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In Vitro Assembly of Coiled Bodies in *Xenopus* Egg Extract

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When demembranated sperm nuclei are placed in a *Xenopus* egg extract, they become surrounded by a nuclear envelope and then swell to form morphologically typical pronuclei. Granules ranging from <1.0 to ~3.0 μm in diameter appear within such nuclei. Bell *et al.* identified four nucleolar proteins in these "prenucleolar bodies" by immunofluorescent staining (fibrillarin, nucleolin, B23/NO38, 180-kDa nucleolar protein). By in situ hybridization we show that these bodies also contain U3 and U8 small nuclear RNAs (snRNAs), known to be involved in pre-rRNA processing. Moreover, they contain all the snRNAs involved in pre-mRNA splicing (U1, U2, U4, U5, and U6), as well as U7, which is required for histone pre-mRNA 3′ end formation. In addition to the nucleolar antigens previously identified, we demonstrated staining with antibodies against the Sm epitope, trimethylguanosine, and coilin. Because the composition of these prenucleolar bodies is closer to that of coiled bodies than to nucleoli, we propose that they be referred to as coiled bodies. The existence of large coiled bodies in transcriptionally inactive pronuclei suggests that they may play a role in the import, assembly, and storage of RNA processing components but are not themselves sites of processing. In transcriptionally active nuclei coiled bodies could serve as sites for initial preassembly and distribution of snRNP complexes for the three major RNA processing pathways: pre-mRNA splicing, pre-rRNA processing, and histone pre-mRNA 3′ end formation.

INTRODUCTION

The pioneering experiments of Lohka and Masui (1983, 1984) showed that morphologically typical pronuclei are formed in vitro when demembranated sperm nuclei are incubated in an appropriate amphibian egg extract. In its simplest form the extract consists of egg cytoplasm lightly centrifuged to remove yolk and lipids. The nuclei assembled under these conditions have nuclear envelopes with pores and an internal lamina, and they specifically import nuclear proteins stored in the cytoplasm of the egg (Newmeyer *et al.*, 1986; Newport, 1987; Newport and Forbes, 1987; Newmeyer and Wilson, 1991; Almouzni and Wolffe, 1993). At the light microscope level, the chromatin has a somewhat fibrous appearance, but otherwise these nuclei appear relatively structureless except for small granules that vary from barely detectable to a maximum of ~3 μm diameter (e.g., Figure 1b in Newmeyer *et al.*, 1986; Figure 4 in Newmeyer and Forbes, 1988). Similar granules occur in early cleavage nuclei of *Xenopus* and other amphibians, where they have been called nucleolar bodies or prenucleolar bodies (Hay and Gurdon, 1967; Hay, 1968; Steele *et al.*, 1984). Bell *et al.* (1992) were the first to study the composition of prenucleolar bodies in nuclei assembled in vitro. They showed strong and highly specific immunofluorescent staining with antibodies against four nucleolar proteins—fibrillarin, nucleolin, B23/NO38, and a 180-kDa protein found in *Xenopus* nucleoli (Schmidt-Zachmann *et al.*, 1984). They were unable to demonstrate either rRNA or U3 small nuclear (sn) RNA[1] by in situ hybridization with digoxigenin-labeled riboprobes. Because rRNA synthesis does not begin until late in embryogenesis (Brown and Gurdon, 1964; Brown and Littna, 1964), the absence of rRNA was expected. On the other hand, U3 snRNA is asso-

[1] Abbreviations used: GV, germinal vesicle; sn, small nuclear.

Table 1. Antibodies used for immunofluorescence

Antibody	Specificity or antigen used for production	Reference
mAb 17C12	Fibrillarin	K.M. Pollard, pers. comm.
Rabbit serum R2D2	*Xenopus* nucleolin	Heine *et al.*, 1993
mAb No-114	180-kDa nucleolar protein from *Xenopus*	Schmidt-Zachmann *et al.*, 1984
mAb Y12	Sm epitope of snRNPs	Lerner *et al.*, 1981
mAb K121	Trimethylguanosine cap of snRNAs	Krainer, 1988
mAb anti-SC35	Human SR protein SC35 (non-snRNP splicing factor)	Fu and Maniatis, 1990
mAb 104	*Xenopus* SR proteins (non-snRNP splicing factors)	Roth *et al.*, 1990
Rabbit serum R288	Human p80-coilin (14-kDa carboxy-terminal fragment)	Andrade *et al.*, 1993
mAb H1	SPH-1 (*Xenopus* coilin)	Tuma *et al.*, 1993
Rabbit sera R31 and R80	*Xenopus* coilin peptide	This paper
mAb iD2	A and B group hnRNPs	Leser *et al.*, 1984
mAb 4D11	L hnRNP	Piñol-Roma *et al.*, 1989
Rabbit serum penta	Acetylated histone H4	Lin *et al.*, 1989
mAb 37-1A9	*Xenopus* xnf7 protein	Dreyer *et al.*, 1981

ciated with fibrillarin under some conditions (Tyc and Steitz, 1989), and one would not have been surprised to find it in these nuclei, despite the absence of rRNA synthesis. We therefore decided to examine prenucleolar bodies more closely using ^{3}H-labeled antisense probes for snRNAs. Here we show that prenucleolar bodies do, in fact, contain U3 snRNA, as well as U8, another snRNA associated with fibrillarin. Moreover, they contain all the pre-mRNA splicing snRNAs (U1, U2, U4, U5, and U6) and U7 snRNA, which is involved in histone pre-mRNA 3′ end processing. Finally, they react with antibodies against coilin, a protein originally described from the coiled body of somatic nuclei (Raška *et al.*, 1991). On the basis of their composition, we suggest that prenucleolar bodies formed during nuclear assembly in egg extracts are more closely related to coiled bodies than to typical nucleoli. In this paper, we refer to them as coiled bodies.

MATERIALS AND METHODS

Animals and Egg Collection

Adult *Xenopus laevis* were purchased from Xenopus 1 (Ann Arbor, MI). They were maintained in fiberglass aquaria and fed on beef heart. Three or four days before an experiment, a female was primed with an injection of 100 IU of pregnant mares' serum (Sigma, St. Louis, MO). The evening before eggs were to be collected, the female was given a single dose of 300 IU of human chorionic gonadotropin (Sigma) into the dorsal lymph sac and was placed in a 1-gal aquarium containing about 2 l of 0.1 M NaCl. Unfertilized eggs laid in the saline solution do not spontaneously activate (Leno and Laskey, 1991). Furthermore, the jelly does not swell, and the eggs do not adhere to each other. The female usually laid eggs overnight and continued to lay in the morning, if the aquarium was left undisturbed and in the dark at 16–20°C; it was generally not necessary to squeeze the animal for additional eggs.

Nuclear Assembly Extracts

Eggs from a single female were dejellied by gently swirling in 2% cysteine, pH 7.8 for no more than 10 min. For preparing crude interphase egg extract, we used S-lysis buffer according to the protocol described by Newmeyer and Wilson (1991). In brief, eggs were centrifuged at 4°C for 12 min at 9000 × *g* (10 000 rpm in the Beckman SW56 rotor [Fullerton, CA]), after which the yolk and lipid layers were discarded. The crude extract was held on ice for ≤4–5 h without appreciable decay of nuclear assembly activity. Demembranated *Xenopus* sperm were prepared with lysolecithin and stored at −70°C in small aliquots containing 80 000 sperm heads per microliter (Newmeyer and Wilson, 1991). For nuclear assembly 1–4 μl of sperm heads were added to 50 μl of egg extract and held at room temperature. Nuclei generally reached their maximal size by ~1 h but were usable for cytological preparations for ≤3–4 h.

Cytology

For immunofluorescence and in situ hybridization studies a sample of 2–3 μl of egg extract was placed in the center of a siliconized 18-mm^2 coverslip and inverted over a "subbed" slide (prepared by dipping standard 3″ × 1″ microscope slides in 0.5% gelatin, 0.05% $CrK(SO_4)_2 \cdot 12H_2O$, drying in air, and baking overnight at 65°C). In most cases capillarity drew the solution evenly to the edges of the coverslip; additional pressure on the coverslip was not used, because the fragile nuclei were easily broken. The slide was placed in liquid N_2 until frozen, the coverslip was flipped off with a razor blade, and the preparation was placed in 2% paraformaldehyde in 95% ethanol for 30–60 min. Slides were stored in 95% ethanol until further use. In some cases nuclei were prefixed by adding an equal volume of egg extract to 4% paraformaldehyde in OR2 buffer (Wallace *et al.*, 1973). Preparations were made as with unfixed extract, except that squashing was possible, because the nuclei were less fragile.

