U bodies are cytoplasmic structures that contain uridine-rich small nuclear ribonucleoproteins and associate with P bodies

Ji-Long Liu and Joseph G. Gall*

Department of Embryology, Carnegie Institution of Washington, 3520 San Martin Drive, Baltimore, MD 21218

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Uridine-rich small nuclear ribonucleoproteins (U snRNPs) are involved in key steps of pre-mRNA processing in the nucleus of eukaryotic cells. U snRNPs are enriched in the nucleus in discrete organelles that include speckles, Cajal bodies, and histone locus bodies. However, most U snRNPs are assembled in the cytoplasm, not in the nucleus. Despite extensive biochemical information, little is known about the spatial organization of U snRNPs in the cytoplasm. Here we show that U snRNPs in *Drosophila* are concentrated in discrete cytoplasmic structures, which we call U bodies, because they contain the major U snRNPs. In addition to snRNPs, U bodies contain essential snRNP assembly factors, suggesting that U bodies are sites for assembly or storage of snRNPs before their import into the nucleus. U bodies invariably associate with P bodies, which are involved in RNA surveillance and decay. Genetic disruption of P body components affects the organization of U bodies, suggesting that the two cytoplasmic bodies may cooperate in regulating aspects of snRNP metabolism. The identification of U bodies provides an opportunity to correlate specific biochemical steps of snRNP biogenesis with structural features of the cytoplasm.

Cajal body | histone locus body | *Drosophila* | SMN | snRNP

RNAs synthesized in the nucleus of eukaryotic cells almost invariably undergo processing before they are sent to their final destination. Processing can involve cleavage of a precursor molecule, removal of parts of the precursor, or modification of specific bases or sugars. The processing machinery consists of small RNAs (usually <300 nucleotides) associated with specific proteins. The U1, U2, U4/U6, and U5 small nuclear ribonucleoproteins (snRNPs) are major components of the spliceosome, the complex that removes introns from the majority of pre-mRNAs (1–3). The U7 snRNP specifically carries out 3′ cleavage of intronless histone pre-mRNAs (4, 5). Each snRNP contains a small RNA associated with a ring of seven Sm/Lsm proteins and one or more snRNP-specific proteins (4, 6, 7). As their name implies, snRNPs function in the nucleus, where they are enriched in discrete nuclear organelles that include speckles (8, 9), Cajal bodies (10, 11), and histone locus bodies (12). However, it has been known for a long time that most snRNPs are assembled in the cytoplasm (13), and properly assembled Sm cores are required for snRNP import into the nucleus (14). Nevertheless, little is known about the spatial organization of snRNPs in the cytoplasm.

We used the *Drosophila* ovary as a model system to investigate the organization of cellular snRNPs. The *Drosophila* ovary contains egg chambers in various stages of development, from the beginning of meiosis to the fully formed oocyte ready for fertilization and egg laying. Each egg chamber contains a developing oocyte and 15 associated nurse cells. For our purposes, the large size of the nurse cells and their polyploid nuclei facilitates study of the snRNPs by immunofluorescent staining and *in situ* hybridization. Ovarian development has been extensively studied both genetically and cytologically (15), providing a rich background of information and experimental procedures.

Results

We began our study with the U7 snRNP, which contains two unique Sm core proteins, dLsm10 and dLsm11 (4). To determine the localization of the U7 snRNP, we made transgenic flies that express yellow fluorescent protein (YFP) fusions of dLsm11. In somatic follicle cells of the ovary, YFP-dLsm11 was concentrated in one or two nuclear foci that colocalized with the histone gene cluster on chromosome 2. We called these foci the histone locus bodies (12). In the giant polyploid nuclei of the nurse cells, there were multiple histone locus bodies associated with multiple histone gene clusters. Surprisingly, we observed foci of even stronger labels in the cytoplasm of all cells, especially in the nurse cells and oocyte (Fig. 1*A*). To exclude the possibility that the cytoplasmic dLsm11 foci might be artifacts of overexpression, we made antibodies against dLsm10 and dLsm11 and stained ovaries from wild-type flies. Both antibodies stained nuclear and cytoplasmic foci in the same pattern as the YFP-dLsm11 foci in the transgenic flies (Fig. 1*B*). We then combined immunofluorescence and FISH to determine the localization of U7 snRNA. U7 snRNA displayed the same pattern as dLsm10/dLsm11 in both the nucleus and the cytoplasm (Fig. 1*C*), suggesting that both types of foci contain assembled U7 snRNPs.

