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Short Communication

Gem formation upon constitutive Gemin3 overexpression in *Drosophila*

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Abstract

Gems or 'Gemini of Cajal bodies' are spherical nuclear aggregates of SMN (survival of motor neurons) complexes that frequently overlap Cajal bodies. Although described and characterized in mammalian tissues, gems have not been reported in invertebrates. Stimulation of gem formation in the fruitfly *Drosophila melanogaster* was investigated through the constitutive overexpression of a fluorescently tagged transgene of DEAD-box SMN complex member, Gemin3, in wild-type tissues. Although expression was predominantly cytoplasmic in the larval brain cells, Gemin3 was found enriched in multiple discrete bright foci in the nuclei of several tissues including epidermis, muscle and gut. Similar to their mammalian counterparts, *Drosophila* gems contained endogenous SMN and at times overlapped with Cajal bodies. These findings support the hypothesis that gems are storage sites for excess nuclear SMN complexes and their frequent association with Cajal bodies might imply recruitment for nuclear ribonucleoprotein assembly reactions.

Keywords: Cajal body; *Drosophila*; Gemin3; gem; spinal muscular atrophy; survival of motor neurons

1. Introduction

First described more than a decade ago (Liu and Dreyfuss, 1996), gems or 'Gemini of Cajal bodies' are spherical nuclear concentrates of SMN (survival of motor neurons) complexes that frequently overlap partially or completely Cajal bodies (formerly 'coiled bodies'). The human SMN complex is composed of at least nine members including the name-giving member SMN, Gemins 2-8 and UNRIP (Unr-interacting protein), (Cauchi, 2010). The composition of the SMN complex varies across species with the human version being the most complex and that described in the yeast *Schizosaccharomyces pombe* (composed of only SMN and Gemin2) being the most primitive (Cauchi, 2010; Kroiss et al., 2008). The identification of SMN as the determining factor for SMA (spinal muscular atrophy), a recessively inherited neuromuscular degenerative disorder, led to a large array of works aimed at deciphering the function of SMN. In this respect, assembly of UsnRNPs [uridine-rich small nuclear RNPs (ribonucleoproteins)], which form the building blocks of the spliceosome, remains by far the best-described role for the SMN complex. Nonetheless, recent years saw a surge in reports describing novel and hence non-canonical functions for the SMN complex in neuromuscular tissues, which could explain the elusive tissue specificity of SMA pathology (Briese et al., 2005; Burghes and Beattie, 2009; Cauchi, 2010; Simic, 2008).

Although first identified via immunofluorescence microscopy, immunoelectron microscopy later revealed that gems are devoid of Coilin and Fibrillarin (the signature markers of Cajal bodies) but are rich in SMN complexes to form round granular bodies of intermediate electron density (Malatesta et al., 2004; Navascues et al., 2004). Besides being detected in a small proportion of rapidly proliferating cells in culture (Carvalho et al., 1999), gems separate from Cajal bodies were described in fetal tissues although not adult tissues in which gems and Cajal bodies assumed a singular nuclear entity (Young et al., 2001; Young et al., 2000). Cajal bodies are universal structures which probably host several steps in the biogenesis of diverse RNP classes, and in this respect, the association with gems is thought to implicate a role for SMN complexes in the nuclear phases of RNA metabolism (Cauchi, 2010; Morris, 2008; Nizami et al., 2010).

Gems have so far been described only in mammalian tissues and never in invertebrates including the fruitfly *Drosophila melanogaster* (Cauchi, 2010). Despite co-localizing with Coilin and Fibrillarin in Cajal bodies, SMN has never been reported to form a separate structure in the *Drosophila* nucleus (Chang et al., 2008; Liu et al., 2006; Liu et al., 2009), hence leading to the assumption that gems may be absent in the fruitfly or in the Arthropoda phylum in general. Nevertheless, SMN complexes and UsnRNPs co-localize in discrete spherical bodies in the cytoplasm of fly (and mammalian) tissues (Cauchi et al., 2010; Lee et al., 2009; Liu and Gall, 2007). The jury is still out on the raison d'être of gems although one hypothesis revolves around the possibility that these nuclear bodies are storage sites for excess nuclear SMN complexes. Should this hypothesis be true, high levels of SMN complexes are thought to increase the formation of gems. Furthermore, in view of the stoichiometric nature of the SMN complex, an increase in just a single component is hypothesized to be enough to increase SMN complex concentration to levels necessary for inducing gem formation. In this context, aiming at stimulating the formation of the elusive *Drosophila* gem, an attempt at increasing the cellular levels of the SMN complex member Gemin3 was made through the constitutive overexpression of a functional fluorescently tagged transgene in wild-type flies via the GAL4-UAS (upstream activating sequence) bipartite

