Molecular Biology of the Cell
Vol. 10, 3425–3434, October 1999

# Coilin Shuttles between the Nucleus and Cytoplasm In *Xenopus* Oocytes

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Submitted June 18, 1999; Accepted July 29, 1999
Monitoring Editor: Pam Silver

Coiled bodies are discrete nuclear organelles often identified by the marker protein p80-coilin. Because coilin is not detected in the cytoplasm by immunofluorescence and Western blotting, it has been considered an exclusively nuclear protein. In the *Xenopus* germinal vesicle (GV), most coilin actually resides in the nucleoplasm, although it is highly concentrated in 50–100 coiled bodies. When affinity-purified anti-coilin antibodies were injected into the cytoplasm of oocytes, they could be detected in coiled bodies within 2–3 h. Coiled bodies were intensely labeled after 18 h, whereas other nuclear organelles remained negative. Because the nuclear envelope does not allow passive diffusion of immunoglobulins, this observation suggests that anti-coilin antibodies are imported into the nucleus as an antigen–antibody complex with coilin. Newly synthesized coilin is not required, because cycloheximide had no effect on nuclear import and subsequent targeting of the antibodies. Additional experiments with *myc*-tagged coilin and *myc*-tagged pyruvate kinase confirmed that coilin is a shuttling protein. The shuttling of Nopp140, NO38/B23, and nucleolin was easily demonstrated by the targeting of their respective antibodies to the nucleoli, whereas anti-SC35 did not enter the germinal vesicle. We suggest that coilin, perhaps in association with Nopp140, may function as part of a transport system between the cytoplasm and the coiled bodies.

## INTRODUCTION

In 1903 the Spanish neurobiologist Ramón y Cajal described small silver-staining organelles in the nuclei of pyramidal cells of the brain, which he called nucleolar accessory bodies, because they frequently associated with the prominent nucleolus (Cajal, 1903). The same structures were rediscovered more than 60 years later in nuclei of liver and other mammalian tissues by Monneron and Bernhard (1969), who named them coiled bodies, based on their appearance in electron micrographs. Very little was learned about the composition of these organelles until Raška *et al.* (1991) discovered autoimmune sera that stained them specifically. Using these sera, Andrade *et al.* (1991) cloned a gene encoding a protein, p80-coilin, that occurs in high concentration in coiled bodies. Once coilin became available as a molecular marker, coiled bodies were found to contain many RNA transcription and processing components, including all five of the splicing small nuclear ribonucleoprotein particles (snRNPs), U3 snRNA, U7 snRNA, and several nucleolar proteins, such as fibrillarin and Nopp140 (reviewed by Gall *et al.*, 1995; Lamond and Earnshaw, 1998; Matera, 1998). Possible functions of coiled bodies have been discussed extensively. We have presented evidence that coiled bodies recruit the U7 snRNP and the stem–loop-binding protein (SLBP1) to the chromosomal sites of histone gene transcription (Wu *et al.*, 1996; Bellini and Gall, 1998; Abbott *et al.*, 1999). In addition, they almost certainly play some role in splicing and pre-rRNA processing, such as assembly, modification, or storage of processing components, although it is unlikely that processing itself takes place in coiled bodies.

In the *Xenopus* oocyte nucleus or germinal vesicle (GV), coilin is concentrated in 50–100 structures long known as spheres or sphere organelles (Gall, 1954; Callan and Lloyd, 1960; Callan, 1986). Spheres and somatic coiled bodies share not only coilin (Tuma *et al.*, 1993; Wu *et al.*, 1994), but other components as well, demonstrating their essential homology (Gall *et al.*, 1995).

Although coiled bodies contain the highest concentration of coilin in the nucleus, it has been noted for some time that coilin also occurs throughout the nucleoplasm (Andrade *et al.*, 1993; Carmo-Fonseca *et al.*, 1993; Puvion-Dutilleul *et al.*, 1995; Matera, 1998). This is especially clear in the GV, where as much as 85–90% of coilin is in the soluble nucleoplasm outside of the coiled bodies (Bellini and Gall, 1998). However, neither immunofluorescence nor fractionation studies suggested that any coilin occurs in the cytoplasm, except during mitosis, when the nuclear envelope breaks down

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Abbreviations used: CHX, cycloheximide; GV, germinal vesicle; mAb, monoclonal antibody; NLS, nuclear localization signal; PK, pyruvate kinase; snRNP, small nuclear ribonucleoprotein particle.

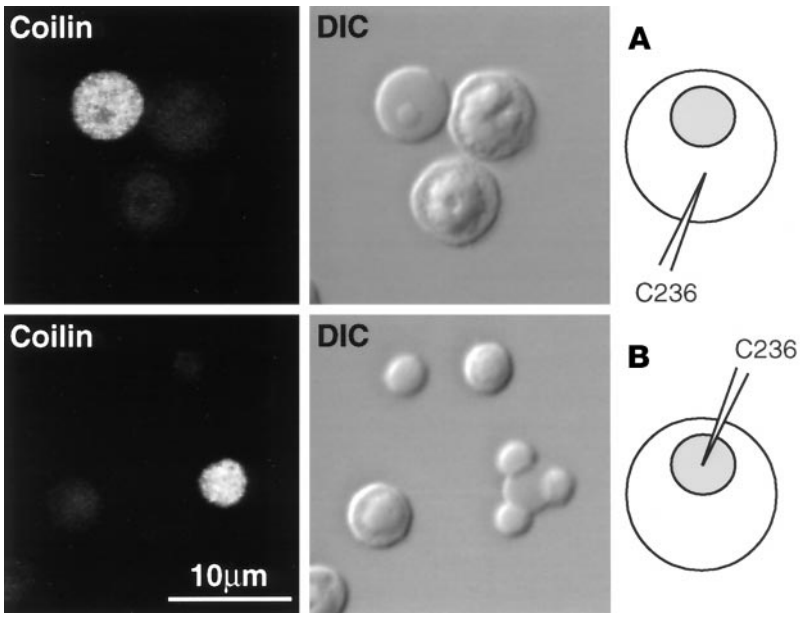


**Figure 1.** Anti-coilin antibody C236 was injected into the cytoplasm (A) or the GV (B) of *Xenopus* oocytes. After 18 h of incubation, GVs were isolated, and the nuclear contents were spread on slides. Preparations were stained with Cy3-conjugated goat anti-rabbit antibody. In both cases strong label was limited to the matrix of the coiled bodies. In A, a single B-snurposome lies within the coiled body. In B, three B-snurposomes are at the surface of the coiled body.

