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Coiled Bodies Are Predisposed to a Spatial Association With Genes That Contain snoRNA Sequences in Their Introns

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Abstract Small nucleolar RNAs (snoRNAs) constitute a group of more than 100 different stable RNA molecules that are found concentrated in the nucleolus where they are involved in the maturation of ribosomal RNA. Most snoRNAs are not produced from their own genes but are encoded in the introns of other genes, referred to as snoRNA host genes. Little is known about the mechanisms by which the snoRNAs are produced from introns and how the snoRNAs mature to functional snoRNP complexes in the nucleolus. One class of intron-encoded snoRNAs binds with high specificity to the protein fibrillarin which is found concentrated in the nucleolus, but also in small nuclear domains known as coiled bodies. It has become clear that genes that code for small stable RNAs, e.g., U1, U2 snRNA, and the U3 snoRNA, are often found adjacent to coiled bodies. High concentrations of transcription factors and RNA processing factors in and around coiled bodies indicate that they may be involved in the expression of the adjacent genes. In order to investigate whether coiled bodies could play a role in the synthesis of intron-encoded snoRNAs the distribution of coiled bodies was studied relative to three different snoRNA host genes, i.e., *hsc70, RPS3, UHG*. All three were found adjacent to coiled bodies at significantly high frequencies (11–19%), compared to control sequences (0–2%), to conclude a preferential association between the snoRNA host genes and coiled bodies. This association could point to a possible role for coiled bodies in the synthesis and/or maturation of snoRNAs. An involvement in snoRNA production could explain the presence of transcription factors, splicing factors, and fibrillarin in coiled bodies. J. Cell. Biochem. 75:393–403, 1999. © 1999 Wiley-Liss, Inc.

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Eukaryotic cells contain a large and complex population of small RNAs that are primarily found associated with the nucleolus, referred to as small nucleolar RNAs (snoRNAs) [for a review see Maxwell and Fournier, 1995]. Over 100 different snoRNAs have now been identified and they share several characteristics. SnoRNAs are usually a few hundred bases in length and contain conserved sequence elements, e.g., C/D boxes or H/ACA boxes [Tollervey and Kiss, 1997]. In addition, these snoRNAs contain sequences that are complementary to specific sites on the ribosomal RNA primary transcripts. Although snoRNAs and their association with rRNA have been known since the late 1960s, the exact function of snoRNAs has only recently been discovered. It was shown that snoRNAs are essential for rRNA maturation and ribosome synthesis. SnoRNAs are involved in endonucleolytic cleavage of the primary rRNA and in guiding specific 2′-O-methylation and pseudouridine formation of the rRNA [Kiss-László et al., 1996; Nicoloso et al., 1996; for a review see Bachellerie and Cavaillé, 1997]. These rRNA processing-functions explain the accumulation of snoRNAs in nucleoli [Matera et al., 1994; Beven et al., 1996] since the nucleoli are the sites where ribosome production takes place.

Most snoRNAs are produced in an unorthodox way; they are not synthesized from their own individual genes but are encoded in the introns of other genes, referred to as snoRNA host genes. Only the U3, U8, and U13 snoRNAs are known to be synthesized from their own genes and their own distinct promotors. The

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other snoRNAs are processed from specific introns in the primary transcripts of the host genes, while the exons form normal mRNA molecules that code for proteins. Strikingly, many of the host genes encode proteins that are associated with the nucleolus and/or ribosomes, e.g., the ribosomal proteins S3, S8, L1, and L5, and the nucleolar protein nucleolin. It seems tempting to speculate that this concerted synthesis of mRNA and snoRNA could be part of a regulatory mechanism for the expression of factors involved in ribosome production [Sollner-Webb, 1993; Tycowski et al., 1993].

Little is yet known about the exact mechanism by which the snoRNAs are produced from introns. It has been shown for a few snoRNAs that only internal snoRNA sequences are required to direct the processing of snoRNAs from the introns and that flanking sequences do not play a role in this. The intron encoded snoRNAs possess an unmodified phosphate group at their 5′ terminus and a simple 3′ hydroxyl group at their 3′ end. It has been proposed that this is the result of exonuclease activity which may be an important component of the snoRNA-production process [Tycowski et al., 1993; Kiss and Filipowicz, 1995; Cavaillé and Bachellerie, 1996]. However, little is known about the proteins, the regulation, or the nuclear organization of snoRNA production.

SnoRNAs occur as ribonucleoprotein complexes rather than as free RNAs. The most ubiquitous and best characterized protein in the snoRNP complex is fibrillarin (known as NOP1 in yeast) [Maxwell and Fournier, 1995]. Fibrillarin has been shown to bind to the category of snoRNAs that contain C and D boxes [Caffarelli et al., 1998]. Consistent with RNA binding, fibrillarin contains RNA recognition motifs (RRM) and glycine-arginine rich sequences. As can be expected from its association with snoRNA, fibrillarin is mainly found concentrated in the nucleolus. However, fibrillarin is also found accumulated in small nucleoplasmic structures, known as coiled bodies [Raška et al., 1990]. This concentration of fibrillarin in coiled bodies has been one of the first indications that snoRNPs are not restricted to the nucleolus.