Immunofluorescence

Slides were hydrated through a descending alcohol series, rinsed in phosphate-buffered saline (0.14 M NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.0), and blocked with 10% horse serum in PBS for 15–30 min. Preparations were reacted with the primary antibody for ~1 h, after which they were rinsed in 10% horse serum and treated with a secondary antibody for another hour. Secondary antibodies were affinity-purified rhodamine- or fluorescein-labeled goat anti-mouse or goat anti-rabbit IgG (Cappel/Organon Teknika, Durham, NC). Slides were mounted in 50% glycerol containing 1 mg/ml of phenylenediamine, pH 9, and sometimes 1.0 μg/ml of 4′, 6-diamidino-2-phenylindol; when not being observed, they were stored at −20°C. Antibodies used in this study are listed in Table 1. The monoclonal antibodies (mAbs) were used as undiluted culture supernates, whereas the rabbit sera were diluted 1/200 to 1/500 with 10% horse serum.

Table 2. Clones used for synthesis of ^{3}H-labeled riboprobes

RNA encoded	Reference
Human U1, U2 snRNAs	Wu *et al.*, 1991
Human U4, U5, U6 snRNAs	Black and Pinto, 1989
Xenopus U3 snRNA	Jeppesen *et al.*, 1988
Xenopus U7 snRNA	Wu and Gall, 1993
Xenopus U8 snRNA	Peculis and Steitz, 1993
Human U11, U12 snRNAs	Wassarman and Steitz, 1992
Human 7SK RNA	Wassarman and Steitz, 1991
Xenopus MRP RNA	Bennett *et al.*, 1992
Xenopus rRNA	Bakken *et al.*, 1982

We also produced two new polyclonal sera, R31 and R80, by immunizing rabbits with a synthetic 21 residue peptide that includes amino acids 429–448 from the SPH1 protein of *Xenopus* (Tuma *et al.*, 1993). The sequence of this peptide, LPLLAAAPQVGKLIAFKLLE(K), differs by only two residues from amino acids 464–483 of human p80-coilin. The antigen used for injection consisted of four copies of the indicated sequence held together by a branched lysine backbone (Tam, 1988).

The immunofluorescence analysis of coiled bodies involved a total of >300 stained preparations.

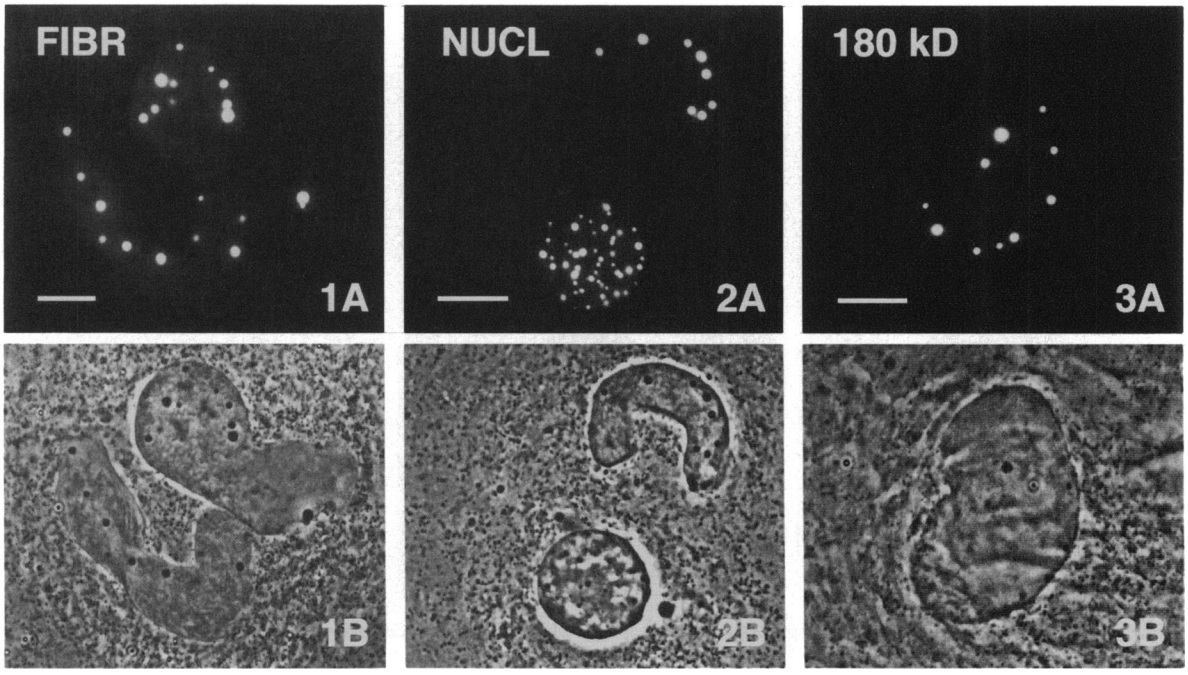
In Situ Hybridization

Slides of egg extract were dehydrated in 100% ethanol, air-dried from acetone, baked for 1 h at 65°C, and cooled. The area occupied by the extract was covered with 7–8 μl of hybridization probe under a 22-mm^2 coverslip; the edges of the coverslip were sealed with rubber cement, and the slide was incubated for ~16 h at 42°C. After removal of the coverslip under 1× SSC (SSC is 0.15 M NaCl + 0.015 M Na citrate, pH 7.0), the slides were incubated in 0.1× SSC for 1 h at 65°C. They were dehydrated, air-dried from acetone, and covered with Kodak (Rochester, NY) NTB-2 liquid emulsion diluted one-half with water. Probes were sense or antisense transcripts copied from cloned snRNA genes with SP6, T3, or T7 RNA polymerase, depending upon the clone and orientation of the insert. ^{3}H-UTP (40–48 Ci/mmol) (Amersham, Arlington Heights, IL) was used as the sole UTP source, resulting in transcripts with specific activities of ~10^8 dpm/μg. Probes were used at 10^5 cpm/μl in a hybridization mix consisting of 40% formamide, 4× SSC, 100 mM Na_3PO4, pH 7.0, 300 μg/ml of *Escherichia coli* tRNA, and 300 μg/ml of *E. coli* DNA. DNA clones used in this study are listed in Table 2. The in situ hybridization analysis of coiled bodies involved ~250 autoradiographs.

RESULTS

Nucleolar Proteins

Bell *et al.* (1992) studied the immunofluorescent staining of prenucleolar bodies found in pronuclei assembled in vitro in *Xenopus* egg extract, hereafter referred to as coiled bodies. They used antibodies against four nucleolar proteins: fibrillarin (human serum S4, Reimer *et al.*, 1987), nucleolin (rabbit serum anti-pp105, Pfeifle and Anderer, 1983), B23/NO38 (mAb No-185, Schmidt-Zachmann *et al.*, 1987), and a protein of M_r = 180 kDa (mAb No-114, Schmidt-Zachmann *et al.*, 1984). We confirmed the presence of fibrillarin in the



Figures 1–3. Pronuclei assembled in vitro from *Xenopus* sperm nuclei after incubation in *Xenopus* egg extract for 1–2 h. Immunostained with antibodies against nucleolar proteins. Figures 1A, 2A, and 3A are immunofluorescent images; 1B, 2B, and 3B are phase contrast images of the same nuclei. (Figure 1) mAb 17C12 against fibrillarin (K. Michael Pollard, personal communication). (Figure 2) Rabbit serum R2D2 against *Xenopus* nucleolin (Heine *et al.*, 1994). (Figure 3) mAb No-114 against a 180-kDa *Xenopus* nucleolar protein (Schmidt-Zachmann *et al.*, 1984). Bar, 10 μm.

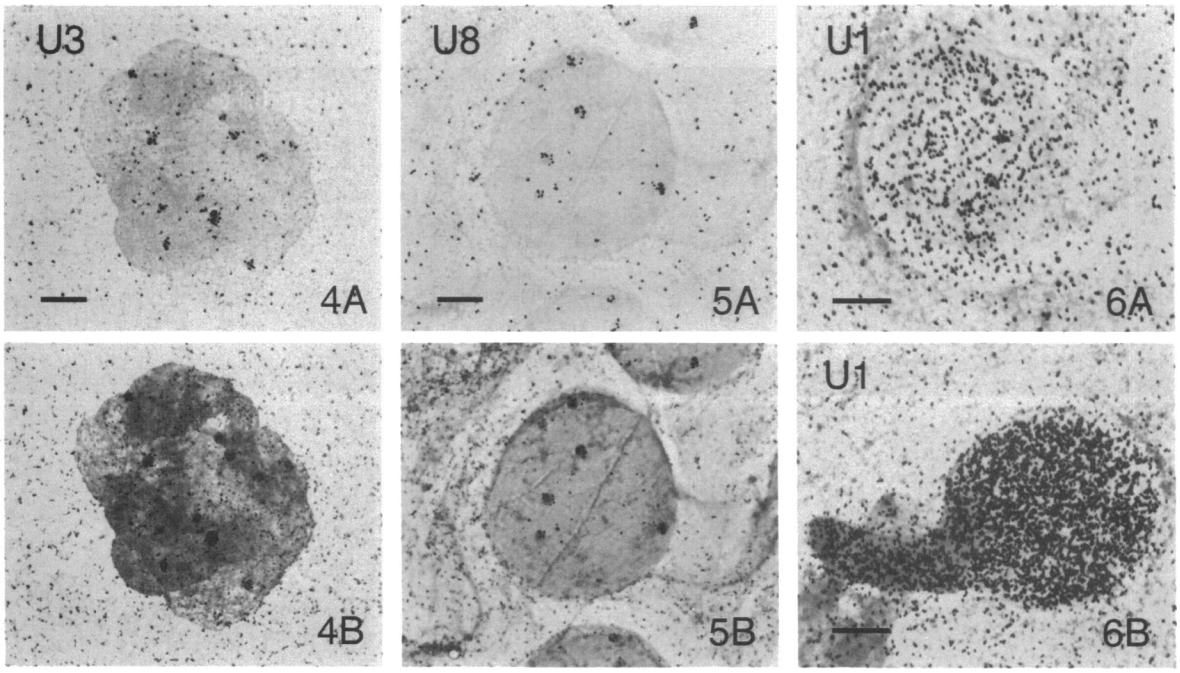
coiled bodies (Figure 1) using mAb 17C12, a previously undescribed antibody derived from a mouse injected with $HgCl_2$ (Pollard, personal communication). We also demonstrated nucleolin (Figure 2) using rabbit serum R2D2 against *Xenopus* nucleolin (Heine *et al.*, 1993), and we confirmed the staining by mAb No-114 with an antibody sample kindly provided by P. Bell and U. Scheer (University of Würzburg) (Figure 3).