FISH with antisense probes against *Drosophila* U1, U2, U4, U5, and U6 snRNA was used to examine the localization of spliceosomal snRNAs. Consistent with previous observations, spliceosomal snRNAs were enriched in one or more specific nuclear foci, the Cajal bodies, along with a lower level throughout the nucleus. Double FISH with a probe for U7 snRNA and any one of the spliceosomal snRNAs showed that Cajal bodies were physically distinct from the histone locus bodies, although the two structures were often in contact with each other (12). The situation in the cytoplasm was quite different. In this case, all of the snRNAs occurred together in the same cytoplasmic foci (Fig. 1 *E–H*), including U6 snRNA, which is not usually thought to transit between the nucleus and cytoplasm. As a control for the specificity of the FISH observations, we showed that the small Cajal body-specific RNA, U85 (16), is strictly limited to the Cajal body in the nucleus (Fig. 1*D*). An antibody that reacts with several core Sm proteins (mAb Y12) showed the same pattern as the spliceosomal snRNAs, again suggesting that the foci contain assembled snRNPs (Fig. 2*C*). We refer to these cytoplasmic foci of high U snRNP concentration as U bodies (cytoplasmic U snRNP bodies).

Because snRNP core proteins occur together with snRNAs in the U bodies, it is possible that U bodies represent sites where snRNPs are assembled and/or stored before transport to the

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*To whom correspondence should be addressed. E-mail: gall@ciwemb.edu.

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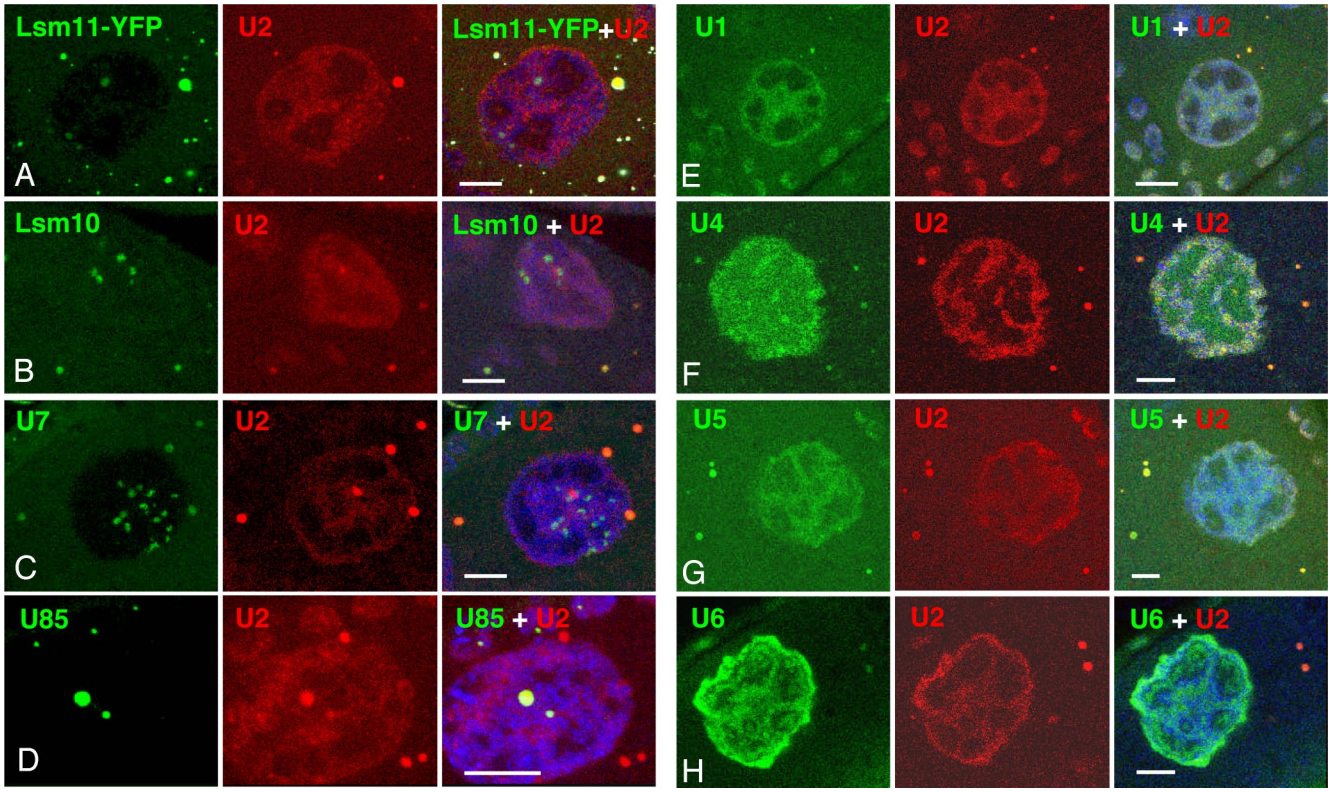