Coiled bodies are spherical structures, one to five per nucleus, with a diameter of 0.2–1.0 μm. They are evolutionary conserved from plants to mammals, indicating that they have a crucial role in the nucleus [for a review see Gall et al., 1995]. Over the years, many factors have been found concentrated in coiled bodies. Among these are nucleolar constituents, such as fibrillarin, ribosomal protein S6 [Raška et al., 1991], and Nopp140 [Meier and Blobel, 1992], but also nucleoplasmic factors, such as the transcription factors TBP, TFIIH, TFIIF [Schul et al., 1998a; Grande et al., 1997; Jordan et al., 1997], the RNA 3′ cleavage and polyadenylation factors CstF and CPSF [Schul et al., 1996], and the splicing factors snRNPs U1, U2, U4/U6 [Carmo-Fonseca et al., 1992]. The protein p80-coilin is especially enriched inside coiled bodies [Raška et al., 1991; Andrade et al., 1991] and is a hall mark for this nuclear domain. Despite the large amount of data available on coiled bodies, their exact function is still unknown [for a review see Lamond and Carmo-Fonseca, 1993].

Various studies have indicated a spatial relationship between coiled bodies and the nucleolus. Electron microscopical images have demonstrated that coiled bodies can be intimately connected with the nucleolar periphery, often appearing to bud off from or fusing with the edge of the nucleolus [Raška et al., 1990; Malatesta et al., 1994a]. Hepatocytes from estradiol injected roosters and hibernating dormice were found to have increased numbers of coiled bodies at the periphery of or inside nucleoli [Ochs et al., 1995; Malatesta et al., 1994b]. A comparable accumulation of coiled bodies in the nucleolus was achieved by treating HeLa cells with the phosphatase inhibitor okadaic acid [Lyon et al., 1997], and in certain human breast carcinoma cells these nucleolar coiled bodies form spontaneously [Ochs et al., 1994]. However, coiled bodies lack a number of typical nucleolar proteins such as RNA polymerase I, nucleolin, and B23 [Raška et al., 1991] and coiled bodies are also often found in the nucleoplasm. In addition, the accumulation of RNA polymerase II transcription factors, polyadenylation factors, and splicing factors inside coiled bodies suggests an involvement of coiled bodies in mRNA production. This is confirmed by the finding that inhibition of RNA polymerase II activity causes the disruption of coiled bodies [Carmo-Fonseca et al., 1992]. However, coiled bodies have not been found to contain any of the essential arginine/serine splicing factors [Raška et al., 1991], and they do not contain newly synthesized RNA [Moreno Diaz de la Espine, 1983; Schul et al., 1996; Jordan et al., 1997]. This dualistic character makes the

coiled bodies one of the most enigmatic nuclear constituents.

Important new insights have come from in situ hybridization studies in human cells which have shown that genes coding for U1 and U2 snRNA, U3 snoRNA, and histone mRNA can be preferentially found positioned adjacent to coiled bodies [Frey and Matera, 1995; Smith et al., 1995; Gao et al., 1997]. This was already described for the histone genes on lampbrush chromosomes in amphibian oocytes which were found associated with sphere organelles that represent the amphibian equivalent of coiled bodies [Callan et al., 1991; Gall et al., 1995]. Additonal findings have indicated that the association between coiled bodies and snRNA genes is dependent on the transcriptional activity of the genes [Frey et al., 1999] and that coiled bodies could be involved in facilitating and/or regulating the production of snRNAs [Schul et al., 1998a]. The association of the U3 snoRNA genes with coiled bodies is of special interest here. It points to an involvement of coiled bodies in the production of snoRNAs, possibly including the production of intron-encoded snoRNAs. Coiled bodies may thus play a central role in the efficient manufacturing of the millions of snRNA and snoRNA molecules that have to be produced from genes and introns each cell cycle. We therefore set out to test this possible involvement of coiled bodies in the processing of snoRNAs from the introns of snoRNA hosting genes.

Since the mechanisms and factors involved in the processing of intron-encoded snoRNAs are hardly known, we decided to test whether the genes that contain the intron-encoded snoRNAs are preferentially found associated with coiled bodies. Genomic in situ hybridization in combination with immunofluorescent labeling and confocal laser scanning microscopy revealed that all three tested snoRNA host genes were predisposed to being associated with coiled bodies. Control experiments and statistical analysis showed this association to be significant and not coincidental. The implications towards a possible relationship between coiled bodies and snoRNA synthesis are discussed.