rRNA Processing Components

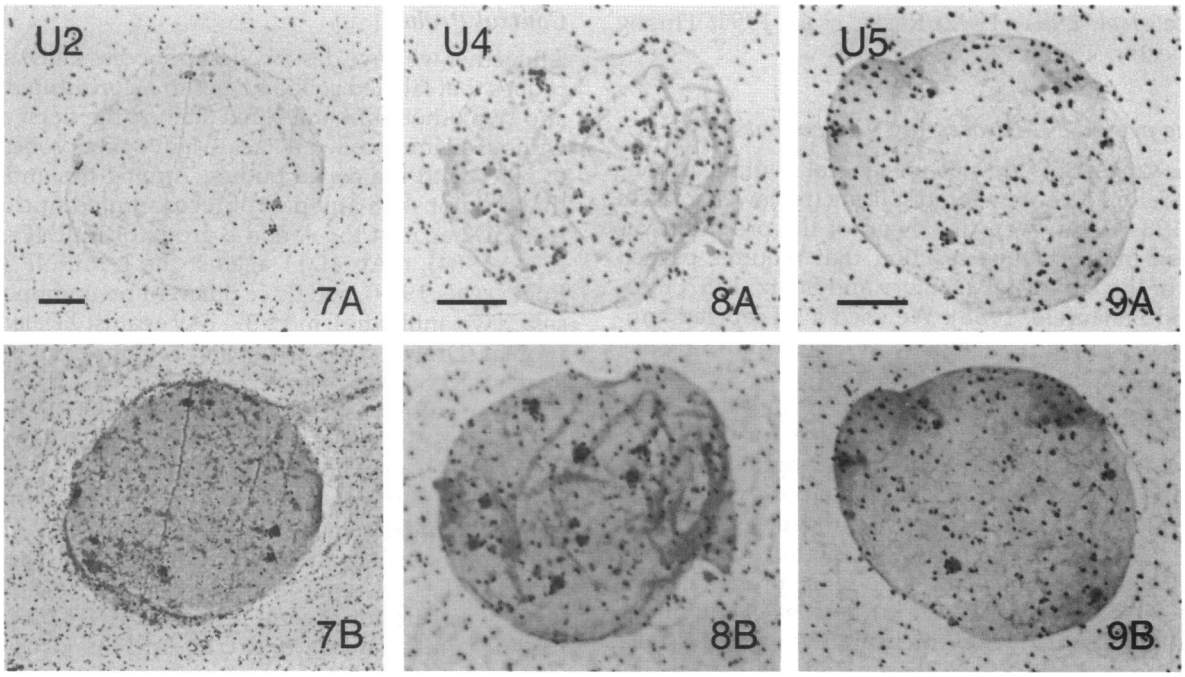
Bell *et al.* (1992) were unable to demonstrate U3 snRNA in the coiled bodies by in situ hybridization using a digoxigenin-labeled antisense riboprobe detected by immunofluorescence. We felt that a ^{3}H-labeled probe might be more sensitive, even though autoradiography provides less spatial resolution than immunofluorescence. U3 was readily demonstrable in the coiled bodies using a ^{3}H-labeled antisense riboprobe ($\sim 10^8$ dpm/μg) with exposure times of 10–20 d (Figure 4). Encouraged by this result, we probed for U8, another snRNA involved in rRNA processing (Peculis and Steitz, 1993) and known to form a complex under some circumstances with fibrillarin and U3 (Tyc and Steitz, 1989). U8 was detectable with a ^{3}H-labeled probe of similar specific activity to that used for U3 and after comparable autoradiographic exposure times (Figure 5). These results suggest that U3 and U8 are present at roughly similar concentrations in the coiled bodies.

Pre-mRNA Splicing Components

The results so far described demonstrated the presence of nucleolar proteins and two snRNAs involved in rRNA processing. Standing alone, these data would suggest that the name prenucleolar bodies was appropriate for these structures and perhaps for the similar bodies found in cleavage nuclei of *Xenopus* and other amphibia (Karasaki, 1964; Hay and Gurdon, 1967). However, it turns out that many nonnucleolar components are also present, prominent among which are the snRNPs involved in pre-mRNA splicing. ^{3}H-labeled probes for U2, U4, U5, and U6 gave results essentially like those already described for U3 and U8, namely a few silver grains over the coiled bodies after autoradiographic exposures of 10–20 d (Figures 7–10). U1 gave different results, depending upon whether the material was lightly or heavily squashed during the initial spreading of the egg extract under the coverslip. In lightly squashed preparations the nuclei were densely labeled throughout



Figures 4–6. Pronuclei assembled in *Xenopus* egg extract. Autoradiographs stained with Coomassie blue after in situ hybridization with ^{3}H-labeled antisense snRNA probes. Figures 4A, 5A, 6A, and 6B were photographed in blue light (470 or 488 nm) to suppress the stain and accentuate the silver grains in the emulsion; Figure 4B was photographed in white light and 5B in blue-green (510 nm) to show morphological details, especially the darkly stained coiled bodies. (Figure 4) Hybridized with antisense U3 snRNA, 18-d exposure; A and B are the same nucleus. (Figure 5) Hybridized with antisense U8 snRNA, 12-d exposure; A and B are the same nucleus. (Figure 6) Hybridized with antisense U1 snRNA. (A) A nucleus with an overall low level of hybridization, probably because of leakage of U1 snRNP during specimen preparation. Clusters of label over coiled bodies, 17-d exposure. (B) A heavily labeled nucleus in which it is impossible to discern specific labeling of the coiled bodies, 17-d exposure. Bar, 10 μm.



Figures 7–9. Nuclei assembled in *Xenopus* egg extract. Autoradiographs after in situ hybridization with ^{3}H-labeled antisense snRNA probes. Figures 7A, 8A, and 9A were photographed at 470 or 488 nm to accentuate the silver grains; 7B, 8B, and 9B are the same nuclei photographed at 510 nm to show the stained coiled bodies. (Figure 7) Hybridized with antisense U2 snRNA, 12-d exposure. (Figure 8) Hybridized with antisense U4 snRNA, 18-d exposure. (Figure 9) Hybridized with antisense U5 snRNA, 18-d exposure. Bar, 10 μm.

(Figure 6B), preventing an assessment of the coiled bodies themselves. In more heavily squashed material, many lightly labeled nuclei were seen in which the coiled bodies appeared to be specifically labeled (Figure 6A). We think that the more weakly labeled nuclei are simply those that have lost their fluid contents through tears in the nuclear envelope. These results suggest that U1 snRNA is associated with the other splicing snRNAs in the coiled bodies but is present in much larger amounts in a "soluble" form throughout the nucleus.

We also used antibodies to probe for the splicing snRNPs. U1, U2, U4, and U5 snRNAs have a trimethylguanosine cap at their 5′ end, which they acquire in the cytoplasm shortly after their synthesis (Mattaj, 1988). Antibodies against the trimethylguanosine cap give strong immunofluorescent staining of nuclear speckles (Reuter *et al.*, 1984) and coiled bodies (Raška *et al.*, 1991) in tissue culture nuclei. One such antibody, mAb K121 (Krainer, 1988), stained the coiled bodies in reconstituted sperm nuclei (Figure 13). Many nuclei also exhibited a generalized stain throughout the nucleoplasm, which was most evident in lightly squashed preparations. These results were expected from the distribution of the splicing snRNAs: strong staining for trimethylguanosine in the coiled bodies because of U1, U2, U4, and U5 and generalized nuclear staining primarily because of U1 snRNA. As in the in situ hybridization experiments, generalized label was presumably lost when nuclei were broken during specimen preparation.

