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Human p80-Coilin Is Targeted to Sphere Organelles in the Amphibian Germinal Vesicle

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Cultured vertebrate cells often display one or more coiled bodies in their nuclei. These are spherical structures ~0.5–1.0 μm in diameter that contain high concentrations of small nuclear ribonucleoproteins (snRNPs); they are distinct from nuclear speckles and nucleoli, the other major sites of snRNP concentration. Coiled bodies in human cells contain a unique protein, p80-coilin, that has an M_r = 80 kDa. Cloned p80-coilin cDNA encodes 576 amino acids with a calculated molecular weight of 62.6 kDa. To determine which of several snRNP-containing structures in the amphibian germinal vesicle (GV) might be the homologue of coiled bodies, we injected *myc*-tagged transcripts of full-length human p80-coilin into the cytoplasm of *Xenopus* oocytes and followed the fate of the translated proteins with an antibody specific for the tag. Western blots of GV proteins showed rapid appearance of both full-length and truncated p80-coilin in the nucleus. Immunofluorescent staining of spread GV contents demonstrated specific uptake of p80-coilin by the sphere organelle within 1 h after injection. Similar experiments were performed with a series of deletion constructs that lacked progressively longer segments from the carboxy terminus. A construct that contained only the first 102 amino acids (18% of the molecule) was specifically targeted to the sphere organelle. Conversely, a construct lacking the first 92 amino acids failed to localize, although it was imported into the GV. Thus, a relatively short region at the amino terminus of human p80-coilin is both necessary and sufficient for localization in the sphere organelle. Sphere organelles in the GV and coiled bodies in somatic nuclei are clearly related in composition. We suggest that they are homologous organelles with similar functions in preassembly and sorting of RNA processing components. Differences in their composition suggest functional specialization in the two cell types.

INTRODUCTION

Nearly all RNA molecules transcribed in the nucleus undergo extensive processing before they are exported to the cytoplasm. These processing steps require participation of the small nuclear ribonucleoproteins (snRNPs[1] or "snurps"), usually as multicomponent complexes. Pre-mRNA splicing involves the five splicing snRNPs U1, U2, U4/U6, and U5 (Steitz *et al.*, 1988; Green, 1991); histone pre-mRNA 3'-end processing requires U7 (Mowry and Steitz, 1987; Birnstiel and Schaufele, 1988); and rRNA processing is known to involve U3 and U8 in vertebrates (Kass *et al.*, 1990; Savino and Gerbi, 1990; Peculis and Steitz, 1993) and several additional snRNAs in yeast (Fournier and Maxwell, 1993). In recent years immunofluorescence and in situ hybridization studies have begun to define the intranuclear localization of snRNPs, particularly in cultured vertebrate cells, thereby allowing speculation about the sites of RNA processing (reviewed in Spector, 1993). Our group has concentrated on the localization of snRNPs in the oocyte nucleus or germinal vesicle (GV) of amphibians (Gall and Callan, 1989; Wu *et al.*, 1991, 1993; Wu and Gall, 1993), taking advantage of the extraordinary spatial resolution and wealth of structural detail afforded by this material.

Here we are concerned with determining which organelle in the GV is homologous to the coiled body of somatic nuclei. Coiled bodies are of unusual interest because they contain components from all three RNA

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

processing systems just mentioned: splicing, histone mRNA processing, and rRNA processing (evidence reviewed in the DISCUSSION). In addition, coiled bodies contain a high concentration of a unique protein called p80-coilin (Raška *et al.*, 1991). Andrade *et al.* (1991) obtained a partial cDNA clone for human p80-coilin from which they deduced the amino acid sequence of the protein exclusive of the amino terminus. They also produced several rabbit polyclonal sera against fusion proteins of p80-coilin, of which serum R288 has been used extensively as an immunofluorescent marker for coiled bodies (Andrade *et al.*, 1993).

We found that R288 stained the C snurposome part of the sphere organelle in GVs of several amphibian species, including the toad *Xenopus laevis*, the frog *Rana temporaria*, and the newt *Notophthalmus viridescens*. Spheres or sphere organelles are complex structures consisting of three parts: a remarkably spherical body, the C snurposome; one or more smaller spherical structures on the surface, called B snurposomes (these may be absent); and internal inclusions that resemble the Bs on the surface. The C snurposome portion contains U7 snRNA (Wu and Gall, 1993), whereas the Bs on the surface and the B-like inclusions contain splicing snRNAs (Wu *et al.*, 1991). U7 has been detected only in C snurposomes, whereas splicing snRNAs are also found in thousands of free B snurposomes independent of the spheres (Wu *et al.*, 1991). To determine if the C snurposome part of the sphere organelle, in fact, contained p80-coilin or a related protein, we injected transcripts of human p80-coilin into *Xenopus* oocytes and localized the translation products by immunofluorescence. For this purpose it was first necessary to obtain a full-length cDNA clone of human p80-coilin. We found that p80-coilin transcripts were efficiently translated in oocytes and that epitope-tagged products accumulated rapidly in the C snurposome component of the sphere organelles. While these experiments were in progress, Tuma *et al.* (1993) identified a coilin-related protein in the sphere organelle of *Xenopus*, which they named SPH-1. It is clear, therefore, that the coiled body in somatic nuclei and the sphere organelle in the GV are related structures. Because there are significant differences in composition between the two organelles, we suggest that functional specialization occurs in different cell types.

