Journal of Cellular Biochemistry 70:181–192 (1998)

Of Coiled Bodies, Gems, and Salmon

A. Gregory Matera

Department of Genetics, Center for Human Genetics, Center for RNA Molecular Biology and Program in Cell Biology, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106-4955

Abstract Coiled bodies (CBs) are nuclear organelles whose morphology and composition have been conserved from plants to animals. They are highly enriched in components of three different RNA processing pathways. Small nuclear RNAs (snRNAs) involved in pre-mRNA splicing, rRNA processing, and histone mRNA 3′ end maturation all take up residence in CBs. However, CB function(s) remain obscure. This review will focus on recent developments in several aspects of CB structure and function, including exciting new results on their twin organelles, called gems. In particular, the reader will be introduced to a novel hypothesis called the "salmon theory of snRNP biogenesis." Questions arising from and experiments necessary to test this hypothesis will be discussed. J. Cell. Biochem. 70:181–192, 1998.

Key words: coiled bodies (CBs); gems; p80 coilin; RNPs; RNA processing

Elucidation of the functional diversity of the mammalian nucleus has been hampered by its apparent structural homogeneity. The lack of membranous compartments within nuclei makes biochemical fractionation difficult. In fact, nuclei *are* highly organized and contain numerous subdomains. These substructures are involved in various aspects of cellular metabolism including: replication, transcription, and RNA processing [for a review see de Jong et al., 1996]. One of the emerging principles of nuclear organization is that many individual domains are associated with specific genetic loci. Another emerging principle is that associations between these various domains and loci are dynamic and can change in response to cellular signals.

Although the nucleolus is certainly the most obvious nuclear organelle, both immunofluorescence and immunoelectron microscopic studies demonstrate that it is not the only identifiable substructure. Examples of other nuclear subdomains include: focal sites of pol II transcription [Iborra et al., 1996, and references therein], DNA replication centers [Berezny, 1991; Hozák et al., 1994], interphase chromosome domains [Cremer et al., 1993], interchromatin granules and perichromatin fibrils [Fakan, 1994], hnRNP protein clusters [Pinol-Roma et al., 1989; Ghetti et al., 1992], and focal concentrations of transcription factors [van Steensel et al., 1995; Elefanty et al., 1996; Grande et al., 1997].

In addition to the domains mentioned above, the nuclei of higher eukaryotes contain various electron-dense structures that are collectively called nuclear bodies [for reviews see Brasch and Ochs, 1992; de Jong et al., 1996]. Elucidating the molecular composition of nuclear bodies has been the subject of a great deal of research in recent years. One such nuclear body is called the "coiled" body, from its characteristic ultrastructural morphology [Monneron and Bernhard 1969]. Coiled bodies (CBs) appear as a tangle of coiled, electron-dense threads ranging from 0.1 to 1.0 μm in diameter. Although the name derives from studies in the electron microscope, the CB was originally identified in the light microscope using silver staining [Ramon y Cajal, 1903]. However, despite ultrastructural analyses which showed that anti-Sm epitopes concentrated in CBs [Elicieri and Ryerse 1984; Fakan et al. 1984], it was not until E. Tan's laboratory isolated a CB marker protein (Andrade et al., 1991; Raska et al., 1991] and A. Lamond's lab showed that hybridizable snRNAs

Contract grant sponsor: NIH; Contract grant number: GM53034; Contract grant sponsor: American Cancer Society; Contract grant number: JFRA-570.

*Correspondence to: Dr. Greg Matera, Department of Genetics, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106-4955.
E-mail: gxm26@po.cwru.edu

Received 5 December 1997; Accepted 8 December 1997

were present within them [Carmo-Fonseca et al., 1991, 1992] that CBs began to attract the attention of the RNA processing community.