The splicing snRNAs can be immunoprecipitated as a group from nuclear extracts with antibodies of the "anti-Sm" type, originally named from a human autoimmune serum (Tan and Kunkel, 1966). Detailed studies show that antibodies of this type recognize several proteins common to the U1, U2, U4/U6, and U5 snRNPs (Lerner *et al.*, 1979; Steitz *et al.*, 1988). mAb Y12 has been used extensively to probe for the Sm group of proteins by immunofluorescence (Lerner *et al.*, 1981), and it gave intense staining of the coiled bodies in reconstituted sperm nuclei (Figure 14). Like mAb K121, it also stained the nucleoplasm in lightly squashed preparations.

Finally, we probed for the SR group of non-snRNP splicing factors using mAb anti-SC35 (Fu and Maniatis, 1990) and mAb 104 (Roth *et al.*, 1990, 1991). Both antibodies gave strong staining of the nucleoplasm in a fraction of nuclei, but neither stained the coiled bodies. As shown in Figure 15, the coiled bodies sometimes could be detected as dark "holes" in an otherwise brightly stained nucleus. These results demonstrate that one or more SR proteins are present in the pronuclei but are not specifically associated with the coiled bodies. In this respect these nuclei are similar to tissue culture nuclei, in which the speckles are well stained by anti-SC35 but the coiled bodies are not (Carmo-

Fonseca *et al.* 1991, 1992; Raška *et al.* 1991; Huang and Spector, 1992).

Histone pre-mRNA Processing Components

Histone transcripts do not in general contain introns and therefore do not undergo splicing (reviewed in Stein *et al.*, 1984). However, the 3′ end of the pre-mRNA is removed in a cleavage reaction that requires participation of the U7 snRNP (Mowry and Steitz, 1987; Birnstiel and Schaufele, 1988). We used a ^{3}H-labeled antisense transcript of *Xenopus* U7 (Phillips and Birnstiel, 1992) to look for U7 in the coiled bodies. As shown in Figure 11, a clear signal was obtained after a 10–20-d autoradiographic exposure. As a control for this experiment, we also hybridized with a full length sense transcript and, to our surprise, obtained a positive signal of equal intensity to that from the antisense transcript (Figure 12). At this time we have no adequate explanation for this finding. The same sense probe failed to hybridize to C snurposomes in the *Xenopus* germinal vesicle (GV), which label intensely with the antisense probe (Wu and Gall, 1993). It is unlikely, therefore, that the sense probe was contaminated with antisense sequences or hybridized to U7 snRNA because of intrastrand complementarity.

Coilin

The only nuclear organelle so far described that contains both splicing and pre-rRNA processing components is the coiled body (Raška *et al.*, 1991; Jiménez-García *et al.*, 1993; reviewed in Lamond and Carmo-Fonseca, 1993). It was of considerable interest, therefore, to stain pronuclei with antibodies against p80-coilin, a recently described protein that occurs at high concentration in the coiled body (Andrade *et al.*, 1991). We began with serum R288, a rabbit polyclonal serum that recognizes a 14-kDa fragment at the carboxy terminus of human p80-coilin (Andrade *et al.*, 1993). This serum gave a clearly positive immunofluorescent signal in the coiled bodies (Figure 16). We next used mAb H1 that gives highly specific staining of C snurposomes in the *Xenopus* GV and recognizes a protein, SPH1, with two regions of sequence similarity to human p80-coilin (Tuma *et al.*, 1993). The staining with mAb H1 was among the strongest of any antibody we have used (Figure 17). In addition to the coiled bodies themselves, the nuclear contents as a whole were well stained in a fraction of the nuclei. Finally, we used two sera, R31 and R80, from rabbits immunized with a synthetic 21 amino acid peptide whose sequence is from a region of *Xenopus* SPH1 that differs by only two amino acids from the corresponding region in human p80-coilin (amino acids 429–448 in SPH1 and 464–483 in p80-coilin). Both sera gave strong staining of the coiled bodies and generalized staining of the nuclear contents (Figure 18).

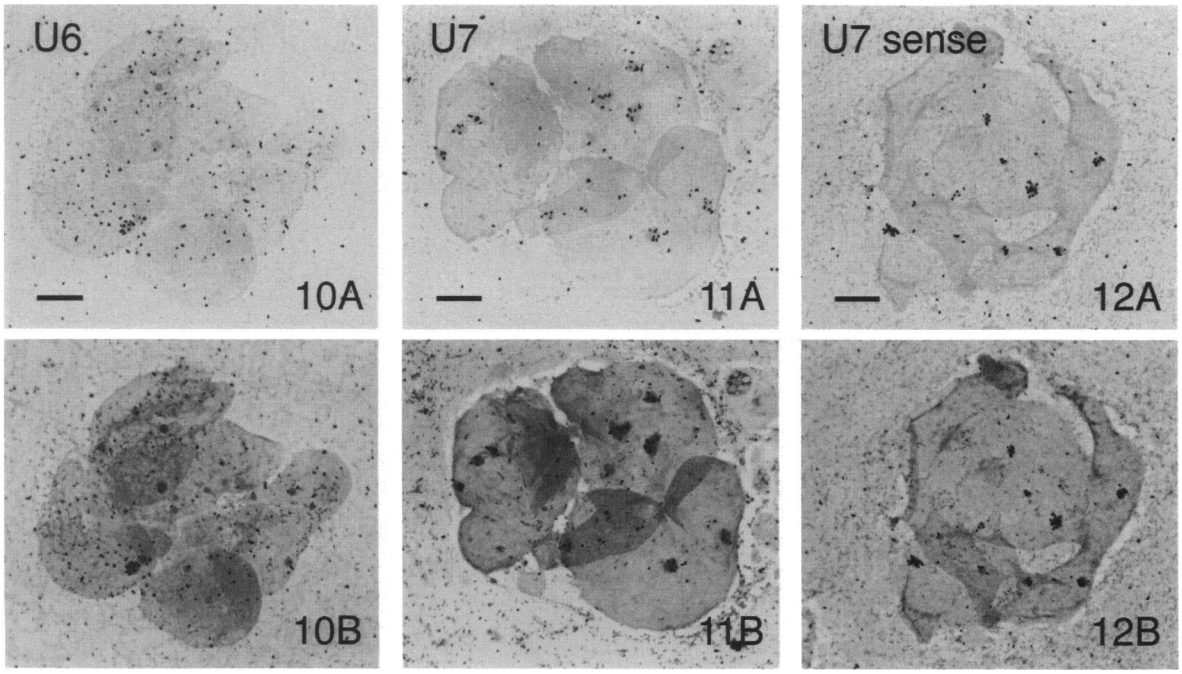
Control Probes

Altogether we have described 9 antibodies and 9 snRNA probes that labeled coiled bodies in reconstituted nuclei and two other antibodies that stained the nucleoplasm without staining coiled bodies. Many other probes failed to react with the coiled bodies. Among the antibodies that did not stain any part of reconstituted nuclei were mAb iD2 against the A and B group of hnRNPs (Leser *et al.*, 1984), mAb 4D11 against the L hnRNP (Piñol-Roma *et al.*, 1989), and three different preimmune rabbit sera. Two antibodies, mAb 37-1A9 against *Xenopus* xnf7 protein (Dreyer *et al.*, 1981) and rabbit serum "penta" against acetylated histone H4 (Lin *et al.*, 1989), gave the same pattern as mAbs 104 and α-SC35; that is, they stained the nucleus more or less uniformly but left the coiled bodies unstained.