(Andrade *et al.*, 1993; Carmo-Fonseca *et al.*, 1993). Therefore, we were surprised to find that an anti-coilin antibody injected into the oocyte cytoplasm was imported into the GV and targeted to the coiled bodies, a strong suggestion that the antibody interacted with coilin in the cytoplasm and traveled to the nucleus as an antigen–antibody complex. We have examined this issue in more detail and conclude that coilin shuttles continuously between the nucleus and cytoplasm of the oocyte.

## MATERIALS AND METHODS

### *In Vitro Translation*

*Myc*-tagged coilin and *myc*-tagged pyruvate kinase (PK) were produced using an in vitro transcription–translation coupling system (Promega, Madison, WI) under the conditions suggested by the manufacturer. Translation products were analyzed by Western blots to ensure that full-length proteins were produced and to estimate their concentration. No further purification of newly translated proteins was performed before injection into oocytes. The *Xenopus* coilin cDNA clone was kindly provided by Z. Wu (Carnegie Institution). The PK clone was produced as follow: the DNA sequence encoding residues 20–410 of PK was amplified by the PCR (primers A and B) from the cDNA clone NPK (Peculis and Gall, 1992) and subcloned into the MT6 vector (Roth *et al.*, 1991), in which an SV40 nuclear localization signal (NLS) had been inserted downstream of six copies of the c-*myc* epitope (Wu *et al.*, 1994).

Primers used for PCR: A, 5′-CTGCACGCGGATCCAGACACCTTTCTGG-3′; B, 5′-GGAGCCTGCTGATGCGGCCGCAGCAGGC-3′.

### *Oocytes and Injections*

A fragment of ovary was surgically removed from an adult *Xenopus*. Oocytes with a diameter of ~1 mm (stage IV–V) (Dumont, 1972) were manually separated and kept at 18°C in OR2 saline (Wallace *et al.*, 1973). All injections were performed using a Nanoject microinjection apparatus (Drummond, Broomall, PA). For nuclear injections, oocytes were first centrifuged at 500 × *g* for 20 min to position the GV immediately under the cortex of the animal pole, thus increasing the accuracy of injection. Volumes of 20 and 5 nl were injected into the cytoplasm and the GV, respectively. For cytoplasmic injection, the concentration of antibody was ~5–10 μg/ml. For nuclear injection, antibodies were concentrated to 20–40 μg/ml with a centrifugal filter device that excluded proteins of >5 kDa (Biomax-5K; Millipore, Bedford, MA).

### *Cycloheximide*

In some experiments cycloheximide (CHX) was used to inhibit protein synthesis. Typically, oocytes were held in OR2 containing 50 μg/ml CHX at 18°C for 3 h before injection and for 3–21 h after injection. To demonstrate that CHX blocks translation, 200 nCi of [$^{35}$S]methionine (New England Nuclear, Boston, MA) were injected into the cytoplasm of control or CHX-treated oocytes. After 21 h of incubation in OR2 or OR2 with CHX, GV and cytoplasmic proteins were isolated from 15 oocytes and separated on a 10% polyacrylamide gel. The gel was fixed and dried, and labeled proteins were detected by autoradiography.

### *Immunofluorescent Staining and Microscopy*

GV spreads were prepared as described (Gall, 1998). Fixation was in 2% paraformaldehyde in PBS for 1 h. After fixation, preparations were rinsed in PBS, blocked in 10% horse serum, and stained for 1 h with antibody in 10% horse serum. Antibodies used in this study were goat anti-mouse immunoglobulin G (IgG) or goat anti-rabbit IgG labeled with fluorescein or Cy3 (Jackson ImmunoResearch, West Grove, PA). Confocal laser microscopy was performed with the Leica TCS NT system (Leica Microsystems, Deerfield, IL). Fluorescence quantitation was done as described by Abbott *et al.* (1999).

### *Immunoprecipitations and Western Blots*

Fifty GVs were isolated by hand in 100 μl of 5:1 buffer (83 mM KCl, 17 mM NaCl, 6.5 mM $Na_2HPO_4$, 3.5 mM $KH_2PO_4$, 1 mM $MgCl_2$, 1 mM DTT). GVs were mechanically disrupted, and NP40 was added to a final concentration of 0.5%. The insoluble material was pelleted by centrifugation at 20,000 × *g* for 15 min at 4°C. The supernate was then incubated with 20 μl of agarose beads coated with protein G (Life Technologies, Gaithersburg, MD), previously blocked in 10

mg/ml BSA for 1 h and equilibrated in an equal volume of 5:1 buffer with NP40. After 2 h of incubation at 4°C under constant agitation, the beads were washed five times for 5 min with 1 ml of 5:1 buffer, and bound material was eluted in 40 μl of sample buffer (Laemmli, 1970) with boiling for 5 min. Western blots were performed as described (Bellini and Gall, 1998).

