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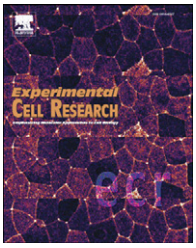

Research Article

Drosophila SMN complex proteins Gemin2, Gemin3, and Gemin5 are components of U bodies

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ABSTRACT

Uridine-rich small nuclear ribonucleoproteins (U snRNPs) play key roles in pre-mRNA processing in the nucleus. The assembly of most U snRNPs takes place in the cytoplasm and is facilitated by the survival motor neuron (SMN) complex. Discrete cytoplasmic RNA granules called U bodies have been proposed to be specific sites for snRNP assembly because they contain U snRNPs and SMN. U bodies invariably associate with P bodies, which are involved in mRNA decay and translational control. However, it remains unknown whether other SMN complex proteins also localise to U bodies. In *Drosophila* there are four SMN complex proteins, namely SMN, Gemin2/CG10419, Gemin3 and Gemin5/Rigor mortis. *Drosophila* Gemin3 was originally identified as the *Drosophila* orthologue of human and yeast Dhh1, a component of P bodies. Through an *in silico* analysis of the DEAD-box RNA helicases we confirmed that Gemin3 is the bona fide *Drosophila* orthologue of vertebrate Gemin3 whereas the *Drosophila* orthologue of Dhh1 is Me31B. We then made use of the *Drosophila* egg chamber as a model system to study the subcellular distribution of the Gemin proteins as well as Me31B. Our cytological investigations show that Gemin2, Gemin3 and Gemin5 colocalise with SMN in U bodies. Although they are excluded from P bodies, as components of U bodies, Gemin2, Gemin3 and Gemin5 are consistently found associated with P bodies, wherein Me31B resides. In addition to a role in snRNP biogenesis, SMN complexes residing in U bodies may also be involved in mRNP assembly and/or transport.

Introduction

In living cells, RNAs associate with proteins to form ribonucleoproteins (RNPs) which migrate among various compartments in the nucleus and the cytoplasm [1]. Recently a number of cytoplasmic compartments populating different classes of RNPs have been identified under the light microscope [2–4]. For example, proteins involved in mRNA degradation are concentrated within cytoplasmic processing bodies (P bodies) [5], while U bodies contain uridine-rich snRNPs [6]. Despite being distinct, U bodies and P bodies are physically and functionally related, indicating they contribute to a common cellular pathway, termed the U body–P body pathway [7].

Reduction of survival motor neuron (SMN) protein levels causes spinal muscular atrophy (SMA), the most common genetic cause of childhood mortality [8]. In humans, SMN oligomerises and forms a stable macromolecular complex with at least eight other proteins [9,10]. Among them, four proteins – SMN, Gemin2, Gemin3 and Gemin5 – have orthologues in *Drosophila* [11–14]. Biochemical studies have demonstrated that the SMN complex

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facilitates the assembly of snRNPs [9,15]. Cytological studies have shown that SMN is enriched in U bodies, suggesting that the U body contributes to snRNP assembly [6,7]. However, key evidence as to whether U bodies contain other SMN complex proteins is currently lacking.

SMN complex components were also found to be part of SMN-independent multiprotein complexes and likely perform important cellular functions outside the SMN complex [16]. Notably, in addition to their SMN complex membership, various Gemin proteins were reported to associate with P body components: Gemin3 was isolated in a low abundant complex with Gemin4, Argonatue2 and numerous miRNAs [17–19] whereas Gemin5 was consistently identified as an eIF4E-binding partner [20,21]. In this context, it was highly desirable to clarify whether the Gemin proteins localise either in U bodies or P bodies or both.

We and others independently identified CG6539 as the *Drosophila* orthologue of human Gemin3 [11–13]. Nevertheless, CG6539 was originally identified as the *Drosophila* orthologue of human and yeast Dhh1, a component of P bodies [5,22]. To clarify whether CG6539 is the *Drosophila* orthologue of Gemin3 or else of Dhh1, we have undertaken an *in silico* analysis of the DEAD-box RNA helicases paying particular attention to the Gemin3 and Dhh1 subfamilies. Our analysis supports CG6539 as the *Drosophila* orthologue of vertebrate Gemin3, and Me31B as the *Drosophila* orthologue of human and yeast Dhh1. Subsequently we made use of the *Drosophila* ovary as an *in vivo* model system to investigate the subcellular localisations of the SMN complex proteins, Gemin2 (CG10419), Gemin3 and Gemin5 (previously named Rigor mortis) [23], as well as Me31B. *Drosophila* egg chambers are ideal for the study of RNA granules because the nurse cells and the oocyte contain abundant U bodies and P bodies, and because they are very large at late stages. Our results show that Gemin2, Gemin3 and Gemin5 colocalise with SMN in U bodies. Despite being excluded from P bodies, SMN complex proteins as U body components are invariably associated with P bodies that contain Me31B.

Materials and methods

Computational analysis of protein sequences

Gemin3 homologous protein sequences were collected using BLAST sequence similarity searches [24] of various protein sequence databases: UniProt [25] and GenBank [26]. In some instances (Table S1), homologous sequences were used to produce improved gene predictions from genomic DNA using FGENESH+ [27]. The input for the phylogenetic analysis was a multiple sequence alignment (MSA) corresponding to the DEAD-box family conserved region. The MSA was generated using T-Coffee software [28] and default parameters. The dendrogram was calculated with the neighbour-joining method [29] using ClustalW [30] and was edited with Treetool (http://iubio.bio.indiana.edu/soft/molbio/unix/treetool). Support for the topology of the phylogenetic tree was investigated by bootstrapping experiments (1000 replicates) [31]. We also generated trees using a Bayesian and Maximum Likelihood methods, using the Phylogeny.fr server [32–34] and default parameters, and found that all three gave an identical topology strongly suggesting the overall structure of the tree to be correct. For illustrative purposes only the Neighbour Joining tree is shown (See Fig. 1.).

Fly stocks and genetics

Drosophila melanogaster stocks were cultured on standard molasses/maize meal and agar medium in plastic vials or bottles at 25 °C. The *y w* stock was utilised as the wild-type fly strain. The UAS-ECFP-Gemin3 was described previously [12] and its expression was driven by Act5C-GAL4. The UAS-Lsm11-YFP flies were provided by Joe Gall, Carnegie Institution Department of Embryology, Baltimore, MD, USA [6,35], and expression of Lsm11-YFP was driven by da-GAL4. The GFP-Me31B and eIF4E-GFP flies are protein trap lines generated by Mike Buszczak and Allan Spradling, Carnegie Institution Department of Embryology, Baltimore, MD, USA [36].