MATERIALS AND METHODS

Cloning and Sequencing of Full-length p80-Coilin

To extend the sequence of human p80-coilin into the 5′ region, we synthesized two nested primers based on the partial sequence of Andrade *et al.* (1991). One primer, 5′ AGGAATTCGCCTGTACTTTCGG 3′ (AG + *Eco*RI + complement of nucleotides 607–620) (Figure 1), was used with reverse transcriptase (Life Sciences, St. Petersburg, FL) to produce cDNA copies of the 5′ end of p80-coilin mRNA. Template for the reverse transcriptase reaction was poly A(+) RNA isolated from HeLa cells. A poly C tail was added to the cDNA, which was then amplified by the anchored polymerase chain reaction (PCR) method (Frohman *et al.*, 1988), using a second primer at the 3′ end based on the coilin sequence, 5′ AGGAATTCCTTCGTTATCATCACCCACTGTG 3′ (AG + *Eco*RI + complement of nucleotides 513-535) and an anchor primer at the unknown 5′ end, 5′ CUACUACUACUAGGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG 3′ (5′ RACE System, Life Technologies, Gaithersburg, MD). The PCR product was cloned into the pCRII vector (Invitrogen, San Diego, CA) and subsequently recloned into pBluescript KS (+) (Stratagene, La Jolla, CA). The entire insert was sequenced in both directions using the dideoxy chain termination procedure (Sanger *et al.*, 1977).

After the sequence of the 5′ end had been determined, primers were constructed for PCR synthesis of a clone that contained the entire coding region and most of the 3′ untranslated region. The primers were 5′ AGGAATTCCACCAAGCAAGATGGCAGCTTC 3′ (AG + *Eco*RI + nucleotides −11 to +11) and a pair of nested primers, 5′ AGGAATTCAGTTCAATATGATTTTATTTAAAAATGT 3′ (AG + *Eco*RI + complement to nucleotides 2573–2600) and 5′ AGGAATTCTACAGCTGAAGGAGGGATCTGATC 3′ (AG + *Eco*RI + complement to nucleotides 2548–2571) at the 3′ end. The initial PCR product was cloned in pCRII and recloned as a *Bam*HI/*Xba*I fragment in MT6. MT6 consists of pBluescript KS (+) containing six tandem copies of a 10–amino acid epitope from the c-*myc* gene (Roth *et al.*, 1991). Inserts that are in-frame with the *myc* sequences give translation products that can be detected with monoclonal antibody (mAb) 9E10 against the *myc* epitope (Evan *et al.*, 1985). The full-length p80-coilin clone was designated MYC-1 (Figure 3).

Another full-length clone was produced by subcloning the insert of MYC-1 into a modified MT6 vector in which the SV40 nuclear localization sequence (NLS), PPKKKRKV, was placed downstream of the *myc* epitopes. This clone was designated MYC + NLS. Various deletion clones were derived from MYC+NLS by cutting at *Xba* I (5′ overhang) and *Sac* I (3′ overhang) sites at the 3′ end of the insert, digesting into the coding region with exonuclease III and exonuclease VII (Life Technologies), and religating (Figure 3).

Synthesis of Transcripts for Injection

Capped sense-strand transcripts were copied with T3 polymerase (Stratagene) from 1 μg of various cDNA clones after linearization with restriction enzymes (*Xba* I for MYC-1, *Sac* I for MYC + NLS, and *Pvu* II for all other clones). The reaction products were analyzed on a 1% agarose/1.3% formaldehyde gel to determine the size of the transcript and its relative yield. The amount of RNA was roughly estimated by comparison with the molecular weight standards. Aliquots were precipitated as needed and resuspended in H_2O for injection. The final concentration was estimated to be ~25 ng/μl.

Oocyte Injections

A sample of ovary was removed from an anesthetized *Xenopus laevis* and held at 18–20°C in OR2 saline (Wallace *et al.*, 1973). Individual oocytes with their surrounding follicle cells were separated with jeweler's forceps and stored for 2–3 h before injection. Oocytes that acquired a mottled or stippled pigment distribution in the animal hemisphere during this time were discarded. Injections were performed with a glass needle using air pressure from a 50-ml plastic syringe (Kay, 1991). Approximately 20 nl of solution containing 0.5 ng of RNA was injected into the cytoplasm of each oocyte. After injection oocytes were incubated in OR2 at 18–20°C for 15 min to 96 h.

Western Blots

To determine the nature of the products translated from various transcripts, 10–30 GVs were isolated from injected oocytes in a medium containing 83 mM KCl, 17 mM NaCl, 6.5 mM Na_2HPO_4, 3.5 mM KH_2PO_4, 1 mM $MgCl_2$, and 1 mM dithiothreitol pH 7.0. After a 10-s sonication, proteins were precipitated with 17% trichloroacetic acid (TCA) for 15 min on ice and centrifuged to give a readily visible pellet.

The pellet was washed once with 5% TCA and three times with 80% acetone. After removal of the acetone, the sample was boiled in 10–20 μl of sample buffer (Laemmli, 1970). Proteins were separated on a 10% polyacrylamide-sodium dodecyl sulfate (SDS) gel and electroeluted to an Immobilon membrane (Millipore, Bedford, MA). Western blotting was performed with various primary antibodies detected with horseradish peroxidase (Vectastain ABC kit, Vector Laboratories, Burlingame, CA). In the case of proteins tagged with the *myc* epitope, the primary antibody was unfractionated culture supernate from hybridoma cell line 9E10 (Evan *et al.*, 1985).

