Small Nuclear Ribonucleoproteins and Heterogeneous Nuclear Ribonucleoproteins in the Amphibian Germinal Vesicle: Loops, Spheres, and Snurposomes

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Abstract. We have examined the distribution of snRNPs in the germinal vesicle (GV) of frogs and salamanders by immunofluorescent staining and in situ nucleic acid hybridization. The major snRNAs involved in pre-mRNA splicing (U1, U2, U4, U5, and U6) occur together in nearly all loops of the lampbrush chromosomes, and in hundreds to thousands of small granules (1–4 μm diameter) suspended in the nucleoplasm. The loops and granules also contain several antigens that are regularly associated with snRNAs or spliceosomes (the Sm antigen, U1- and U2-specific antigens, and the splicing factor SC35). A second type of granule, often distinguishable by morphology, contains only U1 snRNA and associated antigens. We propose the term "snurposome" to describe the granules that contain snRNPs ("snurps"). Those that contain only U1 snRNA are A snurposomes, whereas those that contain all the splicing snRNAs are B snurposomes.

GVs contain a third type of snRNP granule, which we call the C snurposome. C snurposomes range in size from <1 μm to giant structures >20 μm in diameter. Usually, although not invariably, they have B snurposomes on their surface. They may also contain from one to hundreds of inclusions. Because of their remarkably spherical shape, C snurposomes with their associated B snurposomes have long been referred to as spheres or sphere organelles. Most spheres are free in the nucleoplasm, but a few are attached to chromosomes at specific chromosome loci, the sphere organizers (SOs). The relationship of sphere organelles to other snRNP-containing structures in the GV is obscure.

We show by immunofluorescent staining that the lampbrush loops and B snurposomes also react with antibodies against heterogeneous nuclear ribonucleoproteins (hnRNPs). Transcription units on the loops are uniformly stained by anti-hnRNP and anti-snRNP antibodies, suggesting that nascent transcripts are associated with hnRNPs and snRNPs along their entire length, perhaps in the form of a unitary hnRNP/snRNP particle. That B snurposomes contain so many components involved in pre-mRNA packaging and processing suggests that they may serve as sites for assembly and storage of hnRNP/snRNP complexes destined for transport to the nascent transcripts on the lampbrush chromosome loops.

Because of its large size and ease of experimental manipulation, the amphibian oocyte has been useful for studies on small nuclear ribonucleoproteins (snRNPs).[1] In *Xenopus*, the total amount of snRNA in the germinal vesicle (GV) increases during stages 1 and 2 of oogenesis, levels off at the beginning of vitellogenesis, and remains constant thereafter, despite growth of the oocyte as a whole. The mature GV contains 4,000–20,000 times as much U1 and U2 snRNA as a somatic nucleus, enough to supply the cleavage nuclei of the embryo before resumption of snRNA synthesis at the mid-blastula transition (Zeller et al., 1983; Forbes et al., 1983; Fritz et al., 1984; Lund and Dahlberg, 1987). In addition to the store of snRNPs in the GV, the oocyte contains a large excess of snRNP proteins in the cytoplasm. When snRNA is injected into the cytoplasm, additional snRNP complexes are formed that then migrate into the GV (De Robertis et al., 1982; Forbes et al., 1983; Fritz et al., 1984).

The general features of snRNA synthesis and snRNP assembly in the oocyte are similar to those in somatic nuclei (see reviews by Dahlberg and Lund, 1988; Mattaj, 1988; Zieve and Sauterer, 1990). The snRNAs involved in splicing (U1, U2, U4, U5, and U6) are synthesized in the nucleus with an inverted monomethyl Gppp cap at the 5′ end, except for U6, which has a γ-monomethyl phosphate on the terminal

1. *Abbreviations used in this paper*: DAPI, 4′,6-diamidino-2-phenylindol; DIC, differential interference contrast; GV, germinal vesicle; hn, heterogeneous nucleus; sn, small nuclear; TU, transcription unit.

guanosine triphosphate (Singh and Reddy, 1989). Again except for U6, they are exported to the cytoplasm, where they form complexes with the Sm proteins and acquire the characteristic trimethylguanosine cap. They are then reimported into the GV. Nucleotide sequences essential for these steps have been identified by use of various deleted or modified snRNAs (Hamm and Mattaj, 1990; Hamm et al., 1990; Neuman de Vegvar and Dahlberg, 1990).

The splicing components of the GV are apparently in excess of the ongoing needs of the oocyte, or in any case can be recruited for splicing of exogenously supplied pre-mRNA (Wickens et al., 1980; Miller et al., 1982; Wickens and Gurdon, 1983). The whole system is remarkably resilient to experimental manipulation. Pan and Prives (1988, 1989) depleted *Xenopus* GVs of their endogenous U1 or U2 snRNA by injecting antisense deoxyribonucleotides, reestablished a population of U1 or U2 snRNPs by later injecting human snRNAs, and then demonstrated accurate splicing of SV-40 transcripts.

Despite the wealth of experimental studies on snRNPs and splicing in the oocyte, very little information is available concerning the localization of snRNPs within the GV or the cytological concomitants of splicing. Recently we used two mAbs to study the distribution of snRNPs in lampbrush chromosome preparations (spread GV contents) from oocytes of the newt *Notophthalmus viridescens* and the frog *Xenopus laevis* (Gall and Callan, 1989). The two mAbs were Y12, specific for the Sm epitope common to the major snRNPs (Lerner et al., 1981), and K121, specific for the trimethylguanosine cap (Krainer, 1988). Both antibodies stained the majority of lampbrush chromosome loops, several dozen organelles known as spheres, and hundreds to thousands of small granules in the nucleoplasm. These mAbs did not stain the multiple nucleoli, chromomeres, or certain specific loops.

Here we confirm this distribution of snRNPs by use of additional antibodies and by in situ hybridization with snRNA-specific probes. We distinguish three types of snRNP granules, which we designate A, B, and C snurposomes. The A snurposomes contain only U1 snRNA and associated antigens, whereas the Bs contain the five major splicing snRNAs (U1, U2, U4, U5, U6) and a variety of associated proteins. C snurposomes stain with mAbs Y12 and K121, and hence presumably contain snRNPs, but we have been unable to identify specific snRNAs in them by in situ hybridization. C snurposomes vary greatly in size and are usually associated with one or more B snurposomes. Large C snurposomes with their associated Bs have been known for many years as spheres or sphere organelles in the cytological literature (reviewed in Callan, 1986).

We have also examined the distribution of hnRNPs in the GV by immunofluorescence microscopy. As shown several times before, antibodies against known or presumed hnRNPs stain the majority of lampbrush chromosome loops (Sommerville et al., 1978; Sommerville, 1981; Martin and Okamura, 1981; Lacroix et al., 1985; Leser and Martin, 1987; Roth and Gall, 1987; Piñol-Roma et al., 1989; Roth et al., 1990). Here we show that they also stain B snurposomes, but not As and Cs.

Because B snurposomes and lampbrush chromosome loops share so many snRNP and hnRNP components, we suggest that some of the hnRNP/snRNP packaging and splicing machinery may be preassembled in B snurposomes before transport to the nascent transcripts.

Materials and Methods

GV Spreads

The general technique for spreading GV contents is described in Callan (1986), Macgregor and Varley (1988), and Gall et al. (1991). Here we give details of our most current procedure, which includes modifications essential for immunofluorescence studies on snRNP and hnRNP components. The basics remain the same: dissection of a GV in a suitable "isolation solution," removal of the nuclear envelope, transfer of the nuclear contents to a "spreading solution," and centrifugation onto a glass slide. A piece of ovary is removed from a newt or frog and placed in a salt solution (OR2) originally developed for culturing *Xenopus* oocytes (Wallace et al., 1973). In this solution oocytes remain in satisfactory condition for hours or days. A few oocytes are transferred to the isolation solution consisting of 83 mM KCl, 17 mM NaCl, 6.5 mM Na_2HPO_4, 3.5 mM KH_2PO_4, 1 mM $MgCl_2$, 1 mM DTT, pH 7.2. Under the dissecting microscope a GV is removed with two pairs of jeweler's forceps; it is then cleaned of yolk by pipetting up and down in a pipette whose inner diameter is ~ 0.8 mm, somewhat larger than the GV diameter. The envelope is removed, again with two pairs of forceps or one pair of forceps and a fine tungsten needle. In this solution, the nuclear contents of *Notophthalmus* and *Xenopus* will remain indefinitely as a relatively tough, gelatinous ball. The nuclear contents are next picked up with the same pipette, washed briefly in a dish of spreading solution and transferred to a well slide previously filled with the same solution. The spreading solution consists of 21 mM KCl, 4 mM NaCl, 1.6 mM Na_2HPO_4, 0.9 mM KH_2PO_4, 1 mM $MgCl_2$, 5 μM $CaCl_2$, 1 mM DTT, and 0.01% paraformaldehyde, pH 7.2. This solution differs in two important respects from that used in our earlier experiments (Gall et al., 1981). The first is inclusion of Mg^{2+}, which is essential for preservation of spheres and nucleoplasmic granules; the second is lowering of the paraformaldehyde concentration, which greatly improves adherence of the nuclear contents to the glass slide. After the nuclear contents have been transferred to the well slide, they are allowed to disperse for 30–60 min, or until the chromosomes are flat against the glass slide. The whole preparation is then centrifuged in a special holder in the rotor (model HS4, Sorvall Instruments, Norwalk, CT) at 4–5,000 rpm for 1 h. Before immunofluorescent staining, slides are treated in one of three ways: (*a*) they are transferred immediately to 70% ethanol and stored, (*b*) they are postfixed 1–2 h in 2% paraformaldehyde in PBS and are then transferred to 70% ethanol for storage, or (*c*) they are postfixed in 2% paraformaldehyde and are then washed and held in PBS. Some antibodies fail to stain after paraformaldehyde fixation, whereas others require it for maximal intensity. Similarly, some antibodies do better with 70% ethanol fixation, either alone or after paraformaldehyde. Some type of postfixation is required after centrifugation, since the nuclear contents are sensitive to nucleases and proteases that are often present in the antibody or blocking solutions.

Immunofluorescence

Preparations were treated for 10–15 min in a blocking solution consisting of 10% horse serum in PBS with 0.02% NaN_3 as preservative. The primary antibody was applied for ~ 1 h. mAbs were applied as undiluted culture medium from hybridoma lines, as ascites fluid diluted 1:100 or 1:200, or as purified mouse IgG diluted to 5–50 μg/ml in blocking solution. Rabbit and human polyclonal sera were usually diluted 1:100 to 1:500 in blocking solution. The secondary antibody consisted of fluorescein- or rhodamine-labeled, affinity-purified antibodies of appropriate specificity (Cappel Laboratories, Cochranville, PA); it was applied for 1 h. Preparations were mounted in 50% glycerol, 1 mg/ml phenylenediamine, 0.02% NaN_3. Except during observation, they were stored at −20°C. Antibodies used in this study are listed in Table I.