Generation of antibodies against Drosophila Gemin3 and Gemin2

New Zealand white rabbits were co-immunised according to standard procedures at Sigma-Genosys Ltd. (Suffolk, UK) with two polypeptides, one of which represents an internal region (SHNNKNLRVKEKE) whereas the other is found within the C-terminal region (CRSKKAHHLRKRHVY) of the *Drosophila* Gemin3 protein. Briefly, following collection of the pre-immune serum, animals were immunised with 200 µg of polypeptides in complete Freund's adjuvant followed by subsequent injections of 100 µg with incomplete adjuvant. Antisera used in this study are derived from test bleed 5 (after eight injections). To make the antibody against *Drosophila* Gemin2 (CG10419), a polypeptide in the N-terminal region (EPDSSFDPQKPPES) of *Drosophila* Gemin2 was synthesized and injected in rabbits and the immunised sera were affinity-purified according to standard procedures at Genscript Corp. (Piscataway, NJ, USA).

Immunostaining of ovaries

Ovaries were dissected from adult female flies in Grace's insect medium (Invitrogen Ltd., Paisley, UK), fixed in 4% paraformaldehyde in PBS and then washed in 1× PBS + 0.1% Triton® X-100 + 3% Normal Goat Serum (PBT). The tissues were next subjected to overnight staining by primary antibodies. The next day, tissues were washed in PBT and stained overnight with either anti-mouse or anti-rabbit Alexa Fluor-conjugated secondary goat antibodies and Hoechst 33342 nuclear stain. Following washing, the tissues were mounted and viewed with a Zeiss LSM 510 META confocal microscope. Primary antibodies used include mouse anti-GFP (Roche Diagnostics Ltd., West Sussex, UK), affinity-purified rabbit polyclonal antibody against *Drosophila* SMN kindly provided by J. Zhou, University of Massachusetts Medical School, Worcester, MA, USA [37], mouse monoclonal antibody against *Drosophila* SMN kindly provided by S. Artavanis-Tsakonas, Harvard Medical School, Cambridge, MA, USA [38], affinity-purified rabbit polyclonal antibody against *Drosophila* Gemin5/Rig kindly provided by C. S. Thummel, University of Utah School of Medicine, Salt Lake City, UT, USA [23], and rabbit anti-Cup kindly provided by A. Nakamura, Riken Centre for Developmental Biology, Kobe, Hyogo, Japan [39]. To reduce bleed-through, secondary antibodies conjugated with fluorochromes that have well-separated excitation and emission spectra (Alexa Fluor 488 and Alexa Fluor 633; Invitrogen) were used for co-localisation experiments.

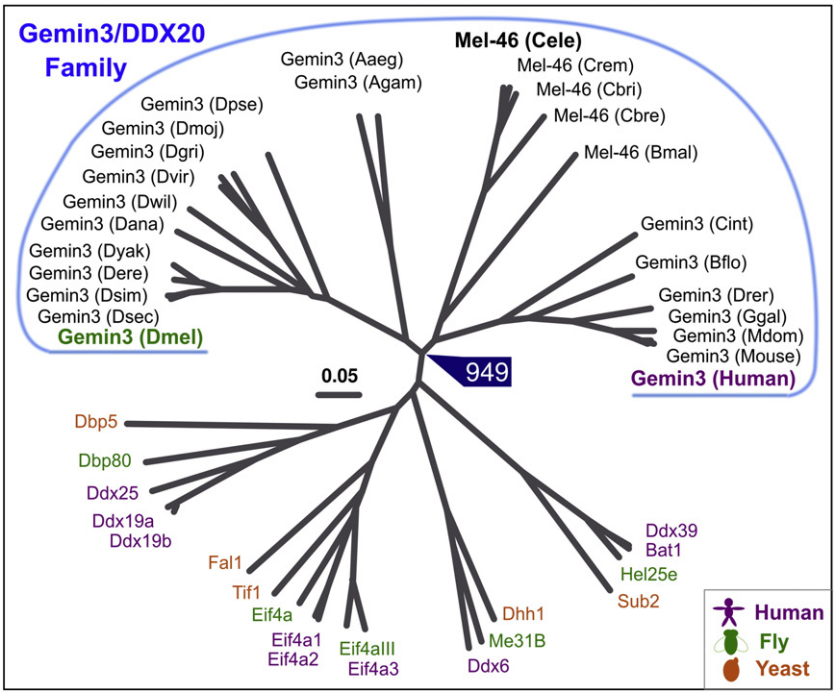

Fig. 1 – Representative phylogeny of the Gemin3/DDX20 family. The Gemin3/DDX20 family is indicated by a blue line. Selected human, *Drosophila melanogaster* and yeast (*Saccharomyces cerevisiae*) DEAD-box proteins are coloured in purple, green and orange, respectively. The sequences of the Gemin3/DDX20 family and selected members of the DEAD-box family shown in the tree are named as described in Table S2. The scale bar shows the average number of amino acid substitutions per site (0.05). The main bootstrap value that supports the Gemin3 family is labelled in blue. Gemin3 (Dmel) = CG6539. The affinity of this family was strongly supported in bootstrapping experiments (94.9%) (see Materials and Methods).

Confocal microscopy

Images were taken under 40× or 63× objectives on a laser-scanning confocal microscope (Zeiss LSM 510 META, Oberkochen, Germany) and processed using Zeiss Lsm Image Browser. The original confocal images were exported as TIF files and processed using Photoshop CS2 (Adobe Systems, Mountain View, CA, USA).

Results

Drosophila DEAD-box RNA helicases

DEAD-box RNA helicases are distinguished by the presence of nine conserved motifs including the Asp-Glu-Ala-Asp or DEAD (in one-letter code) motif, which gives the protein family its name. Although DEAD-box proteins show considerable sequence and structural similarities within their conserved 'helicase' core, their flanking N- and C-terminal domains are highly divergent and are thus thought to provide specificity of function through interaction with specific RNA substrates or other interacting factors [40,41].