GV Spreads

Preparations of spread GV contents were made as described earlier (Gall *et al.*, 1991) with the following modifications. Oocytes were removed from the female and held in OR2 solution at 18°C for 18–24 h before use. After a GV had been removed in the 100 mM isolation medium, it was placed within 20 s into the 25 mM spreading medium. The nuclear envelope was removed with forceps, and the nuclear gel was pipetted quickly into the spreading chamber. Because dispersal of the nuclear gel occurred within minutes, centrifugation of the preparation could begin almost immediately. With these modifications it is possible to obtain well-spread *Xenopus* GV preparations in which all components (chromosomes, nucleoli, snurposomes, spheres) remain attached firmly to the glass slide.

Immunofluorescence

GV spreads were blocked with 10% horse serum in phosphate-buffered saline for 15–30 min and then reacted with primary antibody for ~1 h. They were rinsed with 10% horse serum and treated with a secondary antibody for another hour. Primary antibodies were mAb 9E10 (Evan *et al.*, 1985) against the *c-myc* epitope and rabbit serum R31 (Bauer *et al.*, 1994) against a synthetic 20-amino acid peptide derived from protein SPH-1 of *Xenopus* (Tuma *et al.*, 1993). 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 stored at −20°C when not being observed.

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-31                                A TCT CTC GGC TTC CGT TGA GCA CCA AGC AAG

  1  ATG GCA GCT TCC GAG ACG GTT AGG CTA CGG CTT CAA TTT GAT TAC
  1  M   A   A   S   E   T   V   R   L   R   L   Q   F   D   Y

 46  CCG CCG CCA GCT ACC CCG CAC TGT ACG GCC TTC TGG CTT CTG GTC
 16  P   P   P   A   T   P   H   C   T   A   F   W   L   L   V

 91  GAC TTG AAC AGA TGC CGA GTC GTC ACA GAT CTC ATT AGT CTC ATC
 31  D   L   N   R   C   R   V   V   T   D   L   I   S   L   I

136  CGC CAG CGC TTC GGC TTC AGT TCT GGG GCC TTC CTA GGC CTC TAC
 46  R   Q   R   F   G   F   S   S   G   A   F   L   G   L   Y

181  CTG GAG GGG GGG CTC TTG CCC CCC GCC GAG AGC GCG CGC CTT GTG
 61  L   E   G   G   L   L   P   P   A   E   S   A   R   L   V

226  AGA GAC AAC GAC TGC CTC AGA GTT AAA TTA GAA GAG AGA GGA GTT
 76  R   D   N   D   C   L   R   V   K   L   E   E   R   G   V

271  GCT GAG AAT TCT GTA GTC ATC AGT AAT GGT GAC ATT AAT TTA TCT
 91  A   E   N   S   V   V   I   S   N   G   D   I   N   L   S

316  CTT AGA AAA GCA AAG AAG CGG GCA TTT CAG TTA GAG GAG GGT GAA
106  L   R   K   A   K   K   R   A   F   Q   L   E   E   G   E

361  GAA ACT GAA CCA GAT TGC AAA TAT TCA AAG AAG CAT TGG AAG AGT
121  E   T   E   P   D   C   K   Y   S   K   K   H   W   K   S

406  CGA GAG AAC AAT AAC AAT AAT GAG AAG GTC TTG GAT CTG GAA CCA
136  R   E   N   N   N   N   N   E   K   V   L   D   L   E   P

451  AAA GCT GTC ACA GAT CAG ACT GTC AGC AAA AAA AAC AAG AGA AAA
151  K   A   V   T   D   Q   T   V   S   K   K   N   K   R   K

496  AAT AAA GCA ACC TGT GGC ACA GTG GGT GAT GAT AAC GAA GAG GCC
166  N   K   A   T   C   G   T   V   G   D   D   N   E   E   A

541  AAA AGA AAA TCA CCA AAG AAA AAG GAG AAA TGT GAA TAT AAA AAA
181  K   R   K   S   P   K   K   K   E   K   C   E   Y   K   K

586  AAG GCT AAG AAT CCC AAG TCT CCG AAA GTA CAG GCA GTG AAA GAC
196  K   A   K   N   P   K   S   P   K   V   Q   A   V   K   D

631  TGG GCC AAT CAG AGA TGT AGT TCT CCA AAA GGT TCT GCT AGA AAC
211  W   A   N   Q   R   C   S   S   P   K   G   S   A   R   N
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Figure 1. Sequence of the 5′ end of human p80-coilin cDNA with conceptual translation. Nucleotides −31 to 512 in bold type are new. The rest of the molecule, beginning with nucleotide 513, was sequenced by Andrade *et al.* (1991). The open reading frame extends to a TGA stop codon at nucleotide 1729, for a total of 576 amino acids. This sequence is available as GenBank Accession U06632 submitted by E.K.L. Chan.

RESULTS

Cloning of Full-length p80-Coilin

In their original study of human p80-coilin, Raška *et al.* (1991) described several human autoimmune sera that stained coiled bodies in tissue culture nuclei and reacted on Western blots with a protein of M_r = 80 kDa. Subsequently, Andrade *et al.* (1991) used a high titer serum to recover a partial cDNA clone from an expression library of human sequences. Additional clones were obtained by hybridization, using probes based on the sequence of the first clone. Their longest cDNA clone contained 2088 bases, encoding 405 amino acids (48 kDa) of p80-coilin followed by a long 3′-untranslated region. From the published sequence we constructed primers for anchored PCR extension (Frohman *et al.*, 1988) into the 5′ region of the molecule, using as the template cDNA copied from HeLa cell mRNA. Our longest clone added 543 new bases to the sequence for a total of 2631 bases (Figure 1). Northern blots show that p80-coilin mRNA has a length of 2.65 kilobases (kb) (Figure 2), suggesting that the cDNA sequence is essentially complete. The new sequence extends the open reading frame to a single TGA stop codon near the 5′ end (Figure 1). Twelve bases downstream from the stop is a methionine codon, which is probably the translation initiation site, because its context is reasonable (G at +4, A at −3) (Kozak, 1989) and the next methionine is at amino acid position 329. Full-length p80-coilin is 576 amino acids in length with a calculated molecular weight (M.W.) of 62.6 kDa, well below the 80 kDa predicted from its mobility on SDS gels. The translation products derived from our clones are similarly retarded on SDS gels (next section), supporting the conclusion that the true molecular weight of p80-coilin is <80 kDa. While we were carrying out this cloning, a similar strategy was used by Takano and Chan to obtain the 5′ end of p80-coilin cDNA (personal communication). Their sequence (GenBank Accession U06632) is identical to ours.