Fig. 1. U bodies contain U snRNPs. Nurse cells from the ovary of transgenic (*A*) and wild-type flies (*B–H*). (*Center*) FISH for U2 snRNA (red), which defines the cytoplasmic U bodies. (*Left*) Second probe (green). (*Right*) Overlay stained with DNA-specific DAPI (blue). (*A*) Lsm11-YFP expressed from a transgene. (*B*) Antibody stain for Lsm 10. (*C*) FISH for U7 snRNA. (*D*) FISH for U85 scaRNA, specific for the Cajal body in the nucleus. The three bright dots in *Upper Left* are Cajal bodies in three follicle nuclei. (*E–H*) FISH for U1, U4, U5, and U6 snRNAs. (Scale bars: 10 μm.)

nucleus. To explore this possibility, we examined the distribution of the survival of motor neurons (SMN) protein. SMN occurs in a complex that is required for efficient cytoplasmic assembly of the Sm core onto snRNAs (6, 17, 18). We asked whether SMN might be localized in U bodies by examining the distribution of YFP-SMN in transgenic flies and by staining with an antibody against SMN. Consistent with this idea, we observed SMN protein in one or a few discrete cytoplasmic foci, similar to U bodies. Double-labeling experiments with Lsm10, Lsm11, mAb Y12, and U2 snRNA confirmed that these foci of SMN concentration are, in fact, U bodies (Fig. 2 *A–D*). SMN also occurs diffusely throughout the cytoplasm.

The interaction between SMN and Sm proteins is enhanced by methylation, specifically by conversion of arginine residues to symmetrical dimethylarginine (sDMA) in the Sm proteins B/B′, D1, and D3 (19, 20). In *Drosophila*, this conversion is carried out primarily by *Drosophila* arginine methyl transferase (Dart) 5 and, to a lesser extent, by Dart 7. Flies homozygous for a piggyBac transposon insertion in the *dart5* gene show almost complete lack of sDMA in their Sm proteins (21). We examined U bodies in ovaries of heterozygous and homozygous *dart5* mutant flies. In heterozygotes, there were fewer U bodies in the nurse cells and oocytes than in wild-type flies. In homozygous *dart5* flies, U bodies were not detectable by staining for SMN or by *in situ* hybridization with probes against U snRNAs (Fig. 2 *E* and *F*). Concomitantly, homozygous *dart5* mutants displayed a dramatic loss of staining with mAb Y12, which is specific for sDMA modification [supporting information (SI) Fig. 5]. Our results suggest that U bodies may be related to specific steps in snRNP assembly, such as sDMA modification, or to the overall rate of assembly.

To determine the generality of U bodies, we extended our studies to other tissues. We found U bodies in all cell types that we examined, including brain, gut, and testis (SI Fig. 6). We observed similar structures in *Drosophila virilis* ovaries and in cultured human and *Xenopus* cells (SI Fig. 7). Given the fundamental role of snRNPs in RNA processing, it seems possible that U bodies are conserved structures in most, if not all, eukaryotic cells. We are aware of only one earlier study in which U bodies may have been observed. Zieve (22) showed that snRNP proteins are localized in punctate structures in the cytoplasm of cultured hamster fibroblasts, especially after hypertonic treatment. His study did not include an examination of snRNAs or other proteins involved in snRNP assembly.

We next asked whether U bodies are related to previously identified cytoplasmic organelles. Double-labeling experiments were performed with markers for the U body and markers for Golgi bodies, mitochondria, the endoplasmic reticulum (ER), centrioles, and P bodies (Fig. 3 and SI Fig. 8). U body markers do not strictly colocalize with markers for any of these structures. However, U bodies invariably associate with P bodies (Fig. 3 *A–C*) and the ER (SI Fig. 8*A*), and they are most abundant in regions of the cytoplasm that are rich in mitochondria (SI Fig. 8*C*).