By undertaking a domain architecture analysis using SMART (simple modular architecture research tool) [42] and Pfam [43], we identified 70 different members of the DEAD-box helicase family encoded in the *Drosophila melanogaster* genome (Table S1). Using human and mouse Gemin3, we and two other groups previously identified CG6539 as the *Drosophila* orthologue of vertebrate Gemin3 [11–13]. In Flybase, CG6539 was first identified as an orthologue of human and yeast Dhh1. Identification of orthologues among diverse species is essential to provide accurate functional predictions, because orthologues usually retain comparable functions over the course of evolution [44]. To clarify the true evolutionary relationships of these genes, we determined from phylogenetic analyses of Gemin3 protein sequences that CG6539 and human Gemin3 belong to the DDX20 subfamily of DEAD-box RNA helicases; by contrast, *Drosophila* Me31B and, human and yeast Dhh1 belong to the DDX6 subfamily (Fig. 1; Table S2).

DEAD-box RNA helicases Gemin3 and Me31B localize at distinct but related structures

Drosophila ovaries are organised into several hollow tubular structures (ovarioles) containing progressively maturing egg chambers. Each egg chamber is composed of a single oocyte and 15 nurse cells, and is surrounded by a monolayer of follicle cells. Nurse cells synthesize large amounts of RNAs and proteins that are transported to the developing occyte (Supplemental Fig. 1; reviewed in [45]. In order to investigate the subcellular localisation of Gemin3 in *Drosophila* egg chambers, we generated a polyclonal anti-Gemin3 antibody directed against a peptide located internally in its sequence and a second peptide found at its C-terminus. To compare the subcellular localizations of the two DEAD-box RNA helicases, Gemin3 and Me31B, we used the anti-Gemin3 antibody to stain egg chambers derived from a Me31B-YFP protein trap line. Although both Gemin3 and Me31B show granular staining in the egg chamber, the granules differ in size and distribution. While

Me31B exhibits irregular granules in the cytoplasm of nurse cells and oocytes, Gemin3 labels a few bright spherical foci against a background with lower staining intensity (Fig. 2). In the same egg chamber, Me31B granules show a higher density in the oocyte than in nurse cells. However, Gemin3 particles exhibit comparable densities in nurse cells and in the oocyte. During oogenesis, egg chambers from later stages contain more Gemin3 and Me31B granules than those from earlier stages.

Gemin3 is a component of U bodies

Gemin3 shows the same staining pattern in egg chambers derived from wild-type (Figs. 3A–C) or from an Me31B-YFP protein trap line (Fig. 2). The observed Gemin3 staining pattern was similar to that reported for U bodies which are known to be enriched in U snRNPs and in SMN [6]. Consequently, we investigated whether Gemin3 is a novel component of U bodies by performing double-

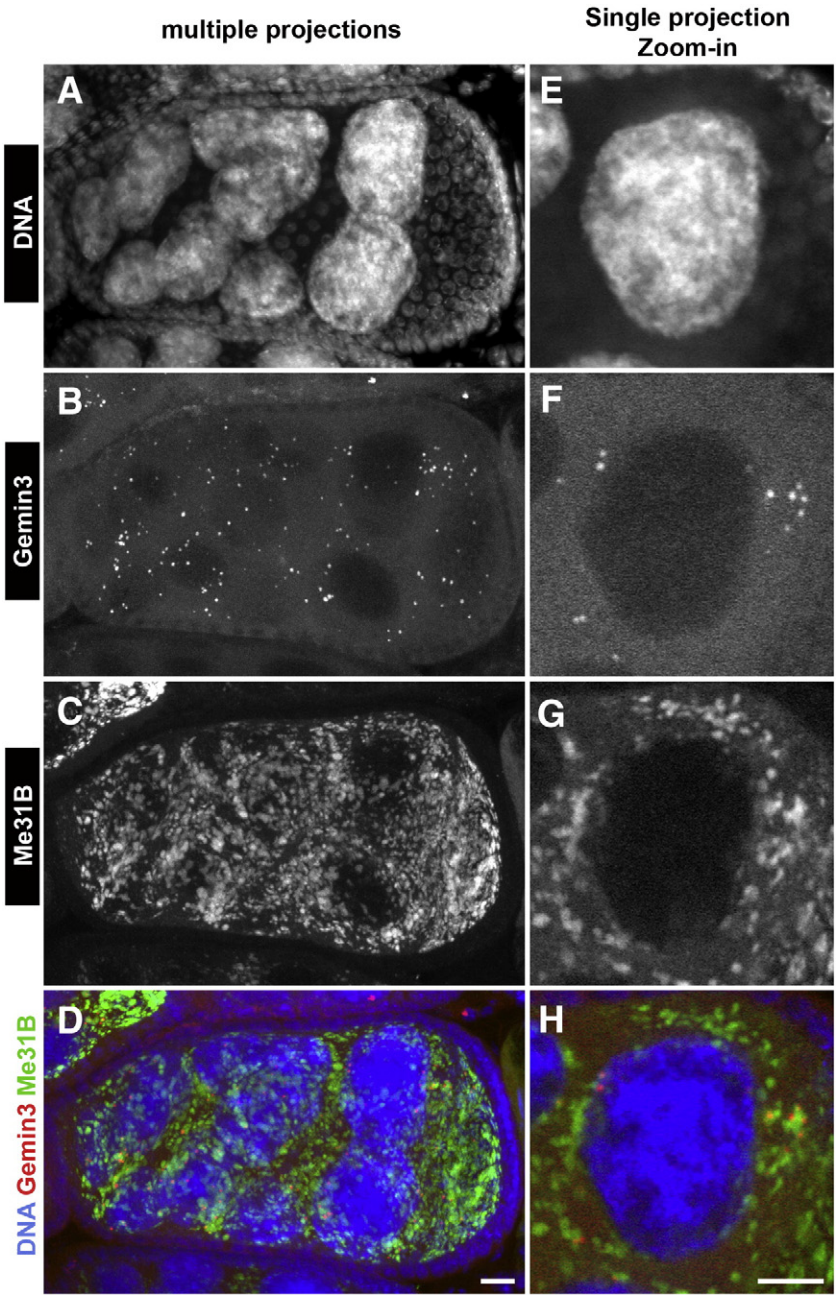

Fig. 2 – RNA helicases Gemin3 and Me31B in a *Drosophila* egg chamber. DNA is stained with Hoechst 33342 (white in A, E and blue in D, H). Gemin3 (B, F) and Me31B (C, G) show distinct localisation in nurse cells and the oocyte which is located at the posterior end (to the right in A–D). (D, H) Gemin3 stains spherical structures (red), which are close to, but do not overlap, irregular structures enriched with Me31B (green). (E–H) Magnified images of A–D. Note that the images in A–D are overlays of 13 projections, whilst images in E–H are from one projection. Scale bars, 10 μm.