A COILED BODY-SPECIFIC PROTEIN

Antibodies raised against recombinant p80 coilin or anti-p80 peptide antibodies strongly and specifically label coiled bodies [Andrade et al., 1991; Chan et al., 1994; Wu et al., 1994; Bohmann et al., 1995a]. However, it is important to note that not all of the p80 coilin in the nucleus is concentrated within CBs (Fig. 1). There may well be a free pool of protein [Andrade et al., 1993; Carmo-Fonseca et al., 1993]. As can be seen in Figure 1, numerous smaller foci can be detected, as well as a general diffuse staining that appears to be above background levels. The human p80 coilin gene was cloned, sequenced, and mapped to chromosome 17q22-23 [Chan et al., 1994]. While anti-coilin antibodies stain similar structures in a wide variety of species [Beven et al., 1995; Gall et al., 1995; Yannoni and White, 1997], the only other coilin homologue reported to date is the SPH-1 protein from the sphere organelles of *Xenopus* oocytes [Tuma et al. 1993]. This protein is highly similar to human p80 coilin on its N- and C-terminal regions, but differs markedly throughout the middle of the protein [Chan et al., 1994; Bohmann et al., 1995a].

The best evidence that spheres and CBs are homologous organelles comes from the fact that myc-tagged p80 coilin is specifically targeted to spheres when transcripts of the human protein are injected into *Xenopus* oocytes [Wu et al., 1994]. Targeting of coilin to spheres and CBs in frog oocytes and human somatic cells, respectively, is complex and requires sequences near the N-terminus as well as more conventional nuclear localization signals [Wu et al., 1994; Bohmann et al., 1995b]. Precise CB localization signals have not been described. Transient overexpression of mutant p80 coilin also causes a number of interesting cellular phenotypes, including the redistribution of Nopp140 and fibrillarin [Bohmann et al., 1995b]. Coilin contains two types of "classical" nuclear localization signals [reviewed in Dingwall and Laskey, 1991; Görlich, 1997], a simple one and a bipartite one [Chan et al., 1994; Wu et al., 1994; Bohmann et al., 1995b]. Import of p80 coilin into the nucleus is therefore likely to proceed through the importin α pathway [Weis et al., 1995].

Following import into the nucleus, details concerning coilin's route to the CB are sketchy at best. However, it seems likely that Nopp140, a primarily nucleolar protein which is known to contain NLS-binding motifs and sublocalize in CBs [Meier and Blobel, 1992, 1994], is involved. Additional hints to the intranuclear trafficking question come from elegant experiments by Lyon et al. [1997], who showed that coilin's phosphorylation state is important for its localization. Inhibition of Ser/Thr dephosphorylation using okadaic acid or transient transfection of a coilin point mutation that mimics a constitutively phosphorylated protein results in accumulation of p80 coilin and splicing snRNPs *within* the nucleolus [Lyon et al., 1997]. Although CBs are frequently detected at the periphery of nucleoli (they were originally dubbed "nucleolar accessory bodies" by Cajal), intranucleolar CBs are not normally detected. Curiously, this intranucleolar phenotype had only been observed twice previously: in certain breast cancer cell lines [Ochs et al., 1994] and in hepatic cells of hibernating dormice [Malatesta et al., 1994]. The observations of Lyon et al. [1997] provide a testable hypothesis for the relocation of CBs in the hibernating dormice and breast cancer cells, e.g., that mutations or alterations in the (de)phosphorylation pathway of p80 coilin result in the observed intranucleolar accumulations. These observations are consistent with the idea that newly assembled splicing snRNPs and p80 coilin traffic through nucleoli on their way to the nucleoplasm [Lyon et al., 1997] (see The Salmon Theory).

Coiled bodies are dynamic structures; they disassemble during mitosis and reassemble in mid-G1 after the reformation of nucleoli and the resumption of transcription [Andrade et al., 1993; Ferreira et al., 1994]. The mitotic disassembly is concomitant with a hyperphosphorylation of coilin on at least two additional serine residues [Carmo-Fonseca et al., 1993]. Although CBs appear to be somewhat larger during S phase, there is no obvious cell-cycle regulated expression of coilin and the total amount of protein remains approximately the same throughout the cell cycle [Andrade et al., 1993]. These and other experiments (see below) illustrate the dynamic nature of the coiled body. However, in order to discuss possible functional role(s) for CBs, we first need to explore their molecular composition.