## RESULTS

### *Anti-Coilin Antibodies Are Imported from the Cytoplasm to the GV*

The first experiment to suggest that coilin shuttles between the nucleus and the cytoplasm involved the injection of anti-coilin antibodies into the cytoplasm of *Xenopus* oocytes. We used two affinity-purified antibodies, mAb H1 against *Xenopus* coilin (also called SPH-1; Tuma *et al.*, 1993) and C236, a rabbit polyclonal serum raised against bacterially expressed *Xenopus* coilin. In each case ~25 pg of antibody were injected into the cytoplasm or into the GV of stage IV–V oocytes. Spread preparations of GV contents were made 30 min to 24 h later and stained with fluorescein- or Cy3-tagged secondary antibody. When injected into the GV, the antibody was readily detectable in coiled bodies within 30 min, with very little variation in signal intensity over time. In contrast, when the antibody was injected into the cytoplasm, staining was first detectable ~2 h after injection and increased in intensity over time. Both antibodies localized strictly in the matrix of the coiled bodies (Figure 1). As expected, GV spreads from control uninjected oocytes did not stain when treated with secondary antibody only. These observations demonstrate that 1) in the GV, an anti-coilin antibody can form an immune complex with coilin in the matrix of the coiled bodies; and 2) an anti-coilin antibody can be imported from the cytoplasm into the GV, where it localizes in the same pattern as endogenous coilin (Tuma *et al.*, 1993; Wu *et al.*, 1994). Because antibodies do not normally cross the nuclear envelope (Bonner, 1975; Einck and Bustin, 1984; Stacey and Allfrey, 1984), the probable interpretation is that the injected antibody binds to coilin in the cytoplasm and is targeted as an antigen–antibody complex to the coiled bodies in the GV.

To demonstrate that coilin shuttles between the GV and the cytoplasm, it must be shown that import of anti-coilin antibody does not depend on synthesis of new coilin in the cytoplasm, which is known from previous experiments to be targeted to coiled bodies in the GV and in somatic nuclei (Tuma *et al.*, 1993; Wu *et al.*, 1994; Bohmann *et al.*, 1995). Oocytes were incubated in CHX for 3 h, injected with anti-coilin antibody, and returned to CHX. GV preparations from these oocytes appeared normal in all respects, and their coiled bodies stained with secondary antibody alone (Figure 2A). To demonstrate that CHX was effective in blocking translation at the concentration used, oocytes were injected with [$^{35}$S]methionine, and their proteins were analyzed by PAGE (Figure 2B). [$^{35}$S]Methionine was not incorporated into proteins from CHX-treated oocytes but was readily detectable in proteins from control oocytes.

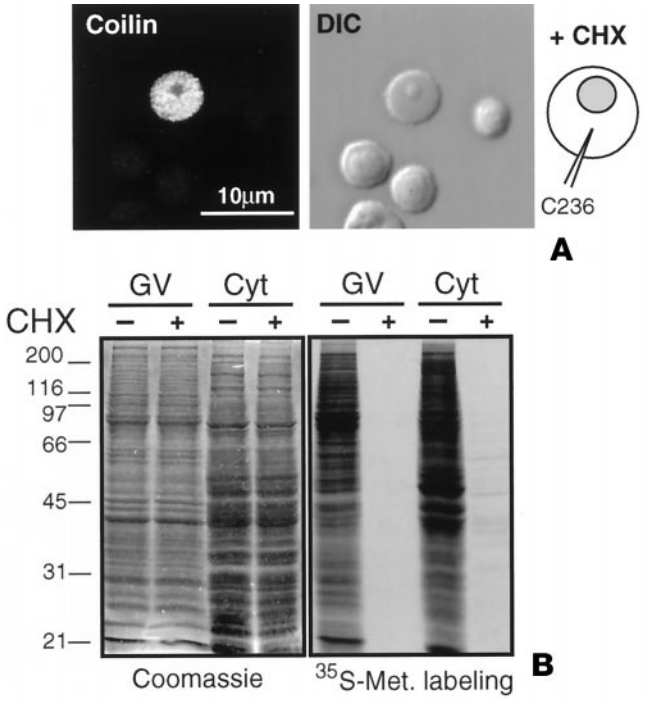


**Figure 2.** (A) Anti-coilin antibody C236 was injected into the cytoplasm of oocytes treated with CHX. After 18 h of incubation in CHX-containing OR2, Cy3-conjugated goat anti-rabbit antibody was used to detect C236 on GV spread preparations. C236 was specifically localized in the matrix of coiled bodies. (B) [$^{35}$S]Methionine was injected into the cytoplasm of control oocytes or oocytes treated with CHX as in A. After 24 h of incubation, cytoplasmic and nuclear proteins were separated by SDS-PAGE. The gel was stained with Coomassie blue to demonstrate that similar amounts of protein were loaded (left panel), and incorporation of [$^{35}$S]methionine in newly synthesized proteins was detected by autoradiography (right panel). Essentially no label occurs in the GV and cytoplasm of CHX-treated oocytes.

### *Myc-tagged Coilin Shuttles between the GV and the Cytoplasm*

To study shuttling in more detail, we examined the behavior of *Xenopus* coilin that was tagged with six copies of the c-*myc* epitope (Roth *et al.*, 1991; Wu *et al.*, 1994). The tagged protein was synthesized in vitro in a coupled transcription–translation reaction and was detected with mAb 9E10, which is specific for the c-*myc* epitope (Evan *et al.*, 1985). The anti-*myc* antibody was injected into the cytoplasm of oocytes, followed 1 h later by injection of *myc*-tagged coilin. GV spreads were prepared 3–18 h after the second injection and stained with secondary antibody alone (Cy3-labeled goat anti-mouse IgG). Staining of coiled bodies was readily detectable (Figure 3A). On the other hand, when anti-*myc* mAb 9E10 was injected alone into the cytoplasm of control oocytes, it was not subsequently detected in the GV. These results imply that the antibody cannot enter the nucleus by itself, but when coinjected with *myc*-tagged coilin into the cytoplasm, it forms an antigen–antibody complex that enters the GV and is targeted to the coiled bodies. Shuttling is demonstrated by injection of *myc*-tagged coilin into the GV, fol-

