Chromosoma (2014) 123:515–527
DOI 10.1007/s00412-014-0470-0

REVIEW

Emerging Roles for hnRNPs in post-transcriptional regulation: what can we learn from flies?

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Received: 8 January 2014 / Revised: 21 May 2014 / Accepted: 23 May 2014 / Published online: 10 June 2014

Abstract Heterogeneous nuclear ribonucleoproteins (hnRNPs) are a highly conserved family of RNA-binding proteins able to associate with nascent RNAs in order to support their localization, maturation and translation. Research over this last decade has remarked the importance of gene regulatory processes at post-transcriptional level, highlighting the emerging roles of hnRNPs in several essential biological events. Indeed, hnRNPs are key factors in regulating gene expression, thus, having a number of roles in many biological pathways. Moreover, failure of the activities catalysed by hnRNPs affects various biological processes and may underlie several human diseases including cancer, diabetes and neurodegenerative syndromes. In this review, we summarize some of hnRNPs' roles in the model organism *Drosophila melanogaster*, particularly focusing on their participation in all aspects of post-transcriptional regulation as well as their conserved role and involvement in the aetiology of human pathologies.

Keywords Drosophila · hnRNPs · Post-transcriptional gene regulation · Omega speckles · Nucleoplasmic organization · Proteinopathies

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Introduction

Heterogeneous nuclear ribonucleoproteins (hnRNPs) belong to a large RNA-binding protein family, which share common structural domains. Historically, hnRNPs were classified as proteins involved in processing heterogeneous nuclear RNAs (hnRNAs) into mature messenger RNAs (mRNAs), to carry biological functions that include mRNA export, localization, translation and stability (Dreyfuss et al. 1993; Chaudhury et al. 2010; Dreyfuss et al. 2002; Busch and Hertel 2012; Han et al. 2010). However, works conducted in several research groups showed that hnRNPs play different key roles not only in RNA processing but also in cell signalling, telomere biogenesis, DNA repair as well as in the regulation of gene expression at both transcriptional and translational levels (Krecic and Swanson 1999; He and Smith 2009; Singh 2001). Generally, the most common structural motif shared between all hnRNPs is the RNA-binding domain (RBD) or the RNA recognition motif (RRM), located at the N-terminus. Nevertheless, RNA-protein recognition is not only mediated by RRM domain, but also by other structures as double-stranded RNA-binding motif (dsRBM), Pumilio homology domain (PUF), RGG repeats, Zinc-binding domains and KH domains (Auweter et al. 2006; Chang and Ramos 2005). Thus, the definition of some hnRNPs present in the literature is not strictly unambiguous, and it is possible to speculate that several hnRNPs may not yet be classified as such. Indeed, given their structural diversity and the fact that the hnRNPs seem to participate in several cellular processes rather than to be seldom "fixed" in a unique role, probably the number of proteins belonging to hnRNPs family will increase.

The model system *Drosophila melanogaster* encodes at least 14 major hnRNPs, which have structural and functional homologs in mammals (Table 1) (Buchenau et al. 1997; Haynes et al. 1991; Hovemann et al. 2000; Matunis et al. 1992a; Matunis et al. 1992b; Reim et al. 1999; Blanchette

Table 1 List of *Drosophila* hnRNPs function and conservation

Gene	Name	Synonyms	Process	*H. sapiens* homolog	References
caz	cabeza	SARFH, P19	Neuromuscular development	FUS (or HNRNPP2), EWSR1, T AF15	[Zinszner et al. 1997; Wang et al. 2011]
glo	glorund	p67	Cell polarity	HNRNPH2, GRSF1, HNRNPH3, RBM12, HNRNPH1, HNRNPF	[Kalifa et al. 2006]
heph	hephaestus	PTB, CG2094, l(3)03429, dmPTB, ema	Cell polarity	RBM20, PTBP1, PTBP3, PTBP2	[Davis et al. 2002]
Hrb57A	Heterogeneous nuclear ribonucleoprotein at 57A	bancal, Q18	Nucleoplasmic compartment organization	HNRNPK	[Hovemann et al. 2000]
Hrb87F	Heterogeneous nuclear ribonucleoprotein at 87 F	hrp36, P11	Nucleoplasmic compartment organization, interaction with chromatin component, regulated by covalent modifications and small metabolites, neuromuscular development	HNRNPA0, HNRNPA3; HNRNPA1; HNRNPA2B1; HNRNPA1L2	[Haynes et al. 1991]
Hrb98DE	Heterogeneous nuclear ribonucleoprotein at 98DE	Hrp38	Regulated by covalent modifications and small matabolites, neuromuscular development	HNRNPA0	[Kim et al 2013]
Hrb27C	Heterogeneous nuclear ribonucleoprotein at 27C	Hrp48, p50, RRM7, Hrb27-C	Nucleoplasmic compartment organization, cell polarity	DAZAP1	[Goodrich et al. 2004]
nonA	no on or off transient A	diss, nonA, Bj6	Nucleoplasmic compartment organization, neuromuscular development	SFPQ, PSPC1, NONO	[Stanewsky et al. 1996; Reim et al. 1999]
Pep	Protein on ecdysone puffs	PEP/X4	Nucleoplasmic compartment organization, interaction with chromatin component	CIZ1	[Reim et al. 1999]
rump	rumpelstiltskin	hrp59, hnRNP M	Nucleoplasmic compartment organization, interaction with chromatin component	MYEF2, HNRNPM	[Sinsimer et al. 2011]
sm	smooth	smo	Neuromuscular development	HNRNPL, HNRPLL	[Mackay 1985]
Syp	Syncrip	Syp	Cell polarity, neuromuscular development	RBM47, RBM46, DND1, SYNCRIP, A1C, FHNRNPR	[McDermott et al. 2012]
sqd	squid	hrp40, l(3)j4B4, Squid, RRM3	Nucleoplasmic compartment organization, cell polarity, regulated by covalent modifications and small metabolites, neuromuscular development	HNRPDL, HNRNPAB,	[Krecic and Swanson 1999]
TBPH	TDP-43	TAR DNA-binding protein, TDP-43	Neuromuscular development	TDP-43 or TARDBP	[Buratti and Baralle 2009; Strong 2010; Buratti and Baralle 2010]