COILED BODY COMPONENTS

Small RNPs

As discussed above, coiled bodies were first identified morphologically. The CB autoantigen, p80 coilin, was the first marker protein specific to these structures [Raska et al., 1991]. Since then, the list of molecules that are reportedly enriched within CBs has steadily grown. Most prominent on the list are the major and minor splicing snRNPs U1, U2, U4, U5, U6, U11, and U12 [Carmo-Fonseca et al., 1992; Huang and Spector, 1992; Matera and Ward, 1993]. These snRNAs were shown to localize throughout the nucleoplasm in a speckled pattern, excluding nucleoli and concentrating in CBs (Fig. 2). The absence of polyA$^+$ RNA within CBs makes it unlikely that they are sites of actual pre-mRNA splicing [Matera and Ward, 1993; Huang et al., 1994]. In this context, it is important to note that although splicing snRNPs are highly enriched within CBs, only a small fraction of their overall number is present in CBs at a given time [Matera and Ward, 1993]. Both common (e.g., TMG-cap and Sm proteins) and specific (e.g., U2B″) snRNP epitopes have been demonstrated to accumulate within these organelles [Raska et al., 1991; Carmo-Fonseca et al., 1992; Huang and Spector, 1992; Matera and Ward, 1993; Fakan, 1994]. Thus, the snRNPs that accumulate in CBs are likely to be mature ones, since TMG-cap and Sm particle assembly takes place after snRNA synthesis and export to the cytoplasm [reviewed in Izaurralde and Mattaj, 1992].

Following import back into the nucleus, at least some of these snRNPs must make their way to the CB. However, the presence of snRNPs in CBs requires ongoing transcription [Carmo-Fonseca et al., 1992]. When transcription is blocked, snRNPs no longer concentrate in CBs and the IGCs (speckles) become enlarged. A similar phenotype is observed either when transcription is shut off during terminal differentiation [Antoniou et al., 1993] or when splicing is inhibited using antisense oligonucleotides [O'Keefe et al., 1994]. Taken together, these data suggest that CBs are not simply storage sites for inactive snRNPs. If CBs *were* merely storehouses for snRNPs, then one might reasonably expect CBs to be enlarged when transcription and processing were shut down [Lamond and Carmo-Fonseca, 1993].

In addition to the splicing snRNPs, somatic CBs and oocyte spheres contain relatively high concentrations of U7 snRNA [Wu and Gall, 1993; Frey and Matera, 1995]. Interestingly, the sphere organelles (C snurposomes) are compartmentalized [Wu et al., 1991]. Coilin and U7 snRNA are located in the matrix of the C snurposomes whereas splicing snRNPs and SR proteins are detected only on the surface of the spheres in so-called B snurposomes and in B-like inclusions within the matrix of the sphere [Wu et al., 1991; Tuma et al., 1993; Wu and Gall, 1993; Gall et al., 1995]. Coilin and U7 are not detected in the B-like inclusions. There is no evidence for or against such a compartmentalization of somatic CB components. However, due to the fact that spheres are much larger organelles (10 μm in diameter), it may be that somatic CBs are simply too small to see subcompartments. Furthermore, Gall and co-workers have shown that the number of U7 binding sites within spheres is saturable [Wu et al., 1996]. Microinjection of labeled U7 RNA shows that the injected constructs replace endogenous U7 snRNA. Thus, there is likely to be a flux of snRNPs through CBs.

Nucleolar Proteins and snoRNPs

Ultrastructural studies have since confirmed Cajal's original observations that coiled bodies are somehow related to nucleoli [reviewed in Lamond and Carmo-Fonseca, 1993]. Numerous electron micrographs clearly show CBs and nucleoli in close juxtaposition. Whether they actually bud off of or dock onto nucleoli is not known, but it is hard to believe CBs are static structures. The presence of nucleolar epitopes such as fibrillarin, Nopp140, NAP57, and topoisomerase I and the absence of others such as nucleolin and numatrin is curious [Andrade et al., 1991; Meier and Blobel, 1994]. While fibrillarin is easily visualized within CBs, snoRNAs such as U3 and U8 are extremely hard to detect [Carmo-Fonseca et al., 1993; Bauer et al., 1994; Jiménez-García et al., 1994; Matera et al., 1994]. This suggests that either U3 sequences are somehow masked within the CB or that other fibrillar snoRNPs may accumulate there. Or perhaps there is a free pool of fibrillarin within CBs. In any event, CBs contain elements from three major RNA processing pathways: pre-mRNA splicing, histone mRNA 3′ maturation, and pre-rRNA processing [Gall et al., 1995].