In Situ Hybridization with ^{3}H-labeled Probes

GV preparations were stored in 70% ethanol after postfixation in 2% paraformaldehyde for 1–2 h. Before hybridization they were dehydrated with 95% and 100% ethanol and then placed for several hours in xylene to remove the paraffin that originally held the plastic spreading chamber in

Table I. Antibodies Used for Immunofluorescence

Antibody	Specificity or reactivity	M_r of bands in immunoblots of GV proteins*
		kD
mAb Y12	Sm epitope of snRNPs (Lerner et al., 1981)	13, 17, 30, 50, 53, 59, 66, 75, 83 (X.l.)* 17, 19, 21, 57, 4 bands 67–73 (N.v.)
mAb K121	Trimethylguanosine cap of snRNAs (Krainer, 1988)	ND
Human serum 361	Immunoprecipitates U1 snRNP (Wassarman, D., unpublished)	46, 68, 125 (strong), 142 (X.1) 145 (N.v.)
Human serum U1AG	A, C, and 70K proteins of U1 snRNP (Pettersson et al., 1984)	68 (X.1.) 14, 22, 26 (strong), 142 (N.v.)
Human serum U2GA	Immunoprecipitates U2 snRNP (Bruzik and Steitz, 1990)	17 (strong), 86, 117, 167 (X.1.) 28, 43, 88 (N.v.)
mAb αSC35	Non-snRNP splicing factor SC35 (Fu and Maniatis, 1990)	42, 49, 85 (N.v.)
mAb iD2	A and B group hnRNPs (Leser et al., 1984)	Three to four bands between 35–40 (X.1.)
mAb 4D11	L hnRNP (Piñol-Roma et al., 1989)	60 (N.v.)
Mouse serum A1	Raised against fusion protein of *Xenopus* A1 hnRNP and *E. coli* β-gal (Kay, B., unpublished)	ND
mAb SE5	90-kD nuclear protein of *N. viridescens* (Roth and Gall, 1987)	90 (N.v.)
mAb 104	Phosphorylated epitope on 43-kD nuclear protein of *X. laevis* (Roth et al., 1990)	43 + smear >100 (X.1.)
Rabbit serum "penta"	Acetylated histone H4. Raised against A(GGK)$_5$GGC in which Ks are acetylated (Lin et al., 1989)	14 (N.v. and X.1.)

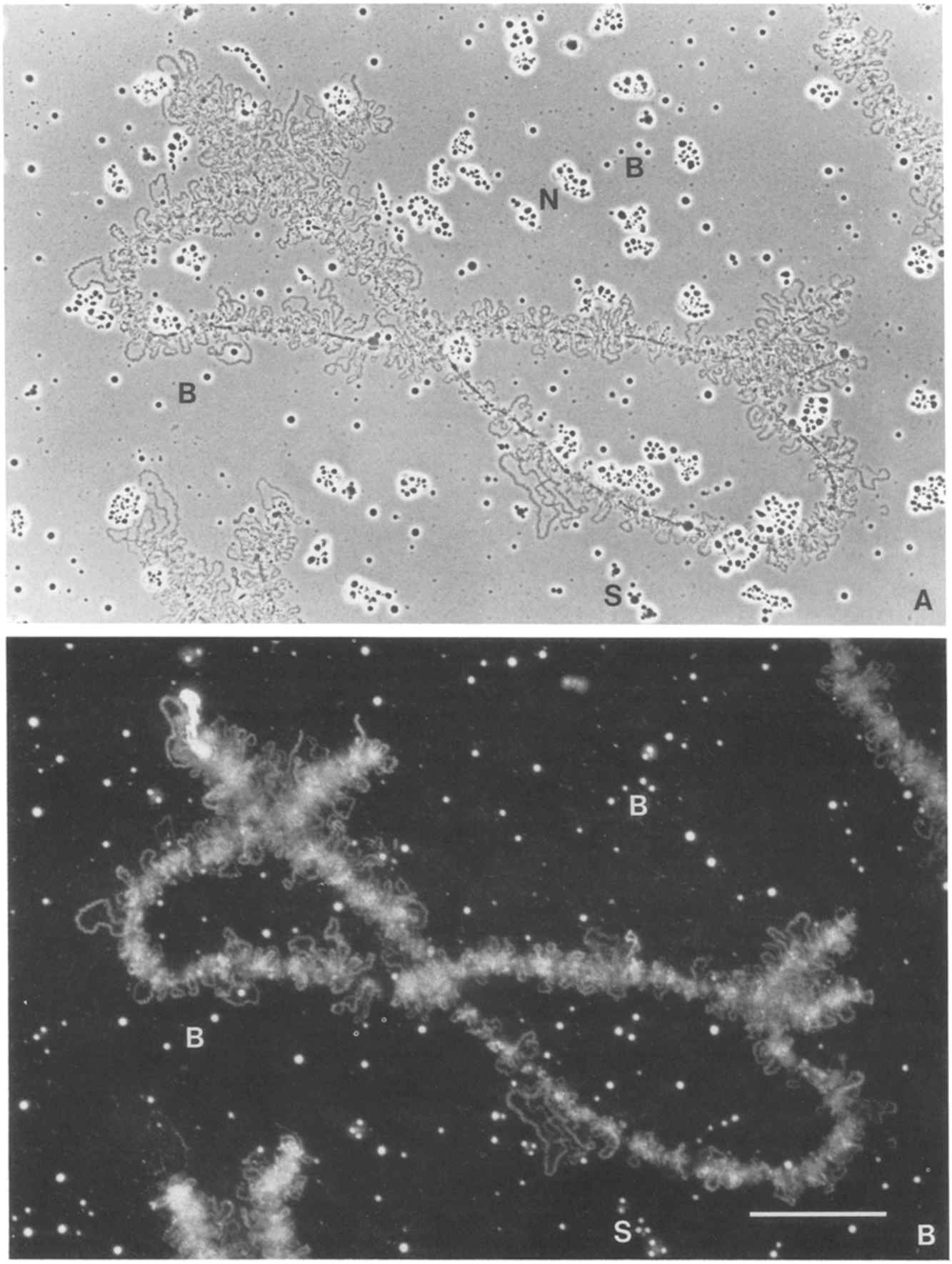
* X.1. = *Xenopus laevis*; N.v. = *Notophthalmus viridescens*.

place. They were then passed through 100% ethanol, acetone, and air dried. The hybridization solution (4–5 μl) consisted of 40% formamide, 4× SSC (SSC is 0.15 M NaCl, 0.015 M Na citrate, pH 7.0), 0.1 M PO_4^{3-}, 300 μg/ml *Escherichia coli* DNA, 300 μg/ml *E. coli* tRNA, and a ^{3}H-labeled probe. Probes were used at 5–15 × 10^4 cpm/μl. Counts were determined by spotting a small amount of probe on a nitrocellulose filter, washing in 5% TCA, rinsing in 70% ethanol, and counting in scintillation fluor (counter efficiency ~50% for ^{3}H in solution). Hybridization was carried

Table II. Plasmids Used for Synthesis of ^{3}H-labeled Riboprobes for In Situ Hybridization

snRNA	Plasmid	^{3}H-labeled riboprobe
U1	685-bp BamHI fragment of human U1 gene and flanking sequences cloned in pBluescript KS(+)	T3 transcript = antisense U1 T7 transcript = sense U1
U2	610-bp EcoRI/SalI fragment of human U2 gene and flanking sequences cloned in pBluescript KS(+)	T3 transcript = antisense U2 T7 transcript = sense U2
U3	179 bp (nucleotides 35–213) from *Xenopus laevis* U3 gene cloned in EcoRI/SmaI site of pBluescript KS(+)	T3 transcript = antisense U3 T7 transcript = sense U3
U4	90-bp oligonucleotide from U4 gene cloned in SacI/HindIII site of pSP65	SP6 transcript = antisense U4
U5	79-bp oligonucleotide from U5 gene cloned in SacI/BamHI site of pSP65	SP6 transcript = antisense U5
U6	342-bp BamHI/PstI fragment of mouse U6 gene and flanking sequences	T7 transcript = antisense U6 T3 transcript = sense U6

Clones U4-U6 are described in Black and Pinto (1989).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out overnight at 42°C. Preparations were rinsed in 0.1× SSC at 60°C for 1 h to remove unhybridized probe. NTB-2 liquid emulsion (Eastman Kodak Co., Rochester, NY) was used for autoradiography. In some cases they were also treated with RNase A (20 μg/ml) for 30 min at room temperature. ^{3}H-labeled probes consisted of T3, T7, or SP6 transcripts copied from linearized clones using ^{3}H-UTP (40–45 Ci/mmol). The specific activity of the probes was ~10^8 dpm/μg. Clones used in this study are listed in Table II.

Microscopy

Phase contrast, DIC (Nomarski), and fluorescence observations were made with a Zeiss Axiophot microscope. Filter combinations 09 and 14 were used for fluorescein and rhodamine respectively. Filter combination 17, which excludes rhodamine fluorescence from the fluorescein channel, was used for fluorescein in double-labeled specimens. Photographs were taken on sensitized Kodak Technical Pan Film (Microfluor, Stony Brook, NY) developed in D-19 for 12 min (effective ASA about 300 for white or green light, somewhat faster for red), or on Kodak Tri-X film developed with Diafine (effective ASA ~1,000 for both green and red). Confocal images were taken with a Zeiss confocal laser scan microscope LSM-10.

Results

(a) Morphology of Snurposomes and Spheres

A GV from a medium or large-sized oocyte of *Notophthalmus* or *Xenopus* contains, in addition to the lampbrush chromosomes and multiple nucleoli, several dozen spheres (for review, see Callan, 1986) and hundreds to thousands of smaller granules suspended in the nucleoplasm (Fig. 1). We recently showed that the spheres and granules contain snRNPs (Gall and Callan, 1989). We believe it would be useful to have a more descriptive term for these extrachromosomal structures that contain snRNPs, and we propose the term *snurposome* as one that emphasizes composition, but is neutral with respect to function. The first part of the term is laboratory jargon for snRNP (an otherwise unpronounceable acronym); the latter part is derived from the Greek *soma* (body), and is commonly used as a suffix for subcellular components.

In *Notophthalmus* GVs there are three types of snurposome, which we designate A, B, and C. Most of the free granules in the nucleoplasm with diameters in the range of 0.5 μm to ~4 μm are either As or Bs. Their maximum size and number vary from oocyte to oocyte, suggesting that physiological factors may influence the total amount of snurposome material. As and Bs can often be distinguished morphologically. The A snurposomes resemble miniature mammalian red blood cells, because their rim is of greater optical path than the center (Fig. 2, *A* and *F*; Fig. 3 *A*). Since they always appear circular in outline, they are probably not flattened, but instead are spherical with a low density core. B snurposomes are more or less homogeneous by DIC and phase contrast (Fig. 2, *A* and *F*; Fig. 3, *A*, *D*, and *F*). Generally they are larger than As and therefore more evident in low magnification views of GV spreads (Fig. 1). The morphological distinction just described is not always apparent, especially with smaller granules, so that A and B snurposomes are more easily told apart by their reactions to antibodies. The B snurposomes of *Xenopus* (Fig. 5 *A*) are similar to those of *Notophthalmus*, but we have not identified the *Xenopus* equivalent of A snurposomes.

C snurposomes present a more complicated situation, since they are variable in size and usually occur in association with B snurposomes. Large C snurposomes with their associated Bs (Fig. 4, *A–D*) have long been referred to in the cytological literature as spheres or sphere organelles, and we will continue that usage. However, Cs are not always associated with Bs, and they may be much smaller than the structures usually called spheres; in these cases, the new term C snurposome is helpful. The larger sphere bodies usually contain one to many roughly spherical inclusions (Figs. 4 and 5). When these are small and numerous, the sphere body has a finely granular texture when viewed by phase contrast or DIC microscopy (Fig. 4 *A*).

Most spheres occur free in the nucleoplasm, but a few are attached at one or more specific chromosomal loci called sphere organizers (SOs). Two such loci exist in *Notophthalmus* on chromosomes 2 and 6 (Gall, 1954; Gall et al., 1981; Callan, 1986) and three in *Xenopus* on chromosomes 8, 9, and 16 (Callan et al., 1987).