Cytoplasmic P bodies contain numerous components involved in mRNA degradation and RNA silencing (both miRNA and siRNA pathways) (23–27). U bodies in the cytoplasm of *Drosophila* nurse cells and oocytes differ from P bodies in size, shape, and distribution. U bodies are discrete, more or less spherical structures, whereas P bodies have various shapes and often form large irregular aggregates in the cytoplasm (Fig. 3 *A–C*). The concentration of P bodies is higher in the oocyte than in its accompanying nurse cells, whereas U bodies tend to be evenly distributed between these two cell types. To determine the

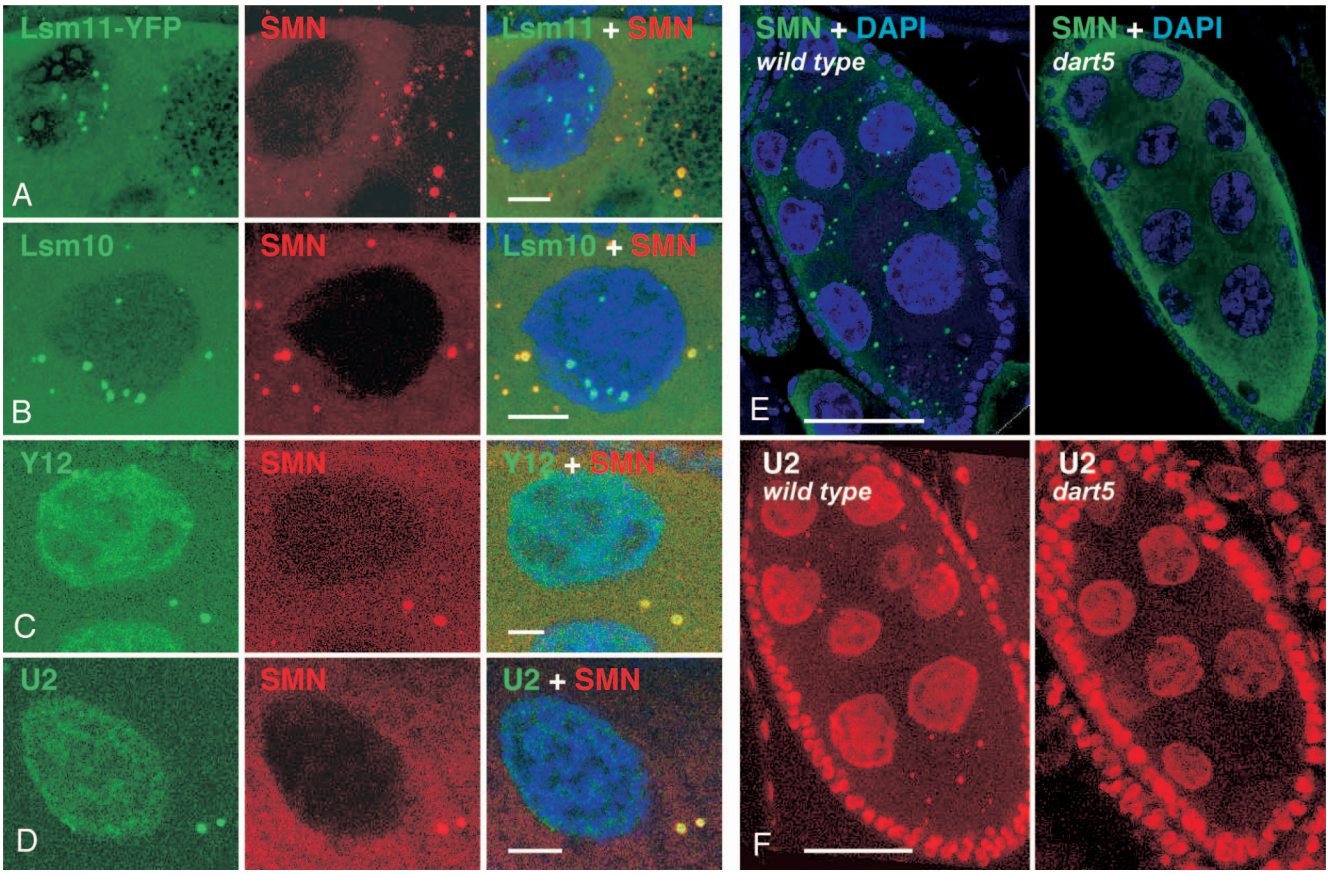

Fig. 2. U bodies and the snRNP assembly machinery. (*A–D*) Colocalization of SMN protein with U body components in nurse cells of transgenic (*A*) and wild-type (*B–D*) flies. (*Center*) Antibody staining for SMN (red). (*Left*) U body component (green). (*Right*) Overlay plus DAPI stain for DNA (blue). SMN staining is present in the nuclear Cajal bodies but is not visible without overexposing the intensely stained cytoplasmic U bodies. (*A*) Lsm11-YFP expressed from a transgene. (*B*) Antibody stain for Lsm10. (*C*) mAb Y12 stain, specific for symmetrical dimethylarginine residues. (*D*) FISH for U2 snRNA. (Scale bars: 10 μm.) (*E* and *F*) Comparison of egg chambers from wild-type females and *dart5* mutant females, which lack DART5, the major enzyme that methylates snRNP proteins. U bodies are detectable with an antibody against SMN or by FISH for U2 snRNA in wild-type flies (*Left*) but are missing from *dart5* mutant flies (*Right*). (Scale bars: 50 μm.)

relationship, if any, between U and P bodies, we performed double-labeling experiments by using various combinations of markers for the U and P bodies. We consistently observed that every U body is attached to one or more P bodies, but not every P body is associated with a U body (Figs. 3 *A–C* and 4). In many cases, several U and P bodies are joined together to form a string of alternating bodies.