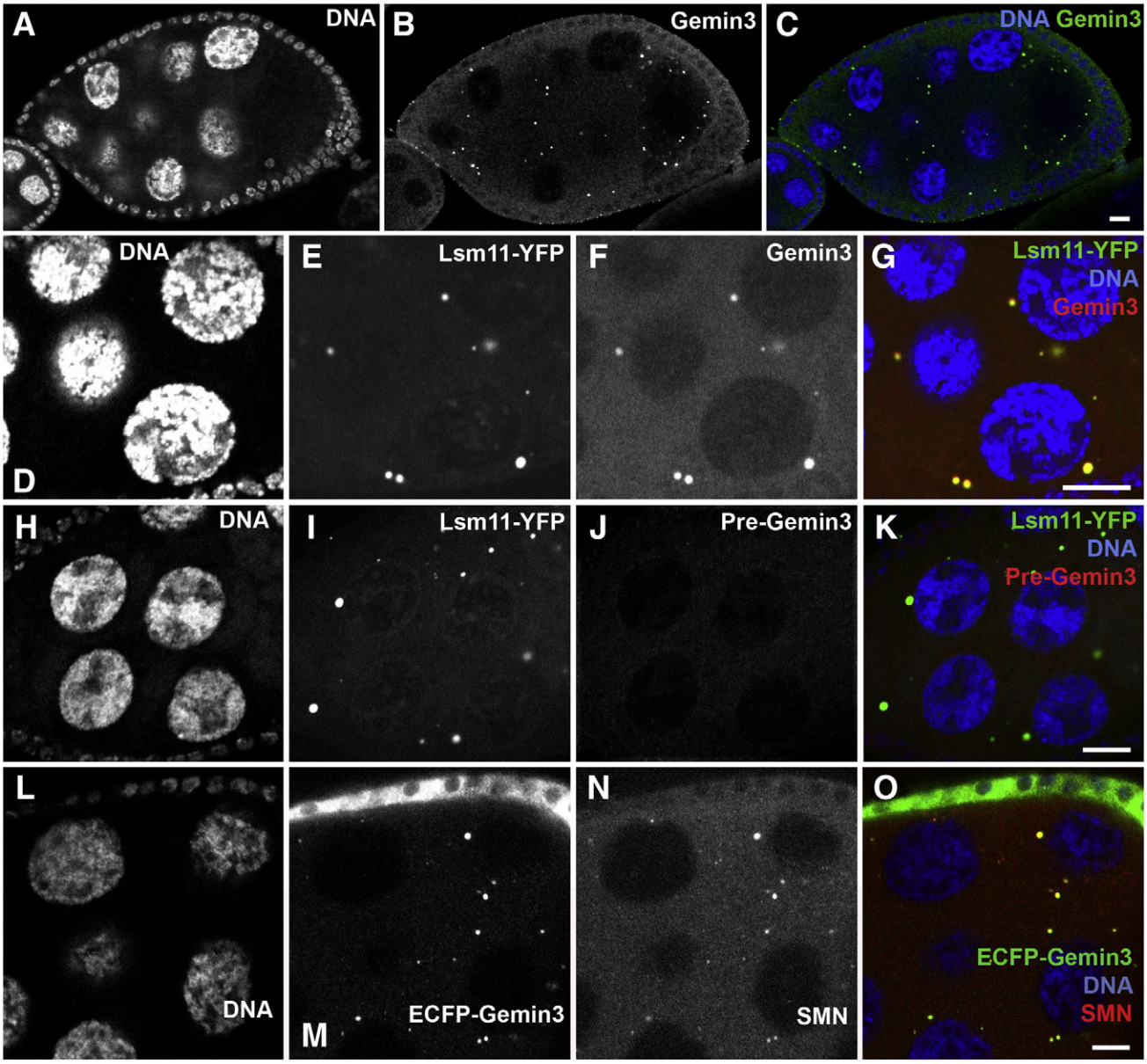

Fig. 3 – Gemin3 localises to U bodies. (A–C) *Drosophila* wild-type egg chamber stained with anti-Gemin3 antibody. Gemin3 is enriched in several discrete spherical foci in the cytoplasm of germline cells. (D–G) Antibody against Gemin3 stains U bodies labelled by Lsm11-YFP. (H–K) Pre-immune serum from the same animal in which the Gemin3 antibody was raised (pre-Gemin3) does not stain U bodies labelled by Lsm11-YFP. (L–O) Antibody against SMN, a component of U bodies, stains cytoplasmic foci in the germline cells formed by constitutively expressing ECFP-Gemin3. Note that SMN staining is weak in follicle cells even when they are overexpressing ECFP-Gemin3. Scale bars, 10 μm.

labelling experiments using egg chambers expressing a YFP fusion of Lsm11 (Lsm11-YFP), a U7 snRNP-specific protein that localises to U bodies. U bodies in Lsm11-YFP are very large, presumably owing to overexpression of Lsm11. Gemin3 antiserum stained all U bodies labelled by Lsm11-YFP (Figs. 3D–G). As a negative control, no signal was observed when egg chambers expressing Lsm11-YFP were stained with pre-immune serum from the same animal from which the Gemin3 antibody was obtained (Figs. 3H–K). When egg chambers expressed an ECFP-Gemin3 fusion protein, many foci appeared in the cytoplasm of nurse cells and oocytes. Those ECFP-Gemin3 foci appear to be U bodies because they also contain SMN, a marker for U bodies (Figs. 3L–O). Taken together, these results demonstrate that Gemin3 is a novel component of U bodies.