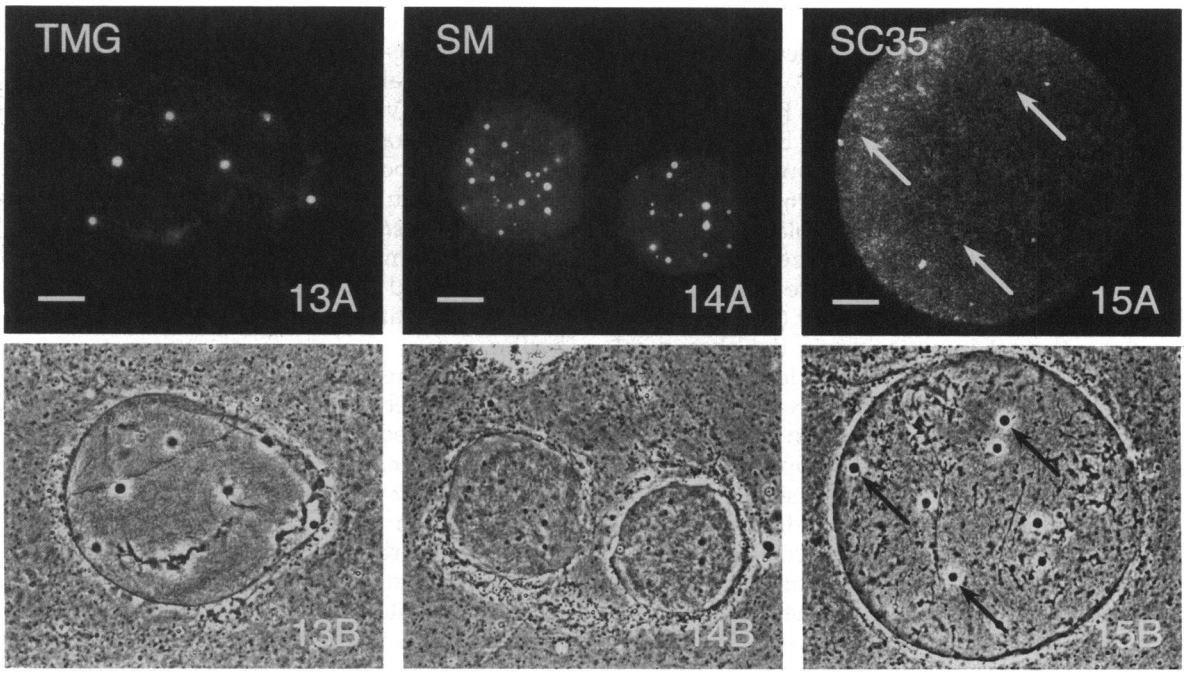
Negative controls for the in situ hybridization experiments included sense-strand probes for U1, U2, U3, U4, and U8. In most cases, reconstituted nuclei and their coiled bodies were not labeled above background. The same was true for several unrelated RNA probes, including antisense U11 and U12 snRNAs (Wassarman and Steitz, 1992), sense and antisense 7SK RNA (Wassarman and Steitz, 1991), sense and antisense *Xenopus* RNase MRP RNA (Bennett *et al.*, 1992), and sense-strand rRNA (Bakken *et al.*, 1982). The antisense rRNA probe gave such an intense signal because of rRNA in the extract that no specific statement about the nuclei was possible. In some slides exposed for periods of >2 wk, a few silver grains were seen over a small fraction of the coiled bodies, suggesting that the background binding of some probes is higher over coiled bodies than over the rest of the nucleus. The situation is comparable to that seen in preparations of spread GV contents, where the extrachromosomal nucleoli bind essentially all ^{3}H-labeled riboprobes at a low level, even under stringent hybridization conditions. It could well be that nucleolar proteins in the coiled bodies are responsible for this very low, but detectable background binding.

DISCUSSION

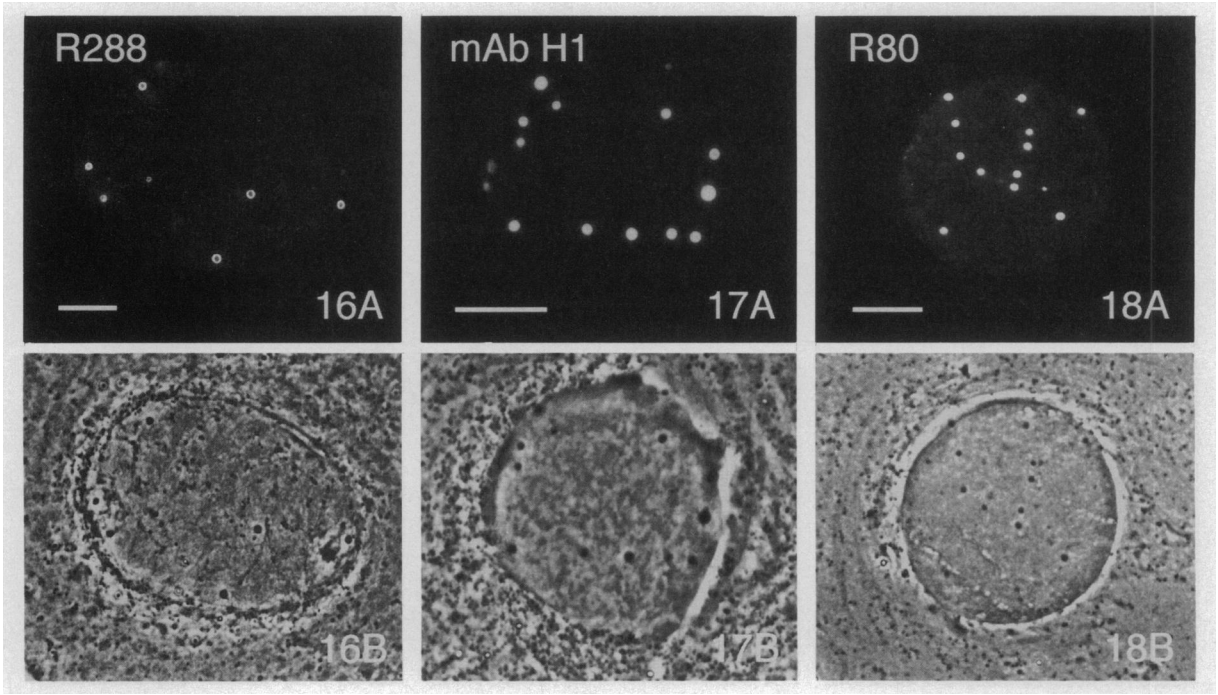
The term *prenucleolar body* has been used to describe two structures that, in light of the results presented here, are quite different. The term usually refers to small bodies that appear within the newly formed nucleus at the end of mitosis and the beginning of interphase (Ochs *et al.*, 1985; Jiménez-García *et al.*, 1989, 1994; Scheer *et al.*, 1993). These bodies contain some typical nucleolar components such as fibrillarin, nucleolin, and U3 snRNA. However, they do not contain rDNA, and their number is greater than the number of nucleolus organizers. As the definitive nucleoli reappear and grow in size, these bodies disappear, presumably by incorporation into the nucleoli. They are prenucleolar in the sense that they contain materials destined for the nu-



Figures 10–12. Nuclei assembled in *Xenopus* egg extract after in situ hybridization with ^{3}H-labeled snRNA probes. Figures 10A, 11A, and 12A were photographed at 488 nm to accentuate the silver grains; 10B, 11B, and 12B are the same nuclei photographed at 510 nm (10B and 12B) or in white light (11B) to show the stained coiled bodies. (Figure 10) Hybridized with antisense U6 snRNA, 18-d exposure. (Figure 11) Hybridized with antisense U7 snRNA, 13-d exposure. (Figure 12) Hybridized with *sense* U7 snRNA, 21-d exposure. Bar, 10 μm.



Figures 13–15. Pronuclei assembled in vitro from *Xenopus* sperm nuclei after incubation in *Xenopus* egg extract for 1–2 h. Immunostained with antibodies against pre-mRNA splicing components. Figures 13A, 14A, and 15A are immunofluorescent images; 13B, 14B, and 15B are phase contrast images of the same nuclei. (Figure 13) mAb K121 against the trimethylguanosine cap (Krainer, 1988). (Figure 14) mAb Y12 against the Sm group of snRNP proteins (Lerner *et al.*, 1981). (Figure 15) mAb anti-SC35 against protein SC35, a non-snRNP essential splicing factor (Fu and Maniatis, 1990). Note that the stain is excluded from the coiled bodies (arrows). Bar, 10 μm.



Figures 16–18. Pronuclei assembled in vitro from *Xenopus* sperm nuclei after incubation in *Xenopus* egg extract for 1–2 h. Immunostained with antibodies against human p80-coilin and the coilin-related protein SPH1 from *Xenopus*. Figures 16A, 17A, and 18A are immunofluorescent images; 16B, 17B, and 18B are phase contrast images of the same nuclei. (Figure 16) Rabbit serum R288 against a fusion protein containing 14 kDa from the carboxy terminus of human p80-coilin (Andrade *et al.*, 1993). (Figure 17) mAb H1 against *Xenopus* SPH1, a protein originally described from C snurposomes of the GV (Tuma *et al.*, 1993). (Figure 18) Rabbit serum R80 against a synthetic 21–amino acid peptide containing residues 429–448 from *Xenopus* SPH1. Bar, 10 μm.

cleoli; they are not true nucleoli, however, because they are not formed at the chromosomal nucleolus organizers and do not synthesize rRNA.

The second use of prenucleolar body has been to describe small round structures seen in both male and female pronuclei and in cleavage stage nuclei (Karasaki, 1965; Hay and Gurdon, 1967; Hay, 1968; Steele *et al.*, 1984; Bell *et al.*, 1992). These prenucleolar bodies differ from typical nucleoli in their smaller size (generally <1 μm diameter vs. 3–4 μm), in the absence of fibrillar and granular zones by electron microscopy (Hay and Gurdon, 1967), and, most importantly, in the absence of rRNA synthesis. In *Xenopus*, typical nucleoli appear first in the dorsal ectoderm at about stages 8–10, somewhat later in the mesoderm, and last of all in the endoderm (Hay and Gurdon, 1967), and their appearance correlates with the onset of rRNA synthesis (Brown and Littna, 1964). Because prenucleolar bodies in the early cleavage nuclei do not fuse to form nucleoli, as they do in tissue culture cells, they are not precursors to nucleoli. Instead the term expresses an undefined hypothetical relationship to nucleoli; namely, that prenucleolar bodies are incomplete nucleoli or in some sense take the place of functional nucleoli.