et al. 2009). On top of being an excellent genetic system, flies have the unique advantage of producing actively transcribing polytene chromosomes, which constitute a powerful cytogenetic tool for the analysis of hnRNPs in situ (Swaminathan et al. 2012; Gilbert 2008). Moreover, research conducted over the last decade has highlighted emerging roles for hnRNPs in gene regulatory processes at a post-transcriptional level also in mammals (Norris and Calarco 2012; Han et al. 2010; He and Smith 2009). Remarkably, a growing body of evidence is indicating that mis-regulation of hnRNPs may underlie a variety of human diseases. Indeed, mis-regulation of hnRNP levels and of the post-transcriptional modifications catalysed by hnRNPs have been reported in cancer (Carpenter et al. 2006; Gao et al. 2013; Dery et al. 2011; Patry et al. 2003), diabetes, hypertension (Lo et al. 2012) and also in neurodegenerative diseases (Hanson et al. 2012; Lee et al. 2012).

Here, we provide a comprehensive overview of the hnRNPs composition, nuclear localization, organization and function in the model system *D. melanogaster*, emphasizing the use of fruit fly as an elective system for studying hnRNPs' biology as well as diseases connected with their mis-function.

hnRNPs, nucleoplasmic compartment and chromatin

hnRNP's complexes are unusually large, and typically contain numerous proteins, with various relative abundance (Markovtsov et al. 2000). Many hnRNPs shuttle between nucleus and cytoplasm, performing fundamental roles in RNA localization in both compartments (Krecic and Swanson 1999; Dreyfuss et al. 2002; He and Smith 2009; Han et al. 2010; Singh 2001). In *D. melanogaster*, some nucleus-localized hnRNPs are associated with the non-coding hsrω-n RNA in the nucleoplasmic omega speckles compartment (Fig. 1a) (Jolly and Lakhotia 2006; Prasanth et al. 2000; Onorati et al. 2011; Lakhotia et al. 1999; Lakhotia et al. 2001; Ji and Tulin 2009). Omega speckles are specialized nuclear compartments, distinct from other nuclear speckles, localizing in the nucleoplasm close to chromatin edges. Omega speckles are believed to function as storage sites for the unengaged hnRNPs and other related RNA-processing proteins. Indeed, since hnRNPs participate in various cellular reactions, their activity is finely controlled, and it is believed that omega speckles function as a hub for hnRNPs storage and bioavailability control in normal as well as stressed cell (Prasanth et al. 2000; Jolly and Lakhotia 2006; Onorati et al. 2011; Lakhotia et al. 1999; Lakhotia 2011).

The hsrω-n non-coding (ncRNA) is essential for the assembly and organization of omega speckles, acting as an organizer molecule that regulates the intranuclear trafficking and availability of hnRNPs. In fact, loss of *hsrω* function causes the disorganization of omega speckles and a diffused distribution of hnRNPs in the nucleoplasm (Prasanth et al. 2000; Lakhotia et al. 2012; Mallik and Lakhotia 2011). Intriguingly, the heat-induced non-coding transcripts named *Sat III* identified in human cells display striking functional similarities with the fly hsrω transcripts (Jolly and Lakhotia 2006). Like the hsrω transcripts, several splicing factors and hnRNPs such as the human hnRNP M (the *Drosophila* homolog of Rumpelstiltskin) associate with the Sat III transcripts in the nuclear stress bodies (nSBs), a unique subnuclear organelle which forms in response to heat shock in human cells (Jolly and Lakhotia 2006). In conclusion, hsrω and Sat III transcripts could dynamically regulate RNA-processing proteins, possibly working through a common paradigm (Jolly and Lakhotia 2006).

Interestingly, several mechanisms regulating RNA processing are related to transcription, indicating a functional connection between chromatin dynamics, transcription and RNA processing (Onorati et al. 2011; Tyagi et al. 2009; Allemand et al. 2008). Indeed, recent findings highlighted the central role of hnRNPs in the post-transcriptional regulation of several genes by direct and/or indirect interaction with factors affecting chromatin structure and nucleosomes dynamic. For example, ISWI, the catalytic subunit of several ATP-dependent chromatin remodeling complexes, is essential for omega speckles organization (Onorati et al. 2011), providing the first in vivo and in vitro example of a chromatin remodeler involved in the organization of a nucleoplasmic compartment. In particular, ISWI interacts physically and functionally with the hsrω ncRNA (Fig. 1a), and *ISWI-null* mutants show severe omega speckle organization defects (Onorati et al. 2011). In details, in *ISWI*-null mutants, instead of the typical speckle structures, hsrω localizes in nuclear compartments forming "trail"-like structures. Moreover, the distribution of the omega speckle-associated hnRNPs is also compromised. This phenotype was interpreted as severe defects in the maturation and organization of omega speckles caused by loss of the chromatin remodeler ISWI. In conclusion, it was hypothesized that ISWI could "remodel" speckles by structurally helping the assembly or release of specific hnRNPs with the hsrω ncRNA to generate mature omega speckles (Onorati et al. 2011).

Another interesting interaction between chromatin remodelers and hnRNPs has been shown in the Visa laboratory (Tyagi et al. 2009) (Fig. 1b). Indeed Visa and co-workers have characterized, in *D. melanogaster* as well as in *C. tentans*, the interaction between Brahma (Brm), the catalytic subunit of the SWI/SNF chromatin remodeling complex, and some hnRNPs. The authors have shown that Brm is incorporated into nascent pre-mRNPs during transcription (Fig. 1b). Brm, interacting directly with several protein factors, regulates the processing of pre-mRNAs. Among these factors, at least two are hnRNPs, such as Hrb87F and Rump (Tyagi et al. 2009). Interestingly, the association of Brm with the RNPs is conserved from insects to mammals. Moreover, the authors have shown that the post-transcriptional role