Me31B is a component of P bodies but not U bodies

Human and yeast Dhh1 localise to P bodies [5,46]. As indicated by our phylogenetic analyses, Me31B is the *Drosophila* orthologue of Dhh1. In support of this and consistent with previous studies [6,7], we observed that Me31B colocalised with other P body components such as Cup (Figs. 4E–H) and eIF4E (Figs. 4I–L). By contrast, Me31B expression does not coincide with U body markers such as

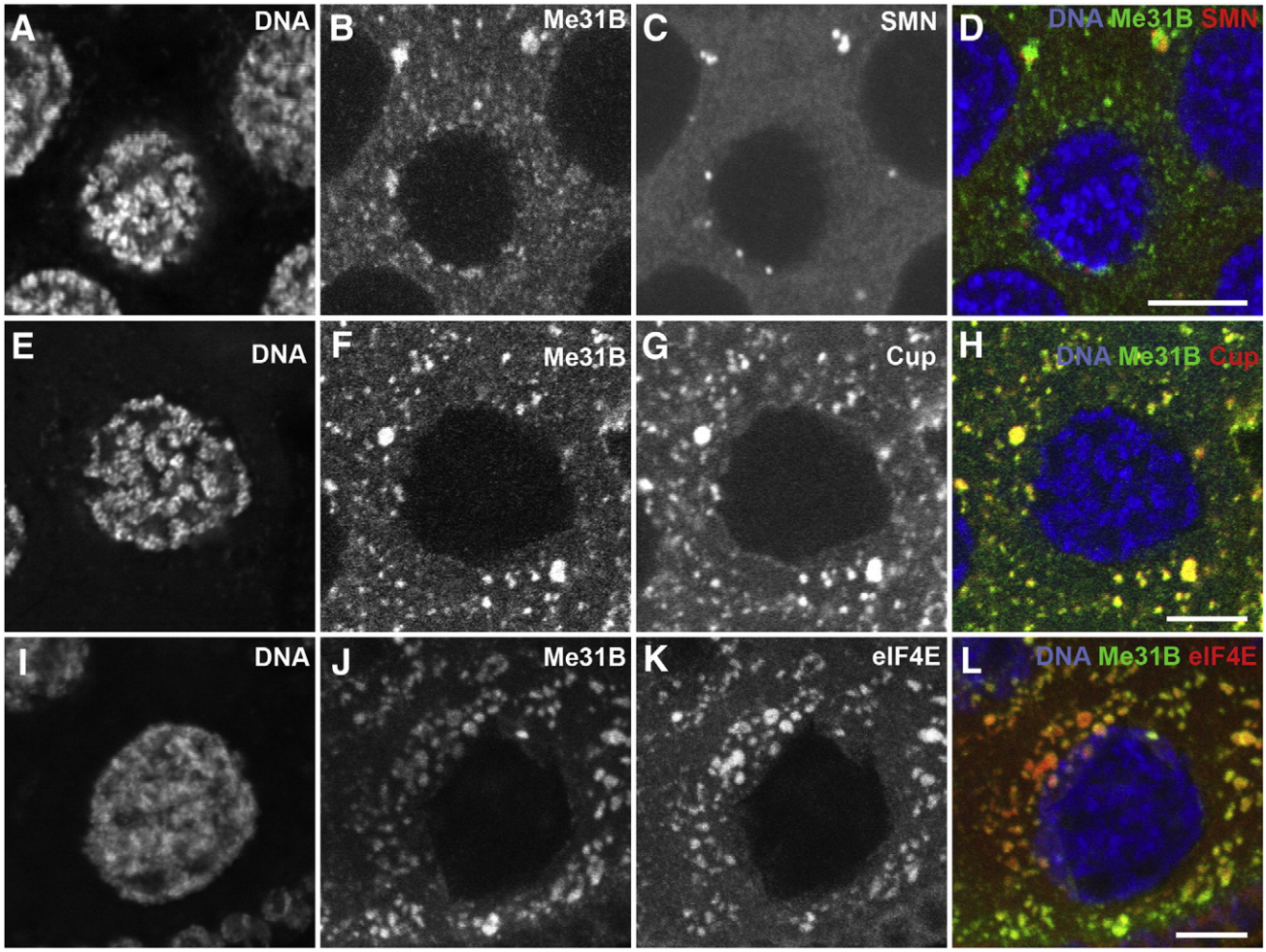

Fig. 4 – Me31B localizes to P bodies but not to U bodies. (A–D) Double labelling of Me31B and SMN in nurse cells. U bodies labelled by SMN are close to, but do not overlap, structures labelled by Me31B. (E–H) Double labelling of Me31B and Cup in nurse cells. Me31B precisely colocalises with Cup, a component of P bodies. (I–L) Double labelling of Me31B and eIF4E in nurse cells. Me31B precisely colocalises with eIF4E, another component of P bodies. Scale bars, 10 μm.

SMN (Figs. 4A–D) and Gemin3 (Fig. 2), although consistent with results in earlier studies [6,7], both SMN and Gemin3 were associated with Me31B-containing P bodies (Figs. 2D–H; Figs. 4A–D).

Gemin5 is enriched in U bodies

Gemin5 was identified as the factor that allows the SMN complex to specifically recognise and bind snRNAs, as well as being a probable mediator of Sm protein transfer onto snRNAs [47,48]. In *Drosophila*, a protein called Rigor Mortis (Rig) is the orthologue of human Gemin5. Rig was identified as a nuclear receptor cofactor that is required for ecdysone signalling during larval development [23]. For simplicity, we shall use the name Gemin5 for this protein hereafter.

We made use of an antibody directed against Gemin5 to investigate the localisation of Gemin5 in *Drosophila* egg chambers [23]. Staining of wild-type *Drosophila* egg chambers with anti-Gemin5 revealed several discrete foci that resembled the staining pattern of U bodies (Figs. 5A–C). To test whether *Drosophila* Gemin5 is a component of U bodies, we performed double-labelling experiments using egg chambers with a constitutive expression of Lsm11-YFP or ECFP-Gemin3. Our results show that the anti-Gemin5 antibody stains U bodies labelled either by Lsm11-YFP (Figs. 5D–F) or by ECFP-Gemin3 (Figs. 5G–I) in germline cells. The staining of Gemin5 in U bodies is specific as the Gemin5 signal is weak in follicle cells even when ECFP-Gemin3 is overexpressed (Figs. 5G–I). Double labelling with the P body marker Me31B confirms that all U bodies stained by Gemin5 associate with P bodies (Fig. 6).