In *Notophthalmus* the attached spheres at the SOs are typically 7–10 μm in diameter, and the same is true for many of the free spheres. An occasional free sphere may have a diameter as great as 20 μm, especially in mature oocytes ready for ovulation (Fig. 4 *A*). Large spheres are easy to recognize morphologically because of the numerous B snurposomes on their surface. Small spheres (C snurposomes) associated with two or three granules are also easy to identify (Fig. 4 *E*; Fig. 5). However, still smaller C snurposomes exist, and some of these are not associated with Bs. The ability to recognize small C snurposomes became possible only when sera were found that stain them preferentially (Fig. 4, *E–H*). Many of the smaller C snurposomes are the same size as the B snurposomes with which they are associated (Fig. 4, *G* and *H*), and some are recognizable only as a small brightly staining dot on one side of a B snurposome. Although C snurposomes are commonly associated with more than one B snurposome, we have only rarely seen the converse, a B associated with two Cs.

(b) Antibody Studies

Spheres and Snurposomes. Our earlier antibody study (Gall and Callan, 1989) showed that spheres and numerous granules in the nucleoplasm contain snRNPs, since all are intensely stained by mAb Y12, specific for several proteins

Figure 1. Low magnification view of GV contents of the newt *N. viridescens*. The field shown here includes roughly 5–10% of one nucleus. (*A*) Phase contrast image of a single lampbrush bivalent, a few dozen extrachromosomal nucleoli in the form of beaded necklaces (*N*), several spheres (*S*), and numerous B snurposomes (*B*). (*B*) Immunofluorescent image of the same field after staining with mAb αSC35 followed by fluorescein-labeled goat anti-mouse IgG. mAb αSC35 recognizes a non-snRNP splicing factor (Fu and Maniatis, 1990). It stains most lampbrush loops, but leaves the chromomere axis unstained (note the apparent gaps along the axis, which correspond to prominent chromomeres in the phase contrast image). The B snurposomes stain intensely. The sphere bodies (= C snurposomes) are unstained, but the B snurposomes on their surface stain like the free B snurposomes. Nucleoli are negative. Bar, 50 μm.

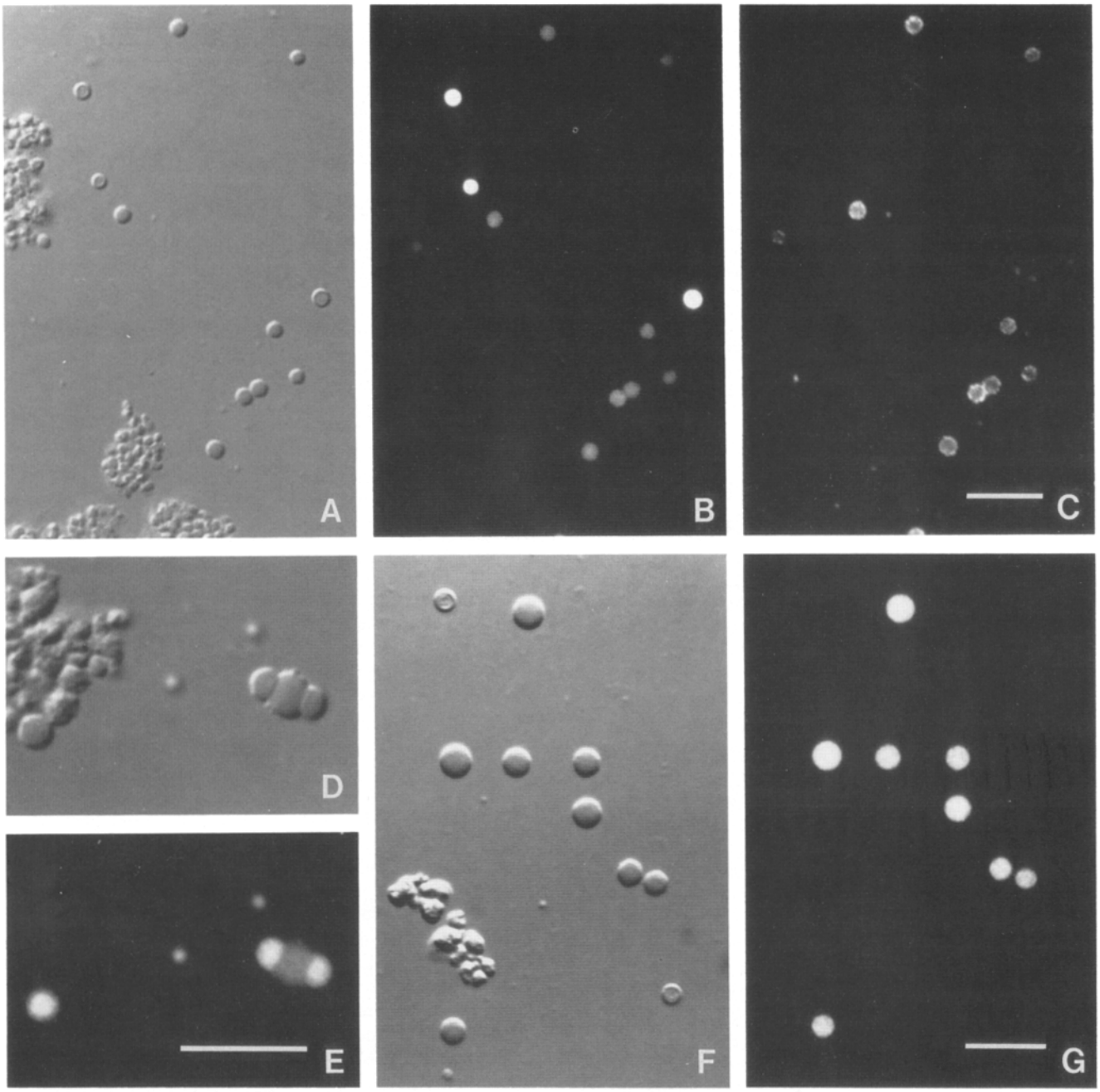


Figure 2. (*A*) A field containing A and B snurposomes and nucleoli from a GV of *N. viridescens*. The three A snurposomes have an annular appearance, whereas the nine B snurposomes are more homogeneous. Double stained with human serum 361 against U1 snRNPs and mAb SE5 against an abundant loop protein (Roth and Gall, 1987). DIC image. (*B*) Immunofluorescence image in fluorescein channel to show staining with serum 361. B snurposomes stain less intensely than As. (*C*) Immunofluorescent image in rhodamine channel to show staining with mAb SE5. This antibody stains minute particles on the surface of the B snurposomes, but does not stain A snurposomes. (*D*) A small C snurposome with two B snurposomes on its surface (*right*) and part of a nucleolus (*left*), from a GV of *N. viridescens*. DIC image. (*E*) The same field stained with human serum 361 against U1 snRNPs. The C snurposome stains less intensely than the Bs, suggesting a difference in concentration of U1-specific antigen(s). The nucleolus is unstained. Next to the nucleolus is a single B snurposome. (*F*) A field containing two A snurposomes, eight B snurposomes, and a nucleolus, from a GV of *N. viridescens*. DIC image. (*G*) The same area stained with mAb αSC35, which is specific for a non-snRNP splicing component. The B snurposomes stain intensely, but the A snurposomes and nucleolus are negative. Bars, 10 μm.

common to the major snRNPs (Lerner et al., 1981), and by mAb K121, specific for the trimethylguanosine cap at the 5′ end of all the major snRNAs except U6 (Krainer, 1988). Not until we used additional antibodies did we recognize that there are two distinct types of free granules, the A and B snurposomes, and that the protuberances on the spheres are identical in every respect to free B snurposomes.

These features can be illustrated by the staining patterns with three human patient sera: U1AG, 361, and U2GA. U1AG reacts with the A, C, and 70K proteins of U1 snRNPs (Petters-

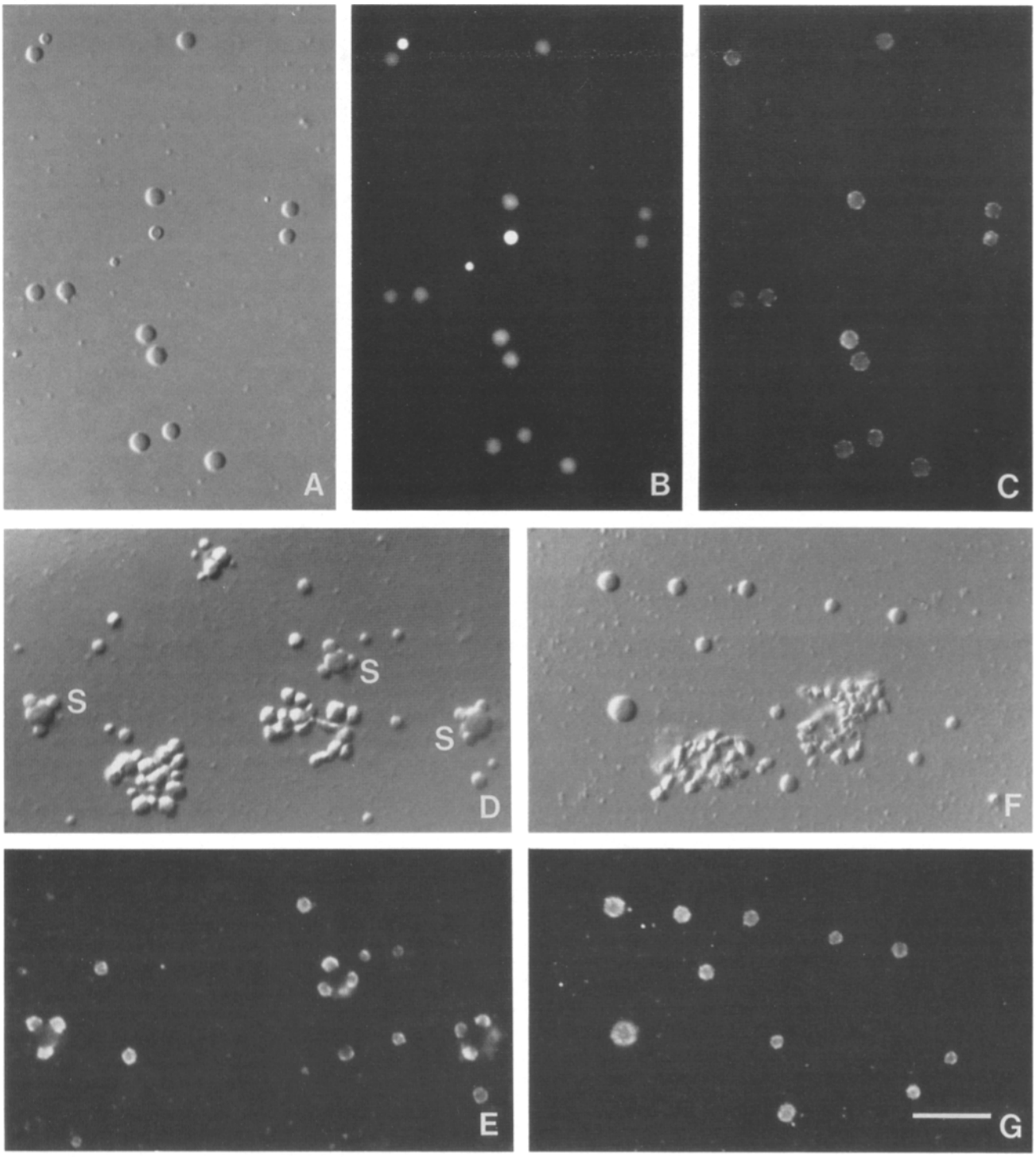


Figure 3. (*A*) A field containing three A and twelve B snurposomes from *N. viridescens.* Double stained with human serum 361 against U1 snRNPs, and mAb iD2 against the A and B group hnRNPs (Leser et al., 1984). DIC image. (*B*) The same field viewed by fluorescence in the fluorescein channel to show staining by serum 361. The three A snurposomes are stained more intensely than the Bs. (*C*) The same field viewed in the rhodamine channel to show staining by mAb iD2. The three A snurposomes are negative; staining of the B snurposomes is limited to minute patches on their surfaces. (*D*) A field containing two large nucleoli, three spheres (S), and about 15 snurposomes from a GV of *N. viridescens.* DIC image. (*E*) Immunofluorescent image of the same field stained with mAb iD2. Note that the sphere bodies (= C snurposomes) are unstained, but the B snurposomes on their surface stain exactly like the free B snurposomes. The nucleoli and a few A snurposomes are unstained. (*F*) A field containing two nucleoli and eleven B snurposomes from a GV of *N. viridescens.* DIC image. (*G*) Immunofluorescent image of the same field stained with mAb 4D11, which recognizes the L hnRNP (Piñol-Roma et al., 1989). The periphery of the B snurposomes is stained, but the nucleoli are negative. Bar, 10 μm.