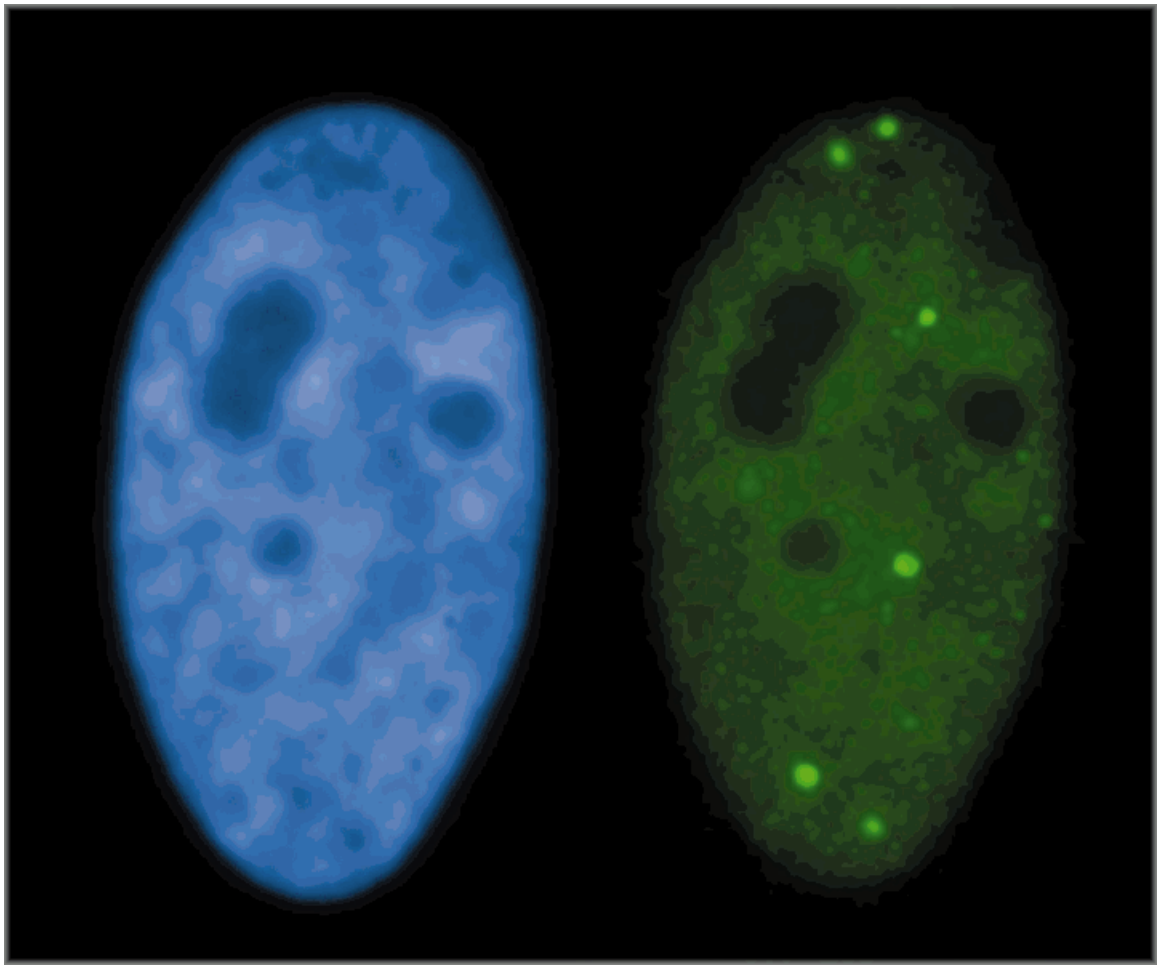

Fig. 1. The same HeLa cell nucleus was stained with DAPI (left, blue) and with anti-p80 coilin antibodies (right, green). Notice the prominent coiled bodies (bright foci), as well as several smaller nucleoplasmic granules in the green channel. Negatively stained regions, best illustrated in the blue channel, are nucleoli. See text for details.