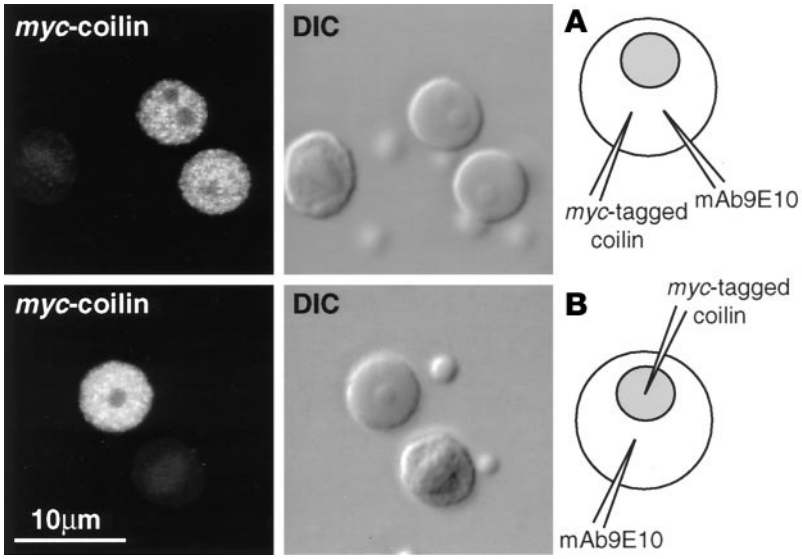


**Figure 3.** (A) mAb 9E10 was injected into the cytoplasm of oocytes 1 h before injection of in vitro-synthesized *myc*-tagged coilin. After 18 h, mAb 9E10 was detected in the coiled body matrix with Cy3-conjugated goat anti-mouse antibody. The interpretation is that mAb 9E10 reacted with the *myc*-tagged coilin in the cytoplasm, and the two were imported together into the nucleus and were targeted to the coiled bodies. (B) M*yc*-tagged coilin was injected into the nucleus 3 h before mAb 9E10 was injected into the cytoplasm. Eighteen hours later, mAb 9E10 was detected in the coiled bodies. Because the antibody alone cannot enter the nucleus, the interpretation is that *myc*-tagged coilin shuttled to the cytoplasm and reacted with mAb 9E10, and the two entered the nucleus as an antigen–antibody complex that was targeted to the coiled bodies.

lowed 3 h later by injection of anti-*myc* mAb 9E10 into the cytoplasm. When GV spreads were prepared 3–18 h later and stained with secondary antibody, coiled bodies were readily detectable (Figure 3B). Thus, the anti-*myc* antibody must have been imported by complexing with *myc*-tagged coilin that shuttled to the cytoplasm and returned to the nucleus. It might be argued that some *myc*-tagged coilin could have leaked into the cytoplasm at the time of injection. However, in these experiments *myc*-tagged coilin was not detectable in the cytoplasm by Western blotting 3 h after the nuclear injection. As already mentioned, coilin is rapidly imported into the GV. Thus, even if leakage occurred at the time of injection, by 3 h coilin has reached its equilibrium distribution, which is essentially all nuclear.

### *Myc-tagged PK Does Not Shuttle*

To demonstrate that not all *myc*-tagged proteins shuttle like coilin in such injection experiments, we compared the behavior of *myc*-tagged coilin with that of *myc*-tagged PK. Because PK is a cytoplasmic enzyme, it was also tagged with an SV40 NLS to ensure its targeting to the nucleus. PK does not localize to any particular structure in the GV, and so the complex of *myc*-tagged PK and anti-*myc* mAb 9E10 could not be assayed by staining a conventional GV spread preparation. Instead, we carried out immunoprecipitations with protein G and asked whether *myc*-tagged PK was present in the immunoprecipitate or in the supenate. The first experiment involved cytoplasmic injection of the antibody followed 1 h later by cytoplasmic injection of *myc*-tagged PK. GV proteins were isolated 3–18 h later and immunoprecipitated with protein G-Sepharose beads. Under these circumstances essentially all PK was nuclear by 3 h and was precipitable as an antigen–antibody complex (Figure 4A), showing that *myc*-tagged PK could import the antibody from the cytoplasm into the GV. However, when *myc*-tagged PK was injected into the GV and anti-*myc* antibody was injected into the cytoplasm, an antigen–antibody complex was not found in the nucleus at 3 h and was barely detectable at 18 h (Figure 4B). The implication of this experiment is that *myc*-tagged PK in the GV does not shuttle and therefore cannot interact with its cognate antibody in the cytoplasm to bring it into the GV. The weak signal observed at 18 h is possibly due to a small amount of passive diffusion of *myc*-tagged PK out of the GV.

The results were quite different when the experiments were carried out with *myc*-tagged coilin. In this case, an antigen–antibody complex was detected in the GV, regardless of whether *myc*-tagged coilin was injected into the cytoplasm or the GV (Figure 4, A and B). The implication, as in the staining experiments, is that nuclear coilin shuttles to the cytoplasm and associates with the anti-*myc* antibody, and the antigen–antibody complex enters the nucleus.

### *Energy but Not Transcription Is Required for Coilin to Shuttle*

The primary sequence of human p80-coilin displays both simple (residues 107–112) and bipartite (residues 181–198) NLSs, which are involved in the nuclear import of coilin in transfected cells (Bohmann *et al.*, 1995). These two NLSs are conserved in *Xenopus* coilin, and because they are potential targets for the importin receptors (Görlich, 1998; Ohno *et al.*, 1998), it is likely that nuclear import of coilin requires energy. Antibody C236 was injected into the cytoplasm of oocytes that were then maintained in OR2 saline at either 4 or 18°C. GV spreads were prepared 21 h later, and the accumulation of C236 in the coiled bodies was quantitated by immunofluorescence analysis. We found a fourfold difference in staining between oocytes maintained at 4 and 18°C (Figure 5). Because active transport processes in cells are inhibited at low temperature, it is likely that coilin shuttles in an energy-dependent pathway.