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Abbreviations: CFP, cyan fluorescent protein; SMA, spinal muscular atrophy; SMN, survival of motor neurons; UAS, upstream activating sequence; RNP, ribonucleoprotein; UsnRNP, uridine-rich small nuclear RNP.

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expression system (reviewed by Cauchi and van den Heuvel, 2006).

2. Materials and methods

2.1. Fly genetics

D. melanogaster stocks were cultured on standard molasses/maize meal and agar medium in plastic vials at 25°C. The wild-type fly strain was the *y w* stock. Synthesis of the *UAS·CFP* (cyan fluorescent protein)-*Gemin3* transgene was described previously (Cauchi et al., 2008) and its expression was driven constitutively by ubiquitously expressing *da-GAL4* or *1032-GAL4* drivers, the former obtained from the Bloomington *Drosophila* Stock Centre at Indiana University.

2.2. Immunofluorescence

Tissues were dissected in PBS, fixed in 4% paraformaldehyde in PBS and then washed in 1 × PBS+0.5% Triton X-100+0.3% (v/v) normal goat serum. The tissues were then subjected to overnight staining by primary antibodies and then stained overnight the following day with either anti-mouse or anti-rabbit Alexa Fluor®-conjugated secondary goat antibodies. Tissues were finally counterstained with Hoechst 33342 nuclear stain and Cy5-conjugated phallodin before washing and mounting. Zeiss LSM 510 META or Bio-Rad Radiance 2100 confocal microscopes were used for imaging tissues. Primary antibodies used include mouse anti-GFP (green fluorescent protein; Roche Diagnostics, West Sussex, U.K.), rabbit anti-Coilin (a gift from Joseph Gall, Carnegie Institution, Baltimore, MD, U.S.A.) and rabbit anti-SMN (a gift from Marcel van den Heuvel, University of Oxford, Oxford, U.K.). The original confocal images were processed using ImageJ software (National Institutes of Health, Bethesda, MD, U.S.A.).

3. Results

3.1. Constitutive Gemin3 overexpression stimulates gem formation in select tissues

Gemin3 is a DEAD-box RNA helicase implicated in a transcriptional and RNA silencing role in addition to its undefined involvement in UsnRNP biogenesis within the SMN complex (Cauchi, 2010). In *Drosophila*, Gemin3 was shown to have a motor function in addition to being required for development (Cauchi et al., 2008; Shpargel et al., 2009). When expression of a *CFP–Gemin3* transgene was driven in wild-type *Drosophila* larval brains, Gemin3 was found to be predominantly cytoplasmic, exhibiting a granular staining pattern (Figure 1A). Remarkably, expression of the epitope-tagged *Gemin3* transgene in additional tissues uncovered a different cellular localization pattern. In this regard, Gemin3 was enriched in a multitude of discrete bright puncta within the nucleus of larval body wall epidermal cells, whereas it adopted a diffuse low-level staining pattern in the cytoplasm (Figure 1B). The Gemin3-enriched nuclear bodies were of different sizes and were distributed evenly throughout the nucleoplasm. A similar expression pattern was observed in larval somatic muscles (Figure 1C). In these tissues, Gemin3 also aggregates in nuclear puncta of variable sizes; however, such structures were usually detected above a background of diffuse low-level nucleoplasmic staining. Cytoplasmic staining was either low or absent. The predominant nuclear staining as well as the bright nuclear puncta persisted through development and could be detected in wild-type adult flight muscles (Figure 1D). Interestingly, the Gemin3 localization pattern in *Drosophila* muscle is predominantly nucleoplasmic in contrast to that observed for SMN, which in addition to its nuclear presence was reported to co-localize with actin and α-actinin at the respective I-band and Z-line of the sarcomere (Liu et al., 2006; Rajendra et al., 2007).

