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U7 small nuclear RNA in C snurposomes of the *Xenopus* germinal vesicle

(RNA processing/histone genes and mRNA/*in situ* hybridization/oocytes)

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ABSTRACT **In the amphibian germinal vesicle small nuclear RNAs (snRNAs) occur in morphologically distinct structures called snurposomes. Three types (A, B, and C) have been distinguished on the basis of cytological appearance and snRNA composition. C snurposomes in *Xenopus* are spherical bodies ranging in diameter from <1 μm to about 10 μm. They stain intensely with antibodies against trimethylguanosine and the small nuclear ribonucleoprotein-specific Sm antigen but give weak or negative *in situ* hybridization reactions for the snRNAs involved in pre-mRNA splicing (U1, U2, U4, U5, and U6). We show here that C snurposomes in the *Xenopus* germinal vesicle contain U7 snRNA, an snRNA of low abundance involved in processing the 3′ end of histone pre-mRNA. *Xenopus* U7 is 58 nucleotides long and is capped at the 5′ end with trimethylguanosine. C snurposomes are often associated with B snurposomes, which contain the splicing snRNAs but not U7; B and C snurposomes together constitute a morphologically complex structure known as a sphere or sphere organelle. Although most spheres and C snurposomes are extrachromosomal, a few are attached at the histone gene loci on chromosomes 8, 9, and 16. Because they contain U7 snRNA and occur at the sites of histone pre-mRNA synthesis, C snurposomes presumably play a role in processing histone transcripts.**

The localization of small nuclear ribonucleoproteins (snRNPs) in the amphibian oocyte nucleus, or germinal vesicle (GV), has been studied by *in situ* nucleic acid hybridization and immunofluorescence (1–5). snRNPs occur on the lampbrush chromosome loops associated with the nascent transcripts and in three morphologically distinct types of extrachromosomal granules that we designate A, B, and C snurposomes. The A snurposomes, so far identified only in GVs of the newt *Notophthalmus*, contain U1 snRNA and associated proteins. B snurposomes occur in all amphibian species examined, both urodele and anuran. They contain the five snRNAs involved in pre-mRNA splicing (U1, U2, U4, U5, and U6) plus several snRNP-specific proteins and the SR group of non-snRNP essential splicing factors (6, 7). Until now the snRNP composition of C snurposomes has remained problematic. Here we demonstrate by *in situ* hybridization that C snurposomes in *Xenopus* GVs contain U7 snRNA. Studies in sea urchins (8) and cultured mammalian cells (9) show that the U7 snRNP is involved in processing the 3′ end of histone pre-mRNAs. C snurposomes often have Bs attached to their surface, the combination being referred to as a sphere or sphere organelle in the cytological literature. It has been known for some time that a few of the several dozen spheres in the GV are attached to the lampbrush chromosomes at the histone gene loci (10, 11). The presence of U7 snRNA and the close association with the sites of histone pre-mRNA synthesis suggest that spheres and their constituent C snurposomes are involved in processing histone transcripts.

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MATERIALS AND METHODS

Probes. The sequence of the *Xenopus* U7 snRNA gene was recently published (12). Complementary oligonucleotides were synthesized that contained the complete sense or antisense sequence of U7 (58 bases) plus 3′ *Sac* I and 3′ *Kpn* I overhangs, respectively, to provide cloning sites. The oligonucleotides were annealed and cloned into the *Kpn* I and *Sac* I sites of pBluescript II KS+, and the product was sequenced by the dideoxy chain-termination method of Sanger *et al.* (13). Antisense probes were made by *in vitro* transcription with T7 RNA polymerase after linearization at the *Kpn* I site in the polylinker; sense probes were made with T3 polymerase after linearization at the *Sac* I site (14). ^{3}H-labeled probes were synthesized with [^{3}H]UTP as precursor (Amersham; 43 Ci/mmol; 1 Ci = 37 GBq) to give a product of about 10^8 dpm/μg. Biotin-labeled probes were also made with T3 or T7 polymerase using as precursor a mixture of UTP and biotin-11-UTP (Enzo Diagnostics, New York).

Cytology. GVs were isolated manually from *Xenopus* oocytes and their contents were spread for cytological analysis as described (15). *In situ* hybridization with ^{3}H-labeled probes followed the procedure outlined in Wu *et al.* (2). Biotin-labeled probes were hybridized and washed under the same conditions as ^{3}H-labeled probes. They were detected with fluorescein-labeled avidin DN or with an unlabeled goat antibody against biotin (Vector Laboratories) followed by fluorescein-labeled rabbit anti-goat IgG (Organon Teknika). Preparations were examined by bright-field, epifluorescence, or confocal microscopy using the Zeiss LS-10 laser scan microscope.