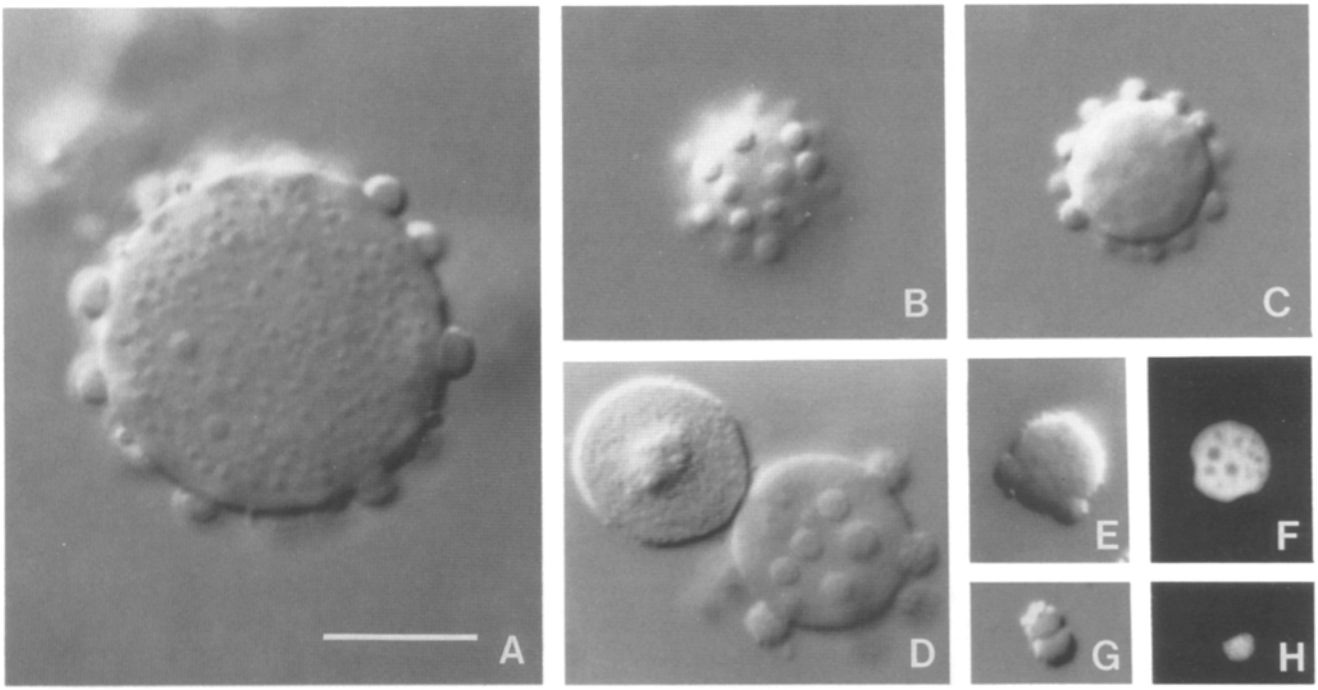


Figure 4. (*A*) A very large sphere from a GV of *N. viridescens*. DIC image focused near the middle of the sphere to show inclusions of various sizes. The surface is covered with B snurposomes. (*B* and *C*) Surface view (*B*) and optical section near the center (*C*) of a sphere from *N. viridescens*. DIC image. (*D*) A sphere from *X. laevis* next to a nucleolus. The sphere contains several inclusions that are nearly as large as the B snurposomes on its surface. DIC image taken with laser scan microscope. (*E*) A medium-sized sphere from *N. viridescens* with two B snurposomes on its surface and several faintly visible inclusions. DIC image, laser scan microscope. (*F*) Immunofluorescent image of the sphere in *E* stained with rabbit polyclonal serum "penta." This antibody stains the matrix of the sphere, but does not stain the inclusions or the B snurposomes. Laser scan microscope in confocal mode. (*G*) A small "sphere" consisting of two equal-sized snurposomes. (*H*) The same pair stained with serum "penta," which identifies the lower snurposome as a C (without inclusions). Bar, 10 μm.

son et al., 1984); 361 immunoprecipitates only U1 snRNPs, but its protein specificity has not been determined (Wassarman, D., personal communication). These two antibodies stain both A and B snurposomes, but the As are usually much brighter (Fig. 2, *A* and *B*; Fig. 3, *A* and *B*; Fig. 7, *D* and *F*; Fig. 8, *E, G, J,* and *L*). The protuberances on the spheres stain exactly like free B snurposomes. The appearance of the sphere body (= C snurposome) depends on its size. Small Cs stain less intensely than the Bs with which they are associated (Fig. 2, *D* and *E*); larger spheres appear brighter, but this is probably a simple consequence of their greater thickness. The third patient serum, U2GA, immunoprecipitates only U2 snRNPs (Bruzik and Steitz, 1990). It fails to stain A snurposomes and it shows the same distinction between strongly stained B snurposomes and weak or negative sphere bodies (not shown). These results suggest that U1-related proteins are present in all three snurposomes, with concentrations in the order A > B > C (= sphere body). On the other hand, U2-related proteins are present in B snurposomes, but are absent from As and possibly from Cs as well. Although U1AG, 361, and U2GA provided the first clear distinction among the three snurposomes, the possibility of multiple specificities in patient sera makes it difficult to assess the significance of the weak staining in the sphere bodies.

An antibody that distinguishes B snurposomes clearly from As and Cs is mAb αSC35 (Fu and Maniatis, 1990). This antibody is directed against a 35-kD protein that is essential for splicing, but is not part of a snRNP. It stains B snurposomes brilliantly, leaving As completely unstained (Fig. 2, *F* and *G*). The staining of spheres is particularly interesting. Like the free B snurposomes in the same preparation, the Bs on the surface of the spheres are brightly stained. The matrix of the C snurposomes is unstained, but the inclusions are moderately stained. This is particularly well shown in small spheres with only one or a few inclusions (Fig. 5, *A* and *B*). Fig. 1 shows a low magnification view of GV contents from *N. viridescens* stained with mAb αSC35. In addition to the features just described, this figure shows strongly stained lampbrush loops, but completely negative nucleoli, chromomeres, and axial granules.

Five other antibodies show an entirely different pattern of staining on snurposomes, reacting only with the periphery of the Bs. These antibodies leave unstained the core of the B snurposomes, the sphere bodies, and the A snurposomes (Fig. 2, *A* and *C*; Fig. 3, *A, C–G*; Fig. 7, *D* and *E*; Fig. 8, *A, B, E, F, J,* and *K*). The peripheral stain on the Bs occurs in patches that are clearly visible by fluorescence, but are barely detectable by DIC and phase contrast. Among the five that show this pattern are three, mAb iD2, mAb 4D11, and mouse polyclonal serum A1, that were raised against known hnRNPs. mAb iD2 reacts with the A and B group hnRNP proteins from a variety of vertebrate species, including *Xenopus* (Leser et al., 1984). mAb 4D11 was raised against the L hnRNP of HeLa cells, but it also reacts with nuclear proteins from *Notophthalmus* (Piñol-Roma et al., 1989). Poly-