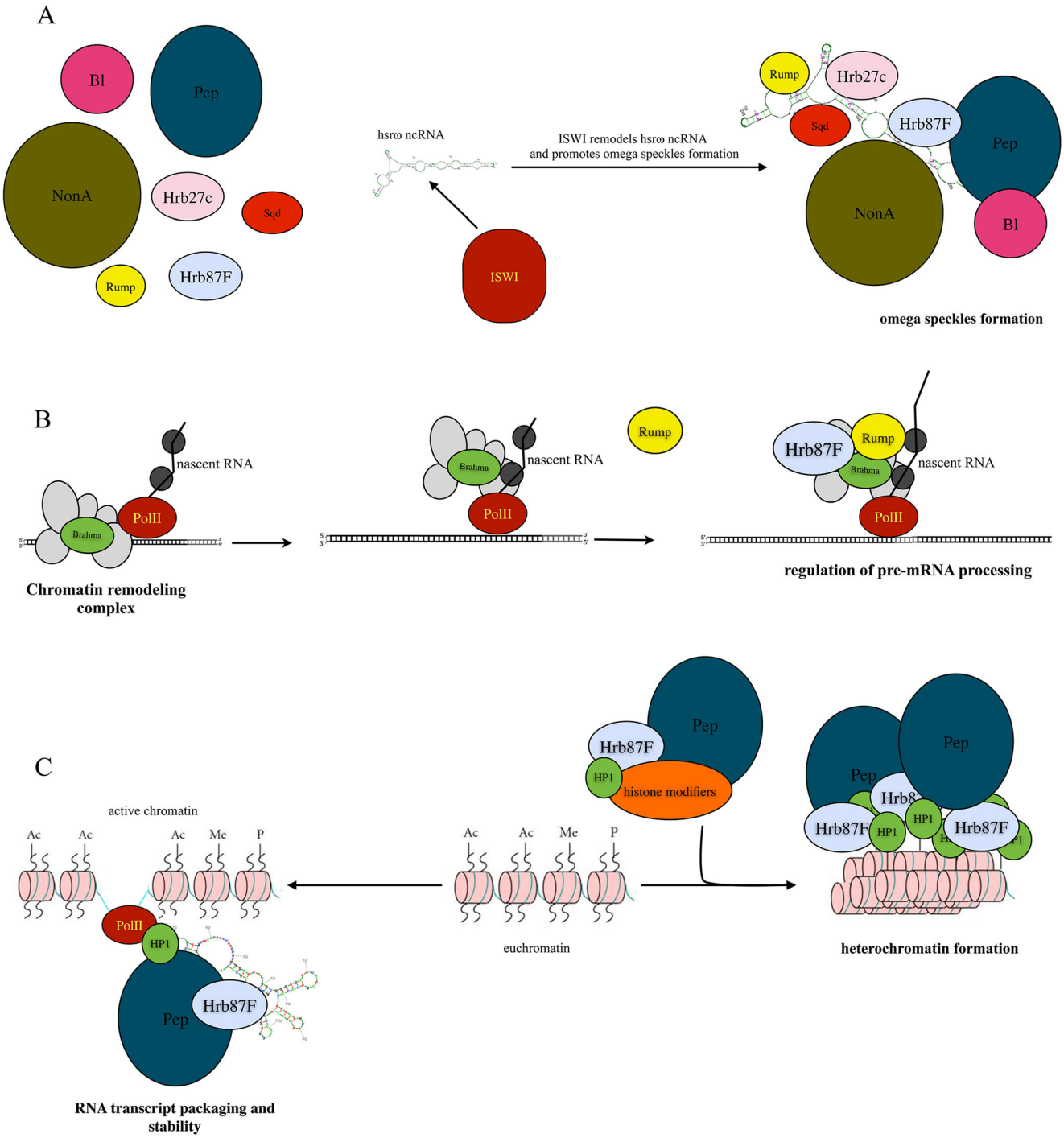

Fig. 1 HnRNPs interaction with chromatin components. Schematic representation of *Drosophila* hnRNPs interacting with chromatin remodeling complexes and other chromatin components. **a** ISWI, interacting with the ncRNA hsr-omega, ensures the correct functioning of nucleoplasmic omega speckles. Indeed the speckles 'remodeling' by ISWI, probably by structurally helping the assembly or release of specific hnRNPs with the hsrω ncRNA, is a fundamental step to ensure the correct organization between RNA and proteins to generate mature omega speckles. **b** A fraction of Brm within the context of SWI/SNF chromatin remodeling complexes, interacting directly with the hnRNPs Hrb87F and Rump, regulates the processing of pre-mRNAs determining the amount and the type of alternative transcripts produced. **c** The interaction between HP1a, histones modifiers and the hnRNPs PEP and Hrb87F regulates heterochromatin formation (*right panel*), while HP1a interaction with hnRNPs leads to the reinforcement of gene expression through RNA packaging (*left panel*)

mediated by the Brm protein is within the context of the SWI/SNF complex, in human as well as insect cells (Fig. 1b). In this manner, SWI/SNF seems to regulate gene expression by determining not only the amount of mRNA synthesized from a given promoter but also the type of alternative transcripts produced (Tyagi et al. 2009).

Piacentini et al. have showed a further example of interaction between a chromatin component and hnRNPs. Indeed, despite the Heterochromatin Protein I (HP1a) being historically associated with heterochromatin function, Piacentini and co-authors revealed that the HP1a seems to have a novel and unexpected role in maintaining chromatin activity through interaction with the hnRNPs. In fact, the interaction between HP1a, modified histones and specific hnRNP proteins induces heterochromatin formation and gene silencing. Moreover, HP1a interaction with RNA-packaging hnRNP proteins may also induce RNA compaction and stabilization that in turn can reinforce gene expression (Fig. 1c) (Piacentini et al. 2009).