MATERIALS AND METHODS

Cell Culture and Synchronization

T24 cells (from human bladder carcinoma) were grown on circular glass coverslips at 37°C under a 10% CO_2 atmosphere in DMEM (Gibco) supplemented with 1% glutamine (Gibco), 10% fetal calf serum (Gibco), and antibiotics (100 IU/ml penicillin and 100 mg/ml streptomycin; Gibco). Cells were fixed at about 70% confluency.

Fluorescent In Situ Hybridization and Immunofluorescence Labeling

All steps were performed at room temperature unless stated otherwise. Coverslips with attached cells were rinsed once in PBS and incubated with 2% paraformaldehyde in PBS for 15 min. After fixation cells were rinsed twice with PBS and permeabilized with 0.5% Triton X-100 (Sigma) in PBS for 5 min. Cells were subsequently rinsed twice in PBS and incubated in PBS containing 100 mM glycine (Sigma) for 10 min. Cells were dehydrated by subsequent incubations in 70%, 90%, and 100% ice cold ethanol for 4 min per incubation, and air dried. Genomic DNA was denatured by incubating the coverslips in 2× SSC containing 70% formamide (pH 7.2) at 80°C for 5 min. Immediately after, the cells were treated with the 70%, 90%, and 100% ice-cold ethanol for 4 min each, and air dried. The cells were incubated overnight in probe solution at 37°C.

The probes were produced from the following human genomic clones: a ≈36 kb fragment of the *RNU1* locus at 1p36, a ≈6 kb fragment of the *RNU2* locus at 17q21 (gifts from drs. A.G. Matera and A.M. Weiner) [Frey and Matera, 1995], a 4.4 kb fragment of the *hsc70* gene locus (gift from Dr. M.E. Mirault) [Dworniczak and Mirault, 1987], a ≈25 kb fragment of the *RPS3* locus at 11q13 (gift from Dr. G.A. Evans) [Smith et al., 1993], a combined 3.3 kb 5′ fragment and 9.0 kb 3′ fragment of the *UHG* gene locus at 11q13 (gifts from Dr. A.G. Matera) [Frey et al., 1997], a ≈1 kb fragment of the centromere of chromosome 1 (*Cen1*; gift from Dr. N. Groot), and a 8.6 kb fragment of the dihydrofolate reductase (*DHFR*) gene locus at 5q12–13 (ATCC clone CHB203). The probes were labeled by nick translation using digoxigenin-labeled dUTP essentially as described by Rigby et al. [1977] and Langer et al. [1981]. The probes were heat denatured in 70% deionized formamide together with COT-1 DNA (Boehringer) at 80°C for 10 min. The final probe solution contained 2× SSC, 50% formamide, 10% dextran sulphate, COT-1, and herring sperm DNA, in addition to the labeled probe.

After incubation with probe solution, the coverslips were washed three times 5 min in 2×

SSC containing 50% formamide (pH 7.2) at 39°C and three times 5 min in 1× SSC at room temperature. The cells were washed twice in PBS and incubated for 30 min in PBH (PBS containing 0.1 mg/ml nuclease free acetylated BSA (Sigma) and 0.1 µg/ml herring sperm DNA).

Cells were subsequently processed for immunofluorescent labeling as follows. The cells were incubated for 2 h with polyclonal antibody 204/5 from rabbit against p80-coilin (gift from Dr. A.I. Lamond) [Bohmann et al., 1995] diluted in PBH. Subsequently, cells were washed four times 5 min in PBH and incubated with FITC conjugated anti-digoxigenin antibody (Sigma) and Cy3 conjugated anti-rabbit IgG antibody (Jackson Immunoresearch Lab.) diluted in PBH for 1.5 h.

After labeling, cells were washed two times 5 min in PBH and two times 5 min in PBS followed by incubation in PBS containing 0.4 mg/ml Hoechst 33258 (Sigma) for 5 min. All coverslips were mounted in Vectashield (Vector Laboratories).

Confocal Laser Scanning Microscopy and Image Analysis

Images of double labeled cells were produced on a Leica confocal laser scanning microscope with a 100×/1.35 oil immersion lens. A dual wavelength laser was used to excite green (FITC) and red (Cy3) fluorochromes simultaneously at 488 nm and 514 nm, respectively. The fluorescence signals from the two fluorochromes were recorded simultaneously. Optical crosstalk was quantified and subtracted as described previously [Manders et al., 1992]. Image analysis was performed using Scil-Image software, developed at the University of Amsterdam [Van Balen et al., 1994]. About 50 three-dimensional images of each genomic in situ hybridization labeling were recorded and analysed. The stacks of optical sections were screened for coiled bodies adjacent to labeled genomic loci by two researchers independently. Only touching nuclear foci were counted as positive, separate foci very close together were considered 'not associated.'

Statistical Analysis

Image analysis yielded the number of nuclei containing zero, one, two, etc., coiled bodies adjacent to a labeled genomic locus. The average number of coiled bodies adjacent to a locus per nucleus was calculated for each genomic probe (Table I). Because of the high number of nuclei analysed, the low average numbers and the restriction that negative values are physically impossible, the numbers follow a Poisson distribution.