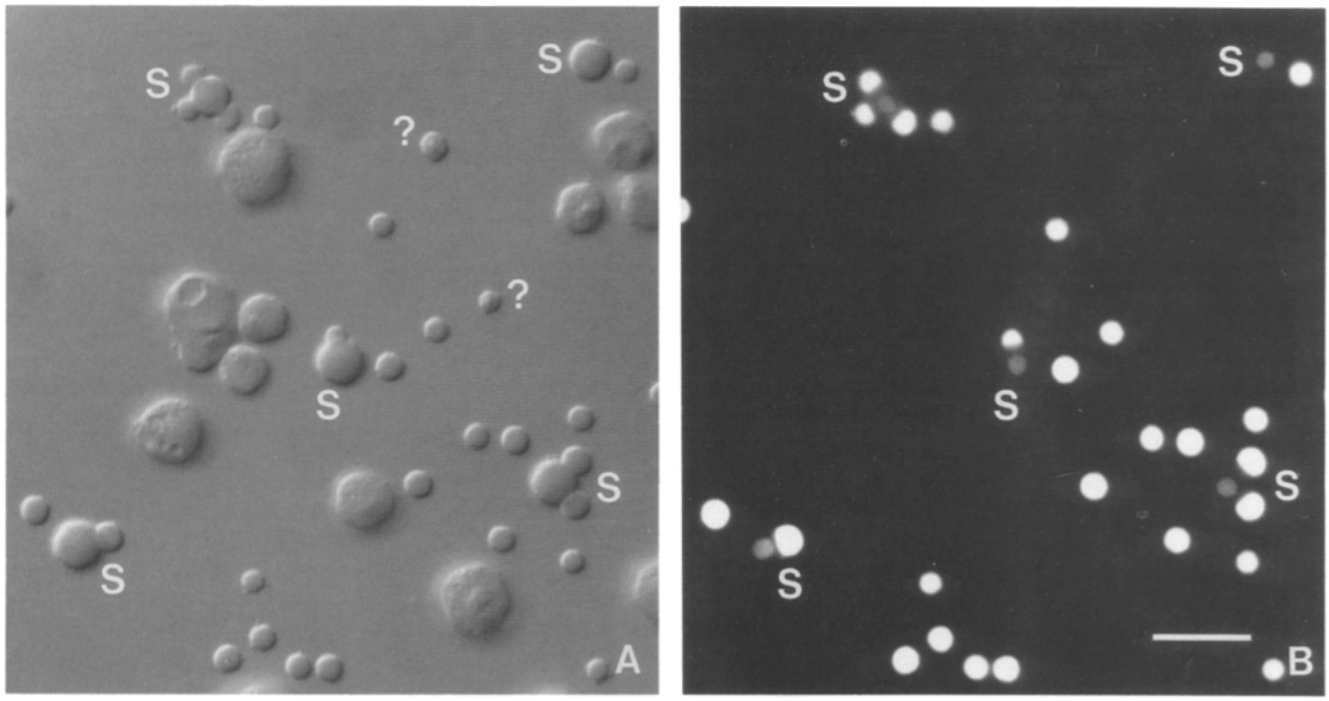


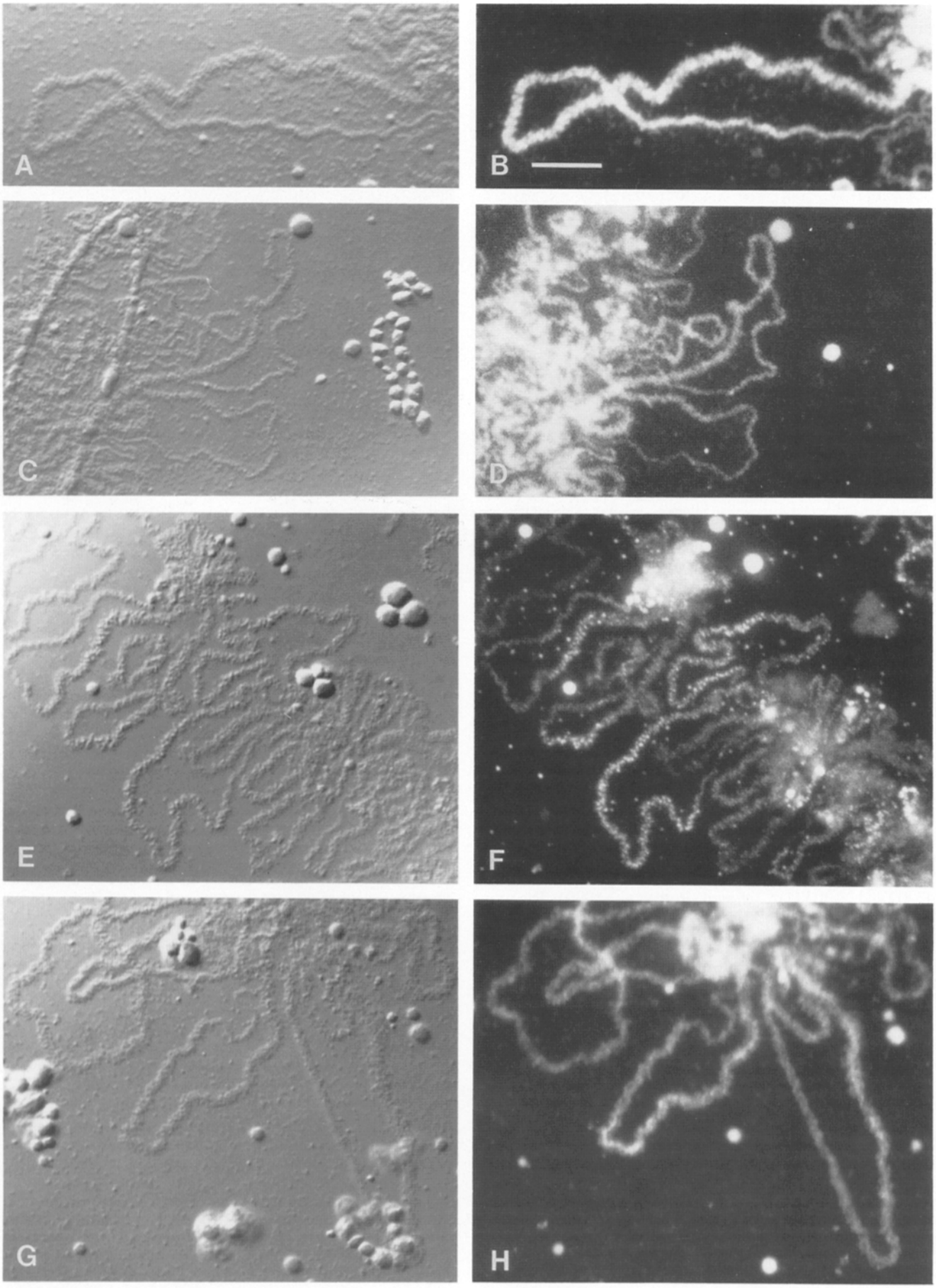
Figure 5. (*A*) Extrachromosomal nucleoli, spheres (*S*), and B snurposomes from a GV of *X. laevis*. Four spheres have at least one B snurposome on their surface; the sphere (= C snurposome) at extreme upper right lies near a B snurposome, but has none on its surface. (*B*) Immunofluorescent image of the same field stained with mAb αSC35, which recognizes a non-snRNP splicing component (Fu and Maniatis, 1990). B snurposomes, both free and on the surface of the spheres, stain intensely. The sphere matrix is negative, but each sphere contains one inclusion that stains moderately well (cf. the inclusions in the larger sphere shown in Fig. 4 *D*). Nucleoli are negative, as are the two granules of distinctive morphology marked "?." Bar, 10 μm.

clonal serum A1 was raised against a fusion protein consisting of *Xenopus* A1 hnRNP linked to *E. coli* β-galactosidase (Kay, B., personal communication). These three antibodies provide evidence that B snurposomes contain hnRNPs in addition to the snRNPs previously demonstrated. The other two antibodies that stain the periphery of Bs were originally selected for their ability to stain lampbrush chromosome loops of *Notophthalmus* and *Xenopus*, respectively. mAb SE5 reacts with a single nuclear protein of M_r = 90 kD in *Notophthalmus* (Roth and Gall, 1987), and mAb 104 reacts with a phosphorylated epitope on nuclear proteins of M_r = 43 kD and >100 kD in *Xenopus* (Roth et al., 1990). The proteins recognized by mAbs SE5 and 104 have not yet been characterized. They are clearly associated with nascent transcripts on the chromosomes, but whether they are hnRNPs, snRNPs, or some other type of protein is not known.

A double-label experiment is possible with any one of the mouse mAbs and any one of the human sera. We have tested several such pairs to verify the simultaneous staining of individual snurposomes. Fig. 3, *A–C* shows an experiment with rhodamine-labeled iD2 and fluorescein-labeled 361. The A snurposomes stain intensely with 361 but are negative with mAb iD2, whereas the Bs are less bright with 361 and are stained peripherally with mAb iD2. A similar double-label experiment involving fluorescein-labeled serum 361 and rhodamine-labeled mAb SE5 is shown in Fig. 2, *A–C*. The distribution of SE5 staining exactly parallels that of iD2.

A few years ago Lacroix and his colleagues described mAb B24, which stains the sphere body but not the B snurposomes on its surface (Lacroix et al., 1985). We have recently tested a rabbit polyclonal serum whose staining pattern is similar to that of mAb B24, but not identical. This serum, designated "penta," gives a weak generalized stain of the nuclear contents, but the sphere bodies stand out brilliantly. Furthermore, the stain is limited to the matrix of the spheres, leaving the inclusions and the B snurposomes on the surface at background level (Fig. 4, *E–H*). In this respect, "penta" and mAb αSC35 give exactly converse pictures. "Penta" was derived from a rabbit injected with the synthetic peptide $A(GGK)_5GGC$, in which the five K residues were acetylated (Lin et al., 1989). mAb B24 and serum "penta" demonstrate that the matrix of the sphere body has a composition different from that of A and B snurposomes, even though all three structures contain snRNPs.

Lampbrush Chromosomes. As reported earlier (Gall and Callan, 1989), most loops on the lampbrush chromosomes stain with mAbs Y12 and K121 (Fig. 6, *E* and *F*), and therefore presumably contain snRNPs. An important feature of the staining pattern is that transcription units (TUs) are stained along their entire length, with the intensity of stain more or less proportional to the amount of RNP matrix visible by phase contrast or DIC microscopy. TUs are recognizable as "thin-to-thick" regions of the RNP matrix on the loops; loops usually contain one or a small number of TUs, and two TUs on the same loop can have opposite polarity (Angelier and Lacroix, 1975; Scheer et al., 1976; Gall et al., 1983). A few specific loops fail to stain, among which the most prominent are the giant loops on chromosome 2 and the so-called sequentially labeling loops on chromosome 11 (see Callan, 1986). The failure of these loops to stain is not due


A
B
C
D
E
F
G
H

to simple technical problems, since other antibodies stain them strongly and specifically (Roth and Gall, 1987; Piñol-Roma et al., 1989).

A similar pattern of loop staining is seen with a variety of other antibodies against snRNPs and hnRNPs. These include the three anti-snRNP human sera U1AG, U2GA, and 361 (Fig. 6, *C* and *D*); also mAb iD2 (Fig. 6, *A* and *B*), which recognize the A and B group hnRNPs, and mAb 4D11, which recognize the L hnRNP. Loops are stained by mAb SE5 (Roth and Gall, 1987) and mAb 104 (Roth et al., 1990); like the anti-hnRNP antibodies iD2 and 4D11, these two antibodies stain the periphery of B granules, but their antigens have not been fully characterized. Finally, mAb αSC35, which recognizes a non-snRNP splicing factor (Fu and Maniatis, 1990) stains the loops (Fig. 6, *G* and *H*). With one exception, none of the antibodies discussed here stain the special loops on chromosomes 2 and 11. The exception is mAb 4D11, which gives an unusually strong reaction on the giant loops of chromosome 2, but does not stain those on chromosome 11 (Piñol-Roma et al., 1989).

One might have expected that the loops that transcribe histone sequences would stain poorly with antibodies against splicing components, since histone genes contain no introns. However, the histone loops are strongly stained by sera 361 and U2GA (not shown). They are also stained by mAb Y12, but in this case one might argue that the reaction is due to the U7 snRNP, which reacts with anti-Sm antibodies (Birnstiel and Schaufele, 1988). Several years ago we found that the histone loops are located at or very close to the SOs on chromosomes 2 and 6 (Gall et al., 1981), but the significance of this observation vis-á-vis the distribution of snRNPs is completely unknown.

Other Structures. None of the antibodies discussed above stain the chromomere axis of the chromosomes (Figs. 1 and 6, *C* and *D*), the structures referred to as axial granules, or prominent spherical bodies often associated with centromeres and telomeres (Gall, 1991). The multiple nucleoli range from completely invisible (Figs. 1, 2, 3, 5, and 6, *D* and *H*) to "readily detectable" but well below the level of loop staining (Fig. 6, *E* and *F*). It is often possible to see that the body of the nucleolus is unstained, whereas its surface is covered with extremely small stained particles (e.g., mAbs SE5 and αSC35).

(c) In Situ Hybridization

We have carried out in situ hybridization studies on spread nuclear contents using antisense probes for all six major snRNAs (U1–U6). As controls, we used sense probes for U1, U2, U3, and U6, all of which were negative under conditions where antisense probes gave strong reactions. A list of all probes is given in Table II. We also treated some preparations with RNase A (100 μg/ml for 1 h at 42°C) to remove RNA before hybridization; such preparations were negative with all six antisense probes.

The antisense probes for the major snRNAs involved in splicing (U1, U2, U4, U5, and U6) gave broadly similar results. Each of them labeled free B snurposomes, the Bs on the spheres, and most of the chromosome loops. They did not label chromomeres, large granules associated with telomeres and centromeres, and certain specific loops (the sequentially labeling loops of chromosome 11 and the giant loops of chromosome 2). For reasons discussed below, we are not yet sure whether any of the probes label the sphere bodies (= C snurposomes). The U4, U5, and U6 antisense probes did not label nucleoli. The U1 and U2 antisense probes gave moderately strong labeling of nucleoli, which was reduced or eliminated by pretreatment with proteinase K. The antisense probe for U3 labeled only nucleoli, whether or not the preparation was treated with proteinase K. Details of these results will now be presented.

A and B Snurposomes. When we carried out our first in situ hybridization experiments, we had not yet distinguished A and B snurposomes, and our results were somewhat ambiguous. Antisense probes except U3 labeled the majority of nucleoplasmic granules in every preparation. In those cases where the labeling level was quite high, there were relatively few unlabeled granules (Figs. 7, *B* and *C*, 8, *A–C, D,* and *I*). Where the overall level was low, there was considerable variability in the number of silver grains per granule, and there were unlabeled granules, as expected on statistical grounds. These experiments established the existence of the major splicing snRNAs in extrachromosomal granules, but they did not permit more detailed deductions.