We used primers from the 5′ and 3′ ends of the full-length p80-coilin sequence to carry out a PCR reaction on cDNA synthesized from HeLa cell mRNA. A 2.6-kb product was cloned initially in pCRII (Invitrogen) and subsequently recloned as a *Bam*HI/*Xba* I fragment in MT6, a vector that includes six repeats of an epitope

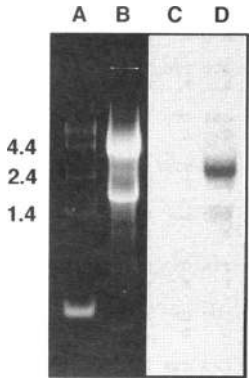

Figure 2. Northern blot of human p80-coilin mRNA. Lanes A and B are markers and unfractionated HeLa cell RNA stained with ethidium bromide after electrophoresis on an agarose-formaldehyde gel. Sizes of markers in kb. C and D are the same samples after transfer to a nitrocellulose filter and hybridization with ^{32}P-labeled human p80-coilin DNA. p80-coilin mRNA is a single band at 2.65 kb.

derived from the human c-*myc* gene (Roth *et al.*, 1991) (Figure 3, MYC-1). Another clone was made that contained the SV40 NLS in addition to the *myc* tag (Figure 3, MYC + NLS).

Translation of Full-length p80-Coilin in Xenopus Oocytes

Capped transcripts were synthesized in vitro from MYC-1 and MYC + NLS. Samples of RNA were electrophoresed on agarose/formaldehyde gels and shown to have the expected length of 2.6 kb. In a typical experiment, groups of 20–40 *Xenopus* oocytes were injected with RNA and were then incubated at 18–20°C for different times in OR2 saline (Wallace *et al.*, 1973). Samples of 15–30 GVs were isolated, and the proteins were separated on SDS-polyacrylamide gels for Western blotting with mAb 9E10. As shown in Figure 4, lane B, proteins of several different sizes were detected in the GV, the pattern of bands being essentially the same whether oocytes were incubated for 24 h or only 2–4 h after injection. The largest protein had an $M_r \sim 100$ kDa. The full-length clones have an open reading frame of 2010 or 2013 base pairs (bp), encoding fusion proteins of 73.5 kDa (10.9 kDa of *myc* tag + 62.6 kDa of p80 coilin). The anomalously slow migration of the fusion protein suggests that p80-coilin itself is retarded in SDS-polyacrylamide gels. This would explain the discrepancy between the observed mobility of p80-coilin (80 kDa) and its calculated molecular weight (62.6 kDa). Several shorter proteins are evident on the blot in the range of 36–48 kDa (Figure 4, lane B). Because the *myc* tag was located at the extreme amino terminus, the shorter proteins must all represent carboxy-terminal truncations of p80-coilin. These could have been produced by premature termination of translation or by breakdown of full-length p80-coilin after synthesis.

The presence or absence of the SV40 NLS made no apparent difference in the nuclear accumulation of full-length translation products, suggesting that p80-coilin contains a functional NLS. A putative bipartite NLS of the type described by Robbins *et al.* (1991) occurs at amino acids 181–198 (KRKSPKKKEKCEYKKKAK).

GV spreads from injected oocytes were prepared for immunofluorescence after an incubation period of 24–48 h. Preparations stained with mAb 9E10 against the *myc* epitope on p80-coilin showed uniformly bright staining of the C snurposome component of the sphere organelles, whereas control GVs from uninjected oocytes were unstained (Figure 5, B and E). Occasionally, the background was elevated on all structures, and a few nuclei contained irregular masses of intensely staining material 10–30 μm in diameter, presumably precipitates of p80-coilin itself. These results suggested that p80-coilin was overproduced by 24–48 h, prompting us to try shorter incubation times. When GV spreads were made 1–4 h after injection of mRNA, C snurpo-