3.2. Association of gems with Cajal bodies is conserved in *Drosophila*

To investigate whether the Gemin3-enriched nuclear puncta were actually the *Drosophila* counterparts of mammalian gems, immunofluorescence co-localization studies were undertaken. To this end, experiments focused on the large cells of the highly accessible gastric caeca, which are four blind-ended tubes that evaginate from the anterior midgut (Figure 2A). Constitutive expression of the CFP–Gemin3 fusion protein leads to the formation of several bright discrete foci ranging from small to large in the polyploid nuclei of gastric caecal cells (Figure 2B; see Supplementary Movie S1 available at http://www.cellbiolint.org/cbi/vvv/cbivvvppppadd.htm). Interestingly, some foci are cytoplasmic although they are always found strictly confined to the perinuclear zone. Notably, double-labelling experiments revealed co-localization of endogenous SMN with CFP–Gemin3, indicating that the discrete cellular bodies observed in caecal cells most probably host aggregates of an SMN–Gemin3 complex (Figure 2B). The relationship between the SMN–Gemin3 puncta and Cajal bodies was also scrutinized. Gut cells were recently reported to host more than one Coilin-positive Cajal body (Liu et al., 2009). On staining for Coilin, Cajal bodies in caecal cells were sometimes found overlapping and/or neighbouring the SMN–Gemin3 nuclear bodies (Figure 2C). In view of the presence of SMN and the relationship with Cajal bodies, the foci formed on constitutive up-regulation of Gemin3 are most probably the *Drosophila* counterparts of mammalian gems.

4. Discussion

Gems and Cajal bodies are kinetically autonomous nuclear structures (Dundr et al., 2004), although the formation and stability of gems is independent of Cajal bodies (Lemm et al., 2006). The findings reported here bring *Drosophila* on a par with vertebrates with respect to the presence of gems and strongly suggest that gems are probably storage depots of excess SMN complexes. An increase in the number of gems on overexpression of SMN has been reported previously in cultured mammalian cells (Hao le

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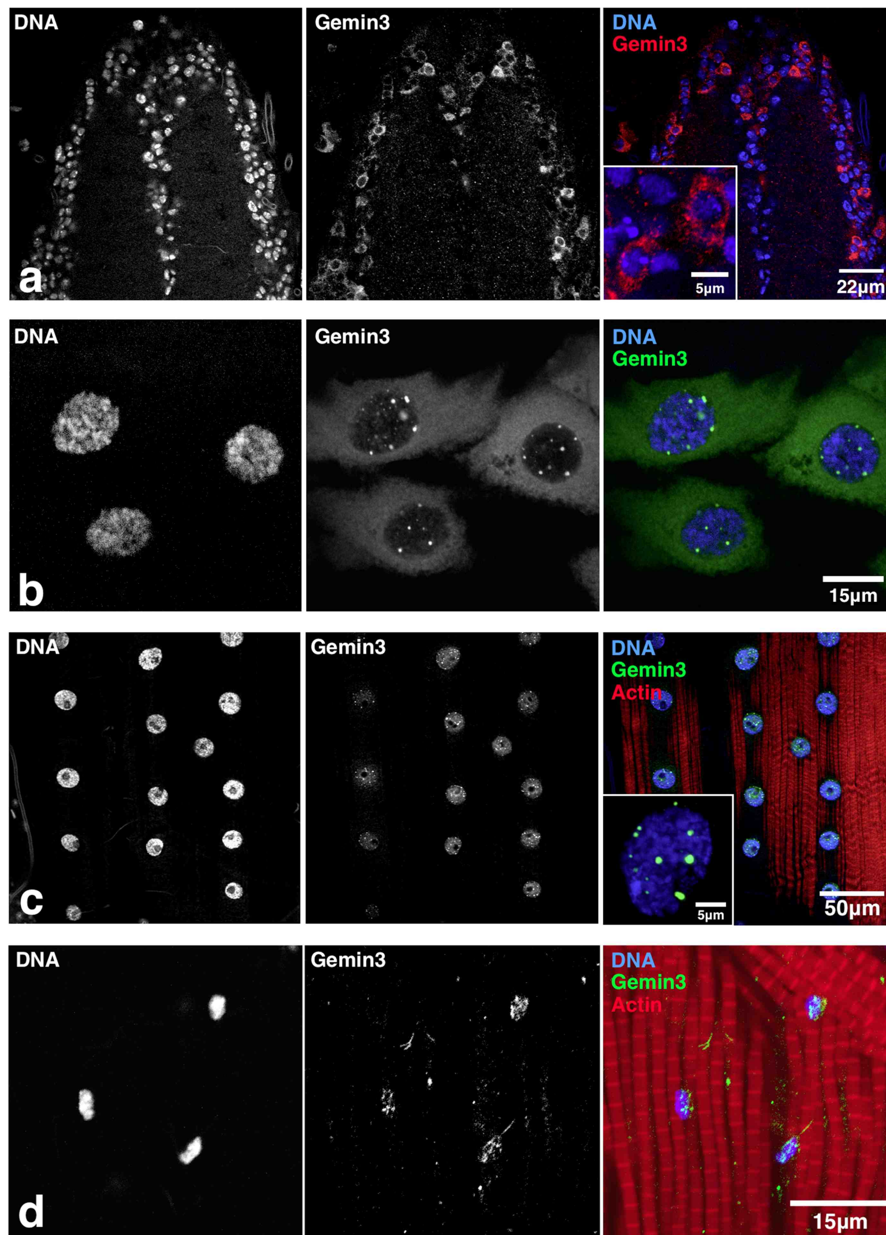