In subsequent experiments we used the following protocol. Suitable areas were located by DIC and photographed. The preparations were then stained with one or two antibodies, and the same areas were rephotographed. Finally, in situ hybridization was carried out with a ^{3}H-labeled antisense probe. The developed autoradiograph was stained with Coomassie blue, and the selected areas were again found and photographed. Although this procedure was laborious, it permitted us to make definitive statements about individual snRNAs. There was one unexpected drawback to the method; namely, prestaining with fluorescent antibodies significantly reduced the sensitivity of the autoradiograph. We have not investigated this phenomenon in detail, since it did not interfere with our particular aims.

In the first experiments with the antisense U1 probe, some granules labeled much more intensely than others. Fig. 7, *B* and *C* shows an autoradiographic field before and after removal of the silver grains from the emulsion. The heavy clusters of label occur over relatively small granules, a fact that is not clear from the autoradiograph alone. These smaller, heavily labeled granules were shown to be A snurpo-

Figure 6. Lampbrush chromosome loops, extrachromosomal nucleoli, and B snurposomes from *N. viridescens*. DIC images (*A, C, E,* and *G*) and corresponding immunofluorescent images (*B, D, F,* and *H*) after antibody staining. In each case the immunofluorescent and DIC images of the loops are superimposable. (*A* and *B*) mAb iD2, which recognizes the A and B group hnRNPs. (*C* and *D*) Human autoimmune serum 361, which recognizes proteins associated with U1 snRNA. Loops and two B snurposomes are well stained; extrachromosomal nucleolus and chromomere axis of the chromosome are unstained. (*E* and *F*) mAb K121, which is specific for the trimethylguanosine cap of snRNAs. Loops and several B snurposomes are well stained; nucleoli are weak but above background level. (*G* and *H*) mAb αSC35, which recognizes an essential non-snRNP splicing protein. Loops and several B snurposomes are stained; nucleoli are negative. Bar, 10 μm.

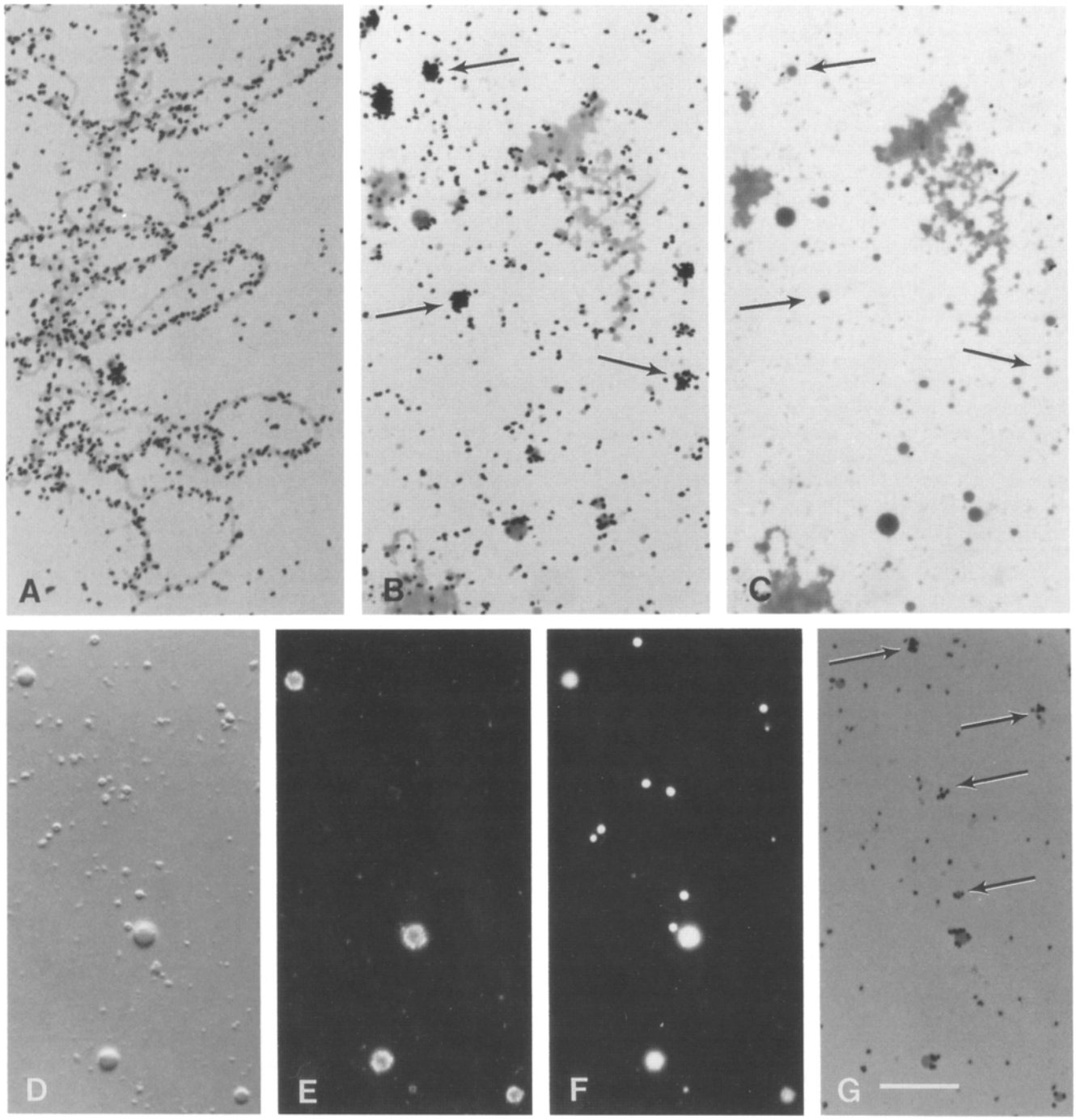


Figure 7. (*A*) Autoradiograph of lampbrush chromosome loops of *N. viridescens* after in situ hybridization with a ^{3}H-labeled antisense U1 snRNA probe. Probe specific activity 10^8 dpm/μg; 10 d exposure. (*B*) A similar autoradiograph showing labeled snurposomes. (*C*) The same field as in *B* after removal of the silver grains from the autoradiographic emulsion with $K_3Fe(CN)_6$. Although it is not possible to distinguish A and B snurposomes in this stained preparation, most of the small, heavily labeled granules (*arrows*) are probably As, whereas the less heavily labeled granules are Bs. Much of the background label, including that over the nucleoli, may be due to U1 snRNA in the very tiny particles visible throughout the preparation. (*D*) A field of A and B snurposomes from a GV of *N. viridescens*, double stained with mAb SE5 and human serum 361. DIC image. (*E*) Immunofluorescent image of the same field in the rhodamine channel to show peripheral staining of the four B snurposomes with mAb SE5. (*F*) Immunofluorescent image in the fluorescein channel to show staining of both A and B snurposomes with human serum 361, specific for U1 snRNPs. The A snurposomes are the small, intensely stained granules. (*G*) The same field after *in situ* hybridization with a ^{3}H-labeled antisense U1 snRNA probe (specific activity 10^8 dpm/μg). 6 d exposure. Both A and B snurposomes are labeled, but relative to their size the As have a disproportionately large number of silver grains (*arrows*). Bar, 10 μm.

somes in a combined immunofluorescence and hybridization experiment. Fig. 7, *D–G* shows the same area by DIC, by immunofluorescence after staining with mAb SE5 and serum 361, and after in situ hybridization with the antisense U1 probe. Both A and B snurposomes are radioactive, but relative to their size the As are much more strongly labeled than the Bs.

The antisense probes for U2, U4, U5, and U6 each labeled B snurposomes, but were completely negative on A snurposomes. Fig. 8 *C* shows a number of strongly labeled B snurposomes in the same field with 3 unlabeled As, after hybridization with the antisense U2 probe. In this case the As are clearly identifiable by their morphology (Fig. 8 *A*) and their failure to stain with mAb SE5 (Fig. 8 *B*). Similar fluorescence and autoradiographic data were obtained for U4 (Fig. 8, *E–H*), U5 (not shown), and U6 (Fig. 8, *J–M*). The antisense U1 and U2 probes gave generally higher "background" labeling than did the U4, U5, and U6 probes (cf. Figs. 7 *B* and 8 *C* with Figs. 8, *D* and *I*). One possibility is that U1 and U2 snRNAs are found in the multitude of submicron particles evident in most of our preparations, but U4, U5, and U6 are limited to the B snurposomes.

Spheres. For geometrical reasons, the labeling pattern is easier to evaluate on small spheres with one or two associated B snurposomes, than on larger spheres with multiple Bs. Examples of such small spheres are shown in Figs. 8, *N–Q*. The B snurposomes attached to the C snurposome were labeled by all the snRNA probes except U3; the intensity of label was comparable to that of free B snurposomes in the same preparation. The C snurposomes, on the other hand, were either unlabeled or had only a few silver grains above them. These few grains could be due to ^{3}H disintegrations that took place within either the C snurposome or its associated Bs. In any case, it is clear that the concentration of U snRNAs in the small C snurposomes, if not zero, is much lower than in the Bs.

It would be particularly useful to have information on the large sphere bodies, because their inclusions resemble B snurposomes in certain respects. However, there are severe geometrical problems involved in the case of larger spheres, which cause a bulge in the overlying autoradiographic emulsion. Silver grains can be seen above such spheres and around their sides (not shown). Sometimes the pattern of silver grains follows the pattern of B snurposomes on the surface rather closely. From this we can conclude that the Bs are labeled, but we cannot make deductions about the sphere body itself. Electrons arising within the sphere body cannot penetrate the B snurposomes on the surface, which are >1 μm in diameter. Evidence for label within the sphere body must, therefore, come from silver grains located between the Bs. However, the Bs are so closely spaced (e.g., Fig. 4, *A* and *B*) that at least some of the silver grains in these regions are due to disintegrations from within the Bs themselves. Thus we are reluctant to make any definitive statement about the sphere body based on autoradiographs of large spheres.

We tried to carry out in situ hybridization of snRNA probes on sections of GVs, but so far our results have not been satisfactory.

Lampbrush Chromosomes. The lampbrush chromosomes were labeled by all the antisense snRNA probes except U3. Most loops were labeled to roughly the same intensity (Fig. 7 *A*). As with the fluorescent antibodies, label was absent from the giant loops on chromosome 2 and the sequentially labeling loops on chromosome 11. In the case of the U1 snRNA probe, the histone loops on chromosomes 2 and 6 were more strongly labeled than other loops of comparable morphology (not shown). This observation is consistent with the strong immunofluorescent staining of these loops by U1 specific sera (U1AG and 361).

Discussion

In a previous study we used mAbs Y12 and K121 to determine the distribution of snRNPs within the amphibian GV (Gall and Callan, 1989). mAb Y12 reacts with several proteins associated with the major splicing snRNAs (Lerner et al., 1981), whereas mAb K121 detects the trimethylguanosine cap found on all but U6 of these same snRNAs (Krainer, 1988). Both antibodies stain the majority of lampbrush chromosome loops, the sphere organelles, and hundreds to thousands of smaller granules in the nucleoplasm. We here confirm this distribution of snRNPs by use of additional antibodies and by in situ hybridization with ^{3}H-labeled antisense probes. We define two types of free granule by morphology and composition, which we designate A and B snurposomes. A third type of granule, the C snurposome, sometimes exists independently, but is usually associated with one or more B snurposomes. C snurposomes come in a wide range of sizes (1–20 μm diam); large Cs with multiple B snurposomes on their surface have long been referred to as spheres or sphere organelles (see Callan, 1986).