Some shuttling proteins, such as the heterogeneous nuclear RNP A1, require transcription for their return to the nucleus. When cells are treated with inhibitors of RNA synthesis, such as actinomycin D or 5,6-dichloro-β-D-ribofuranosyl benzimidazole (DRB), these proteins accumulate in the cytoplasm,

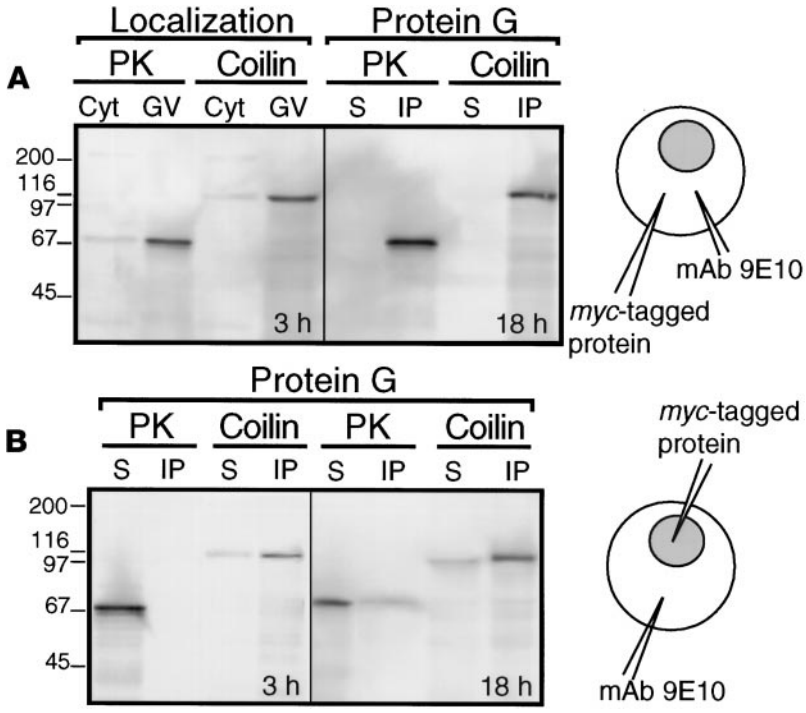


**Figure 4.** mAb 9E10 was injected into the cytoplasm of oocytes 1 h before injection of either *myc*-tagged coilin or *myc*-tagged PK. (A) Three hours later, a Western blot with mAb 9E10 as probe shows that both tagged proteins had translocated to the GV (left panel). At 18 h, GVs were isolated, and protein G-Sepharose beads were used to immunoprecipitate mAb 9E10. The presence of coprecipitated *myc*-tagged coilin or PK was demonstrated by Western blotting with mAb 9E10 as probe (right panel). (B) At 3 h (left panel) and 18 h (right panel) after nuclear injection, GVs were isolated, and proteins were precipitated as in A. *Myc*-tagged coilin was immunoprecipitated, whereas *myc*-tagged PK was not. The interpretation is that *myc*-tagged PK injected into the GV does not shuttle to the cytoplasm to react with mAb 9E10, whereas coilin does. Cyt, cytoplasm; S, supernate; IP, immunoprecipitate.

where they can be detected by immunostaining (Piñol-Roma and Dreyfuss, 1991; Cáceres *et al.*, 1998). Because coilin, like heterogeneous nuclear RNP A1, is an RNA-binding protein in vitro (Bellini and Gall, 1998), we tested whether transcription was necessary for its shuttling. Using the antibody assay, we followed the shuttling of coilin in oocytes treated with actinomycin D (50 $\mu$g/ml). Transcription was completely inhibited after 3 h, as visualized by the collapse of the lampbrush chromosome loops. However, there was no effect on the nuclear import of an anti-coilin antibody injected into the cytoplasm. We therefore conclude that transcription is not required for coilin to shuttle.

### *Nucleolin, NO38 (B23), and Nopp140 Shuttle between the GV and the Cytoplasm, but SC35 Does Not*

We carried out additional antibody experiments to explore the general usefulness of GV spreads for detecting shuttling proteins. Nopp140 was of particular interest for several reasons. First, Nopp140 is found in coiled bodies but, unlike coilin, occurs primarily in the dense fibrillar component of the nucleolus (Meier and Blobel, 1990). Second, Nopp140 has been shown to shuttle in tissue culture cells (Meier and Blobel, 1992). And last, Nopp140 and coilin can form a complex in vitro and in a yeast two-hybrid assay, suggesting that they may normally

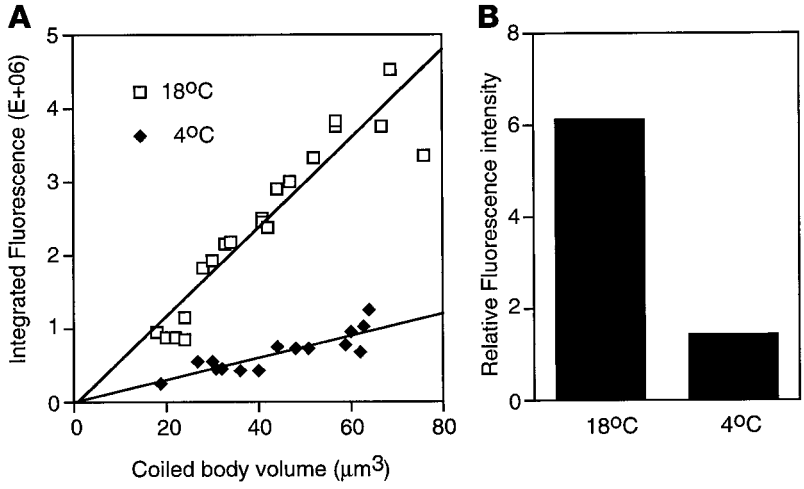


**Figure 5.** Import of anti-coilin antibody C236 into coiled bodies at 4 and 18°C. (A) C236 was injected into the cytoplasm of *Xenopus* oocytes. After 18 h of incubation in CHX-containing OR2, GV spreads were stained with Cy3-conjugated goat anti-rabbit antibody. The total fluorescence of individual coiled bodies was measured with a charge-coupled device (CCD) camera and plotted against coiled body volume. The linear relationship indicates that the amount of imported antibody is directly proportional to volume. At 4°C, import of C236 into coiled bodies was noticeably reduced, suggesting that shuttling is an energy-dependent process. (B) The slopes of the lines in A represent the concentration of C236 in coiled bodies (fluorescence intensity per unit volume). The concentration is four times higher at 18 than at 4°C.