Figure 1 Gemin3 subcellular localization in *Drosophila* brain, epidermal and muscle tissues
(**A**) Motor neuron soma showing CFP–Gemin3 expression confined to the cytoplasm surrounding Hoechst-stained nuclei. The zoomed inset demonstrates that cytoplasmic staining has a granular pattern. (**B**) Constitutive overexpression of CFP-tagged Gemin3 in epidermal cells leads to a diffuse low-level cytoplasmic staining and multiple bright nuclear puncta of various sizes within the nucleoplasm. (**C**) On constitutive overexpression of fluorescently tagged *Gemin3* transgene in wild-type larval body wall muscles, Gemin3 is enriched in several bright nuclear foci. The inset shows a *Z*-stack projection of a typical muscle cell nucleus showing that the Gemin3-enriched bodies can assume a range of different sizes. (**D**) The predominantly nuclear CFP–Gemin3 staining is maintained in wild-type adult flight muscles and multiple discrete foci remain visible.

et al., 2007; Jarecki et al., 2005; Shpargel et al., 2003; Young et al., 2000) although this was not confirmed in other studies (Navascues et al., 2004; Pellizzoni et al., 1998). To the author's knowledge this study is, however, the first to assess gem dynamics on over-expression of a Gemin member of the SMN complex. Gem numbers are significantly reduced when SMN levels are depleted either via knockdown (Feng et al., 2005) or in cells from SMA patients (Coovert et al., 1997; Jarecki et al., 2005; Lefebvre et al., 1997), whereas only moderate or no significant effects were observed on knockdown of several Gemins (Feng et al., 2005).

The coupling of gems with Cajal bodies is probably influenced by several factors including the symmetrical dimethylation of the arginine- and glycine-rich SMN-interacting domain present on Coilin (Hebert et al., 2002). The conserved association of gems