Snurposome is intended as a general morphological term for subcellular structures that contain snRNPs, regardless of function. We emphasize that it is not a synonym for spliceosome. Spliceosome refers to a molecular complex involved in the removal of introns from pre-mRNA, and is defined primarily by in vitro biochemical reactions (Brody and Abelson, 1985; Maniatis and Reed, 1987; Steitz et al., 1988). Snurposomes and spliceosomes differ greatly in size. Purified mammalian spliceosomes are 400–600 Å in diameter (Reed et al., 1988), whereas snurposomes are some 10–500 times greater in size; i.e., <1 μm to ~20 μm in diameter.

In this study we used 12 antibodies, including mAbs Y12 and K121, to study the distribution of snRNP and hnRNP proteins. To examine individual snRNAs, as opposed to snRNP proteins, we carried out in situ hybridization experiments with ^{3}H-labeled antisense snRNA probes. The results can be summarized briefly as follows.

Lampbrush Chromosome Loops

The majority of loops labeled with all of the antisense probes for the splicing snRNAs U1, U2, U4, U5, and U6, and they stained with an antibody against the trimethylguanosine cap. They also stained with antibodies against U1- and U2-specific snRNP proteins, the common snRNP antigen Sm, the non-snRNP splicing factor SC35, the A and B group hnRNPs, and the L hnRNP. They also stained with antibodies against other partially characterized loop proteins. In all cases the hybridization or immunofluorescence extended throughout the length of the TUs. A very small number of specific loops failed to label with these antisense probes and antibodies. In general, therefore, we conclude that most nascent transcripts on lampbrush chromosome loops are associated with all of

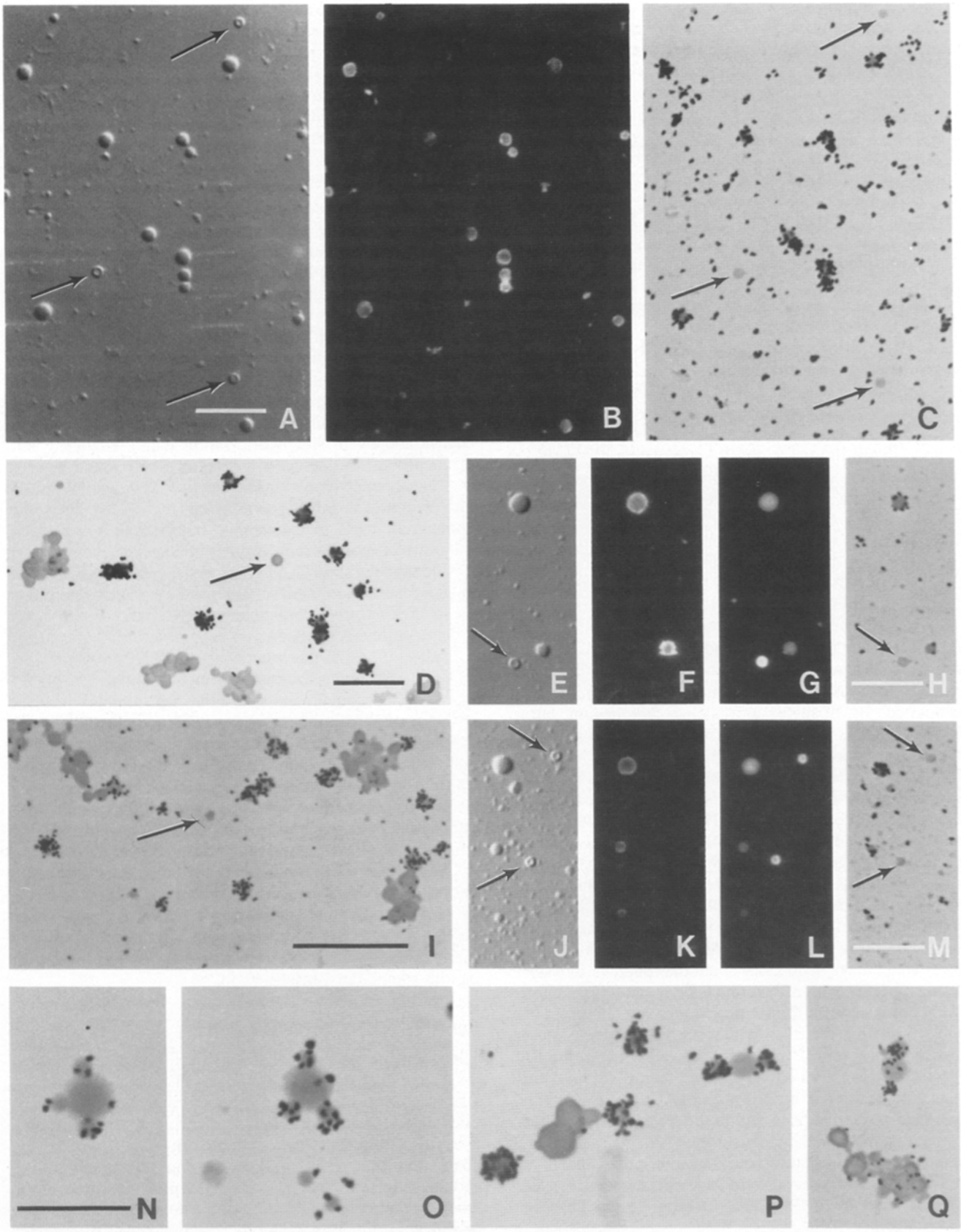


Figure 8. (*A*) A field with three A snurposomes (*arrows*) and numerous Bs from a GV of *N. viridescens.* DIC image (the white streaks are imperfections in the photographic negative). (*B*) Immunofluorescent image of the same field stained with mAb SE5, which reacts with the periphery of B snurposomes, but leaves the As unstained. (*C*) The same field after in situ hybridization with a ^{3}H-labeled antisense U2 probe (specific activity 10^{8} dpm/μg). 34 d exposure. The A snurposomes (*arrows*) are completely negative, whereas the Bs are well labeled. (*D*) A field showing A and B snurposomes and nucleoli from a GV of *N. viridescens*, after in situ hybridization with a ^{3}H-labeled

the splicing snRNAs, and with various snRNP and hnRNP proteins. Until additional antibodies have been tried, we will not know whether all snRNP and hnRNP proteins occur on the loops, but this is a distinct possibility.

A Snurposomes

The A snurposomes were labeled by the antisense U1 probe, but not by any other antisense probes we tested. They stained brilliantly with two different human sera against U1 snRNP proteins, as well as with mAbs Y12 and K121, but were unstained by any of the other antibodies. Of the components for which we tested, therefore, they contain only U1 snRNPs.

B Snurposomes

The B snurposomes reacted with all snRNP and hnRNP probes that also reacted with lampbrush loops. The snRNP components, and the non-snRNP splicing factor SC35, occur throughout the body of the B snurposomes, whereas the hnRNP proteins, and the antigens recognized by mAbs SE5 and 104, are limited to minute patches on the periphery. The level of labeling in the B snurposomes was approximately the same for all of the antisense probes (Figs. 7 and 8). Since the probes were of similar specific activity, we conclude that the splicing snRNAs are present in roughly equivalent concentrations in B snurposomes. A detailed statistical analysis would be necessary before we could make a more quantitative statement, and this would be complicated by the variable size of B snurposomes. We believe, however, that we would have detected order-of-magnitude differences in individual snRNA concentrations.

C Snurposomes

By C snurposome we mean the sphere body in the case of the larger sphere organelles, as well as many smaller granules that are usually, but not always associated with B snurposomes. They consist of two components, a matrix and inclusions, both of which stain with mAb K121. The matrix usually stains stronger than the inclusions with mAb Y12, but a distinction between the two components is not always possible. Only the inclusions are stained by mAb αSC35. The human immune sera against U1 and U2 snRNPs give weak and variable results, and the antisense snRNA probes probably do not react. Because of the consistent staining with mAbs Y12 and K121, we believe that C snurposomes contain snRNPs, but we have not been able to demonstrate convincingly any of the five major splicing snRNAs.

The antibody and in situ hybridization data are summarized in Table III. Information about the distribution of snRNPs and hnRNPs in the GV allows us to address several important questions. Where does pre-mRNA splicing take place? What is the relationship between snRNP and hnRNP components? What is the function of A, B, and C snurposomes? What is the relationship between B snurposomes and spheres? And finally, do structures comparable to A, B, and C snurposomes occur in other cell types? We will consider these questions in turn.

Where Does Pre-mRNA Splicing Take Place?

Our studies show that many components essential for splicing are associated with the nascent RNA transcripts on the lampbrush loops. It seems reasonable to conclude, therefore, that the splicing machinery is in place before the pre-mRNA transcripts leave the DNA of the chromosome. A similar conclusion was reached earlier by Sass and Pederson (1984) and Vazquez-Nin et al. (1990), who studied the polytene chromosomes of *Chironomus*, and by Fakan et al. (1986), who examined the distribution of snRNPs on spread transcription complexes from tissue culture cells. The strongest evidence that splicing occurs, while the nascent transcripts are still attached to the DNA template comes from the electron micrographs of *Drosophila* TUs published by Osheim et al. (1985) and Beyer and Osheim (1988), which show what can be interpreted as splicing complexes on the nascent transcripts, as well as reduction in length of transcripts before the end of a TU. Electron micrographs of TUs from newt lampbrush chromosomes provide additional evidence. Angelier and Lacroix (1975) described looped out regions ("annelets") on individual transcripts, and Scheer et al. (1976) reported similar ring-like structures lying alongside nascent transcripts. Such configurations could well represent stages in intron removal.

Although the EM data are highly suggestive, it is conceivable that splicing takes place after the transcripts leave the lampbrush loops. Specifically, because B snurposomes contain the same splicing components as the loops, one could imagine that unspliced transcripts leave the chromosomes, travel to the B snurposomes, and there undergo splicing. One could test this hypothesis by looking for poly A RNA in B

antisense U4 snRNA probe (specific activity 10^8 dpm/μg). 10 d exposure. B snurposomes are well labeled. The larger unlabeled structures are nucleoli; an unlabeled A snurposome is indicated by the arrow. (*E*) One A snurposome (*arrow*) and two Bs from a GV of *N. viridescens*, double stained with human serum 361 and mAb SE5. DIC image. (*F*) Immunofluorescent image of the same field in the rhodamine channel to show peripheral staining of the B snurposomes with mAb SE5. (*G*) The same field in the fluorescein channel to show staining of both A and B snurposomes with human serum 361. (*H*) The same field after in situ hybridization with a ^{3}H-labeled antisense U4 snRNA probe (specific activity 10^8 dpm/μg). 32 d exposure. The B snurposomes are labeled, but the A is not (*arrow*). (*I*) Several A and B snurposomes and nucleoli from a GV of *N. viridescens* after in situ hybridization with a ^{3}H-labeled antisense U5 snRNA probe (specific activity 10^8 dpm/μg). 10 d exposure. The B snurposomes are labeled; nucleoli and an A snurposome (*arrow*) are negative. (*J*) A and B snurposomes from a GV of *N. viridescens*, double stained with mAb SE5 and human serum 361. Arrows point to As. DIC image. (*K*) Immunofluorescent image of the same field in the rhodamine channel to show peripheral staining of B snurposomes with mAb SE5. (*L*) The same field in the fluorescein channel showing staining of both A and B snurposomes with serum 361. (*M*) The same field after in situ hybridization with a ^{3}H-labeled antisense U6 probe (specific activity 10^8 dpm/μg). 32 d exposure. Only the B snurposomes are labeled. Arrows indicate unlabeled As. (*N–Q*) Small spheres consisting of C snurposomes with two or three B snurposomes on their surface, after in situ hybridization with ^{3}H-labeled antisense U2 (*N* and *O*), U4 (*P*), or U5 (*Q*) snRNA probes (specific activity 10^8 dpm/μg). Exposure 5 d (*N* and *O*) or 10 d (*P* and *Q*). The B snurposomes are well labeled, but the C snurposomes are either negative or very weakly labeled. Nucleoli are negative. Bar, 10 μm.