RESULTS AND DISCUSSION

Structure and Composition of C Snurposomes. C snurposomes in the GV of *Xenopus* are spherical structures ranging in diameter from <1 μm to >10 μm (in the newt *Notophthalmus* they may be as large as 20 μm). Often, but not invariably, they have one or more B snurposomes tightly attached to their surface and one or more inclusions that resemble B snurposomes (Fig. 1). The entire complex of C snurposome, inclusions, and attached Bs constitutes the structure referred to as a *sphere* or *sphere organelle* in the cytological literature (reviewed in ref. 16). Here we confine the term C snurposome to the matrix of the sphere or to the whole organelle when it lacks associated B snurposomes and inclusions.

Abbreviations: GV, germinal vesicle; mAb, monoclonal antibody; sn, small nuclear; RNP, ribonucleoprotein.

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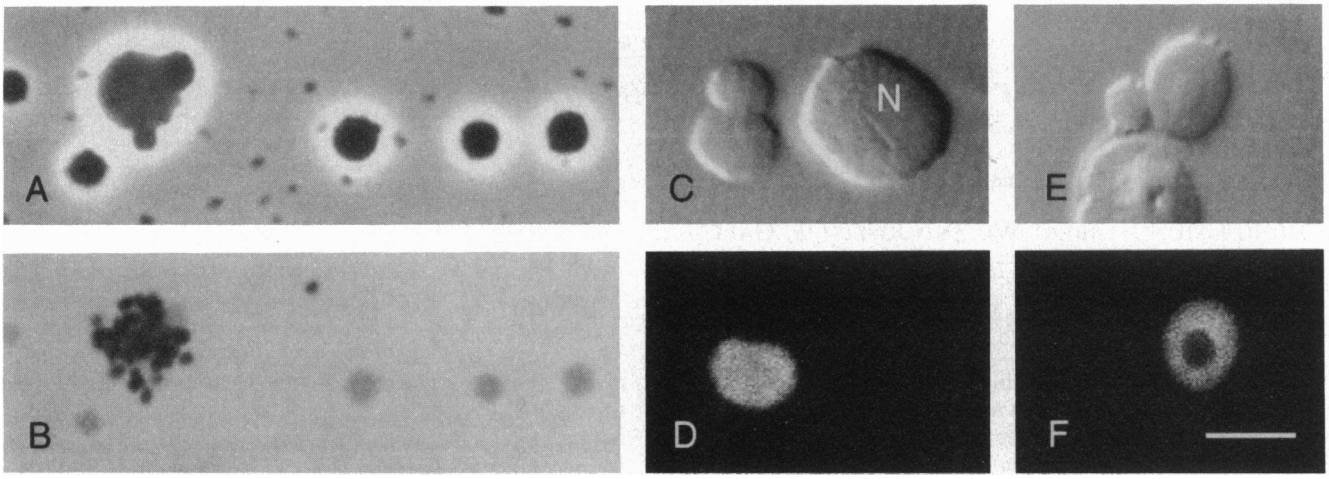

FIG. 1. Localization of U7 snRNA in C snurposomes of *Xenopus*. (*A*) Five B snurposomes and a C snurposome with two attached Bs. Phase contrast. (*B*) Same field after hybridization with a ^{3}H-labeled antisense U7 riboprobe. Autoradiographic exposure for 4 days; stained with Coomassie blue. Silver grains are numerous above the C snurposome. (*C*) A field containing a C snurposome with a single attached B snurposome, next to a nucleolus (N). Differential interference contrast. (*D*) The same field after hybridization with a biotin-labeled antisense U7 riboprobe followed by unlabeled goat anti-biotin and fluorescein-labeled rabbit anti-goat IgG. Only the C snurposome is labeled. Confocal image. (*E*) Another field showing a C snurposome with an attached B snurposome, next to a nucleolus. In this case the C has a B-like inclusion. Differential interference contrast. (*F*) The same field after hybridization with a biotin-labeled antisense U7 riboprobe detected with fluorescein-labeled avidin DN. The inclusion is not labeled. Confocal image. (Bar = 5 μm.)

B and C snurposomes stain strongly with monoclonal antibody (mAb) K121 against the trimethylguanosine cap of snRNAs (17) and mAb Y12 against the Sm epitope of snRNA-associated proteins (18). Hence the entire sphere organelle is uniformly stained by these antibodies (1, 2); in some cases the inclusions may be detectable because they stain slightly more or slightly less intensely than the matrix. The situation is quite different with mAb αSC35 against the non-snRNP essential splicing factor SC35 (6), which stains the B snurposomes and inclusions but not the matrix (2). ^{3}H-labeled antisense probes against the major splicing snRNAs (U1, U2, U4, U5, and U6) hybridize strongly with B snurposomes but weakly or not at all with Cs (2).

Demonstration of U7 snRNA. Using antisense probes against the entire *Xenopus* U7 snRNA, we find strong and exclusive *in situ* hybridization to C snurposomes (Fig. 1). Differential labeling of the parts of a sphere organelle are most clearly seen with biotinylated probes detected by fluorescence. Whereas the C snurposome stains strongly, neither the B snurposomes on the surface of the C (Fig. 1 *C* and *D*) nor the B-like inclusions (Fig. 1 *E* and *F*) are labeled. We have also used a ^{3}H-labeled antisense probe against *Xenopus* U7 with similar results, although in this case the heavy label above the sphere organelle prevents any statement about the inclusions (Fig. 1 *A* and *B*). Sense strand probes, both biotinylated and ^{3}H-labeled, gave only background levels of stain or autoradiographic signal (data not shown).