These associations might simply be coincidental because both U and P bodies are associated with the ER (SI Fig 8*A*) (25). Alternatively, there may be a functional relationship between the two types of cytoplasmic structures. To explore this possibility, we examined the distribution of U bodies in ovaries of flies that were mutant for two P body components, Trailer Hitch (Tral) and Argonaute2 (Ago2). In *tral*^{1} ovaries, Tral protein is not detectable by immunoblotting (25). Other markers show that P bodies are still present, although they are somewhat smaller than in wild-type flies. In *tral*^{1} mutants, U bodies are reduced to one or a few unusually large foci (Fig. 3*E*) in stages 8–10 egg chambers, compared to those from *tral*^{1}/+ ovaries of the same stage (Fig. 3*D*). U bodies in ovaries of heterozygous *ago2* flies (*ago2*^{51B}/+) are distributed evenly in the nurse cells and the oocyte as they are in wild-type flies. However, in the homozygous mutant *ago2*^{51B}, we found few or no U bodies in the nurse cell cytoplasm, whereas U bodies were still clearly present in the ooplasm (SI Fig. 9). These observations suggest that the formation and/or organization of U bodies may depend on proper functioning of P bodies.

Discussion

Based on these data, we propose the following model, which incorporates some well known features of snRNP assembly (Fig. 4). U snRNAs are transcribed in the nucleus and exported to the cytoplasm, where they acquire a trimethylguanosine cap and associate with the Sm/Lsm proteins. U bodies may be sites for some steps in this assembly or they may serve primarily for storage of snRNPs after their assembly but before import into the nucleus. The close physical association between U and P bodies suggests a functional relationship between the two organelles. Possibly some steps in snRNP assembly require exchange of molecules between the two bodies. For instance, the Lsm1–Lsm7 complex might be assembled in the U body, but stored in the P body (28). Alternatively, the U body–P body association may reflect a feedback mechanism that maintains the proper flow of snRNPs to the nucleus. This feedback could involve regulated release of snRNPs from the U bodies depending on the rate of mRNA degradation in the P bodies, or snRNP assembly/storage in the U bodies might be balanced by snRNP degradation in the associated P bodies. Further biochemical and genetic studies will help to establish the precise role of U bodies in snRNP biogenesis and the relationship between U bodies and P bodies.

Materials and Methods

Fly Stocks. *Drosophila melanogaster* strains were maintained at 21–23°C on a standard cornmeal-based medium. A *y w* stock was