The study of Bell *et al.* (1992) seemed to justify the designation prenucleolar body, at least in the case of male pronuclei, by showing the presence of four typical nucleolar proteins—fibrillarin, nucleolin, B23/NO38, and a nucleolus-specific 180-kDa protein. However, the situation is clearly more complex, because these bodies also contain a variety of non-nucleolar components. In terms of overall molecular composition, they most closely correspond to coiled bodies, as described from a variety of cultured cells (Monneron and Bernhard, 1969; Hervás *et al.*, 1980; Raška *et al.*, 1991; Spector *et al.*, 1992; Carmo-Fonseca *et al.*, 1992, 1993; reviewed in Lamond and Carmo-Fonseca, 1993). For this reason, we propose that these structures be referred to as coiled bodies.

Currently the single most definitive marker for coiled bodies in vertebrate nuclei is p80-coilin, a protein originally identified on the basis of human autoimmune sera (Raška *et al.*, 1991). Human p80-coilin was cloned by Andrade *et al.* (1991), who used a bacterially expressed fusion protein to produce a polyclonal serum against the carboxy-terminal 14 kDa of the molecule. This polyclonal serum, R288, has been widely used to distinguish coiled bodies from other intranuclear inclusions, particularly the so-called nuclear speckles (Raška *et al.*, 1991; Spector *et al.*, 1992; Andrade *et al.*, 1993; Carmo-Fonseca *et al.*, 1993; Matera and Ward, 1993). R288 gave strong staining of the coiled bodies in our study (Figure 16), suggesting that coilin or a coilin-related protein is present. A good candidate for the actual

protein in these coiled bodies is SPH1, recently described by Tuma *et al.* (1993) from the sphere organelles of the *Xenopus* GV. SPH1 was identified on the basis of mAb H1, which stains the C snurposome component of the sphere organelle. It also stains the coiled bodies formed in egg extracts (Figure 17). It should be noted that R288 against human p80-coilin likewise stains C snurposomes (Wu and Gall, 1993). In other words, polyclonal serum R288 against human p80-coilin and mAb H1 against *Xenopus* SPH1 both stain C snurposomes in the GV and coiled bodies formed in egg extracts. Although human p80-coilin and SPH1 differ considerably in overall amino acid sequence, they share short regions of identity in both the amino and carboxy terminal parts of the molecule (Tuma *et al.*, 1993; Takano and Chan, unpublished data; Z. Wu, unpublished data). The 14-kDa region of human p80-coilin recognized by serum R288 contains the longest stretch of amino acid identity between coilin and SPH1; thus it is likely that serum R288 recognizes the shared region, accounting for its ability to stain SPH1. To investigate this issue further, we produced two polyclonal sera (R31 and R80) against a 21 amino acid peptide that includes amino acids 429–448 of SPH1 and differs by only two residues from amino acids 464–483 of human p80-coilin. Both sera stain C snurposomes of *Xenopus* GVs and coiled bodies in egg extracts (Figure 18).

In addition to p80-coilin, coiled bodies in tissue culture cells are characterized by their high concentration of the splicing snRNPs U1, U2, U4, U5, and U6. The same is true of the coiled bodies formed in vitro in egg extracts, as shown by in situ hybridization with probes against these snRNAs, and by immunofluorescent staining with antibodies against trimethylguanosine and the Sm epitope. A striking feature of coiled bodies in tissue culture cells is the absence of the non-snRNP splicing factor SC35 (Fu and Maniatis, 1990). Elsewhere in the nucleus, particularly in the nuclear speckles, SC35 is colocalized with the splicing snRNPs. Staining with anti-SC35 was quite easy to demonstrate in the nucleoplasm of nuclei assembled in egg extracts, but the coiled bodies were unstained. Similar results were obtained with mAb 104 (Roth *et al.*, 1990), which recognizes several members of the SR group of proteins to which SC35 belongs. Thus with respect to localization of major splicing factors, coiled bodies in tissue culture nuclei and in egg extracts are similar.

It has been known for several years that coiled bodies in tissue culture cells stain strongly with antibodies against fibrillarin (Raška *et al.*, 1991). This was an unexpected finding, because fibrillarin is a nuclear protein that derives its name from its high concentration in the fibrillar zone of the nucleolus (Ochs *et al.*, 1985). Antibodies against fibrillarin immunoprecipitate U3, U8, U13, U14, and U15, of which U3 and U8 have been definitely implicated in the processing steps that convert pre-rRNA into mature 18S, 28S, and 5.8S ribosomal RNAs (Tyc and Steitz, 1989; Kass *et al.*, 1990; Savino and Gerbi, 1990; Peculis and Steitz, 1993; reviewed in Fournier and Maxwell, 1993). If fibrillarin itself occurs in coiled bodies (and not simply a cross-reacting protein), one or more of the pre-rRNA processing snRNAs might also be found in this organelle. Raška and Dundr (1993) reported the presence of U3 in coiled bodies of HeLa nuclei using an oligonucleotide probe for in situ hybridization on cryosections. Carmo-Fonseca *et al.* (1993) studied the localization of U3 snRNA in HeLa cell nuclei using a biotin-labeled 2′-O-alkyl-oligoribonucleotide probe. Although U3 was readily demonstrated in the nucleoli, it was not detected in the coiled bodies. More recently, using a full length nick-translated probe, Jiménez-García *et al.* (1994) were able to demonstrate U3 in the coiled bodies of both HeLa and normal rat kidney cells. In our study of coiled bodies assembled in vitro, we confirmed the presence of fibrillarin, as earlier reported by Bell *et al.* (1992). We also had no difficulty demonstrating both U3 and U8 snRNAs. Our results, along with those of Raška and Dundr (1993) and Jiménez-García *et al.* (1994), suggest that the fibrillarin-associated snRNAs may be regular components of coiled bodies in various cell types. It will be of considerable interest to look for the remaining fibrillarin-associated snRNAs U13, U14, and U15 in coiled bodies formed in egg extract.

Remarkably, coiled bodies formed in egg extract also contain U7 snRNA, which is required in the third major RNA processing reaction in the nucleus; namely, the removal of the 3′ end of histone pre-mRNA (Mowry and Steitz, 1987; Birnstiel and Schaufele, 1988). Our attempts to demonstrate U7 in the coiled bodies of tissue culture cells have so far been unsuccessful, but we believe that further investigation of this issue is warranted. The most compelling reason to expect U7 in somatic coiled bodies is that U7 and coilin, or a coilin-related protein, are found together not only in coiled bodies formed in egg extract but also in the C snurposomes of oocytes (Tuma *et al.*, 1993; Wu and Gall, 1993). U7 may be similarly colocalized with coilin in the coiled bodies of somatic nuclei but at such a low concentration that detection is difficult. snRNPs are relatively easy to detect in the egg extract system because the nuclei are large, $\leq$50–60 μm in diameter, they are relatively structureless, and they contain multiple coiled bodies that themselves are unusually large, $\leq$3.0 μm in diameter, compared to 0.5–1.0 μm in HeLa nuclei. Under such circumstances, tritium autoradiography offers unique advantages. Because the background is extremely low, convincing localization is possible even when the mean number of silver grains per coiled body is very low.

In summary, published data establish that coiled bodies in tissue culture nuclei contain snRNPs associated with pre-mRNA splicing and pre-rRNA processing. The coiled bodies formed in vitro in egg extract contain these components plus U7, the snRNP associated with histone

pre-mRNA processing. We suggest that coiled bodies in general may contain all of the snRNPs associated with these three processes.

A major question raised by the localization studies is the function of coiled bodies. Despite their content of snRNPs, there is little or no evidence that RNA processing of any type takes place in coiled bodies in somatic cells (reviewed in Lamond and Carmo-Fonseca, 1993). The absence of RNA synthesis in pronuclei and early embryonic nuclei makes it virtually certain that coiled bodies in these cases cannot be sites of RNA processing. One might postulate that they are storage particles for processing components to be used later in embryogenesis, but even this seemingly reasonable role does not stand up to closer inspection. First, the coiled bodies in pronuclei, like those in cleavage nuclei, presumably break down at mitosis and reform during the ensuing interphase. In this respect they do not resemble permanent storage organelles like the lipid and yolk granules of the cytoplasm. More importantly, the number of snRNA molecules in the coiled bodies of the maternal and paternal pronuclei are a minuscule fraction of the total in the egg cytoplasm. For instance, the number of maternally derived U1 and U2 molecules in a *Xenopus* egg is roughly equivalent to the number in 4000–20 000 somatic nuclei (Forbes *et al.*, 1983). Thus, during early embryogenesis the cytoplasm, not coiled bodies, must be the major site for storage of snRNPs.