Gemin2 is a component of U bodies

Gemin2, formerly SMN-interacting protein 1 (SIP1), was the first identified binding partner of SMN [49,50]. Gene silencing of *gemin2* in *C. elegans*, *X. laevis* and zebrafish as well as homozygous knockdown in mice leads to embryonic lethality, thus demonstrating that *gemin2* is an essential gene [51–53]. Ogawa et al. [54] recently reported that Gemin2 stabilises the self-association of human SMN, and hence Gemin2 knockdown in HeLa cells decreased SMN oligomer formation and consequently U snRNP assembly. In this context, Gemin2 is predicted to be a critical player in the SMN complex. To study the subcellular localization of Gemin2 in *Drosophila* egg chambers, we generated a polyclonal

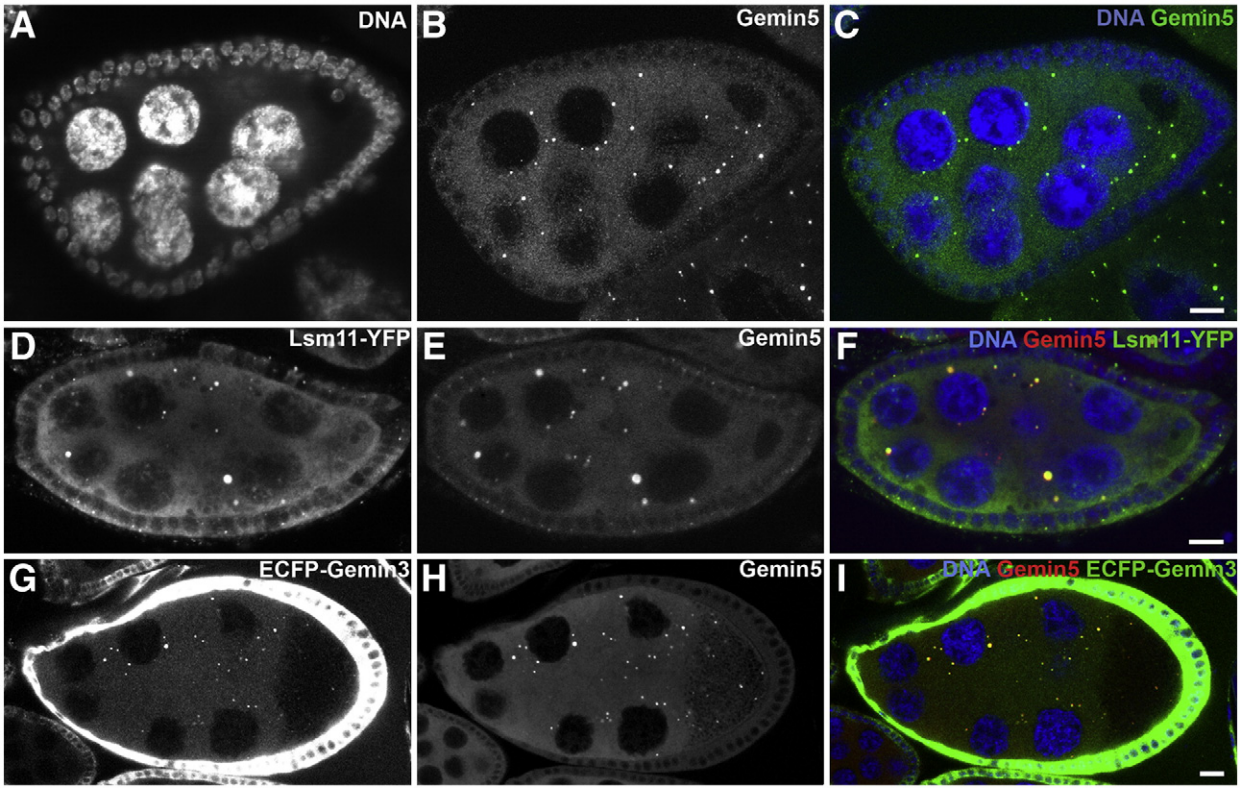

Fig. 5 – Gemin5 localises to U bodies. (A–C) *Drosophila* wild-type egg chamber stained with anti-Gemin5 antibody. Gemin5 is enriched in several discrete spherical foci in the cytoplasm of germline cells. (D–F) Gemin5 localises to U bodies labelled by Lsm11-YFP. (G–I) Antibody against Gemin5 stains cytoplasmic foci in the germline cells formed by constitutively expressing ECFP-Gemin3. Note that Gemin5 staining is weak in follicle cells even when they overexpress ECFP-Gemin3. Scale bars, 10 μm.

antibody against *Drosophila* Gemin2. Using this antibody, we detected punctate structures in wild-type *Drosophila* egg chambers (Figs. 7A–C). To determine whether these Gemin2 positive structures are U bodies, we stained egg chambers expressing Lsm11-YFP, a marker for U bodies, with Gemin2 antibody and found that Gemin2 colocalises with Lsm11-YFP in the cytoplasm of nurse cells and oocytes (Figs. 7D–G). Staining with the pre-immune serum from the same animal, which produced the Gemin2 antibody, showed no signal in U bodies, indicating that the staining of Gemin2 in U bodies is specific (Figs. 7H–K). In addition, a double staining of wild-type egg chambers with antibodies against SMN and Gemin2 showed that SMN and Gemin2 colocalise in U bodies (Figs. 7L–O), while pre-Gemin2 serum gave no signal at all (Figs. 7P–S). Taken together, we demonstrate that Gemin2 is a *bona fide* component of U bodies.

Discussion

In addition to SMN, only Gemin2, Gemin3 and Gemin5 among the vertebrate SMN complex have obvious orthologues in *Drosophila*. *Drosophila smn* and *gemin3* loss-of-function mutants are lethal before pupation, and develop loss of mobility and aberrant neuromuscular junctions before death [12,14,55]. Hypomorphic *smn* mutant flies are viable but have reduced SMN protein levels in the adult thorax causing impaired flight, disorganised muscle fibres and defects in innervating flight muscles [56]. Similarly, expression of a dominant-negative Gemin3 transgene in developing muscles leads to loss of flight and flight muscle degeneration [12]. *Drosophila* Gemin5 was identified as a nuclear receptor interacting protein that is required for ecdysone response during larval development [23]. *Drosophila* Gemin2 remains uncharacterised.

We demonstrate the *in vivo* enrichment of the entire *Drosophila* SMN complex within the U snRNP-rich U bodies. Our evidence suggests that Gemin2, Gemin3 and Gemin5 are core members of the *Drosophila* SMN complex based on their tight co-localisation with the subcellular location of SMN within the *Drosophila* egg chamber. This view is consistent with several reported experiments in higher eukaryotes [48,50,57–59] but it contrasts with that proposed by Kroiss et al. [11] who argued in favour of a simple *Drosophila* SMN complex consisting of only SMN and Gemin2. The differences between our results and those by Kroiss et al. can be explained by the use of different experimental systems, an *in vivo* system in our case and a Schneider2 cell culture system in the case of Kroiss et al. [11]. The presence of the entire SMN complex in U bodies supports the possibility that these cytoplasmic structures are related to snRNP assembly and/or storage (Fig. 8). Although Gemin5 was recently identified as the snRNA recognition and binding component of the SMN complex [47], the specific roles of Gemin2 and Gemin3 in U snRNP assembly remain elusive.