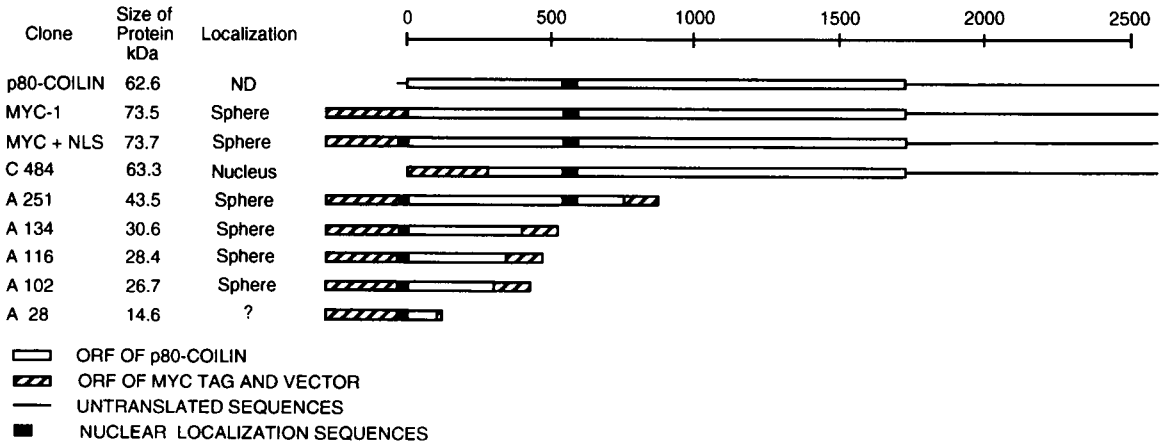

Figure 3. Diagrams of clones used in this study. □, p80-coilin coding sequences; −, untranslated nucleotides; ▨, open reading frames of the *myc* tag (amino ends) or of the vector (carboxy ends); ■ immediately downstream of the *myc* tags denote a synthetic SV40 NLS; ■ within the p80-coilin sequence represent the putative endogenous NLS. Deletion clones are named according to the number of p80-coilin amino acids they encode; A and C designate clones containing amino and carboxy terminal sequences respectively. Scale at the top is in bp.

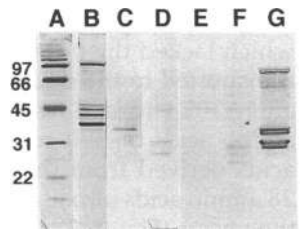

Figure 4. Western blots of GV proteins from oocytes injected with transcripts from full-length and deletion clones of human p80-coilin, detected with mAb 9E10 against the *myc* epitope. (A) Molecular mass markers stained with Coomassie blue; masses in kDa. (B) GV proteins from oocytes injected with a full-length clone (MYC-1) show presumed full-length translation product at ~100 kDa and prominent truncations at 36–48 kDa. (C) Deletion clone A251, single band at 35 kDa. (D) Deletion clone A116, faint bands at 27–30 kDa. Doublet at 45 kDa occurs in all samples, including uninjected controls. (E) Uninjected control oocytes, faint doublet at 45 kDa. (F) Deletion clone A102, faint bands at 24–30 kDa. (G) Deletion clone C484, presumed full-length doublet at about 87 kDa, four prominent truncations at 29–36 kDa.

somes were as well stained as after longer incubation, and the background was lower. In one experiment we detected staining of C snurposomes as early as 15 min after injection.

To determine whether p80-coilin entered all C snurposomes or only a subpopulation, preparations were simultaneously stained with mAb 9E10 to detect human p80-coilin and rabbit serum R31 (Bauer *et al.*, 1994), which recognizes an endogenous protein in C snurposomes called SPH-1 (Tuma *et al.*, 1993). In such cases, there was precise colocalization of the two antibodies (Figure 5, E and F), demonstrating that newly synthesized p80-coilin is targeted to all C snurposomes. In these double label experiments, the intensity of staining with serum R31 was variable (Figure 5F), whereas uninjected controls always showed brightly stained C snurposomes (Figure 5C). The most probable explanation for variable staining is that newly synthesized p80-coilin replaces endogenous SPH-1 in the C snurposomes. To test this hypothesis further, we stained GV preparations from injected oocytes with mAb H1, the antibody originally used to identify SPH-1 (Tuma *et al.*, 1993). Because mAb H1 and mAb 9E10 are both mouse monoclonals, they could not be used together. The C snurposomes from injected oocytes generally stained less brightly with mAb H1 than did those from uninjected controls, again suggesting that human p80-coilin replaced endogenous SPH-1 in injected oocytes.

Transcripts of full-length p80-coilin were also injected into oocytes of the frog *Rana pipiens* and the newt *Notophthalmus viridescens*. In both cases translation products appeared rapidly and specifically in the C snurposomes. In a double label experiment on *Rana* oocytes using mAb 9E10 and rabbit serum R31, the endogenous staining detectable with R31 disappeared completely from the C snurposomes in some GVs.

We conclude from the injection studies that full-length p80-coilin is rapidly targeted to the C snurpo-