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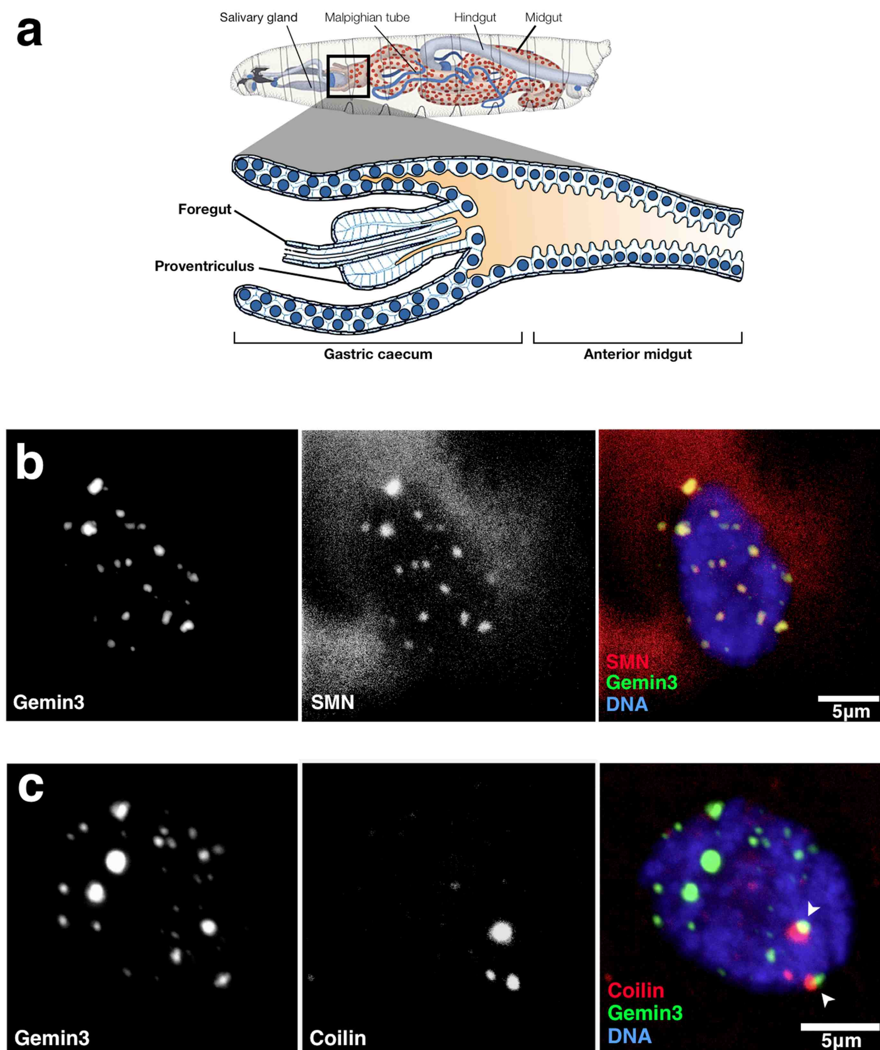

Figure 2 Formation of SMN-positive foci that associate with Cajal bodies on CFP–Gemin3 expression in gastric caecal cells
(**A**) The digestive system of the third instar *Drosophila* larva showing the location and anatomy of the foregut–midgut junction. Four thin blind sacs or gastric caeca (two of which are shown in this view) are located at the junction of the anterior midgut or 'stomach' with the proventiculus. Gastric caeca are thought to secrete digestive enzymes and aid in nutrient uptake (adapted from Hartenstein, 1993; Martin-Bermudo and Brown, 1999). (**B**) Expression of CFP–Gemin3 leads to the formation of several nuclear puncta that contain endogenous SMN as is evident from the degree of co-localization (yellow). Cytoplasmic foci strictly confined to the nuclear periphery are also evident. The image is a *Z*-stack projection of the entire nucleus of a typical gastric caecal cell. (**C**) CFP–Gemin3 puncta are sometimes found to overlap Cajal body structures recognized by the anti-Coilin antibody (arrowhead), hence exhibiting a relationship similar to that of mammalian gems. Images are *Z*-stack projections that span only a small portion of the nucleus (2.25 μm).

with Cajal bodies in *Drosophila* could imply the recruitment of SMN complexes for some late nuclear UsnRNP assembly step in view of the crucial involvement of SMN complexes in the cytoplasmic phase of the reaction and/or the ongoing recycling of UsnRNPs following their participation in pre-mRNA splicing.