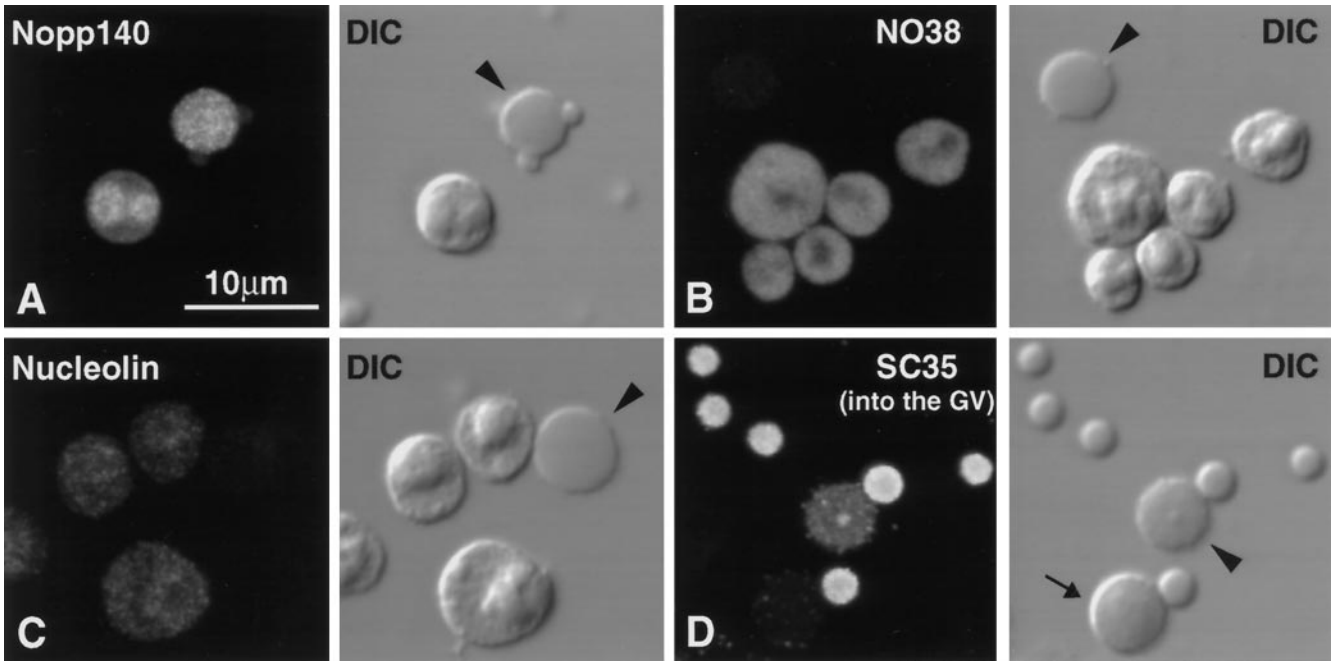


**Figure 6.** (A–C) Three mAbs specific for nucleolar proteins were injected into the cytoplasm of CHX-treated oocytes: mAb No114 against Nopp140, mAb No63 against NO38/B23, and mAb b7–6E7 against nucleolin. After 18 h, GV spreads were prepared for staining with Cy3-conjugated goat anti-mouse antibody. All three antibodies were readily detectable, indicating that their corresponding antigens shuttle. mAb No114 (A) localized in the dense fibrillar component of nucleoli and in the matrix of coiled bodies (arrowhead). In contrast, mAbs No63 (B) and b7–6E7 (C) were distributed uniformly in nucleoli but were absent from the coiled bodies. (D) An antibody against the splicing factor SC35 was not detected in GV spreads after cytoplasmic injection, suggesting that SC35 does not shuttle in the oocyte. As expected, when injected into the GV, the antibody was detected in B-snurposomes and the matrix of coiled bodies (arrowhead) but not in nucleoli (arrow).

associate within the nucleus (Isaac *et al.*, 1998). To examine the behavior of Nopp140 in the oocyte, we used mAb No114, which originally defined a novel *Xenopus* nucleolar protein of apparent molecular mass of 180 kDa (Schmidt-Zachmann *et al.*, 1984). Subsequent studies suggested that this protein, although clearly larger than mammalian Nopp140, is closely related to it in composition and overall structure (Cairns and McStay, 1995). In GV spreads, mAb 114 normally stains the dense fibrillar component of the multiple nucleoli and the matrix of the coiled bodies but does not react with the chromosomes or B-snurposomes. We injected ~25 pg of mAb No114 into the cytoplasm of *Xenopus* oocytes, made spread GV preparations 1–18 h later, and stained with secondary antibody alone. Stain was first detectable after 3 h of incubation and increased over time, simultaneously in the coiled bodies and the multiple nucleoli in a pattern identical to that of the endogenous protein (Figure 6A). As in the earlier experiments with coilin, the nuclear import of mAb No114 was not affected by preincubation of the oocytes in CHX.

We examined two other nucleolar proteins, NO38 (B23) and nucleolin, both of which had been shown to shuttle in cultured cells (Borer *et al.*, 1989). NO38 is localized primarily in nucleoli (Ochs *et al.*, 1983; Schmidt-Zachmann *et al.*, 1987) and was not detected in coiled bodies in cultured cells (Raška *et al.*, 1991) or in the GV (Gall *et al.*, 1995). More recently, we have found that an anti-NO38 antibody, mAb No63 (Schmidt-Zachmann *et al.*, 1987), stains coiled bodies strongly if formaldehyde fixation time is limited to 1–2 h. When mAb No63 was injected into the cytoplasm, it localized exclusively in the nucleoli (Figure 6B). The reason that mAb No63 was not also targeted to coiled bodies remains unclear. Two mAbs against nucleolin, mAb b6-6E7 and P7-1A4 (Wedlich and Dreyer, 1988; Messmer and Dreyer, 1993), were injected into the cytoplasm of *Xenopus* oocytes, and in each case the antibody was readily detectable in nucleoli 21 h later, as shown for mAb b6-6E7 (Figure 6C).