During *Drosophila* oogenesis, nurse cell-derived mRNAs are usually found in large mRNP granules, known as P bodies, where they are repressed and transported to different locations in the oocyte [46]. U bodies invariably associate with P granules within the *Drosophila* egg chambers suggesting that crosstalk between these two organelles might ensure the proper transport of

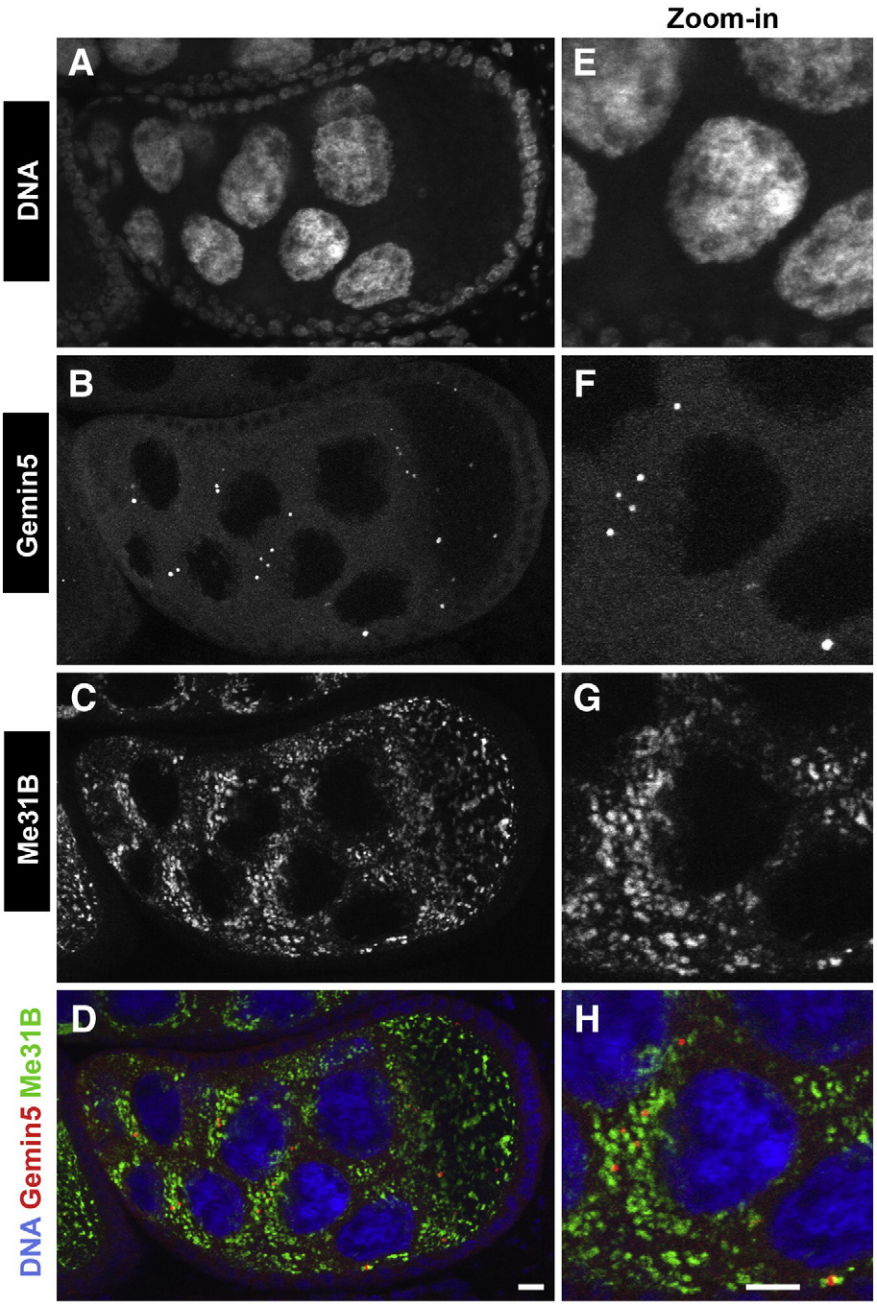

Fig. 6 – Localisation of Gemin5 and Me31B in a *Drosophila* egg chamber. DNA is stained with Hoechst 33342 (white in A, E and blue in D, H). Gemin5 (B, F) and Me31B (C, G) show distinct localisation in nurse cells and the oocyte, which locates at the posterior end (to the right in A–D). (D, H) U bodies stained by Gemin5 (red) are close to, but do not overlap P bodies enriched with Me31B (green). (E–H) Magnified images of A–D. Scale bars, 10 μm.

U snRNPs to the nucleus [6]. Alternatively, in addition to U snRNPs, the SMN complexes resident in U bodies might also be involved in mRNP assembly and/or in mRNP transport towards the maturing oocyte. The possibility that the SMN complex might assemble other RNPs is not remote though the target RNPs remain poorly defined (reviewed in [60]). Complexes formed from SMN, Gemin2 and Gemin3 were shown to be actively transported into neuronal processes and growth cones in cultured neurons of higher eukaryotes [61]. Importantly, in a recent study, Penta et al. [62] demonstrate the presence of SMN in a novel axonal and dendritic mRNP complex. Similar to reports indicating their association with P body components in higher eukaryotes [16,18–20,63], we find that whilst present exclusively in U bodies within *Drosophila* egg chambers, Gemin3 and Gemin5 associate with P bodies. Furthermore, the subcellular distribution patterns of Me31B and Gemin3 are entirely distinct, consistent with our phylogenetic evidence that Gemin3 is not the *Drosophila* orthologue of Dhh1 (Fig. 1). However it is interesting to note that each of the closely associated