The distributions of the snoRNA host genes and the snRNA genes were compared to the different control sequences by a chi-squared test. For greater reliability all nuclei with one or more coiled bodies associated with a genomic locus were grouped together, leaving only one degree of freedom for the chi-squared function. The outcome of the chi-squared test gives the chance of one distribution being part of the other, indicated by a *P*-value (Table II). Commonly, a *P*-value lower than 0.05 is considered to represent a significant difference between two distributions. An example of the calculations for the chi-squared test is given below for the *hsc70* locus when compared to the *Cen1* locus.

The Poisson distribution is described by the formula $P(k) = (\mu^k/k!)\, e^{-\mu}$. The value *P* gives the chance to find 'k' coiled bodies associated with a

TABLE I. Quantitative Analysis of Coiled Bodies and Gene Loci[a]

Probe	Number of nuclei analysed	Average number of CBs per nucleus	Average number of gene loci per nucleus	Percentage of nuclei that contain CBs next to gene loci
hsc70	45	2.2	3.4	13.3%
RPS3	36	1.9	2.7	11.1%
UHG	58	2.1	3.0	19.0%
RNU1	36	2.1	3.7	25.0%
RNU2	69	1.8	3.5	56.5%
DHFR	34	1.7	2.7	0.00%
Cen1	53	2.2	3.0	1.89%

[a]This table shows the observed numbers of nuclei, coiled bodies (CBs), and genomic loci, and the frequencies at which coiled bodies and different genes were found next to each other. The *hsc70, RPS3,* and *UHG* probes detected snoRNA host genes, the *RNU1* and *RNU2* probes served as positive controls, and the *DHFR* and *Cen1* probes were used as negative controls.

TABLE II. Statistical Analysis of Coiled Bodies and Gene Loci[a]

Probe	*P*-values compared to *Cen1*	*P*-values compared to *DHFR*	*P*-values compared to *Cen1* + *DHFR*
hsc70	<0.02	<0.05	<0.01
RPS3	<0.05	<0.05	<0.01
UHG	<0.01	<0.01	<0.01
RNU1	<0.01	<0.01	<0.01
RNU2	<0.01	<0.01	<0.01
Cen1	1	~0.45	~0.50

[a]This table shows the *P*-values that were found when the degrees of association with coiled bodies was compared between the snoRNA host and the control genes by a chi squared test. The *P*-values reflect the probability that two compared distributions do not differ significantly. A *P*-value of 0.05 or less is regarded low enough to conclude a significant difference. All *P*-values for the snRNA and snoRNA genes are below 0.05 indicating that their association with coiled bodies is significantly higher than for a random control sequence.

gene locus in a randomly selected nucleus when the average is 'μ'. The average number for the *hsc70* locus was 0.133 (μ = 0.133). This yields a chance to find no associated coiled bodies (k = 0) in a nucleus of 87.5% ($P[0] = 0.875$). The chance to find one or more associated coiled bodies is $P(\geq 1) = 1 - P(0) = 0.125$. These values are used in the chi-squared test to determine whether the numbers found for a snoRNA host gene locus are significantly higher than the control loci. In this example, the *hsc70* locus is compared with the *Cen1* locus. Of the 53 recorded *Cen1* labeled nuclei, only one contained an associated coiled body. The expected values for this number of nuclei for the *hsc70* locus are: $0.875 \times 53 = 46.375$ nuclei without associated coiled bodies, and $0.125 \times 53 = 6.625$ nuclei with at least one associated coiled body. In the chi-squared test the differences between the expected values (E[k]) and observed values (O[k]) are weighed by the formula: $E(O[k]\text{-}E[k])^2/E(k)$ which in our example comes to: $(52–46.375)^2/46.375 + (1–6.625)^2/6.625 = 5.458$.

When we look at the table for the chi-squared distribution we find that, for one degree of freedom, values higher than 3.841 give a *P*-value < 0.05, higher than 5.412 a *P*-value <0.02, and higher than 6.635 a *P*-value < 0.01. The value in our example is just higher than 5.412 so the *P*-value < 0.02. This means that there is a more than 98% chance that the associations found for the *hsc70* locus are significantly higher than the *Cen1* control locus. A *P*-value of 0.05 or less is generally consider low enough to conclude that the two distributions are significantly different.