We also tested mAb SC35, which detects several members of the SR group of splicing factors but reacts particularly well with SC35 (Fu and Maniatis, 1990). In GV spreads this antibody gives strong immunostaining of the B-snurposomes and the loops of the lampbrush chromosomes (Wu *et al.*, 1991) but also reacts weakly with the matrix of the coiled bodies. In a recent study of tissue culture cells, Cáceres *et al.* (1998) showed that some SR proteins, including SC35, are confined to the nucleus, but that others (ASF/SF2, SRp20, and 9G8) shuttle rapidly between the nucleus and the cytoplasm. We injected ~25 pg of anti-SC35 into the cytoplasm of oocytes, made GV spreads 3–18 h later, and stained them with secondary antibody. We saw no staining of nuclear structures above the background level, suggesting that SC35 does not shuttle in the oocyte. The same amount of antibody injected into the nucleus gave strong staining of the B-snurposomes and chromosome loops and weak staining of the coiled body matrix (Figure 6D). When a much higher dose of antibody (1.8 ng) was injected into the cytoplasm, weak nuclear staining was detectable. Because mAb SC35 is not absolutely specific for SC35, this staining could have been due to import of the antibody by one or more of the shuttling proteins described by Cáceres *et al.* (1998).

### *Nucleoplasmic Coilin*

Although coilin occurs in highest concentration in the coiled bodies, centrifugation experiments establish that up to 90% of coilin in the GV is in the soluble nucleoplasm (Bellini and Gall, 1998). With the demonstration of shuttling, the cytoplasm becomes a third compartment of the oocyte where coilin may be found. To better understand the function(s) of coilin, it would be useful to know more about its movement among these compartments. We have carried out an injection experiment to test whether coilin can move directly from the nucleoplasmic pool to the coiled bodies. One could imagine, for instance, that the nucleoplasmic pool consists exclusively of coilin that has passed through the coiled bodies, and that only cytoplasmic coilin can be targeted to coiled bodies.

We prepared a sample of nucleoplasmic coilin as follows. In vitro transcripts encoding *myc*-tagged coilin were injected into the cytoplasm of 100 oocytes, which were incubated in OR2 saline for 24 h to permit translation and accumulation of tagged coilin in the nucleus. Nuclei were then isolated by hand in a $Ca^{2+}$-free nuclear medium and centrifuged to sediment nuclear organelles (coiled bodies, chromosomes, nucleoli, and B-snurposomes). The supernate contained most of the *myc*-tagged coilin. This soluble nucleoplasmic coilin was then injected into GVs that had been isolated under oil and were essentially free of investing cytoplasm (Paine *et al.*, 1992). Finally, 15 min to 3 h later, the injected GVs were recovered. Cytological spreads were made as usual, and the preparations were stained with anti-*myc* mAb 9E10. Coiled bodies in these GVs were well stained, suggesting that coilin can enter coiled bodies directly from the nucleoplasm without transiting the cytoplasm. A second possibility is that shuttling still takes place between the isolated GV and the very thin layer of cytoplasm surrounding it, and that only coilin that has made this journey can enter the coiled bodies. This second explanation is unlikely for several reasons. 1) Bright staining of coiled bodies was observed as soon as 15 min after injection, whereas in shuttling experiments in whole oocytes, 1–2 h elapsed before stain was detectable in coiled bodies. The kinetics thus favor direct targeting of the nucleoplasmic coilin to the coiled bodies. 2) Because the amount of cytoplasm surrounding the GV under oil is minimal, exchange between the GV and the cytoplasm is probably limited. 3) An ATP-regenerating system is needed to ensure efficient import of NLS-containing proteins into oil-isolated GV (Paine *et al.*, 1992). To distinguish definitely between direct targeting and shuttling, it would be necessary to remove the nuclear envelope and investing cytoplasm from the nuclear contents before making the injection. This would be a technically demanding procedure in itself and would require some way of transferring the free nucleoplasm from oil to an aqueous medium before making a spread preparation.

## DISCUSSION

### *Detection of Shuttling Proteins*

Many proteins are common to both the nucleus and cytoplasm, and still others move between these two compartments on a regular basis. "Shuttling protein" is a somewhat arbitrary designation applied to proteins whose equilibrium distribution during interphase is almost entirely nuclear but that nevertheless enter the cytoplasm transiently and return to the nucleus. The first evidence for this class of proteins came from nuclear transplantation studies on *Ameoba proteus* (Goldstein and Ko, 1981). Amoebae were fed $^{35}$S-labeled methionine for 24 h, and the nucleus from a "hot" animal was then surgically transferred to the cytoplasm of a "cold" animal. After 1 d, radioactivity was detected by autoradiography in the nucleus of the recipient. The discovery of techniques for fusing tissue culture cells from two different sources made similar studies possible on nuclei of higher organisms, with considerably more control over experimental conditions. The transfer of proteins from one nucleus to the other in such heterokaryons can be demonstrated by radioactive label (Rechsteiner and Kuehl, 1979), by species-specific antibodies (Borer *et al.*, 1989; Piñol-Roma and Dreyfuss, 1991), or by epitope tags on transiently expressed proteins (Cáceres *et al.*, 1998). Depending on the species, the nuclei can be distinguished by morphological or biochemical markers. In such experiments it is common to include CHX to inhibit the synthesis of new proteins, which could be targeted to both nuclei independently of shuttling.

A second method for detecting shuttling involves the injection of an antibody into the cytoplasm and testing for its import into the nucleus, usually by immunostaining. This technique depends on the inability of immunoglobulins to cross the nuclear envelope, unless they are complexed with a protein that is itself targeted to the nucleus (Bennett *et al.*, 1983; Madsen *et al.*, 1986; Borer *et al.*, 1989; Meier and Blobel, 1992). Again, it is essential to show that import is independent of new protein synthesis.

*Xenopus* oocytes are one of the most popular cell types for studying the movement of proteins and RNA across the nuclear envelope, both in and out of the nucleus. Injection of precursors is made easy by the large size of the mature oocyte and its GV (1.4 and 0.4 mm diameter, respectively), and clean cytoplasmic and nuclear fractions can be prepared manually from individual oocytes within a matter of seconds. Despite the very large number of biochemical experiments performed on *Xenopus* oocytes in which transport across the nuclear envelope was the major focus, we are unaware of earlier studies in which antibodies were used to follow the shuttling of GV proteins. The specific advantages of the oocyte include the ease of injection, the minute amount of antibody that can be detected (picograms), the detailed cytological localization of the shuttling proteins and antibodies in GV spread preparations, and the ability to perform biochemical fractionation on individually injected oocytes. Antibody injections should provide a useful additional technique for following the traffic of molecules between the nucleus and cytoplasm.