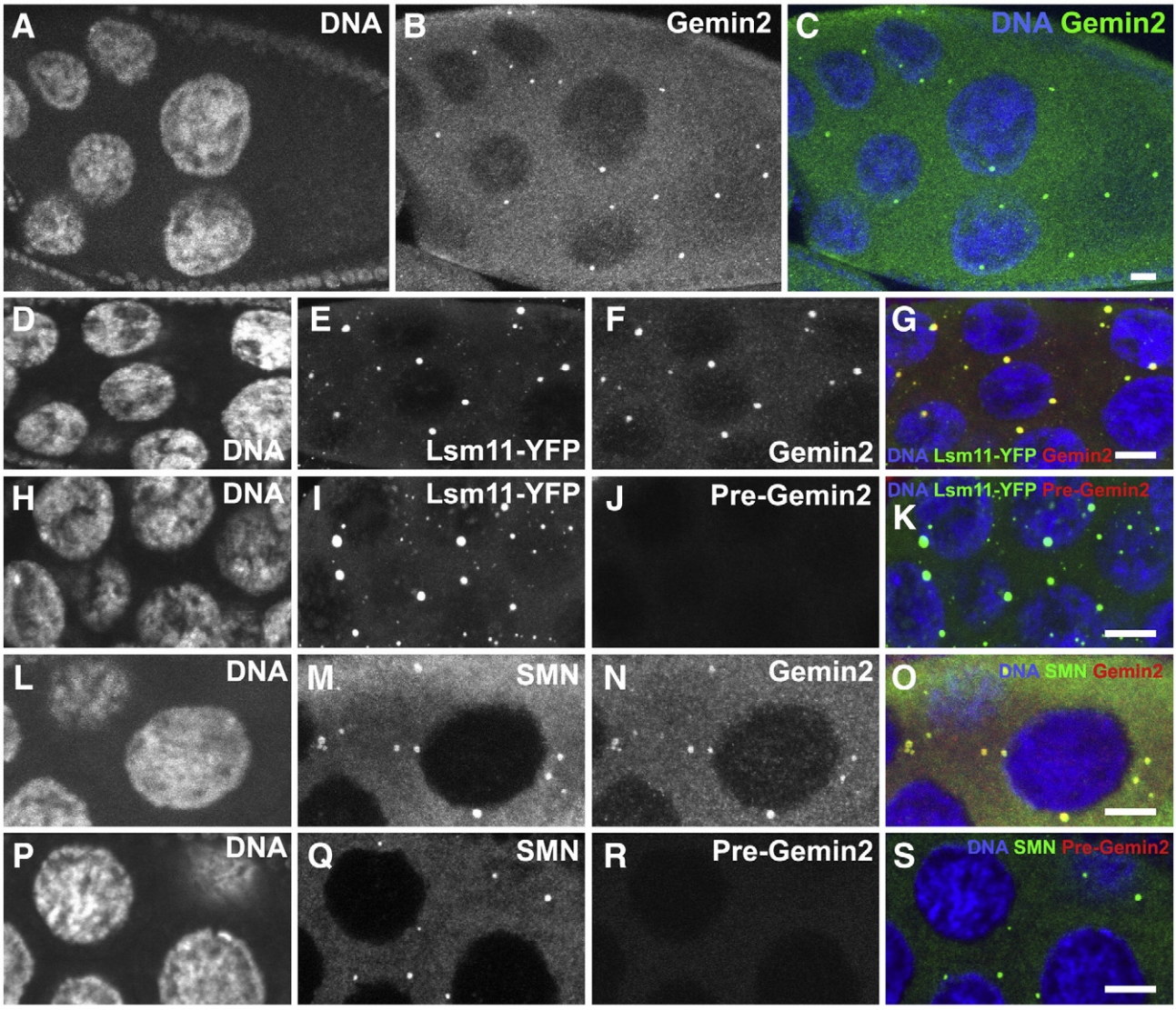

Fig. 7 – Gemin2 localises to U bodies. (A–C) *Drosophila* wild-type egg chamber stained with anti-Gemin2 antibody. Gemin2 is enriched in several discrete spherical foci in the cytoplasm of germline cells. (D–G) Antibody against Gemin2 stains U bodies labelled by Lsm11-YFP. (H–K) Pre-immune serum from the same animal in which the Gemin2 antibody was raised (pre-Gemin2) does not stain U bodies labelled by Lsm11-YFP. (L–O) In a wild-type egg chamber, a mouse antibody against SMN, a component of U bodies, stains the same cytoplasmic foci labelled by a rabbit polyclonal antibody against Gemin2. (P–S) Pre-Gemin2 shows no signal while SMN stains U bodies in a wild-type egg chamber. Scale bars, 10 μm.

U bodies and P bodies can now be seen to host a specific DEAD-box RNA helicase, pointing to a theme of RNA metabolism for the U body–P body pathway [7].

Conclusion

This study confirms that Me31B is the *Drosophila* orthologue of vertebrate and yeast Dhh1, which belongs to the DDX6 subfamily of DEAD-box RNA helicases. *Drosophila* and human Gemin3 are members of the DDX20 subfamily. While Me31B is a component of P bodies, all known *Drosophila* SMN complex proteins, i.e. SMN, Gemin2, Gemin3, and Gemin5, localize in U bodies. In *Drosophila* germline cells, U bodies associate with P bodies. Two RNA helicases, Gemin3 and Me31B, reside in two separate but related organelles in *Drosophila* germline cells, providing a fortuitous opportunity to study RNA compartmentalization in a model organism.

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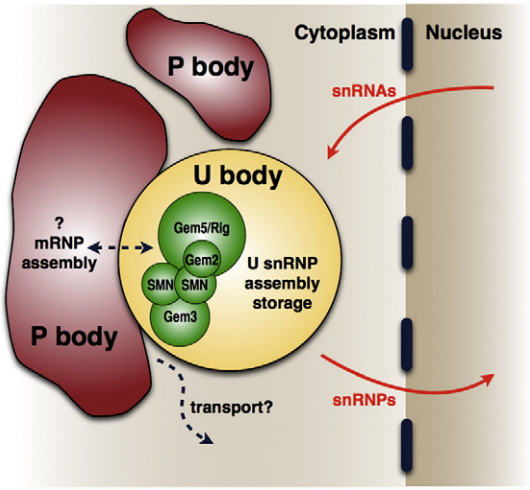

Fig. 8 – A model for the spatial relationship of U and P bodies. Components of the SMN complex including SMN, Gemin2, Gemin3, and Gemin5/Rig are enriched in U bodies, which may in turn affect various functions of P bodies such as assembly and transport of mRNPs.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.yexcr.2010.05.001.

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