Previous failure to detect gems separate from Cajal bodies on overexpression of SMN in *Drosophila* can be explained in light of recent evidence demonstrating that tight regulation of SMN expression is crucial for key developmental events (Grice and Liu, 2011). In this context, SMN excesses that aggregate in nuclear gems may simply not be tolerated on a cellular level. Interestingly, gem formation on constitutive Gemin3 expression occurred only in certain tissues and was absent in others including larval brains and salivary glands (results not shown) as well as ovaries (Cauchi et al., 2010). These results corroborate those by Young et al. (2000), who failed to detect gems (and Cajal bodies) in several mammalian tissues. It is possible that tissues in which gems were prominent fail to degrade excesses of Gemin3 protein or else they express specific factors that drive excess SMN complexes to the nuclear compartment. It is highly unlikely that the lack of gems in certain tissues is due to the lack of transgene expression since the constitutive GAL4 drivers used in this study are used extensively for

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ubiquitous transgene expression; however, it is possible that levels of transgene expression differ in different tissues.

5. Conclusions

The findings reported here extend the phylogenetic distribution of nuclear gems and raise the question as to whether gems are universal structures (subject to certain conditions) similar to their closely associated Cajal bodies. The presence of gems in *Drosophila* augurs well for future studies that exploit the vast genetic tools pertaining to this model organism to gain insights into the enigmatic function of these nuclear organelles.

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References

Briese M, Esmaeili B, Sattelle DB. Is spinal muscular atrophy the result of defects in motor neuron processes? BioEssays 2005;27:946–57.

Burghes AH, Beattie CE. Spinal muscular atrophy: why do low levels of survival motor neuron protein make motor neurons sick? Nat Rev Neurosci 2009;10:597–609.

Carvalho T, Almeida F, Calapez A, Lafarga M, Berciano MT, Carmo-Fonseca M. The spinal muscular atrophy disease gene product, SMN: a link between snRNP biogenesis and the Cajal (coiled) body. J Cell Biol 1999;147:715–28.

Cauchi RJ. SMN and Gemins: 'we are family' ... or are we?: insights into the partnership between Gemins and the spinal muscular atrophy disease protein SMN. BioEssays 2010;32:1077–89.

Cauchi RJ, Davies KE, Liu JL. A motor function for the DEAD-box RNA helicase, Gemin3, in *Drosophila*. PLoS Genet 2008;4:e1000265.

Cauchi RJ, Sanchez-Pulido L, Liu JL. *Drosophila* SMN complex proteins Gemin2, Gemin3, and Gemin5 are components of U bodies. Exp Cell Res 2010;316:2354–64.

Cauchi RJ, van den Heuvel M. The fly as a model for neurodegenerative diseases: is it worth the jump? Neurodegener Dis 2006;3:338–56.

Chang HC, Dimlich DN, Yokokura T, Mukherjee A, Kankel MW, Sen A et al. Modeling spinal muscular atrophy in *Drosophila*. PLoS ONE 2008;3:e3209.

Coovert DD, Le TT, McAndrew PE, Strasswimmer J, Crawford TO, Mendell JR et al. The survival motor neuron protein in spinal muscular atrophy. Hum Mol Genet 1997;6:1205–14.

Dundr M, Hebert MD, Karpova TS, Stanek D, Xu H, Shpargel KB et al. *In vivo* kinetics of Cajal body components. J Cell Biol 2004;164:831–42.

Feng W, Gubitz AK, Wan L, Battle DJ, Dostie J, Golembe TJ et al. Gemins modulate the expression and activity of the SMN complex. Hum Mol Genet 2005;14:1605–11.

Grice SJ, Liu JL. Survival motor neuron protein regulates stem cell division, proliferation, and differentiation in *Drosophila*. PLoS Genet 2011;7:e1002030.

Hao le T, Fuller HR, Lam le T, Le TT, Burghes AH, Morris GE. Absence of Gemin5 from SMN complexes in nuclear Cajal bodies. BMC Cell Biol 2007;8:28.

Hartenstein V. Atlas of *Drosophila* development. Cold Spring Harbor Laboratory Press. Plainview, NY: 1993.

Hebert MD, Shpargel KB, Ospina JK, Tucker KE, Matera AG. Coilin methylation regulates nuclear body formation. Dev Cell 2002;3:329–37.

Jarecki J, Chen X, Bernardino A, Coovert DD, Whitney M, Burghes AHM et al. Diverse small-molecule modulators of SMN expression found by high-throughput compound screening: early leads towards a therapeutic for spinal muscular atrophy. Hum Mol Genet 2005;14:2003–18.