Post-translational modifications of hnRNPs and their regulation by small molecules

In the last few years, an involvement of hnRNPs as a global regulator of alternative splicing has emerged. In particular, work conducted in different groups demonstrated that changes in the abundance of hnRNPs could modulate alternative splicing (Borah et al. 2009; Nichols et al. 2000; Olson et al. 2007; Nilsen and Graveley 2010). Though the molecular mechanism underlying this effect is not clearly understood, Valcarcel and colleagues recently provided evidence that the alternative splicing forms of the hnRNP Squid can contribute to sex-specific splicing during sex determination events (Hartmann et al. 2011). Interestingly, there is also growing evidence that covalent modifications of hnRNPs could regulate their activity/availability modulating their participation in alternative splicing process. In particular, the arginine methylation of hnRNP A1 in mammals was predicted to lock the protein into a non-specific binding mode by preventing the formation of arginine-dependent hydrogen bonds mediating specific interaction between RNA and certain proteins (Calnan et al. 1991; Najbauer et al. 1993). On the other hand, the methylation of hnRNP A2 seems to regulate its nuclear localization (Nichols et al. 2000). Interestingly, the identification of a family of nine arginine methyltransferases (PRMTs) expressed during *Drosophila* development, named DART1 to DART9 (*Drosophila* arginine methyltransferases 1–9), led to the discovery that the hnRNP Squid is also subject to methylation (Boulanger et al. 2004) (Fig. 2a). Although the role of hnRNP methylation in *Drosophila* is still unknown, the conservation of enzymes that catalyzes this post-translational modification strongly indicates a functional conservation with their mammalian counterparts.

Although evidence for other post-translational modifications have not yet been reported for *Drosophila* hnRNPs, the mammalian hnRNPs A1, A3, F, H and K have been shown to undergo modification by sumoylation (Li et al. 2004). However, it is not yet known if this modification affects their RNA-binding affinity. Other post-transcriptional modifications that affect human hnRNPs are phosphorylation and ubiquitination. For example, phosphorylation mediates nuclear/cytosol shuttling, thus providing a regulatory step for hnRNPs cytosolic activity (Habelhah et al. 2001). It has been also found that small molecules and metabolites, such as poly ADP-ribose (pADPr), could influence the activity of some hnRNPs (Gagne et al. 2003). This modification seems to be a conserved mechanism between mammals and *Drosophila*. Indeed, the *Drosophila's* hnRNPs Hrb87F, Hrb98DE and Squid have been shown to be associated with pADPr in vivo (Ji and Tulin 2009; Pinnola et al. 2007; Ji and Tulin 2013). The Tulin laboratory has shown that Squid and Hrb98DE have a putative pADPr-binding domain, homologous to the pADPr-binding motif identified in human hnRNP M (Ji and Tulin 2009). These authors have shown that the poly ADP-ribose regulates, in a non-covalent manner, hnRNPs ability to bind RNA, thus modulating mRNAs alternative splicing. Indeed, there are evidences that increased pADPr binding to hnRNPs alters the RNA-binding ability of hnRNPs in vivo and in vitro, leading to a dissociation of hnRNPs from most transcripts (Ji and Tulin 2009) (Fig. 2b). Furthermore, the ability of pADPr to bind hnRNPs is also upregulated by heat-shock treatment, indicating that pADPr binding to hnRNPs may play a role in regulating hnRNP upon environmental stresses (Ji and Tulin 2009). Moreover, pADPr seems to be responsible for the relocalization of hnRNPs from chromatin to the nucleoplasm (Ji and Tulin 2009), Recent data have shown that PARylation regulates at least two hnRNP-dependent post-transcriptional processes like alternative splicing and translation (Ji and Tulin 2012, 2013). In conclusion, hnRNP regulation by pADPr seems essential to modulate their activity under normal physiological conditions.

hnRNPs and their roles in cell polarity

In *Drosophila* mid-oogenesis, the establishment of the DV axis of the egg and embryo depends on the precise spatial restriction of *grk* mRNA and protein to the dorsal anterior region of the oocyte (Fig. 3). This spatial restriction leads to the localized activation of the epidermal growth factor receptor (EGFR) (Neuman-Silberberg and Schupbach 1993). Among the *Drosophila* hnRNPs, Squid seems to be the leading actor in this process (Fig. 3a). Indeed, as shown in several woks, in *sqd* mutants, the Grk-dependent DV patterning during oogenesis is disrupted (Kelley 1993; Neuman-Silberberg and Schupbach 1993). This disruption is caused by both incorrect localization and translation of *grk* mRNA in the anterior part of the oocyte, leading to ectopic EGFR activation and induction of dorsal cell fates (Kelley 1993; Neuman-Silberberg and Schupbach 1993). Interestingly, other studies have shown evidence that together with Squid, the hnRNP Hrb27C, the alternative splicing factor poly U-binding factor

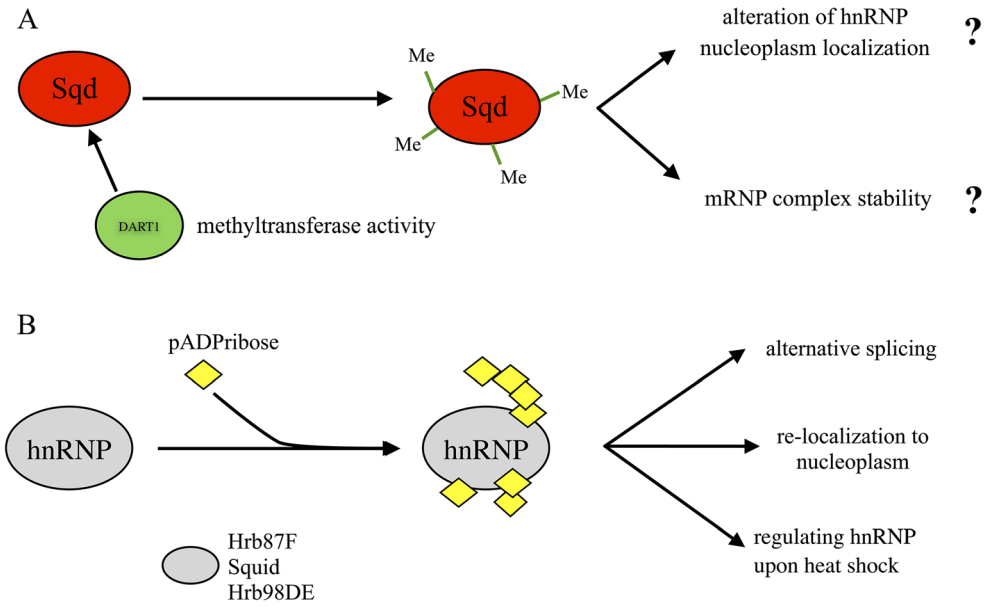

Fig. 2 hnRNPs regulation by covalent modification and small molecules. **a** Outline of covalent modification required for specific Squid activity. Squid is a substrate of DART1, the fly homolog of the human arginine methyltransferase PRMT1 and PRMT4/CARM1. The role of methylation of *Drosophila's* Squid is still unknown, but the data collected lead to hypothesize a functional conservation with the mammalian homolog. **b** pADP-ribose regulates Squid, Hrb98DE and Hrb87F hnRNPs ability to bind RNA (general hnRNP in *grey*), modulating the activity of these hnRNPs at post-transcriptional level

68 kDa (pUf68 also known as Half-pint) and the germline-specific ovarian tumor (Otu) protein are also required for a correct localization of *grk* mRNA in the dorso-anterior side of the oocyte (Norvell et al. 2005; Goodrich et al. 2004).