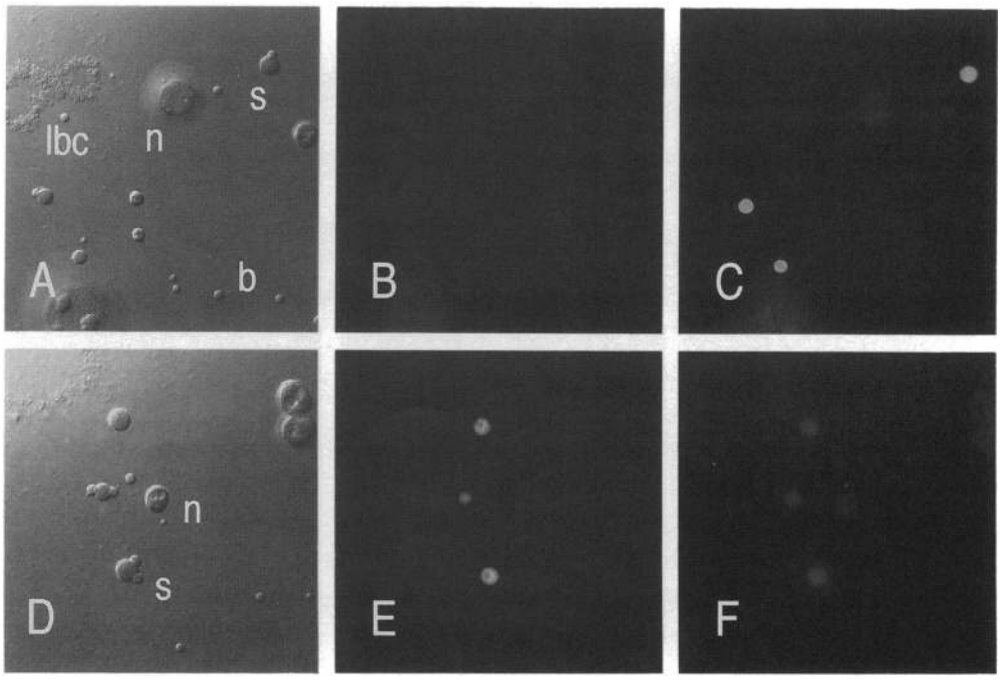

Figure 5. (A–C) A portion of the contents of a single *Xenopus* GV from a control, uninjected oocyte, double-stained with mAb 9E10 against the *myc* tag (fluorescein) and rabbit serum R31 against SPH-1 (*Xenopus* coilin) (rhodamine). (A) Nomarski interference contrast. (B) Same area viewed in the fluorescein channel, showing background level of staining. (C) Same area viewed in the rhodamine channel, showing strong staining of endogenous SPH-1 in the C snurposome portion of the sphere organelles. (D–F) Similar GV contents from an oocyte injected 24 h earlier with transcripts from a full-length human p80-coilin clone (MYC-1). (D) Nomarski interference contrast. (E) Same area in fluorescein channel, showing *myc*-tagged human p80-coilin localized specifically in the C snurposome portion of three sphere organelles. The B snurposomes on the surface (middle and lower sphere) and the B-like inclusions (upper and lower spheres) are unstained. (F) Same area in rhodamine channel, showing colocalization of endogenous *Xenopus* coilin in the sphere organelles. The intensity of endogenous staining is reduced relative to that of the control in C. b, B snurposome; lbc, lampbrush chromosome; n, nucleolus; s, sphere organelle (C snurposome with or without B snurposome(s) on its surface).

some portion of the sphere organelle in GVs of three species of amphibians and that it partially or completely replaces an endogenous protein recognized by serum R31 and mAb H1 (protein SPH-1 in the case of *Xenopus*).

Translation of Truncated p80-Coilin in Xenopus Oocytes

We next made a *myc*-tagged construct that deleted the coding region for the first 92 amino acids of p80-coilin (clone C484, Figure 3). This clone contained the putative bipartite NLS at amino acids 181–198. Transcripts from C484 were efficiently translated after injection into *Xenopus* oocytes; a doublet at ~87 kDa and truncated products at 29–36 kDa were detectable on Western blots of GV proteins (Figure 4, lane G). However, spread GV contents stained with mAb 9E10 failed to reveal any specific morphological structure in which the translation products were accumulated (Figure 6, A–C).

Failure of construct C484 to localize in C snurposomes suggested that an essential binding sequence might be present in the amino terminus of p80-coilin. We therefore made a series of deletion constructs, each of which encoded the amino terminus of the molecule, but progressively less of the carboxy terminus. The starting clone for these constructs was MYC + NLS, in which the *myc* tag is followed by the SV40 NLS (Figure 3). The SV40 NLS assured that the shorter constructs, which lacked the putative endogenous NLS, would be transported to the nucleus. Clones A251, A134, A116, and A102 encoded respectively 251, 134, 116, and 102 amino acids of p80-coilin plus 41 downstream amino acids derived from the vector, whereas A28 encoded 28 amino acids of p80-coilin plus four amino acids from the vector (Figure 3).

Transcripts from these five constructs were injected into oocytes, and 24 h later GVs were collected for Western blots. Except for clone A28, which gave no detectable product, each clone produced one or more bands recognized by mAb 9E10 (Figure 4), indicating that translation products had been synthesized and imported into the GV. The major product of clone A251 ran slightly faster than predicted from its molecular weight (35 kDa vs. 44 kDa), suggesting that it may be truncated at its carboxy terminus, whereas the major products from A134, A116, and A102 ran as predicted.

Clones A251, A134, A116, and A102 gave essentially identical results after immunofluorescent staining of GV spreads with mAb 9E10. Each showed strong staining of C snurposomes but no staining above background in any other structure (Figure 6,D–F). The fact that immunofluorescent staining was strong, despite the generally weak signals given by these clones on Western