RESULTS

Localization of snoRNA Host Genes and Coiled Bodies

The spatial relationship between genes and coiled bodies was investigated by combining in situ hybridization with immunofluorescent labeling. Different genomic fragments, encompassing the various gene loci, were used to generate specific in situ hybridization probes. Antibodies against the protein p80-coilin were used to detect coiled bodies. Three snoRNA host genes were investigated in this study: the *hsc70* gene, containing the U14 snoRNA in three of its introns [Liu and Maxwell, 1990], the gene for ribosomal protein S3, containing the U15 snoRNA in at least one of its introns [Tycowski et al., 1993], and the 'U22 host gene' (*UHG*) which contains eight different snoRNAs, U22, and U25-U31, each in a different intron [Tycowski et al., 1996]. The *UHG* gene is particularly interesting because its function appears primarily focussed on the production of snoRNAs instead of the synthesis of mRNA. Although a spliced and polyadenylated UHG RNA is produced it has little potential for protein coding, it is short lived, and it is poorly conserved between human and mouse. Strikingly, not the exons but the snoRNA-containing introns are the most conserved between the human and murine *UHG* genes, suggesting the intronic sequences may have acquired a more important function than the exons [Moore, 1996]. This makes the *UHG* gene a highly specialized snoRNA host gene and an important subject for our studies.

Probes against the U1 snRNA gene cluster and the U2 snRNA cluster were used as positive controls. These gene loci have previously been found preferentially associated with coiled bodies in various cell types [Frey and Matera, 1995; Smith et al., 1995]. Probes against the *DHFR* gene and the centromere of chromosome 1 (*Cen1*) were used as negative controls. Cells double labeled for coiled bodies and genomic loci were recorded by confocal laser scanning microscopy to allow the analysis of the spatial distribution of the signals in three dimensions. The recorded nuclei were analysed by two researchers independently.

Usually two to four genomic loci and one to three coiled bodies were detected per nucleus. The U2 snRNA gene loci were most frequently found next to a coiled body, but also the U1 snRNA genes and the snoRNA host genes were regularly found accompanied by a coiled body (Fig. 1). Counted numbers are presented in Table I. Although the percentages for the snoRNA host genes were not as high as for the snRNA genes they were considerable higher than may be expected for an arbitrary gene. Comparably, Jacobs et al. [1999] recently showed that the single copy U4, U11, and U12 snRNA loci were significantly associated with coiled bodies, but less frequently than the multicopy snRNA genes like U1 and U2. It has been shown that the chance for a random genomic sequence to be associated with the tiny coiled bodies is very small. None of the random control genes were found associated in the localization studies with the U1 and U2 genes [Frey and Matera, 1995], and comparably low numbers were found for our negative controls *DHFR* and *Cen1* (Table I). A statistical analysis is presented below. The frequency of association seems similar for the *hsc70* and the *S3* genes (13% and 11%, respectively), only the snoRNA-rich *UHG* gene is found adjacent to a coiled