More recently, using a biochemical approach, Squid, IGF-II messenger RNA-binding protein (Imp) and a previously uncharacterized hnRNP named Syncrip (Syp) (Svitkin et al. 2013) were identified as proteins that specifically associate with *grk* in specific nucleotide sequence named *gurken* localization signals (GLS) (McDermott et al. 2012) (Fig. 3a). Syp is the fly homolog of mammalian SYNCRIP/hnRNPQ, a component of RNA transport granules in the dendrites of mammalian hippocampal neurons (Bannai et al. 2004; Svitkin et al. 2013). The new hnRNP Syp may be involved at a step preceding *grk* mRNA final localization in the oocyte, thus influencing its translation (McDermott et al. 2012).

On the other hand, the establishment of the antero-posterior (AP) axis requires posterior localization and translational control of both *osk* and *nos* mRNAs. Osk protein, synthesized from localized *osk* mRNA, nucleates the germ plasm assembly, which determines germ-cell fate in the embryo. This is achieved by the formation of a dynamic *osk* ribonucleoparticle (RNP) complex regulating the transport of *osk* mRNA. Interestingly, this RNP complex also contains the hnRNPs Squid, Hrb27C plus Bruno and Otu proteins (Huynh et al. 2004; Norvell et al. 2005; Yano et al. 2004; Kim-Ha et al. 1995; Gunkel et al. 1998; Goodrich et al. 2004) (Fig. 3b). This large RNP complex represses *osk* translation till its mRNA reaches the posterior pole. Recently, the nucleo-cytoplasmic-shuttling protein Heph/PTBP (polypyrimidine tract-binding protein), a hnRNP homolog of the human hnRNPI/PTB (Davis et al. 2002), has been shown to be a new in vivo component of Osk RNP, essential for the translation repression of *osk* mRNA, but not for its transport (Besse et al. 2009)(Fig. 3b).

The correct patterning of the AP body axis is also mediated by the Nos protein, selectively produced at posterior pole (Fig. 3c). Repression of unlocalized *nos* is mediated by a bipartite translational control element (TCE) at its 3' untranslated region by the interaction with the Smaug repressor (Smg) (Smibert et al. 1999; Forrest et al. 2004) (Fig. 3c). Interestingly, the *Drosophila* hnRNP Glorund (Glo), together with Smg, contributes to the specific translational repression of *nos* mRNA (Kalifa et al. 2006). Glo protein is part of a complex that also contains the hnRNP Hrb27C, the splicing factor Half-pint and the Otu protein (Fig. 3c). Interestingly, Glo seems to play also a role in the alternative splicing of Otu protein, highlighting a Glo role both as a translational repressor as well as a splicing factor (Kalifa et al. 2009). Moreover, two distinct works of the Gavis laboratory elucidated that *nos* localization and translation requires germ plasm formation, initiated by Osk protein during late oogenesis (Sinsimer et al. 2011). In particular, the authors identified two new localization factors, the *Drosophila* hnRNP Rumpelstiltskin (Rump) and the Lost protein as part of a core complex that promotes multiple mRNA localization pathways (Sinsimer et al. 2011) (Fig. 3c).

In conclusion, during *Drosophila* oogenesis, a hierarchy of hnRNP localization is involved in embryo polarity establishment. By a precise temporal- and spatial-regulated interaction with *gurken* (*grk*), *oskar* (*osk*) and *nanos* (*nos*) mRNAs, specific hnRNPs ensure a silent translational state for each of these mRNAs until their release in the correct cell districts, thus contributing indirectly to the binding occurring between each mRNA and the specific motor protein machinery responsible for cell polarity (Fig. 3).

