transcript and target the transcript to the nuclear pore complex via interaction with nuclear pore-associated proteins (Mlp1) for export to the cytoplasm.

To this end, recent studies by Soucek *et al.* on budding yeast Nab2 function^[8] and Wigington *et al.* on human ZC3H14 function^[7] suggest that ZC3H14/Nab2 plays a role in the quality control of mRNA splicing and export. Soucek *et al.* set out to determine whether Nab2 affects the splicing of mRNA transcripts in yeast cells and found that *nab2* zinc finger mutant cells exhibit increased levels of unspliced intron-containing pre-mRNAs, but do not show a strong effect on splicing *in vitro*^[8]. In addition, Soucek *et al.*

identified physical and genetic interactions between Nab2 and splicing factors in yeast cells^[8], most notably the Mud2 and Msl5 proteins - the budding yeast orthologs of human U2 snRNA auxiliary factor 2 (U2AF2)/U2AF⁶⁵ and branchpoint binding protein (BBP)/splicing factor 1 (SF1) - that recognize the branchpoint sequence in introns^[23-26]. Importantly, Soucek *et al.* also identified physical interactions between mouse ZC3H14 and splicing factors in mouse brain, including U2AF2, supporting a conserved link

between ZC3H14 and splicing in neuronal cells^[8]. Finally, Soucek *et al.* observed that the function and pre-mRNA splicing defects of *nab2* mutant cells are rescued by inactivation of the Rrp6 riboexonuclease subunit of the nuclear RNA exosome^[8] - a conserved ribonuclease complex that is critical for RNA processing/degradation of non-coding RNA and pre-mRNA^[27-30].

Combined, these results from Soucek *et al.* suggest a model (Figure 2) for ZC3H14/Nab2 quality control of mRNA splicing and export where ZC3H14/Nab2 binds the poly(A) tail of transcripts and detects improperly spliced and unspliced pre-mRNA via interaction with early splicing factors, such as branchpoint recognition factors, U2AF2/Mud2 and BBP/Msl5. ZC3H14/Nab2 binding to the splicing factors on the pre-mRNA could mark the transcript as unspliced, cause the retention of the transcript, and trigger the recruitment of the RNA exosome to the transcript for degradation. If ZC3H14/Nab2 does not bind to the splicing factors, the transcript could be marked as spliced and ZC3H14/Nab2 and other export factors could stabilize and remodel the transcript and target the transcript to the nuclear pore complex for export to the cytoplasm. Reduced RNA binding by ZC3H14/Nab2 due to disruption of Nab2 zinc fingers or depletion of ZC3H14 would be predicted to impair detection of unspliced pre-mRNA in the nucleus, leading to pre-mRNA escape from degradation, pre-mRNA accumulation and disruption of cellular function.

Complementary to this work, Wigington *et al.* sought to identify target mRNA transcripts regulated by human ZC3H14 in human cells and discovered that ZC3H14 affects the steady-state level of only a small number of transcripts^[7]. For further analysis, Wigington *et al.* selected the *ATP5G1* transcript, encoding a critical subunit of the mitochondrial ATPase synthase F₀ subunit, and found that depletion of ZC3H14 reduces the stability of *ATP5G1* mRNA^[7]. Importantly, Wigington *et al.* showed that ZC3H14 binds to *ATP5G1* mRNA in the nucleus and preferentially binds to unspliced *ATP5G1* pre-mRNA^[7]. In addition, Wigington *et al.* found that depletion of ZC3H14 increases the level of unspliced *ATP5G1* pre-mRNA in the cytoplasm^[7]. Together, these results suggest that ZC3H14 can detect the difference between unspliced pre-mRNA and mature mRNA, facilitate retention of pre-mRNA in the nucleus and protect mature mRNA from degradation. Notably, ZC3H14 interactions with splicing factors, like U2AF2, would allow ZC3H14 to recognize the unspliced pre-mRNA. The data on ZC3H14 from Wigington *et al.* are consistent with the Nab2 data from Soucek *et al.* and previous work on Nab2 and support a role for ZC3H14 in quality control of mRNA splicing and export (Figure 2).

These studies begin to suggest that ZC3H14/Nab2 plays a critical role in ensuring that pre-mRNAs are properly processed in the nucleus before the transcripts are exported to the cytoplasm. This model fits well with previous data showing that Nab2 is important for poly(A) RNA export in yeast cells and suggesting that Nab2 facilitates concentration of properly processed mRNA at the nuclear pore for export^[16, 18, 19, 31]. ZC3H14/Nab2 interaction with splicing factors and pre-mRNA and ZC3H14 localization to pre-mRNA processing nuclear speckles^[6-8, 12] supports a splicing-associated function for ZC3H14/Nab2 that could involve quality control. Genetic interactions between Nab2 and the Rrp6 riboexonuclease of the RNA exosome^[8] and evidence that Nab2 can physically interact with Rrp6^[32, 33] also suggest that Nab2 could affect pre-mRNA degradation via recruitment or regulation of Rrp6. Alternatively, Nab2 interactions with splicing factors could cause a conformational switch in Nab2 that alters the accessibility of the 3'-end of the pre-mRNA to Rrp6 degradation. The kinetics of ZC3H14/Nab2 association with splicing factors could also contribute to the signal of whether to protect or destroy the transcript.

To further understand the molecular functions of ZC3H14/Nab2 in pre-mRNA processing and export, a number of challenges remain. The splicing proteins and other factors that directly bind to ZC3H14/Nab2 need to be defined to gain insight into how ZC3H14/Nab2 recognizes and discerns the difference between unprocessed pre-mRNA and mature mRNA. In addition, the RNA elements/structures in the mRNA targets bound by ZC3H14/Nab2 need to be examined to determine if they contribute to ZC3H14/Nab2-mediated recognition/regulation of the mRNA. Notably, RNA secondary structures, such as stem-loops, have been identified in several yeast introns that impact splicing efficiency^[34-37]. The mechanism by which loss or impairment of ZC3H14/Nab2 leads to extended poly(A) tails on bulk RNA also requires deeper analysis. Whether ZC3H14/Nab2 directly interacts with the polyadenylation machinery or associates with splicing factors or other proteins to regulate polyadenylation needs to be examined. On this note, splicing and polyadenylation steps in pre-mRNA processing are known to be tightly coupled and splicing factors, such as U2AF2/U2AF⁶⁵, which interact with ZC3H14, have been shown to stimulate cleavage and polyadenylation^[38-40].

As neurons appear uniquely sensitive to changes in RNA binding proteins and post-transcriptional processing of RNA^[41], dysregulation of pre-mRNA levels and/or pre-mRNA escape to the cytoplasm due to loss of ZC3H14 could specifically disturb the function of neuronal cells, leading to intellectual disability in patients. Alternatively, the changes

to transcripts that occur when ZC3H14 is lost, such as lengthening of poly(A) tails, could impact the fate or function of the transcript in the cytoplasm. Unraveling the functions of ZC3H14/Nab2 in the future will outline new details on the mRNA processing landscape and shed light on the causes of neuronal dysfunction in intellectual disability.

Conflicting interests

The authors have declared that no conflict of interests exist.

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