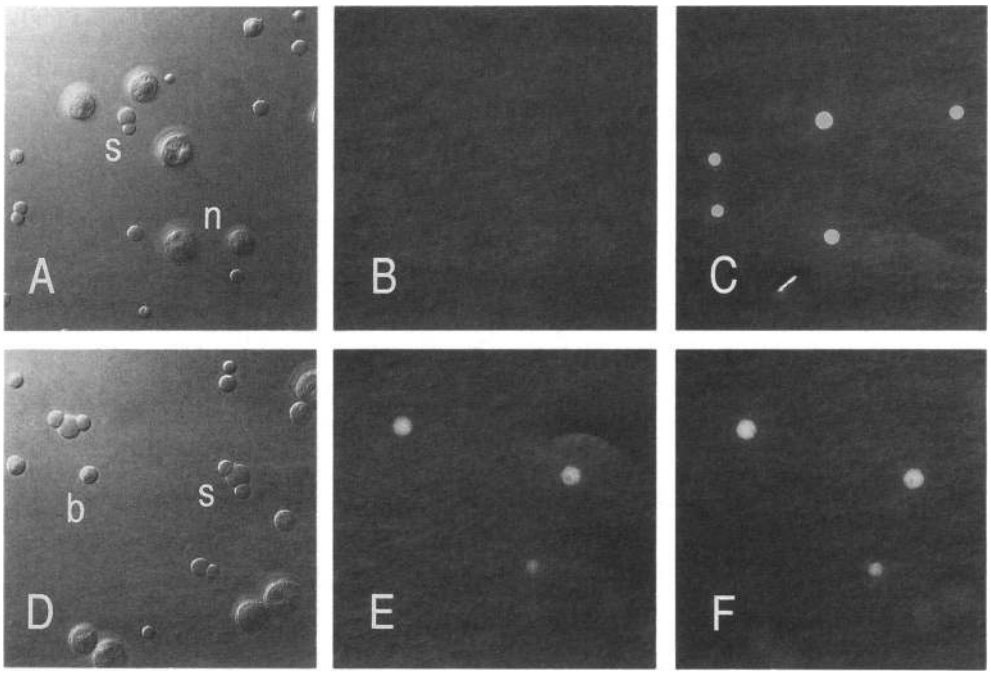

Figure 6. A–C show a portion of the contents of a single *Xenopus* GV from an oocyte injected 24 h previously with transcripts from deletion clone C484, which encodes human p80-coilin lacking 92 amino acids at the amino terminus. Double-stained with mAb 9E10 against the *myc* tag (fluorescein) and rabbit serum R31 against SPH-1 (*Xenopus* coilin) (rhodamine). (A) Nomarski interference contrast. (B) Same area viewed in the fluorescein channel, showing failure of the *myc*-tagged translation product to accumulate in any structure, although Western blots show it is abundantly translated and imported into the GV. (C) Same area viewed in the rhodamine channel, showing strong staining of endogenous SPH-1 in the C snurposome portion of the sphere organelles. (D–F) Similar GV contents from an oocyte injected 24 h earlier with transcripts from deletion clone A116, which encodes the first 116 amino acids of human p80-coilin. (D) Nomarski interference contrast. (E) Same area in fluorescein channel, showing *myc*-tagged translation product localized specifically in the C snurposome portion of three sphere organelles. The B snurposomes attached to the Cs are not stained. (F) Same area in rhodamine channel, showing colocalization of endogenous *Xenopus* coilin in the sphere organelles. b, B snurposome; n, nucleolus; s, sphere organelle (C snurposome with or without B snurposome(s) on its surface).

blots, suggests that the truncated proteins are efficiently targeted to the C snurposomes. Clone A28, for which no translation product was detected on the Western blot, gave no staining by immunofluorescence. Thus the shortest clone to show localization of a product in C snurposomes was A102, which encoded 102 amino acids from the amino terminus of p80-coilin.

DISCUSSION

Using an anchored PCR technique (Frohman *et al.*, 1988), we added 543 bases to the partial sequence of p80-coilin published by Andrade *et al.* (1991), giving a total of 2631 bases. The open reading frame consists of 576 amino acids with a predicted M.W. = 62.6 kDa. On SDS-polyacrylamide gels authentic p80-coilin from tissue culture cells has a mobility corresponding to ~80 kDa (Raška *et al.*, 1991).

Tuma *et al.* (1993) recently described a protein from the sphere organelle of *Xenopus* that they call SPH-1. They pointed out that 185 amino acids at the carboxy terminus of SPH-1 and p80-coilin are 54% identical (75% similar). The amino terminal sequences are likewise closely related (Figure 7). However, a region of ~300 amino acids in the middle of the molecules lacks any evident similarity. It seems likely that SPH-1 is either the *Xenopus* homologue of human p80-coilin or a member of a family of related proteins. The cDNA clone encoding SPH-1 was obtained from an expression library using mAb H1 as a probe. mAb H1 itself was selected for its specific staining of the C snurposome portion of the sphere organelle. As proof that their clone encoded a protein in the sphere organelle, Tuma *et al.* (1993) injected *myc*-tagged transcripts of the clone into *Xenopus* oocytes and showed specific localization of the translation products in C snurposomes. Our finding that human p80-coilin is similarly targeted to C snurposomes supports the view that SPH-1 and p80-coilin are related proteins.

Two features of the localization of p80-coilin in *Xenopus* GVs are noteworthy. First, products can be detected remarkably quickly in the C snurposomes, in some cases by 15 min after injection, and invariably by 1 h. Second, p80-coilin partially replaces endogenous SPH-1, as shown by marked reduction of staining with antibodies for SPH-1 (Figure 5F). Both the rapid localization of p80-coilin and the replacement of endogenous SPH-1 are consistent with the hypothesis that components of the C snurposome are normally turned over rapidly.

Sequence Requirements for Localization

To determine which sequences in p80-coilin are important for its localization in C snurposomes, we made a series of deletion constructs and determined the behavior of their translation products in the GV. Construct C484 lacked the first 276 nucleotides (92 amino acids) at the 5′ end. Its translation products were efficiently imported into the GV but failed to localize in C snurposomes or any other structure. Conversely, a series of constructs that lacked progressively longer segments from the 3′ end of the clone gave products that were specifically localized in C snurposomes. The shortest clone to show localization (A102) encoded only 102 amino acids from the amino terminus. These results were somewhat unexpected, because Tuma *et al.* (1993) showed that a construct of SPH-1 lacking the first 108 amino acids (their B clone) localized in C snurposomes. Our clone C484, which does not localize in Cs, lacks almost precisely the same number of amino acids, including the major region of identity in the amino terminus.

```
p80-coilin  MAASETVRLR LQFDYPPPAT PHCTAFWLLV DLNRCRVVTD        40
            |||   ||.. | |||||||  |    ||||. |  |||||||
    SPH-1   MAAPSPVRVK LLFDYPPPAI PESCMFWLLL DAKRCRVVTD        40

p80-coilin  LISLIRQRFG FSSGAFLGLY LEGGLLPPAE SARLVRDNDC LRVK   84
            | |.|| ..    |. . || .|  ||||.| |  ..|||| .|||
    SPH-1   LASIIRHKYM NGQGGGISLY VEDCLLPPGE SILVIRDNDS IRVK   84
```