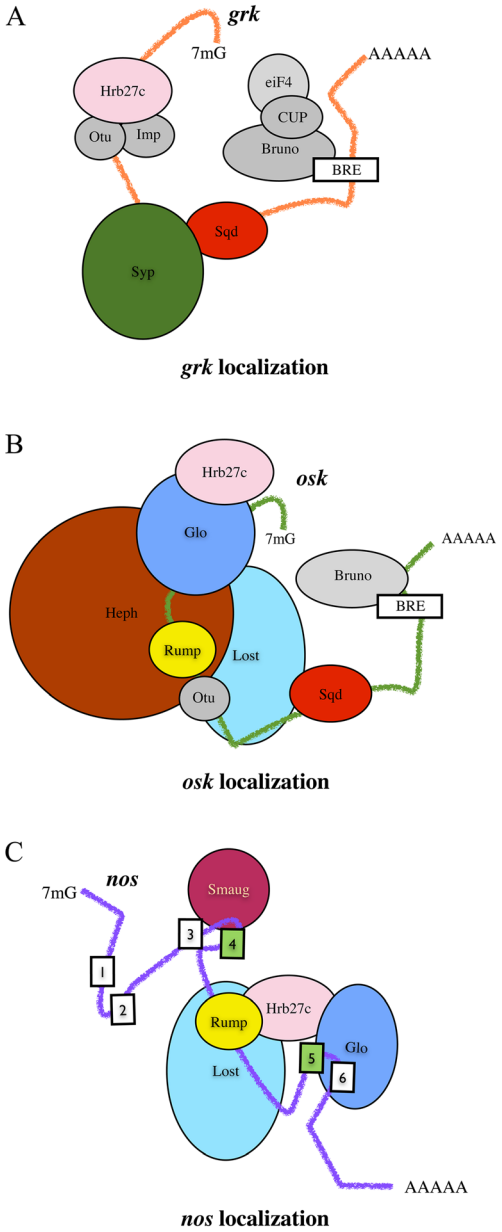

Fig. 3 HnRNPs are involved in antero-posterior (AP) and Dorso-ventral (DV) axis determination. Schematic representation of *Drosophila* hnRNPs involved in AP and DV axis determination. The hnRNP Hrb27c is common in all the complexes that regulate *grk*, *osk* and nos mRNA transcription and translation. In (**a**) and (**b**), Bruno protein, binding BRE sites, and represses *grk* and *osk* translational till its mRNA reaches the posterior pole. Moreover, Bruno, in a complex with Squid, Hrb27c and Syp hnRNPs (**a**), influences the localization and translation of *grk* mRNA. In (**b**), Bruno forms a complex in which the nucleo-cytoplasmic shuttling protein Heph/PTBP is also present, recently identified as a new component of Osk RNP in vivo. In (**c**), *nos* localization and translation are regulated by a complex that contains Glo, Hrb27c and Smaug proteins. In the same complex, the *Drosophila* hnRNP M mammalian homolog Rumpelstiltskin (Rump) and the Lost protein act as new localization factors, promoting multiple mRNAs localization pathway. Depending on the mRNA, other proteins and factors collaborate in maintenance of untranslated status upon its release in correct cell compartment. *Grey figures* are related to proteins that do not belong to hnRNPs' family. *Grk*, *osk* and *nos* and mRNAs are in *orange*, *green* and *violet lines*, respectively.

Roles in neuromuscular development

In the past years, a large body of evidence highlighting the important role of hnRNPs in muscular development has been produced. One of such example is the NonA protein. Although this protein was generally classified as a member of DBHS protein family, here, we considered NonA as an hnRNP. In fact, its structure and function is identical to hnRNPs Hrb87F and Squid. Moreover, NonA is engaged with the non-coding hsrω-n RNA in the nucleoplasmic omega speckles, taking part in RNA processing reactions (Onorati et al. 2011). During early embryogenesis, the hnRNP NonA seems to participate in the transcriptional regulation of key mRNAs needed for muscle development (Stanewsky et al. 1996). Remarkably, the complete loss of NonA is lethal in early embryos, and hypomorphic mutations in the *nonA* gene lead to female sterility, deterioration of vision, impaired movement coordination and behavioural defects (Stanewsky et al. 1996). Although the molecular basis of these pleiotropic defects is unknown, recent studies demonstrated that NonA forms an mRNP complex with the essential nuclear export factor NXF1 in an RNA-dependent manner (Kozlova et al. 2006) (Fig. 4a), thus facilitating the intranuclear mobility of mRNP particles (Fig. 4a) (Kozlova et al. 2006).

A wide range of defects in motor function seems to be under the control of the hnRNP Smooth (*sm*). Smooth is the homolog of the human hnRNP L, whose mutations have been isolated during a genetic screen for quantitative trait loci (QTL) affecting bristle number (Mackay 1985). In the adult stage, *sm* mutations result in decreased motor functions and in defects in tergal depressor of the trochanter (TDT) muscle function. In particular, *sm* homozygous mutants display defective intestinal motility in young flies and reduced flying/jumping capacities (Layalle et al. 2005; Draper et al. 2009). Indeed, the inactivation of *sm* determines axonal defects in the chemosensory neurones, the inability of mutant flies to feed and their precocious death, strongly indicating that Smooth could control axonal guidance and connectivity through the control of mRNA splicing or export (Fig. 4b) (Layalle et al. 2005). Furthermore, in a preliminary screen for the identification of factors associated with *Drosophila* ageing control, *sm* has been identified among genes that in the 'transcription and translation' functional categories influence ageing, although at the moment, the mechanistic details of *sm* functions in longevity control are unknown (Paik et al. 2012).

Recently, the role exerted by the hnRNPs in neuronal development, particularly in post-transcriptional regulation of neuronal mRNAs, has also emerged. Indeed, the hnRNP Glo, expressed in the central nervous system (CNS) at late stages of embryogenesis, seems to act at different developmental