At each successive embryonic mitosis an increasing fraction of the cytoplasmic pool of snRNPs will be imported into nuclei and assembled into coiled bodies. Because this sequence of events is not related to RNA processing, it is perhaps best viewed as an expression of inherent properties of the nucleus and especially of the nuclear envelope. The major nuclear "function" during early embryogenesis is replication of the genome; the entire cell cycle has been streamlined to include only an S phase followed by mitosis, followed by another S phase. Regulated DNA synthesis requires a nuclear envelope (Blow and Laskey, 1988; Leno and Laskey, 1991), and consequently, a typical envelope with pores and an internal lamina is formed during each embryonic cell cycle. Similarly, the nuclei formed during in vitro nuclear assembly are surrounded by typical envelopes, and they import proteins that carry a nuclear localization signal (Newmeyer *et al.*, 1986). It is thus not surprising, as our study shows, that they also import snRNPs. Why, then, are these snRNPs organized into such prominent coiled bodies? Perhaps the answer lies precisely in the lack of RNAs to be processed. In a typical somatic nucleus the coiled bodies may represent a temporary assembly point for snRNPs that are rapidly shuttled to the actual sites of RNA processing. In the in vitro system, the snRNPs have no processing function and remain stalled in the coiled bodies.

In summary, we suggest that the prominent coiled bodies assembled in vitro, as well as those in cleavage nuclei, have no immediate role in RNA processing. Instead, they result from special features of the egg and early embryo: a large pool of snRNPs stored in the cytoplasm, the presence of nuclei with functional envelopes capable of importing snRNPs, and the lack of RNA synthesis, with consequent failure of transport of snRNPs out of the coiled bodies into sites of RNA processing.

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REFERENCES

Almouzni, G., and Wolffe, A.P. (1993). Nuclear assembly, structure, and function: the use of *Xenopus* in vitro systems. Exp. Cell Res. *205*, 1–15.

Andrade, L.E.C., Chan, E.K.L., Raška, I., Peebles, C.L., Roos, G., and Tan, E.M. (1991). Human autoantibody to a novel protein of the nuclear coiled body: immunological characterization and cDNA cloning of p80-coilin. J. Exp. Med. *173*, 1407–1419.

Andrade, L.E.C., Tan, E.M., and Chan, E.K.L. (1993). Immunocytochemical analysis of the coiled body in the cell cycle and during cell proliferation. Proc. Natl. Acad. Sci. USA *90*, 1947–1951.

Bakken, A., Morgan, G., Sollner-Webb, B., Roan, J., Busby, S., and Reeder, R.H. (1982). Mapping of transcription initiation and termination signals on *Xenopus laevis* ribosomal DNA. Proc. Natl. Acad. Sci. USA *79*, 56–60.

Bell, P., Dabauvalle, M.-C., and Scheer, U. (1992). In vitro assembly of prenucleolar bodies in *Xenopus* egg extract. J. Cell Biol. *118*, 1297–1304.

Bennett, J.L., Jeong-Yu, S., and Clayton, D.A. (1992). Characterization of a *Xenopus laevis* ribonucleoprotein endoribonuclease. Isolation of the RNA component and its expression during development. J. Biol. Chem. *267*, 21765–21772.

Birnstiel, M.L., and Schaufele, F.J. (1988). Structure and function of minor snRNPs. In: Structure and Function of Major and Minor Small Nuclear Ribonucleoproteins, ed. M. Birnstiel, Berlin, NY: Springer, 155–182.

Black, D.L., and Pinto, A.L. (1989). U5 small nuclear ribonucleoprotein: RNA structure analysis and ATP-dependent interaction with U4/U6. Mol. Cell. Biol. *9*, 3350–3359.

Blow, J.J., and Laskey, R.A. (1988). A role for the nuclear envelope in controlling DNA replication within the cell cycle. Nature *332*, 546–548.

Brown, D.D., and Gurdon, J.B. (1964). Absence of ribosomal RNA synthesis in the anucleolate mutant of *Xenopus laevis*. Proc. Natl. Acad. Sci. USA *51*, 139–146.

Brown, D.D., and Littna, E. (1964). RNA synthesis during the development of *Xenopus laevis*, the South African clawed toad. J. Mol. Biol. *8*, 669–687.

Carmo-Fonseca, M., Ferreira, J., and Lamond, A.I. (1993). Assembly of snRNP-containing coiled bodies is regulated in interphase and mitosis—evidence that the coiled body is a kinetic nuclear structure. J. Cell Biol. *120*, 841–852.

Carmo-Fonseca, M., Pepperkok, R., Carvalho, M.T., and Lamond, A.I. (1992). Transcription-dependent colocalization of the U1, U2, U4/U6, and U5 snRNPs in coiled bodies. J. Cell Biol. *117*, 1–14.

Carmo-Fonseca, M., Tollervey, D., Barabino, S.M.L., Merdes, A., Brunner, C., Zamore, P.D., Green, M.R., Hurt, E., and Lamond, A.I. (1991). Mammalian nuclei contain foci which are highly enriched in components of the pre-mRNA splicing machinery. EMBO J. *10*, 195–206.

Dreyer, C., Singer, H., and Hausen, P. (1981). Tissue specific nuclear antigens in the germinal vesicle of *Xenopus laevis* oocytes. Roux's Arch. Dev. Biol. *190*, 197–207.

Forbes, D.J., Kornberg, T.B., and Kirschner, M.W. (1983). Small nuclear RNA transcription and ribonucleoprotein assembly in early *Xenopus* development. J. Cell Biol. *97*, 62–72.

Fournier, M.J., and Maxwell, E.S. (1993). The nucleolar snRNAs: catching up with the spliceosomal snRNAs. Trends Biochem. Sci. *18*, 131–135.

Fu, X.-D., and Maniatis, T. (1990). Factor required for mammalian spliceosome assembly is localized to discrete regions in the nucleus. Nature *343*, 437–441.

Hay, E.D. (1968). Structure and function of the nucleolus in developing cells. In: The Nucleus, vol. 3, eds. A.J. Dalton and F. Haguenau, NY: Academic Press, 1–79.

Hay, E.D., and Gurdon, J.B. (1967). Fine structure of the nucleolus in normal and mutant *Xenopus* embryos. J. Cell Sci. *2*, 151–162.

Heine, M.A., Rankin, M.L., and DiMario, P.J. (1993). The Gly/Arg rich (GAR) domain of *Xenopus* nucleolin facilitates in vitro nucleic acid binding and in vivo nucleolar localization. Mol. Biol. Cell *4*, 1189–1204.

Hervás, J.P., Villegas, J., Crespo, D., and Lafarga, M. (1980). Coiled bodies in supraoptic nuclei of the rat hypothalamus during the postnatal period. Am. J. Anat. *159*, 447–454.

Huang, S., and Spector, D.L. (1992). U1 and U2 small nuclear RNAs are present in nuclear speckles. Proc. Natl. Acad. Sci. USA *89*, 305–308.

Jeppesen, C., Stebbins-Boaz, B., and Gerbi, S.A. (1988). Nucleotide sequence determination and secondary structure of *Xenopus* U3 snRNA. Nucleic Acids Res. *16*, 2127–2148.

Jiménez-García, L.F., Rothblum, L.I., Busch, H., and Ochs, R.L. (1989). Nucleologenesis: use of non-isotopic in situ hybridization and immunocytochemistry to compare the localization of rDNA and nucleolar proteins during mitosis. Biol. Cell *65*, 239–246.

Jiménez-García, L.F., Segura-Valdez, M. de L., Ochs, R.L., and Spector, D.L. (1994). Nucleologenesis: U3 snRNA-containing prenucleolar bodies move to sites of active pre-mRNA transcription after mitosis. Mol. Biol. Cell (*in press*).

Karasaki, S. (1965). Electron microscopic examination of the sites of nuclear RNA synthesis during amphibian embryogenesis. J. Cell Biol. *26*, 937–958.

Kass, S., Tyc, K., Steitz, J.A., and Sollner-Webb, B. (1990). The U3 small nucleolar ribonucleoprotein functions in the first step of preribosomal RNA processing. Cell *60*, 897–908.

Krainer, A. (1988). Pre-mRNA splicing by complementation with purified human U1, U2, U4/U6 and U5 snRNPs. Nucleic Acids Res. *16*, 9415–9429.

Lamond, A.I., and Carmo-Fonseca, M. (1993). The coiled body. Trends Cell Biol. *3*, 198–204.

Leno, G.H., and Laskey, R.A. (1991). DNA replication in cell-free extracts from *Xenopus laevis*. In: Methods in Cell Biology, vol. 36, eds. B.K. Kay and H.B. Peng, NY: Academic Press, 561–579.

Lerner, E.A., Lerner, M.R., Janeway, C.A., and Steitz, J.A. (1981). Monoclonal antibodies to nucleic acid-containing cellular constituents: probes for molecular biology and autoimmune disease. Proc. Natl. Acad. Sci. USA *78*, 2737–2741.