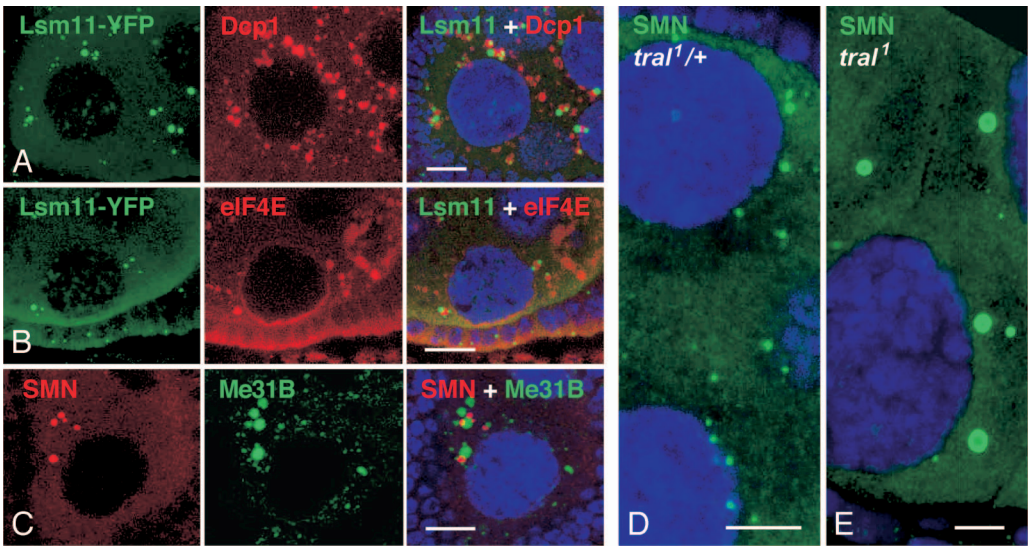

Fig. 3. The U body–P body relationship. (*A–C*) U bodies invariably associate with P bodies. (*Center*) Nurse cells stained with antibodies for three P body markers: Dcp1, eIF4E, and Me31B. (*Left*) U body markers. (*Right*) Overlay with DAPI stain for DNA (blue). (*A* and *B*) Lsm11-YFP expressed from a transgene (green). (*C*) Antibody staining for SMN (red). (Scale bars: 10 μm.) (*D* and *E*) Giant U bodies form in nurse cells of flies that lack Trailer Hitch protein, a P body component. (*D*) Typical U bodies from a heterozygous *tral*^{1}/+ fly. (*E*) Giant U bodies in *tral*^{1} flies, which lack Trailer Hitch protein. (Scale bars: 5 μm.)

used for wild-type control. Lsm11-YFP and SMN-YFP transgenic flies were generated as described previously (12). Other fly strains were as follows: protein traps Me31B-GFP (CB02302 and CB05282), eIF4E-GFP (CC00375), and Tral-GFP (CA06517) (29), provided by M. Buszczak and A. Spradling (Carnegie Institution of Washington); KDEL-GFP (30), provided by M. Lilly (National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD); *dart5* (21), provided by G. Matera (Case Western Reserve University, Cleveland, OH); *tral*^{1} (*tral*^{KG08052}) and Exu-GFP (25, 31), provided by J. Wilhelm (University of California at San Diego, La Jolla, CA); and *ago2*^{51B} (32), provided by F.-B. Gao (University of California, San Francisco, CA).

Cell Culture. HeLa cells were cultured at 37°C in DMEM supplemented with 10% FBS. *Xenopus laevis* kidney cells XLK-WG (ATTC #CRL-2527) were cultured at 32°C in RPMI medium 1640 supplemented with 20% FBS. Cells were seeded on round coverslips, which were then put in 24-well plates.

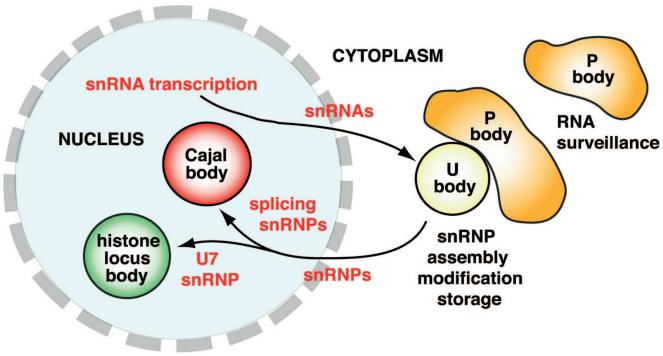

Fig. 4. Nuclear and cytoplasmic bodies involved in snRNP assembly. U snRNAs are transcribed in the nucleus and exported to the cytoplasm, where they form snRNP complexes with Sm and Lsm proteins. U bodies may be sites for assembly, modification, or storage of cytoplasmic snRNPs. On return to the nucleus, snRNPs target to Cajal bodies (splicing snRNPs) or the histone locus body (U7 snRNP). Cytoplasmic U bodies invariably associate with P bodies, which function in RNA surveillance and decay.

Antibodies. The following mouse mAbs were used: mAb Y12 against the Sm epitope (33), provided by J. Steitz (Yale University, New Haven, CT); mAb 2B1 against SMN (34), provided by G. Dreyfuss (University of Pennsylvania, Philadelphia, PA); mAb against γ-tubulin (Sigma–Aldrich, St. Louis, MO); mAb 1B1 against adducin-Hts (Developmental Studies Hybridoma Bank, Iowa City, Iowa); and mAb against complex 5-α (MitoSciences, Eugene, OR). The following polyclonal sera were used: rabbit anti-dLsm10 and dLsm11 (12); rabbit anti-dSMN (35), provided by J. Zhou (University of Massachusetts Medical School, Worcester, MA); rabbit anti-Tral, anti-eIF4E, and anti-Dcp1 (25), provided by J. Wilhelm; rabbit anti-Dcp1 and anti-Dcp2 (26), provided by T. B. Chou (National Taiwan University, Taipei, Republic of China); guinea pig anti-GW (27), provided by A. J. Simmonds (University of Alberta, Edmonton, AB, Canada); and rabbit anti-Lava Lamp (36), provided by J. Sisson (University of Texas, Austin, TX). Secondary antibodies were goat anti-mouse IgG, goat anti-rabbit IgG, and donkey anti-guinea pig IgG labeled with Alexa 488, 568, 594, or 633 (Invitrogen, Carlsbad, CA).