Kroiss M, Schultz J, Wiesner J, Chari A, Sickmann A, Fischer U. Evolution of an RNP assembly system: a minimal SMN complex facilitates formation of UsnRNPs in *Drosophila melanogaster*. Proc Natl Acad Sci USA 2008;105:10045–50.

Lee L, Davies SE, Liu JL. The spinal muscular atrophy protein SMN affects *Drosophila* germline nuclear organization through the U body-P body pathway. Dev Biol 2009;332:142–55.

Lefebvre S, Burlet P, Liu Q, Bertrandy S, Clermont O, Munnich A et al. Correlation between severity and SMN protein level in spinal muscular atrophy. Nat Genet 1997;16:265–9.

Lemm I, Girard C, Kuhn AN, Watkins NJ, Schneider M, Bordonne R et al. Ongoing U snRNP biogenesis is required for the integrity of Cajal bodies. Mol Biol Cell 2006;17:3221–31.

Liu JL, Gall JG. U bodies are cytoplasmic structures that contain uridine-rich small nuclear ribonucleoproteins and associate with P bodies. Proc Natl Acad Sci USA 2007;104:11655–9.

Liu JL, Murphy C, Buszczak M, Clatterbuck S, Goodman R, Gall JG. The *Drosophila melanogaster* Cajal body. J Cell Biol 2006;172:875–84.

Liu JL, Wu Z, Nizami Z, Deryusheva S, Rajendra TK, Beumer KJ et al. Coilin is essential for Cajal body organization in *Drosophila melanogaster*. Mol Biol Cell 2009;20:1661–70.

Liu Q, Dreyfuss G. A novel nuclear structure containing the survival of motor neurons protein. EMBO J 1996;15:3555–65.

Malatesta M, Scassellati C, Meister G, Plottner O, Buhler D, Sowa G et al. Ultrastructural characterisation of a nuclear domain highly enriched in survival of motor neuron (SMN) protein. Exp Cell Res 2004;292:312–21.

Martin-Bermudo MD, Brown NH. Uncoupling integrin adhesion and signaling: the betaPS cytoplasmic domain is sufficient to regulate gene expression in the *Drosophila* embryo. Genes Dev 1999;13:729–39.

Morris GE. The Cajal body. Biochim Biophys Acta 2008;1783:2108–15.

Navascues J, Berciano MT, Tucker KE, Lafarga M, Matera AG. Targeting SMN to Cajal bodies and nuclear gems during neuritogenesis. Chromosoma 2004;112:398–409.

Nizami Z, Deryusheva S, Gall JG. The Cajal body and histone locus body. Cold Spring Harb Perspect Biol 2010;2:a000653.

Pellizzoni L, Kataoka N, Charroux B, Dreyfuss G. A novel function for SMN, the Spinal Muscular Atrophy disease gene product, in pre-mRNA splicing. Cell 1998;95:615–24.

Rajendra TK, Gonsalvez GB, Walker MP, Shpargel KB, Salz HK, Matera AG. A *Drosophila melanogaster* model of spinal muscular atrophy reveals a function for SMN in striated muscle. J Cell Biol 2007;176:831–41.

Shpargel KB, Ospina JK, Tucker KE, Matera AG, Hebert MD. Control of Cajal body number is mediated by the coilin C-terminus. J Cell Sci 2003;116:303–12.

Shpargel KB, Praveen K, Rajendra TK, Matera AG. Gemin3 is an essential gene required for larval motor function and pupation in *Drosophila*. Mol Biol Cell 2009;20:90–101.

Simic G. Pathogenesis of proximal autosomal recessive spinal muscular atrophy. Acta Neuropathol 2008;116:223–34.

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Young PJ, Le TT, Dunckley M, Nguyen TM, Burghes AH, Morris GE. Nuclear gems and Cajal (coiled) bodies in fetal tissues: nucleolar distribution of the spinal muscular atrophy protein, SMN. Exp Cell Res 2001;265:252–61.

Young PJ, Le TT, thi Man N, Burghes AH, Morris GE. The relationship between SMN, the spinal muscular atrophy protein, and nuclear coiled bodies in differentiated tissues and cultured cells. Exp Cell Res 2000;256:365–74.

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