Lerner, M.R., and Steitz, J.A. (1979). Antibodies to small nuclear RNAs complexed with proteins are produced by patients with systemic lupus erythematosus. Proc. Natl. Acad. Sci. USA *76*, 5495–5499.

Leser, G.P., Escara-Wilke, J., and Martin, T.E. (1984). Monoclonal antibodies to heterogeneous nuclear RNA-protein complexes. The core proteins comprise a conserved group of related polypeptides. J. Biol. Chem. *259*, 1827–1833.

Lin, R., Leone, J.W., Cook, R.G., and Allis, C.D. (1989). Antibodies specific to acetylated histones document the existence of deposition- and transcription-related histone acetylation in *Tetrahymena*. J. Cell Biol. *108*, 1577–1588.

Lohka, M.J., and Masui, Y. (1983). Formation in vitro of sperm pronuclei and mitotic chromosomes induced by amphibian ooplasmic components. Science *220*, 719–721.

Lohka, M.J., and Masui, Y. (1984). Role of cytosol and cytoplasmic particles in nuclear envelope assembly and sperm pronuclear formation in cell-free preparations from amphibian eggs. J. Cell Biol. *98*, 1222–1230.

Matera, A.G., and Ward, D.C. (1993). Nucleoplasmic organization of small nuclear ribonucleoproteins in cultured human cells. J. Cell Biol. *121*, 715–727.

Mattaj, I.W. (1988). UsnRNP assembly and transport. In: Structure and Function of Major and Minor Small Nuclear Ribonucleoproteins, ed. M. Birnstiel, Berlin, NY: Springer, 100–114.

Monneron, A., and Bernhard, W. (1969). Fine structural organization of the interphase nucleus in some mammalian cells. J. Ultrastruct. Res. *27*, 266–288.

Mowry, K.L., and Steitz, J.A. (1987). Identification of the human U7 snRNP as one of several factors in the 3' end maturation of histone premessenger RNAs. Science *238*, 1682–1687.

Newmeyer, D.D., Finlay, D.R., and Forbes, D.J. (1986). In vitro transport of a fluorescent nuclear protein and exclusion of non-nuclear proteins. J. Cell Biol. *103*, 2091–2102.

Newmeyer, D.D., and Forbes, D.J. (1988). Nuclear import can be separated into distinct steps in vitro: nuclear pore binding and translocation. Cell *52*, 641–653.

Newmeyer, D.D., and Wilson, K.L. (1991). Egg extracts for nuclear import and nuclear assembly reactions. In: Methods in Cell Biology, vol. 36, eds. B.K. Kay and H.B. Peng, NY: Academic Press, 607–634.

Newport, J. (1987). Nuclear reconstitution in vitro: stages of assembly around protein-free DNA. Cell *48*, 205–217.

Newport, J., and Forbes, D. (1987). The nucleus: structure, function and dynamics. Annu. Rev. Biochem. *56*, 535–565.

Ochs, R.L., Lischwe, M.A., Shen, E., Carroll, R.E., and Busch, H. (1985). Nucleologenesis: composition and fate of prenucleolar bodies. Chromosoma *92*, 330–336.

Ochs, R.L., Lischwe, M.A., Spohn, W.H., and Busch, H. (1985). Fibrillarin: a new protein of the nucleolus identified by autoimmune sera. Biol. Cell *54*, 123–134.

Peculis, B.A., and Steitz, J.A. (1993). Disruption of U8 nucleolar snRNA inhibits 5.8S and 28S rRNA processing in the *Xenopus* oocyte. Cell *73*, 1233–1245.

Pfeifle, J., and Anderer, F.A. (1983). Localization of phosphoprotein pp105 in cell lines of various species. Biochem. Biophys. Res. Commun. *116*, 106–112.

Phillips, S.C., and Birnstiel, M.L. (1992). Analysis of a gene cluster coding for the *Xenopus laevis* U7 snRNA. Biochim. Biophys. Acta *1131*, 95–98.

Piñol-Roma, S., Swanson, M.S., Gall, J.G., and Dreyfuss, G. (1989). A novel heterogeneous nuclear RNP protein with a unique distribution on nascent transcripts. J. Cell Biol. *109*, 2575–2587.

Raška, I., Andrade, L.E.C., Ochs, R.L., Chan, E.K.L., Chang, C.-M., Roos, G., and Tan, E.M. (1991). Immunological and ultrastructural studies of the nuclear coiled body with autoimmune antibodies. Exp. Cell Res. *195*, 27–37.

Raška, I., and Dundr, M. (1993). Compartmentalization of the cell nucleus: case of the nucleolus. Chromosomes Today *11*, 101–119.

Reimer, G., Pollard, K.M., Penning, C.A., Ochs, R.L., Lischwe, M.A., Busch, H., and Tan, E.M.. (1987). Monoclonal autoantibody from a (New Zealand black × New Zealand white) F_1 mouse and some human scleroderma sera target an M_r 34,000 nucleolar protein of the U3 RNP particle. Arthritis Rheum. *30*, 793–800.

Reuter, R., Appel, B., Bringmann, P., Rinke, J., and Lührmann, R. (1984). 5′-terminal caps of snRNAs are reactive with antibodies specific for 2,2,7-trimethylguanosine in whole cells and nuclear matrices. Exp. Cell Res. *154*, 548–560.

Roth, M.B., Murphy, C., and Gall, J.G. (1990). A monoclonal antibody that recognizes a phosphorylated epitope stains lampbrush chromosome loops and small granules in the amphibian germinal vesicle. J. Cell Biol. *111*, 2217–2223.

Roth, M.B., Zahler, A.M., and Stolk, J.A. (1991). A conserved family of nuclear phosphoproteins localized to sites of polymerase II transcription. J. Cell Biol. *115*, 587–596.

Savino, R., and Gerbi, S.A. (1990). In vivo disruption of *Xenopus* U3 snRNA affects ribosomal RNA processing. EMBO J. *9*, 2299–2308.

Schmidt-Zachmann, M., Hügle, B., Scheer, U., and Franke, W.W. (1984). Identification and localization of a novel nucleolar protein of high molecular weight by a monoclonal antibody. Exp. Cell Res. *153*, 327–346.

Schmidt-Zachmann, M.S., Hügle-Dörr, B., and Franke, W.W. (1987). A constitutive nucleolar protein identified as a member of the nucleoplasmin family. EMBO J. *6*, 1881–1890.

Spector, D.L., Lark, G., and Huang, S. (1992). Differences in snRNP localization between transformed and nontransformed cells. Mol. Biol. Cell *3*, 555–569.

Steele, R.E., Thomas, P.S., and Reeder, R.H. (1984). Anucleolate frog embryos contain ribosomal DNA sequences and a nucleolar antigen. Dev. Biol. *102*, 409–416.

Stein, G.S., Stein, J.L., and Marzluff, W.F. (1984). Histone Genes: Structure, Organization, and Regulation, NY: John Wiley.

Steitz, J.A., Black, D.L., Gerke, V., Parker, K.A., Krämer, A., Frendeway, D., and Keller, W. (1988). Functions of the abundant U-snRNPs. In: Structure and Function of Major and Minor Small Nuclear Ribonucleoproteins, ed. M. Birnstiel, Berlin, NY: Springer, 115–154.

Tam, J.P. (1988). Synthetic peptide vaccine design: synthesis and properties of a high-density multiple antigenic peptide system. Proc. Natl. Acad. Sci. USA *85*, 5409–5413.

Tan, E.M., and Kunkel, H.G. (1966). Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus. J. Immunol. *96*, 464–471.

Tuma, R., Stolk, J.A., and Roth, M.B. (1993). Identification and characterization of a sphere organelle protein. J. Cell Biol. *122*, 767–773.

Tyc, K., and Steitz, J.A. (1989). U3, U8 and U13 comprise a new class of mammalian snRNPs localized in the cell nucleolus. EMBO J. *8*, 3113–3119.

Wallace, R.A., Jared, D.W., Dumont, J.N., and Sega, M.W. (1973). Protein incorporation by isolated amphibian oocytes: III. optimum incubation conditions. J. Exp. Zool. *184*, 321–333.

Wassarman, D.A., and Steitz, J.A. (1991). Structural analyses of the 7SK ribonucleoprotein (RNP), the most abundant human small RNP of unknown function. Mol. Cell. Biol. *11*, 3432–3445.

Wassarman, K.M., and Steitz, J.A. (1992). The low-abundance U11 and U12 small nuclear ribonucleoproteins (snRNPs) interact to form a two-snRNP complex. Mol. Cell. Biol. *12*, 1276–1285.

Wu, C.-H.H., and Gall, J.G. (1993). U7 small nuclear RNA in C snurposomes of the *Xenopus* germinal vesicle. Proc. Natl. Acad. Sci. USA *90*, 6257–6259.

Wu, Z., Murphy, C., Callan, H.G., and Gall, J.G. (1991). Small nuclear ribonucleoproteins and heterogeneous nuclear ribonucleoproteins in the amphibian germinal vesicle: loops, spheres, and snurposomes. J. Cell Biol. *113*, 465–483.