### *Coilin Shuttles between the GV and Cytoplasm*

The experiments reported here show that coilin shuttles between the nucleus and cytoplasm in oocytes of *Xenopus*. The basic observation is that antibodies against coilin or an epitope tag on coilin, when injected into the cytoplasm, are imported into the GV and targeted specifically to the coiled bodies. CHX does not interfere with the process, demonstrating that the import of antibodies does not require newly synthesized protein and must depend on shuttling of preexisting nuclear coilin.

In a recent study Almeida *et al.* (1998) demonstrated that a monoclonal antibody against human coilin (mAb 1D4-δ), when injected into the cytoplasm of HeLa cells, was targeted within 1 h to coiled bodies in the nucleus. However, when cells were preincubated for 2.5 h with emetine, an inhibitor of protein synthesis, the antibody did not appear in coiled bodies, suggesting that antibody was imported only when newly synthesized coilin was present in the cytoplasm. Differences in experimental conditions may account for our apparently contradictory observations. One difference may be the effect of protein synthesis inhibitors in the two systems. Emetin, anisomycin, and CHX all induce significant disassembly of coiled bodies in HeLa nuclei after 5 h (Rebelo *et al.*, 1996). In contrast, the morphology of coiled bodies in the oocyte is not significantly affected, even after complete shutdown of protein synthesis by CHX. Furthermore, some anti-coilin antibodies, including mAb 1D4-δ, themselves caused disappearance of coiled bodies from HeLa nuclei after ~24 h (Almeida *et al.*, 1998). This effect may be due to the concentration of injected antibody, 2.5–5.0 mg/ml in the case of HeLa cells compared with 5–10 μg/ml in the oocyte. Thus, in the HeLa experiments, ongoing disassembly of the coiled bodies caused by both the inhibitor and the antibody itself may have affected targeting of the antibody.

### *Why Does Coilin Shuttle?*

Coilin belongs to a growing list of coiled body components that not only occur elsewhere in the nucleus but are known to move between the nucleus and cytoplasm. For instance, splicing snRNAs, although present in coiled bodies, are found predominantly in B-snurposomes in the GV (Wu *et al.*, 1991) and in the speckles of somatic nuclei (reviewed in Spector, 1993). The maturation pathway for splicing snRNAs (except U6) involves export of the newly transcribed snRNA to the cytoplasm, association with Sm proteins, and reimportation to the nucleus (reviewed by Mattaj, 1988). Similarly, B23/NO38 and Nopp140 are primarily nucleolar proteins, although they also occur in coiled bodies. Both shuttle between the nucleus and cytoplasm (Borer *et al.*, 1989; Meier and Blobel, 1992). Here we observed that when Nopp140 reenters the nucleus, it is targeted simultaneously to both coiled bodies and nucleoli. This result contrasts with the finding that in transfected somatic cells, Nopp140 appears first in the nucleolus and then in the coiled bodies (Isaac *et al.*, 1998). One possibility is that newly synthesized Nopp140 differs in some way from Nopp140 that is shuttling, with a consequent effect on targeting. Nopp140 is one of the most heavily phosphorylated proteins in the nucleus, and its state of phosphorylation could be different in the two cases.

Of particular interest is the recent demonstration that Nopp140 and coilin can form a complex in vitro, suggesting that they might be part of a complex within the cell (Isaac *et al.*, 1998). Although coilin is not ordinarily demonstrable in nucleoli by immunofluorescence, it sometimes appears there. For instance, when overexpressed in the oocyte, coilin accumulated in nucleoli (Wu *et al.*, 1994; unpublished experiments of Zheng'an Wu with green fluorescent protein-tagged coilin). Coilin is also found clustered around nucleoli in HeLa cells treated with actinomycin D (Carmo-Fonseca *et al.*, 1992), and certain mutated forms of coilin appeared in the nucleoli of transfected cells (Bohmann *et al.*, 1995). Taken together, these data suggest that coilin and Nopp140, perhaps in association with fibrillarin and NO38, are part of a transport system involving the cytoplasm, nucleoli, and coiled bodies.

One possibility is that coilin is involved in the import of snRNPs into the nucleus or their targeting to intranuclear sites. Earlier we studied the coiled bodies found in pronuclei assembled in *Xenopus* egg extract (Bauer *et al.*, 1994; Bauer and Gall, 1997). These coiled bodies contain coilin and Sm proteins readily demonstrable by immunofluorescent staining. When coilin was immunodepleted from the extract before assembly of pronuclei, coiled bodies still formed within the pronuclei, but they lacked both coilin and Sm proteins. Depletion of Sm proteins from the egg extract gave similar results. These experiments suggested that coilin was involved in either the import of Sm proteins into the nucleus or their targeting to coiled bodies after import. More recently, we have shown that coilin is an RNA-binding protein and that it can form a weak but specific complex with the U7 snRNP (Bellini and Gall, 1998), suggesting that coilin plays a role in the transport of U7 to the coiled body. Because most of the U7 snRNP in the GV is in coiled bodies, whereas most coilin is in the nucleoplasm, it is likely that coilin has other functions as well. One possibility is that coilin is part of a general transport system, only one function of which is to bring the U7 snRNP to coiled bodies.

## ACKNOWLEDGMENTS

We thank the following for antibodies: R. Tuma and M. Roth (mAb H1), M. Schmidt-Zachmann (mAb No63 and mAb No114), C. Dreyer (mAb b6-6E7 and mAb P7-1A4), Z. Wu (C236), S. Munro (mAb 9E10), and X.-D. Fu and T. Maniatis (mAb SC35). This work was supported by research grant GM-33397 from the National Institute of General Medical Sciences. J.G.G. is American Cancer Society Professor of Developmental Genetics.

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