Tissue Preparation. Various tissues (ovary, testis, gut, brain, salivary gland) from *D. melanogaster* third instar larvae and adult flies were examined as whole mounts. Fresh tissues were isolated in Grace's insect medium (37) and fixed at room temperature for 10 min in 4% paraformaldehyde in PBS [135 mM NaCl, 2.5 mM KCl, 4.3 mM Na_2HPO_4, and 1.5 mM KH_2PO_4 (pH 7.2)]. After washing in PBS, samples were used for IF or FISH immediately. Alternatively, fixed samples were stored at 4°C for months in PBT (0.5% horse serum and 0.3% Triton X-100 in PBS) for immunostaining or at −20°C in hybridization mix for FISH. Human or *Xenopus* cultured cells were fixed at room temperature for 10 min in 4% paraformaldehyde in PBS, washed, and stored in PBS at 4°C.

Immunostaining. Whole mounts or cultured cells were stained with a primary antibody overnight, rinsed in PBS, and stained 4 h or overnight with a secondary antibody plus 0.5 μg/ml of the DNA-specific DAPI. To facilitate penetration of reagents into whole tissues, 0.3% Triton X-100 was included in all solutions. Tissues were rinsed in PBT and equilibrated for 2 h or longer in mounting solution (50% glycerol, 1 mg/ml 1,4-diaminobenzene)

before mounting under a coverslip on standard 3 × 1-inch glass slides.

FISH. Fluorescent RNA probes labeled with Alexa-488-UTP or Alexa-546-UTP were prepared by *in vitro* transcription from DNA clones, PCR products, or deoxy-oligonucleotides as described previously (12, 38). One or more probes were prepared for each specific RNA species. We used the following antisense RNA probes in this study (numbers indicate nucleotide positions in the RNA): U1 (1–162, full length), U1 (1–65), U1 (101–162), U2 (1–50), U2 (84–113), U4 (52–81), U5 (12–41), U5 (49–83), U6 (1–107, full length), U6 (1–62), U6 (58–107), U6 (33–62), U6 (70–99), U7 (1–71, full length), U7 (1–32), U7 (21–50), U7 (41–71), U85 (1–316, full length), U85 (56–85), U85 (188–258), U85 (176–240), and U85 (192–210). Probes were diluted in the following hybridization mix: 50% formamide, 5× SSC [1× SSC is 0.15 M NaCl + 0.15 M Na citrate (pH 7)], 10 mM citric acid, 50 μg/ml heparin, 500 μg/ml yeast tRNA, and 0.1% Tween 20. Tissues for *in situ* hybridization were incubated at 42°C for several hours or overnight depending on the probe size. In many cases, tissue was observed while still in the hybridization mix (with 1 μg/ml DAPI). Otherwise tissues were rinsed in PBS and mounted in 50% glycerol-mounting solution. To detect protein and RNA in the same sample, FISH was carried out after immunostaining. After the tissue had been treated with the secondary antibody, it was postfixed for 5 min in 4% paraformaldehyde in PBS. It was then rinsed for 5 min in PBS and equilibrated for 10 min in hybridization mix. FISH was then performed as usual.

Fluorescence Microscopy. Images were taken with a ×40 (N.A. 1.25) or a ×63 (N.A. 1.40) Plan Apochromatic objective on a laser-scanning confocal microscope (NT or SP2; Leica, Exton, PA). Images were taken with the laser intensity and photomultiplier gain adjusted so that pixels in the region of interest were not saturated (glow-over display). In most cases, contrast and relative intensities of the green (Alexa 488), red (Alexa 546, 568, 594, or 633), and blue (DAPI) images were adjusted with Photoshop (Adobe Systems, Mountain View, CA).