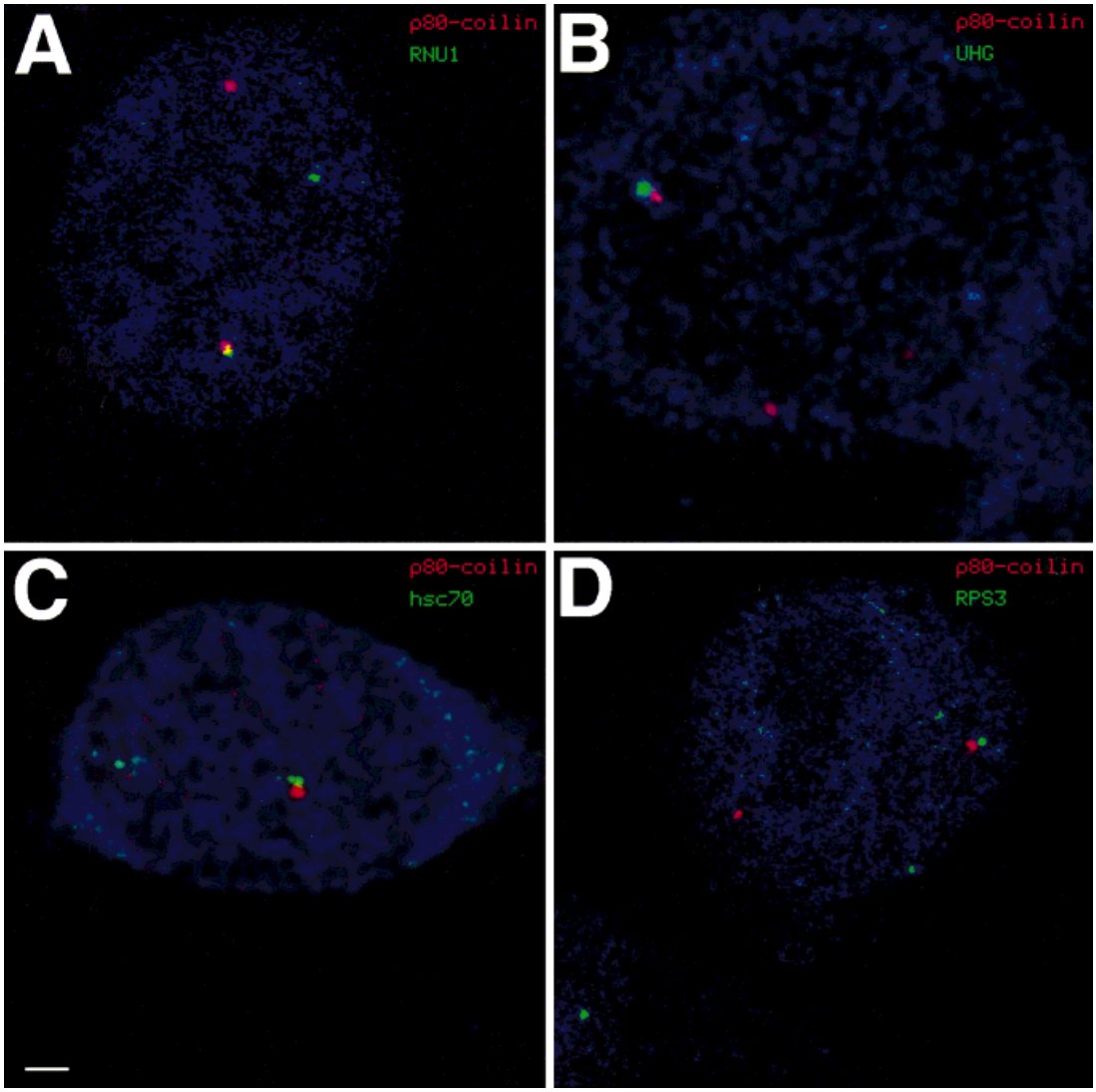

Fig. 1. Confocal optical sections of double labeled cells. Cells were labeled for coiled bodies by immunfluorescent labeling (red) of the protein p80-coilin, and labeled for specific genomic loci by in situ hybridization (green). The genes for (**A**) snRNAs (*RNU1*), (**B**) UHG, (**C**) hsc70, and (**D**) ribosomal protein S3 (*RPS3*) were all regularly found adjacent to a coiled body. The nuclear background is shown in blue. Scale bar = 2 μm.

body more often (19%). The snoRNA host genes are not as frequently positioned next to a coiled body as the U1 and U2 snRNA genes. Only the *UHG* gene comes close the percentage found for the U1 snRNA gene locus. Although the degree of association with coiled bodies is not 100%, there is a distinct spatial relationship between coiled bodies and snoRNA host genes.

Statistical Analysis

The values for cells labeled by a snoRNA host gene-probe and by the control probes were compared by a chi-squared test (explained in Materials and Methods). The results for these chi-squared tests are expressed as *P*-values. The *P*-values represent the chance that the numbers found for the snoRNA host genes are not significantly different from the control genes. A *P*-value of less than 0.05 is usually considered as sufficiently low to conclude a significant difference. The *P*-values we found for the snoRNA host genes were all below 0.05 when they were compared to the separate control genes. This comparison with two independent control genes can be combined to increase the reliability of the test. When the snoRNAs host genes are compared to both control genes together the *P*-values are all below 0.01 (Table II). Comparatively, the control probe *Cen1* scores a *P*-value of about 0.5 in this test (Table II). These findings reveal that the association between snoRNA host genes and coiled bodies is much higher than can be expected for a random genomic sequence. We can therefore conclude that the three snoRNA host genes that we have tested here are preferentially located adjacent to a coiled body. The implications of this result in respect to nuclear organization and snoRNA production are discussed below.

DISCUSSION

Very little is known about the process by which the more than 100 different snoRNAs are produced in several 10,000 copies each cell cycle. The majority is probably produced from the spliced-out introns of transcripts produced from specific snoRNA host genes, but the mechanism by which this occurs is poorly understood. We have investigated the nuclear distribution of three snoRNA host genes; the *hsc70* gene hosting the U14 snoRNA, the *S3* gene hosting the U15 snoRNA, and the *UHG* gene hosting the U22 and U25-U31 snoRNAs. We have found that they are preferentially located adjacent to small nuclear structures known as coiled bodies.

The U1 and U2 snRNA gene clusters, the histone gene clusters and the U3 snoRNA gene clusters have been found adjacent to coiled bodies before [Frey and Matera, 1995; Smith et al., 1995; Gao et al., 1997]. Immunofluorescent localization studies have shown that factors involved in the expression of these genes are concentrated in and around coiled bodies, and that newly synthesized RNA is produced at these sites [Schul et al., 1998a]. It has therefore been put forward that coiled bodies are associated with these genes to facilitate and/or regulate the production of U1 and U2 snRNA, U3 snoRNA, and histone mRNA [Schul et al., 1998b; Matera, 1998]. The association between coiled bodies and snoRNA host genes that we have found here could point to a similar involvement of coiled bodies in the production of intron-encoded snoRNAs. Coiled bodies could function as centers around which efficient transcription, splicing, snoRNA excision and snoRNP formation takes place. First suggestions towards such a function for coiled bodies have already been made by Tycowski et al. [1993] and Lamond and Carmo-Fonseca [1993]. Our results give a first indication that there may indeed be a functional relationship between snoRNA host genes and coiled bodies.