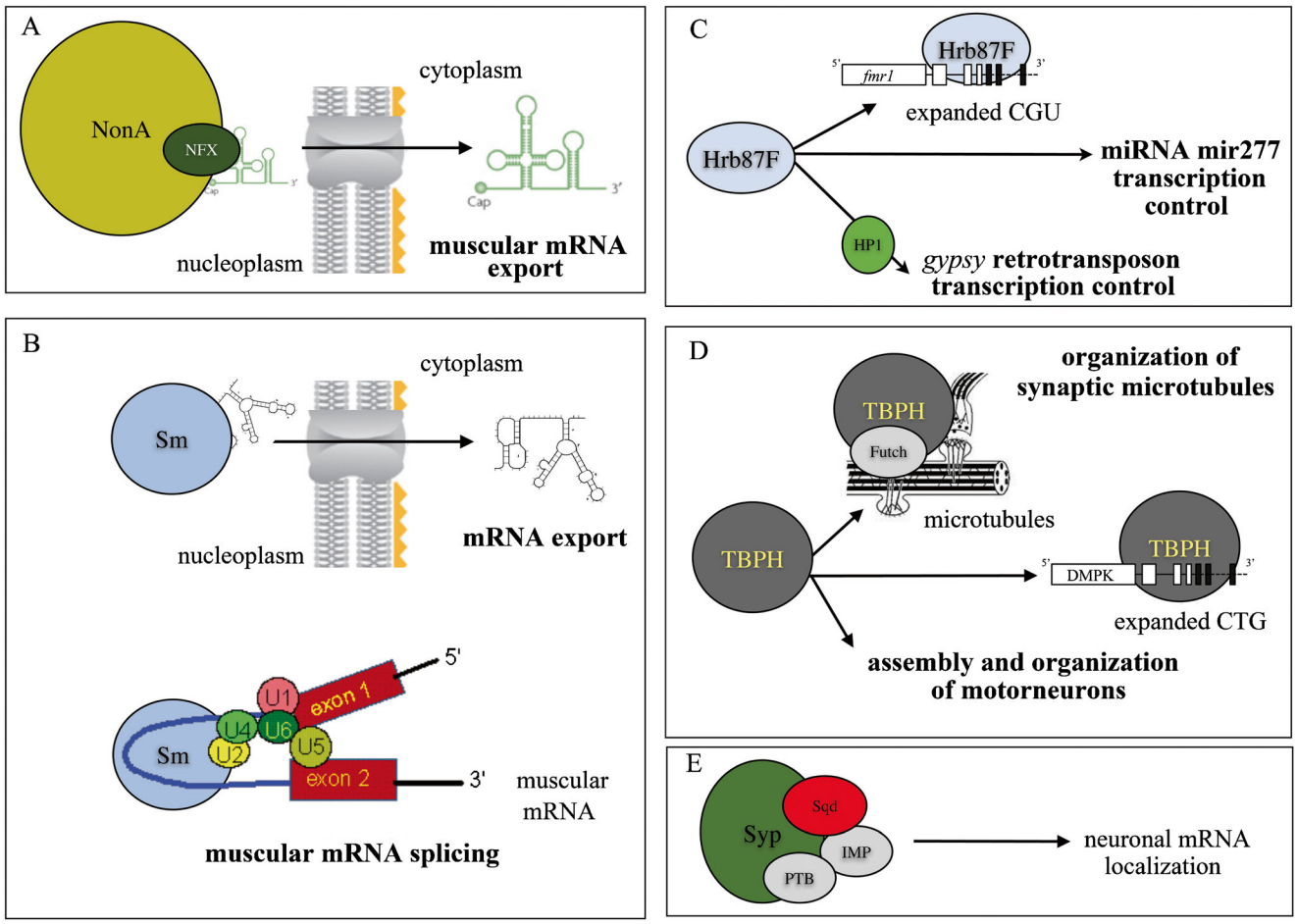

Fig. 4 HnRNPs are involved in neuromuscular development. Schematic outline of the biological processes in which hnRNPs are involved to regulate correct neuromuscular development. (**a**) The hnRNP NonA forms an mRNP complex with the nuclear export factor NXF1, thus facilitating the intranuclear mobility of mRNP particles. (**b**) The hnRNP Smooth (Sm) seems to have a role in mRNA splicing as well as mRNA export. (**c**) Hrb87F directly interacts with the CGU trinucleotide repeat in the 5′ untranslated region of the *FMR1* gene. Hrb87F is also a key factor to block transcription of *mir277* as well as to regulate the transcription of *gypsy* retrotransposons by interaction with HP1 protein. (**d**) TBPH, interacting with the Futsch protein, regulates the organization of synaptic microtubules. Furthermore, in a mechanism similar to Hrb87F, TBPH also binds the CTG trinucleotide repeat in the 3′ untranslated region of the *DMPK* gene. TBPH is also involved in assembly and organization of motoneurons. (**e**) Syp hnRNP intervenes in neuronal mRNA localization, interacting with neuronal mRNA in *Drosophila* nervous system as part of a RNP complex with Imp, PTB and Squid.

stages to regulate the translation of neuronal mRNAs (Piper and Holt 2004; Martin 2005; Kalifa et al. 2006; Olson et al. 2007). Moreover, the hnRNP Hrb87F, in addition to its role in omega speckle formation as well as normal development and stress tolerance (Singh and Lakhotia 2012), is also widely involved in neuronal development. For example, Lakhotia et al. have shown that Hrb87F has a fundamental role in modulating polyglutamine (polyQ) disease toxicity in a *Drosophila* model (Mallik and Lakhotia 2010; Sengupta and Lakhotia 2006). Indeed, data presented suggest that the RNAi-mediated down-regulation of the nuclear hsromega-n transcript leads to an increased availability of Hrb87F and of the transcriptional regulator cAMP response element-binding (CREB) protein (CBP), which in turn suppresses the enhancement of poly(Q) toxicity (Mallik and Lakhotia 2010).

Furthermore, in a *Drosophila* model of fragile X-associated tremor/ataxia syndrome (FXTAS), the Hrb87F hnRNP directly interacts with the CGG trinucleotide repeat in the 5′ untranslated region of the fragile X mental retardation 1 (*FMR1*) gene (Jin et al. 2003), whose expansion over a certain threshold causes the onset of fragile X syndrome (FXS) (Fig. 4c). This interaction is important to reduce Hrb87F bioavailability (Sofola et al. 2007). Moreover, the Jin Laboratory has shown that reduced levels of Hrb87F fail to block the transcription of *mir277*, a miRNA that modulates the neurodegeneration caused by fragile X pre-mutation rCGG repeats. Furthermore, reduced levels of Hrb87F also promote the transcription of *gypsy* retrotransposons, which reinforces neurodegeneration (Fig. 4c) (Tan et al. 2012).

Another *Drosophila* hnRNP that seems to be involved in a neuronal physiological process is TBPH, the *Drosophila* homolog of the human TAR DNA-binding protein (TDP-43 or TARDBP) (Buratti and Baralle 2009; Strong 2010; Buratti and Baralle 2010; Ritson et al. 2010). Despite its unequivocal hnRNP structure, TDP-43 was originally described as a DNA-binding protein with a putative role in HIV transcription (Ou et al. 1995). Recently, TDP-43 has been classified as the major disease protein present in cytoplasmic inclusions in amyotrophic lateral sclerosis (ALS) and fronto-temporal lobar degeneration (FTLD) (Neumann et al. 2006; Arai et al. 2006). Moreover, TDP-43 dysfunction has also been observed in other neurodegenerative disorders like Alzheimer's, Parkinson's and Huntington's disease (Forman et al. 2007; Chen-Plotkin et al. 2010). In *Drosophila*, at least three different fly models elucidated the role of TBPH in neuronal and neuromuscular development. Indeed, flies mutants for TBPH closely reproduce most of the phenotypes observed in ALS patients like decreasing viability, affected synaptic transmission, defective locomotion and also age-related progressive neurodegeneration (Ritson et al. 2010; Hazelett et al. 2012; Li et al. 2010; Neumann et al. 2006). Recently, it has emerged that TBPH interacts with the Futsch protein, the *Drosophila* homolog to human MAP1B (Godena et al. 2011) (Fig. 4d). A reduced interaction between TBPH and Futsch seems to be responsible for the alteration in the organization of synaptic microtubules (Godena et al. 2011).