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1. Steitz JA, Black DL, Gerke V, Parker KA, Kramer A, Frendeway D, Keller W (1988) in *Structure and Function of Major and Minor Small Nuclear Ribonucleoproteins*, ed Birnstiel ML (Springer, Berlin), pp 115–154.
2. Nilsen TW (2003) *BioEssays* 25:1147–1149.
3. Tycowski KT, Kolev NG, Conrad NK, Fok V, Steitz JA (2006) in *The RNA World*, eds Gesteland RF, Cech TR, Atkins JF (Cold Spring Harbor Lab Press, Cold Spring Harbor, NY), pp 327–368.
4. Schümperli D, Pillai RS (2004) *Cell Mol Life Sci* 61:2560–2570.
5. Marzluff WF (2005) *Curr Opin Cell Biol* 17:274–280.
6. Will CL, Lührmann R (2001) *Curr Opin Cell Biol* 13:290–301.
7. Bertrand E, Bordonné R (2004) *Prog Mol Subcell Biol* 35:79–97.
8. Huang S, Spector DL (1992) *Proc Natl Acad Sci USA* 89:305–308.
9. Misteli T, Cáceres JF, Spector DL (1997) *Nature* 387:523–527.
10. Carmo-Fonseca M, Tollervey D, Barabino SML, Merdes A, Brunner C, Zamore PD, Green MR, Hurt E, Lamond AI (1991) *EMBO J* 10:195–206.
11. Gall JG (2000) *Annu Rev Cell Dev Biol* 16:273–300.
12. Liu JL, Murphy C, Buszczak M, Clatterbuck S, Goodman R, Gall JG (2006) *J Cell Biol* 172:875–884.
13. Mattaj IW (1988) in *Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles*, ed Birnstiel ML (Springer, Berlin), pp 100–114.
14. Narayanan U, Achsel T, Lührmann R, Matera AG (2004) *Mol Cell* 16:223–234.
15. Spradling AC (1993) in *The Development of Drosophila melanogaster*, eds Bate M, Martinez-Arias A (Cold Spring Harbor Lab Press, Cold Spring Harbor, NY), pp 1–70.
16. Darzacq X, Jády BE, Verheggen C, Kiss AM, Bertrand E, Kiss T (2002) *EMBO J* 21:2746–2756.
17. Meister G, Eggert C, Fischer U (2002) *Trends Cell Biol* 12:472–478.
18. Paushkin S, Gubitz AK, Massenet S, Dreyfuss G (2002) *Curr Opin Cell Biol* 14:305–312.
19. Brahms H, Meheus L, de Brabandere V, Fischer U, Lührmann R (2001) *RNA* 7:1531–1542.
20. Brahms H, Raymackers J, Union A, de Keyser F, Meheus L, Lührmann R (2000) *J Biol Chem* 275:17122–17129.
21. Gonsalvez GB, Rajendra TK, Tian L, Matera AG (2006) *Curr Biol* 16:1077–1089.
22. Zieve GW (1999) *Exp Cell Res* 247:249–256.
23. Eulalio A, Behm-Ansmant I, Izaurralde E (2007) *Nat Rev Mol Cell Biol* 8:9–22.
24. Nakamura A, Amikura R, Hanyu K, Kobayashi S (2001) *Development* (*Cambridge*, *UK*) 128:3233–3242.
25. Wilhelm JE, Buszczak M, Sayles S (2005) *Dev Cell* 9:675–685.
26. Lin MD, Fan SJ, Hsu WS, Chou TB (2006) *Dev Cell* 10:601–613.
27. Schneider MD, Najand N, Chaker S, Pare JM, Haskins J, Hughes SC, Hobman TC, Locke J, Simmonds AJ (2006) *J Cell Biol* 174:349–358.
28. Ingelfinger D, Arndt-Jovin DJ, Lührmann R, Achsel T (2002) *RNA* 8:1489–1501.
29. Buszczak M, Paterno S, Lighthouse D, Bachman J, Plank J, Owen S, Skora A, Nystul T, Ohlstein B, Allen A, *et al.* (2007) *Genetics* 175:1505–1531.
30. Snapp EL, Iida T, Frescas D, Lippincott-Schwartz J, Lilly MA (2004) *Mol Biol Cell* 15:4512–4521.
31. Wilhelm JE, Hilton M, Amos Q, Henzel WJ (2003) *J Cell Biol* 163:1197–1204.
32. Xu K, Bogert BA, Li W, Su K, Lee A, Gao FB (2004) *Curr Biol* 14:1025–1034.
33. Lerner EA, Lerner MR, Janeway CA, Steitz JA (1981) *Proc Natl Acad Sci USA* 78:2737–2741.
34. Liu Q, Fischer U, Wang F, Dreyfuss G (1997) *Cell* 90:1013–1021.
35. Ilangovan R, Marshall WL, Hua Y, Zhou J (2003) *J Biol Chem* 278:30993–30999.
36. Sisson JC, Field C, Ventura R, Royou A, Sullivan W (2000) *J Cell Biol* 151:905–918.
37. Grace TD (1962) *Nature* 195:788–789.
38. Liu JL, Buszczak M, Gall JG (2006) *Chromosome Res* 14:465–475.