One particularly puzzling aspect of the relationship between coiled bodies and associated genes is that in each single nucleus there are one or more copies of these genes unassociated with a coiled body. Not only the snoRNA host genes we have studied here, but also a substantial percentage of the U1 and U2 snRNA genes, the U3 snoRNA genes, and the histone genes are not next to a coiled body. It is completely unclear what causes this heterogeneity and why the degrees of association vary between cell types. It is possible that the genes are not continuously active, or perhaps coiled bodies are not continuously required for the synthesis and maturation of the RNA. It should be noted, however, that the recorded images of fixed cells only represent a single moment in time. Little is yet known about the dynamic and kinetic properties of coiled bodies. Nonetheless, statistical analysis of many fixed cells does show that the spatial association between snoRNA host genes and coiled bodies is highly significant and represents a specific relationship between these two nuclear constituents. Although there is no

direct evidence that coiled bodies play a role in snoRNA processing it could explain several fundamental properties of coiled bodies, e.g., the accumulation of transcription and splicing factors in coiled bodies, the high concentration fibrillarin in coiled bodies, and the association of coiled bodies with the nucleolus. We will present here a model in which the different properties of coiled bodies are discussed in relation to a possible role in snoRNA production.

I: Coiled bodies contain high concentrations of basal transcription factors, e.g., TBP, TFIIF, and TFIIH. The finding that specific genes, including the snoRNA host-genes, are frequently positioned adjacent to coiled bodies fits with this concentration of transcription factors. Although coiled bodies do not contain newly synthesized RNA, it has been shown that several active genes are found at the periphery of coiled bodies [Schul et al., 1996; Jordan et al., 1997]. Coiled bodies may thus function as sites where the efficient transcription of specific genes, e.g., the snRNA and snoRNA host genes, takes place (Fig. 2A). The sensitivity of coiled bodies to drugs that inhibit RNA polymerase II activity [Carmo-Fonseca et al., 1992] confirms the involvement of coiled bodies in RNA synthesis.