Furthermore, an interaction between TBPH and the dystrophia myotonica-protein kinase (*DMPK*) gene has also been recently identified (Llamusi et al. 2013). Interestingly, the expansion of CTG trinucleotide repeats in the 3′ UTR of the *DMPK* gene (responsible for myotonic dystrophy type 1 (DM1)) could sequester TBPH and other related proteins into nuclear foci, thus depriving cell of these vital protein functions (Llamusi et al. 2013) (Fig. 4d), with a mechanism remembering the previously described interaction between Hrb87F and the CGG trinucleotide repeat at the 5′ untranslated region of *FMR1* gene (Fig. 4c). Moreover, neurodegenerative phenotypes similar to those obtained with TBPH mutants are also caused by mutations in the hnRNP Cabeza (Caz) (Wang et al. 2011; Zinszner et al. 1997), the fly protein homolog to the human FUS protein. Strikingly, it has been shown that the ectopic expression of human FUS restored locomotion disabilities and shorter life span caused by *Drosophila caz* knockout, suggesting that human FUS can compensate Caz function and that the role of both proteins are highly conserved during evolution (Wang et al. 2011; Zinszner et al. 1997).

The work in the Davis laboratory sustained the hypothesis that also the Syp hnRNP intervenes in neuronal mRNA localization with a similar mechanism through which it regulates *grk*, *osk* and *nos* localization (Fig. 4e) (McDermott et al. 2012; Svitkin et al. 2013). Indeed, Syp has been found to interact with neuronal mRNA in *Drosophila* nervous system as part of a RNP complex with other ribonucleoproteins such as IGF-II messenger RNA-binding protein (Imp), polypyrimidine tract-binding protein (PTB) and Squid (Adolph et al. 2009; Davis et al. 2002) (Fig. 4e). Moreover, the work of Davis and colleagues provides the first evidence that Syp associates with RNP granules in the dendrites of hippocampal neurons (McDermott et al. 2012).

Collectively, all these results highlight the fundamental role that hnRNPs have in the muscle and nervous system development (Fig. 4).

Fruitfly as a model system for human hnRNPs-related neurodegenerative proteinopathies

Over this last decade, the fly has been a powerful model system for studying human neurodegenerative diseases, thanks to its high neuronal complexity resulting in an advanced brain able to reproduce fine learning and memory responses (Pandey and Nichols 2011; Bilen and Bonini 2005; Shulman et al. 2003). For example, the use of *Drosophila* has shed light on several aspects of FXTAS disease, as shown above (Fig. 4c) (Tan et al. 2012). Moreover, *Drosophila* gave the opportunity to analyse several aspects of a wide spectrum of neurodegenerative diseases collectively named proteinopathies, characterized by both hnRNPs functions alteration and/or loss (Tsuji et al. 2012; Neumann et al. 2007; Neumann et al. 2006). In particular, the multisystem proteinopathy (MSP) is a rare disease in which inclusion body myopathy (IBM) is associated with Paget's disease of the bone (PDB), fronto-temporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) (Badadani et al. 2010). MSP is characterized by a progressive degeneration of muscle, brain, motor neurons and bones accompanied by prominent TDP-43 protein pathology (Badadani et al. 2010).

Interestingly, in a rare case of MSP, the levels of transcripts corresponding to genes encoding the hnRNP A/B family proteins, in particular hnRNPA2B1 and hnRNPA1, that directly interact with TDP-43 to function cooperatively in RNA metabolism regulation are profoundly altered (Kim et al. 2013; Ramaswami et al. 2013). In another work, making use of a transgenic *Drosophila* model expressing both wild-type or mutant forms of the human hnRNPA2, hnRNPA1 and the fly homolog Hrb98DE, Kim and co-authors found that the MSP-causing mutations fall at the centre of a predicted prion-like domain (PrLD), previously identified at the C-terminal regions of hnRNPA2 and hnRNPA1. Moreover, the authors elucidated that mutation in PrLD act as a gain of function that promotes fibrillation of both hnRNPA2B1 and hnRNPA1 and subsequent toxic cytoplasmic accumulation (Kim et al. 2013). Indeed, disease mutations introduce protein defects into the PrLDs of hnRNPA2 and hnRNPA1, deregulating and

accelerating nucleation and polymerization, altering the dynamics of RNA granule assembly and thus compromising RNA metabolism (Kim et al. 2013). As several hnRNPs have similar PrLDs (Buratti et al. 2005), these class of aggregation-prone RNA-binding proteins might be very good candidates for investigating on ALS and all related neurodegenerative diseases in which protein aggregation cause toxicity and could be a key step for the disease's onset.

Conclusions

In *Drosophila*, at least 14 hnRNPs have been identified. Starting from very early embryo developmental stages, hnRNP's are involved in numerous biological functions affecting RNA biology. We presented a comprehensive overview of all putative factors regulating the nuclear bioavailability of *Drosophila* hnRNPs. Indeed, given the extraordinary dynamic nature of these molecules, assembling of hnRNPs in nucleoplasmic compartments as well as their release could be a key regulatory step for all biological processes in which hnRNPs are involved. Indeed, different signals might regulate directly and/or indirectly hnRNPs nucleoplasmic availability, affecting as a result various aspects of gene regulation. In conclusion, exploring the molecular mechanism underling hnRNPs regulation could allow the understanding of post-transcriptional regulation, including the defects underlying human disease based on the alteration of RNA processing or protein functions like proteinopathies.

Acknowledgments We would like to thank Giulia D'Angelo for the critical reading and feedbacks on this manuscript. We would also like to apologize to all our colleagues whose work was not properly cited due to space restriction. L.L.P. is supported by an AIRC fellowship. This work was supported by grants from Fondazione Telethon, AIRC and Progetto Bandiera Epigen to D.F.V.C and by MFAG grant from AIRC to M.C.O.

Conflict of interest The authors declare no conflict of interest.

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