Table III. Summary of Antibody Staining and In Situ Hybridization with Antisense Probes

	Antibodies												Antisense hybridization probes					
Structure	Y12	K121	361	U1AG	U2GA	αSC35	iD2	4D11	A1	SE5	104	Penta	U1	U2	U3	U4	U5	U6
Chromosome loops	++	++	++	++	++	++	++	++	++	++	++	+/−	++	++	−	+	+	+
A Snurposomes	+++	+++	+++	+++	−	−	−	−	−	−	−	−	+++	−	−	−	−	−
B Snurposomes	++	++	++	++	++	++	+	+	+	+	+	+/−	++	++	−	++	++	++
C Snurposomes	++	++	+	+	+	+*	−	−	−	−	−	+++*	−	−	−	−	−	−
Nucleoli‡	+/−	+/−	+/−	+/−	+/−	−	−	−	−	−	−	+/−	+	+	+++	−	−	−

* Penta stains the matrix, αSC35 the inclusions of C snurposomes.
‡ The nucleoli stain strongly with antibodies not listed here. The weak staining reported here is due, at least in part, to submicron-sized particles on the surface of the nucleoli; the same may be true for the U1 and U2 in situ hybrids.

snurposomes by in situ hybridization, but to our knowledge such experiments have not been carried out. A somewhat weak argument against this hypothesis comes from labeling experiments. The RNA of lampbrush loops labels quickly and strongly when oocytes are incubated in [^{3}H]uridine, but the B snurposomes label only slowly and weakly. If newly synthesized transcripts were traveling to the B snurposomes, one might expect to see more active labeling. We think it more likely that B snurposomes acquire their radioactivity only from newly synthesized snRNA (Callan, H. G., and J. Gall, unpublished).

What Is the Relationship between snRNPs and hnRNPs?

Our immunofluorescence data demonstrate that snRNPs and hnRNPs occur together on the lampbrush loops and in B snurposomes. At the resolution permitted by light microscopy they are strictly colocalized on the loops. Furthermore, both are uniformly distributed along the length of the TUs, in the sense that the immunofluorescent image of a loop is superimposable on its phase contrast or DIC image. We emphasize that typical transcription units on newt lampbrush loops are tens of micrometers long and one to several micrometers wide at the "thick" end. Thus one could easily detect differences in staining of transcripts at the beginning and end of a TU, or even differences between the 5′ end of the transcripts (at the outer edge of the loop) and their attachment points on the DNA (along the axis of the loop). A possible interpretation of this observation is that there is a unitary hnRNP/snRNP particle, which associates with each newly transcribed segment of RNA, and remains with it throughout its sojourn along the transcription unit. Splicing components would be an integral part of such a unitary particle, but would come into play only when juxtaposed with the appropriate splice sites in the RNA. Unless the snRNP components on the loops undergo a complete redistribution during fixation and immunofluorescent staining, they must be associated with many unspliced regions. For instance, the histone loops of *N. viridescens* stain strongly with antibodies against splicing components, yet most of the RNA on these loops is transcribed from a 222-bp simple sequence DNA, and the histone genes themselves do not contain introns (Diaz et al., 1981; Gall et al., 1983).

The model just proposed is based on a static picture of hnRNP and snRNP distribution derived from immunofluorescent and in situ hybridization data. In some respects it is at variance with in vitro studies, which show that hnRNP proteins can assemble on RNAs that lack splice sites but snRNPs require splice sites for proper assembly (e.g., Reed, 1990). However, the conditions for assembly in vitro and in the nucleus may be quite different.

Of particular interest is the fact that hnRNP proteins cover the surface of the B snurposomes. Although such a distribution could arise as an adsorption artifact during specimen preparation, we did not see hnRNP proteins on the A snurposomes, nucleoli, chromomeres, or other structures except the lampbrush loops. For this reason we believe that the association of hnRNPs and snRNPs in B snurposomes reflects a preexisting in vivo condition. One interpretation is that the hnRNP and snRNP components of the unitary particle postulated above come together in B snurposomes before they go to the chromosome loops. It would be useful to have information on the movement of hnRNP and snRNP components from the time they enter the GV until they associate with the nascent transcripts on the lampbrush chromosome loops. Our first attempts to inject ^{3}H-labeled snRNAs and to follow their movement autoradiographically failed because of degradation of the RNA followed by incorporation of the label into other compounds (Jantsch, M., unpublished). Another approach will be to trace the movement of proteins translated from injected transcripts, as we have done for the chromosomal protein SE5 (Roth and Gall, 1989) and the nucleolar protein NO38 (Peculis and Gall, unpublished).

What Is the Function of Snurposomes?

Of the three types of snurposome so far identified, the B snurposomes must bear some special relationship to the lampbrush loops, since these morphologically dissimilar structures share such a variety of RNA packaging and processing components. All of the antisense RNA probes and all of the antibodies that reacted with lampbrush loops also reacted with B snurposomes. Three obvious roles for B snurposomes, not mutually exclusive, are assembly, storage, and recycling of snRNPs and hnRNPs used by the chromosomes during transcription and pre-mRNA processing. In broad outline, one could imagine the following sequence of events. snRNAs are synthesized in the nucleus, then travel to the cytoplasm, acquire the trimethylguanosine cap, and associate with vari-

ous snRNP proteins (Mattaj, 1988; Zieve and Sauterer, 1990). They return to the nucleus as snRNPs and accumulate in the B snurposomes. In the B snurposomes they are assembled into one or more macromolecular complexes that contain all of the splicing snRNPs and other splicing factors such as SC35. These "pre-spliceosome" complexes might associate with hnRNPs at the periphery of the B snurposomes to form hnRNP/snRNP particles, which then travel to the sites of transcription on the chromosome loops. Whatever the details, the basic idea is that snRNPs and hnRNP proteins are preassembled into macromolecular complexes in an extrachromosomal organelle, the B snurposome; these complexes, not free snRNPs and hnRNP proteins, associate with newly transcribed RNA. The proposed scheme is somewhat reminiscent of ribosome assembly. The components of ribosomes are preassembled in a discrete morphological structure, the nucleolus, far from their final destination. They are then transported as macromolecular complexes, the ribosomal subunits, to the sites of protein synthesis in the cytoplasm. The analogy is made even closer by considering that the same RNA transcript associates with hnRNP/snRNP particles during its initial transcription and processing in the nucleus, loses these components when it becomes an mRNA, and then associates with ribosomes in the cytoplasm during its translation. We suggest that the B snurposomes and the nucleolus may play comparable roles as sites for assembly of the major nuclear and cytoplasmic ribonucleoprotein particles respectively.

Our observations show that B snurposomes are abundant in GVs from fully mature oocytes when RNA synthesis in the lampbrush chromosomes is shutting down. We think it likely, therefore, that a second function of B snurposomes is storage of splicing components for the early embryo. Earlier studies showed that the mature *Xenopus* GV contains 4,000–20,000 times as much U1 and U2 snRNA as a somatic nucleus (Forbes et al., 1983; Fritz et al., 1984; Lund and Dahlberg, 1987), and it has been clearly demonstrated that exogenously introduced sequences can be spliced in the amphibian GV (Wickens et al., 1980; Miller et al., 1983; Wickens and Gurdon, 1983; Pan and Prives, 1988, 1989). We do not know what fraction of the stored splicing components is in snurposomes and what fraction may be in some other form.

Finally, since proteins associated with hnRNA do not accompany the mature message to the cytoplasm (reviewed by Dreyfuss, 1986), it is possible that snRNPs and other proteins involved in nuclear RNA processing could be recycled through the B snurposomes.

In discussing possible functions, we have emphasized B snurposomes, because they contain so many of the RNA packaging and processing components. The much simpler composition of A snurposomes precludes an assembly function like that suggested for the Bs. They may be simple storage granules for U1 snRNPs in the GV.

What Is the Relationship between B Snurposomes and the Sphere Organelles?

Large C snurposomes may be studded with a dozen or more B snurposomes, and even the smallest Cs are usually associated with at least one B. Furthermore, the inclusions within Cs have the same fine structure as Bs (Gall, 1991), and react similarly to several antibodies (e.g., both stain with mAb αSC35 but not with "penta"). One is tempted to speculate that a precursor–product relationship exists between the inclusions and the Bs on the surface; i.e., either Bs are formed within the C snurposome and extruded onto its surface, or Bs travel to the surface, where they are taken up by the C. The results of [^{3}H]uridine experiments favor the second hypothesis (Callan, H. G., and J. G. Gall, unpublished observations), but until a good method is found to follow the "life history" of snurposomes, these relationships must remain in the realm of speculation.

Do A, B, and C Snurposomes Occur in Other Cell Types?

It has been known for some time that certain human autoimmune sera, known as anti-Sm and anti-RNP, stain interphase nuclei in a speckled pattern. In most cases the pattern consists of 20–50 bright foci of irregular shape against a more generalized nucleoplasmic staining (Lerner et al., 1981; Nyman et al., 1986; Spector and Smith, 1986; Leser et al., 1989; Spector, 1990). Lerner and Steitz (1979) showed that anti-Sm sera specifically immunoprecipitate U1, U2, U4, U5, and U6 snRNPs; anti-RNP sera have a more restricted specificity limited to U1 snRNPs (Lerner and Steitz, 1981; Fisher et al., 1983; Pettersson et al., 1984). A similar speckled pattern is given by antibodies against the trimethylguanosine cap (Reuter et al., 1984) and by mAb αSC35, which differs in showing relatively little diffuse background (Fu and Maniatis, 1990).

Spector (1990) carried out a careful reconstruction of the speckled regions and concluded that they are part of a reticular network that extends throughout the nucleus exclusive of the nucleolus. He also showed that actively transcribing regions of the nucleus are complementary to, rather than coincident with, the snRNP reticulum. A rather different picture of snRNP localization has recently been proposed by Carmo-Fonseca et al. (1990), based on in situ hybridization with biotin-labeled 2′ *O*-methyl oligonucleotide probes. Using cells that were extracted with Triton X-100 before fixation, they found that U2, U4, U5, and U6 probes hybridized to a small number of discrete foci in HeLa cell nuclei, whereas a U1 probe hybridized to the foci and more generally throughout the nucleus. Immunofluorescent staining with several anti-snRNP antibodies and an antibody against trimethylguanosine gave a more conventional speckled pattern.

At this time it is difficult to homologize the structures in the GV with those in somatic nuclei. Morphology gives few hints, since the chief structural feature of A, B, and C snurposomes in the GV is their nearly spherical shape; nothing of this sort is obvious in the somatic nucleus, with the exception of some of the foci seen by Carmo-Fonseca et al. (1990). From a functional standpoint the transcribing regions in somatic nuclei correspond, at least in part, to lampbrush loops. Thus, the nontranscribing speckled regions and B snurposomes might be equivalent structures. In this case the speckles might represent sites for assembly of splicing components, rather than for splicing itself. Nothing comparable to the U1-specific A snurposome has been reported in somatic nuclei, although U1 snRNPs are more abundant in HeLa nuclei than other snRNPs, and are widely distributed throughout the nucleoplasm. Similarly, spheres (or more specifically, C snurposomes) seem to be limited to oocyte nuclei. Despite the difficulty in homologizing the oocyte and

somatic structures, there is no reason to suppose that RNA packaging and processing are fundamentally different in somatic and germ cells. Our hope is that the oocyte, with its extraordinary degree of morphological complexity, will reveal processes common to all cell types.

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