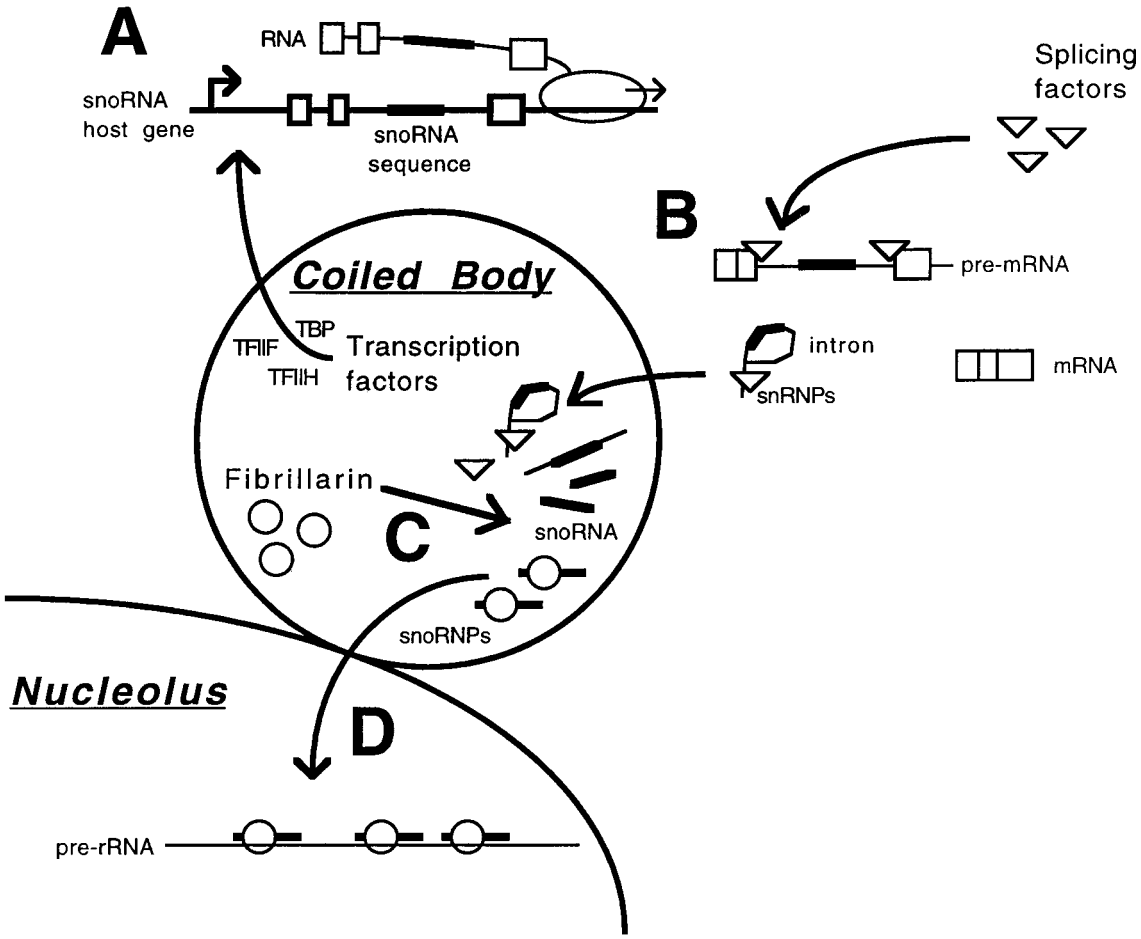

Fig. 2. This cartoon depicts a model of how coiled bodies may be involved in the production of snoRNPs. The model is able to explain many coiled body-characteristics. **A:** Coiled bodies are enriched in transcription factors, e.g., TBP, TFIIF, TFIIH. These can be supplied to genes that are associated with coiled bodies, e.g., snoRNA host genes, to facilitate and regulate RNA synthesis. **B:** Splicing of RNA transcripts takes place in the vicinity of the genes, outside the coiled body. Excised introns that contain snoRNA-sequences can subsequently be taken up by the nearby coiled body. SnRNP splicing-complexes stay associated with the excised introns and thus accumulate in coiled bodies, while other splicing factors dissociate from the introns and are therefore absent from coiled bodies. **C:** SnoRNAs are processed from the introns in coiled bodies where they bind to fibrillarin to form snoRNP complexes. **D:** Coiled bodies can associate with the nucleolus, allowing the delivery of mature snoRNPs to their place of business. This model on a putative role of coiled bodies in the production of snoRNPs unifies the nucleoplasmic functions of coiled bodies with their nucleolar association.

II: The presence of snRNP splicing factors in coiled bodies points to a link with RNA splicing, but the absence of additional essential splicing factors suggests that coiled bodies are not sites of intron excision [Raška et al., 1991]. We propose here that only specific excised introns are present in coiled bodies so they can be processed into snoRNAs (Fig. 2B). Although no nascent RNA can be detected inside coiled bodies, relatively low levels of small intron sequences may escape detection and pass through coiled bodies to be processed.

III: Although coiled bodies contain factors involved in mRNA synthesis and processing, they are also enriched in nucleolar proteins, e.g., fibrillarin, which indicates an unexplained link with the nucleolus. Interestingly, fibrillarin is known to bind specifically to C/D box snoRNAs and is involved in snRNP complex formation (Fig. 2C). Interestingly, recent experiments have indicated that the yeast protein Cbf5p can bind to the H/ACA box snoRNAs [Lafontaine et al., 1998]. Cbf5p has a strong homology with the mammalian NAP57 protein which is, just like fibrillarin, concentrated in nucleoli and coiled bodies [Meier and Blobel, 1994]. The specific accumulation of fibrillarin and NAP57 inside coiled bodies is therefore one of the strongest indications that coiled bodies are involved with snoRNAs. This has been confirmed for the U3 snoRNA which was specifically detected in the nucleolus and in coiled bodies [Jiménez-Garcia et al., 1994; Azum-Gélade et al., 1994].

IV: A spatial and structural relationship between coiled bodies and the nucleolus has repeatedly been reported in many studies. Coiled bodies have been observed inside the nucleolus or appearing to fuse with or bud off the edge of the nucleolus [Raška et al., 1990]. It has remained unclear how this nucleolar association relates to the nucleoplasmic role of coiled bodies in mRNA and snRNA production. However, the production of snoRNAs from the introns of household genes links nucleoplasmic RNA synthesis to a nucleolar function. Since snoRNAs are found concentrated in the nucleolus to perform their function on the rRNA transcripts, they have to travel from the snoRNA host gene to the nucleolus. This function may be performed by coiled bodies as part of a delivery mechanism by which the mature snoRNAs reach the nucleolus (Fig. 2D), as already proposed by Lamond and Carmo-Fonseca [1993]. Based on studies with GFP labeled snRNPs, in which they can visualize coiled bodies in the living cell, Sleeman et al. [1998] suggest that coiled bodies could be part of an "intranuclear trafficking pathway" between the cytoplasm and the nucleolus. Additionally, Isaac et al. [1998] have recently shown that Nopp140, which is found in coiled bodies and nucleoli and is associated with NAP57 [Meier and Blobel, 1994], appears to shuttle between coiled bodies and the nucleoli, possibly chaperoning the transport of other molecules. These finding agree with the proposed involvement of coiled bodies in the maturation and transportation of snoRNAs.

Although there is still little direct proof for the hypothesis that coiled bodies are involved in the production of intron-encoded snoRNAs, it is the only model so far that can explain their many different properties. It is therefore important to note that the findings in this study do indeed show a specific association between snoRNA host genes and coiled bodies, as would be predicted by the hypothetical involvement of coiled bodies in snoRNA synthesis. Further studies will have to show how accurate the proposed hypothesis is.

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