The experiments reported here were made possible by the recent publication of the *Xenopus* U7 snRNA sequence by Phillips and Birnstiel (12). Probes based on the *Xenopus* sequence gave strong positive reactions in C snurposomes of *Xenopus* but not *Notophthalmus*. Earlier we had used probes based on the human U7 sequence without success. The 5′ halves of the *Xenopus* and human sequences are nearly identical (32/34 nucleotides), but the 3′ halves are different in both sequence and length (9, 12). It is probable that our earlier hybridization conditions were too stringent for this degree of divergence.

U7 snRNA is capped at its 5′ end with trimethylguanosine and is associated with Sm proteins (8, 19). Thus, its presence in the C snurposome explains our earlier observation that Cs stain with antibodies against trimethylguanosine and the Sm antigen, both of which are characteristic markers for snRNPs yet are weak or negative by *in situ* hybridization for the five major splicing snRNAs (1, 2).

The Sphere Organelle. Spheres, or sphere organelles, have been recognized as separate cytological entities for many years. What are probably spheres can be seen in drawings of GV contents made around the turn of this century (e.g., figure 50 in ref. 20). By the early 1960s morphological and cytochemical studies had clearly distinguished them from the much more numerous multiple nucleoli in amphibian GVs (refs. 21, 22; reviewed in ref. 16). At that time it was also recognized that a few of the several dozen spheres within a GV were attached at specific sites on the lampbrush chromosome. Attached spheres are morphologically indistinguishable from those free in the nucleoplasm—that is, they may have inclusions and B snurposomes on their surface. The sites of attached spheres were identified as the histone gene loci in newts (10) and more recently in *Xenopus* (11). In *Xenopus* the histone genes are located at three subterminal loci on chromosomes 8, 9, and 16. Each of these loci may bear a single attached sphere, although it is not uncommon for the sphere to be missing from one or more loci or to be represented by a small C snurposome without attached Bs.

There is a striking parallel between the multiple nucleoli and the multiple spheres of GVs: in both cases the majority of organelles are extrachromosomal, whereas a small number are attached at defined chromosomal loci. It is well known that the extrachromosomal nucleoli contain amplified copies of the genes coding for rRNA (23, 24), and so it is reasonable to ask if the extrachromosomal spheres contain some type of amplified DNA. Our earlier *in situ* hybridization studies gave no evidence for histone genes in the extrachromosomal spheres (25–27). Recently Phillips *et al.* (28) asked whether the genes for one or more of the snRNAs might be amplified in the spheres. To test this possibility, they compared DNA from known numbers of GVs and red cell nuclei of *Xenopus*; they found no evidence for amplification of the genes for U1, U2, U4, U5, U6, or U7 in the GV, although the 1000-fold amplification of the rRNA-encoding DNA was readily detectable in their dot-blot experiments. These data rule out any high degree of gene amplification of the snRNA genes in spheres (or elsewhere in the GV, since the biochemical data refer to the entire contents of the nucleus).

Recent data suggest that the sphere organelle of oocytes is related to the coiled body, so far described only from somatic nuclei (29). The coiled body in human cells contains a specific protein, p80-coilin, for which a partial cDNA has been cloned (30). Two antibodies against p80-coilin, one against the fusion protein produced by the clone and one against a carboxyl-terminal peptide, stain C snurposomes of *Xenopus* and *Notophthalmus* (data not shown). In addition, Tuma *et al.* (31) have recently cloned a cDNA for a protein from the C snurposomes of *Xenopus*, and this protein has regions of identity with human p80-coilin. Because the coiled body contains splicing snRNPs in addition to p80-coilin, it cannot be a simple homologue of the C snurposome. Instead, the coiled body may correspond to the entire sphere organelle—C snurposome, inclusions, and attached B snurposomes.

Role of C Snurposomes in Histone Pre-mRNA Processing. Transcription of the histone genes occurs on lampbrush loops immediately adjacent to the attached spheres (25–27). The proximity of histone nascent transcripts to an organelle that contains U7 snRNA strongly suggests a functional relationship. One possibility is that newly completed histone transcripts leave the loops and enter the sphere, where they are processed. Alternatively, the sphere may be a site for preassembly of U7 snRNPs, which move from the sphere to the nascent transcripts on the loops, where processing itself takes place. Neither of these schemes, however, explains the large number of free spheres in the nucleoplasm at considerable distances from the histone loci. The free spheres could be storage organelles for U7 snRNPs destined for use in the embryo after fertilization. The three possible functions of spheres—processing of histone transcripts, assembly of U7 processing complexes, and storage of U7 snRNPs—are not mutually exclusive and could occur concomitantly.

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