Figure 7. Comparison of the first 84 amino acids of human p80-coilin and *Xenopus* SPH-1 (Tuma *et al.*, 1993). Identical amino acids are connected by a line, conservative substitutions by a period. In this region the molecules are 56% identical (71% similar). Beyond amino acid 84 there is little similarity for ~300 amino acids, followed by additional regions of identity in the carboxy terminus, as described by Tuma *et al.* (1993).

The simplest interpretation of these results is that SPH-1 contains two regions that are sufficient for localization, one in the first 108 amino acids and one further downstream. Over evolutionary time, p80-coilin has retained the first localization sequence but has lost the second. This hypothesis predicts that a construct containing only the first 108 amino acids of SPH-1 would localize properly in C snurposomes.

Is the Sphere Organelle a Coiled Body?

p80-coilin has been widely used as a marker for coiled bodies in somatic nuclei (Andrade *et al.*, 1991, 1993; Carmo-Fonseca *et al.*, 1992, 1993; Spector *et al.*, 1992; Wu *et al.*, 1993; Bauer *et al.*, 1994; Matera and Ward, 1993). The discovery of SPH-1, a coilin-related protein, in the sphere organelles of *Xenopus* (Tuma *et al.*, 1993) and our results showing the targeting of human p80-coilin to the same structures suggest that coiled bodies and sphere organelles are related. Both structures also contain splicing snRNPs (U1, U2, U4/U6, and U5), but neither is the major site of snRNP accumulation in the nucleus. Splicing snRNPs occur in the B snurposomes on the surface of the spheres and in the B-like inclusions (Wu *et al.*, 1991), whereas most of the splicing snRNPs in the GV are in the thousands of free B snurposomes. In the nuclei of cultured cells, some of the splicing snRNPs are concentrated in the coiled bodies, but most occur in the so-called nuclear speckles. Coiled bodies

lack the SR group of non-snRNP splicing factors (reviewed in Lamond and Carmo-Fonseca, 1993), whereas these do occur in the B snurposomes and B-like inclusions of sphere organelles (Wu *et al.*, 1991).

U7 snRNA is a prominent constituent of the C snurposome part of the sphere (Wu *et al.*, 1993) but has not so far been reported in coiled bodies from cultured cells. We recently demonstrated U7 in the large coiled bodies in nuclei assembled in vitro in *Xenopus* egg extracts (Wu *et al.*, 1993; Bauer *et al.*, 1994), suggesting that a further search for U7 in cultured cells would be useful.

There is increasing evidence that coiled bodies contain some nucleolar components, particularly the snRNPs involved in pre-rRNA processing. Fibrillarin, a nucleolar protein associated with U3, U8, and U13 snRNAs (Tyc and Steitz, 1989), has been demonstrated in coiled bodies from cultured cells as well as those formed in vitro in egg extracts (Raška *et al.*, 1991; Bell *et al.*, 1992; Wu *et al.*, 1993; Bauer *et al.*, 1994). Although U3 was originally reported as absent from coiled bodies in somatic nuclei (Carmo-Fonseca *et al.*, 1993) and in egg extracts (Bell *et al.*, 1992), more recent studies confirm its presence in both cases, as well as U8 in egg extracts (Raška and Dundr, 1993; Wu *et al.*, 1993; Bauer *et al.*, 1994; Jimeńez-Garciá *et al.*, 1994). The association of coiled bodies with nucleoli was part of the original description (Ramon y Cajal, 1903) and has been noted many times since (e.g. Malatesta *et al.*, 1994).

There is little or no evidence for nucleolar components in sphere organelles. U3 and U8 snRNAs are readily demonstrable by in situ hybridization in the multiple nucleoli of the GV, but the sphere organelles are completely negative. The same is true for immunofluorescent staining with antibodies against fibrillarin and nucleolin (Wu *et al.*, 1993). Therefore, a major difference between coiled bodies in somatic cells and sphere organelles in *Xenopus* oocytes is the presence of rRNA processing snRNPs in the former but their absence from the latter.

In summary, coiled bodies and sphere organelles share a number of components, especially coilin and the splicing snRNPs. Spheres regularly contain U7, as do the coiled bodies formed in egg extracts. On the other hand, coiled bodies contain U3 and U8 that are required for pre-rRNA processing, but sphere organelles apparently do not. We have suggested elsewhere (Wu *et al.*, 1993; Bauer *et al.*, 1994) that coiled bodies serve as the major site for preassembly and sorting of snRNP complexes for three RNA processing pathways—pre-mRNA splicing, histone pre-mRNA 3′ end processing, and rRNA processing. We propose that in the oocyte sphere organelles carry out a similar role in preassembly and sorting of splicing and histone pre-mRNA processing components but are not involved in rRNA processing. In this sense sphere organelles and coiled bodies are homologous organelles with differences in composition related to functional specialization in different cell types. An analysis of coiled bodies in other cell types and organisms should be helpful in defining the multiple functions of these complex organelles.

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