Transcription Factors and Cell-Cycle Control Machinery

In addition to RNA processing components, CBs also contain subunits of several different transcription factors, including TFIIH and TFIIF [Grande et al., 1997; Jordan et al., 1997]. The TFIIH complex is involved in basal transcription as well as DNA excision repair [reviewed in Hoeijmakers et al., 1996]. However, of the nine TFIIH polypeptides, only the kinase subcomplex including p62, cyclin H, cdk7, and MAT1 proteins concentrate in CBs [Jordan et al., 1997]. Other transcription-coupled repair proteins [see van Gool et al., 1997] such as XPB (ERCC3), XPD (ERCC2), XPG, and PCNA are not enriched within CBs [Jordan et al., 1997]. XPB and XPD are helicase subunits of TFIIH; XPG is responsible for the endonucleolytic cut at the 3′ end of the damaged site and PCNA is a subunit of DNA polymerase δ. Thus the absence of these key repair enzymes from CBs makes it unlikely that they are directly involved in repair [Jordan et al., 1997].

The presence of cyclin H, cdk7, p62, and MAT1 in CBs is noteworthy. Three of these proteins (cdk7/cyclin H/MAT1) form a subcomplex with kinase activity. The target(s) of these enzymes within CBs are not known, but one obvious candidate is coilin itself [Jordan et al., 1997]. As mentioned above, coilin is a phosphoprotein whose phosphorylation state varies throughout the cell cycle [Andrade et al., 1993] and very likely controls its intracellular localization [Lyon et al., 1997]. It will be interesting to ascertain the localization of both the kinases and their respective target proteins.

Although some transcription factors (or subunits thereof) are enriched within CBs, SR-proteins, hnRNPs and at least one form of RNA polymerase II are conspicuously absent [Du

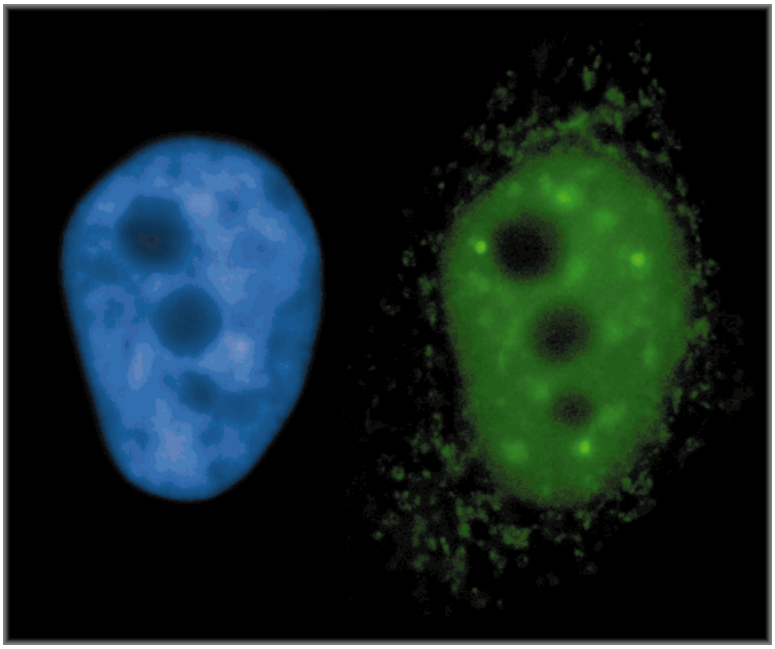

Fig. 2. A biotinylated antisense oligonucleotide complementary to U2 snRNA was hybridized to HeLa cell nuclei. The DAPI image (blue) is on the left, while the anti-U2 oligo hybridization signal is on the right (green). U2 snRNA is distributed diffusely (presumably corresponding to perichromatin fibrils) throughout the nucleoplasm, excluding nucleoli as well as in brighter patches, called speckles (interchromatin granule clusters). The three bright foci are coiled bodies. Depending on fixation methods, U2 RNA can also be detected in the cytoplasm.

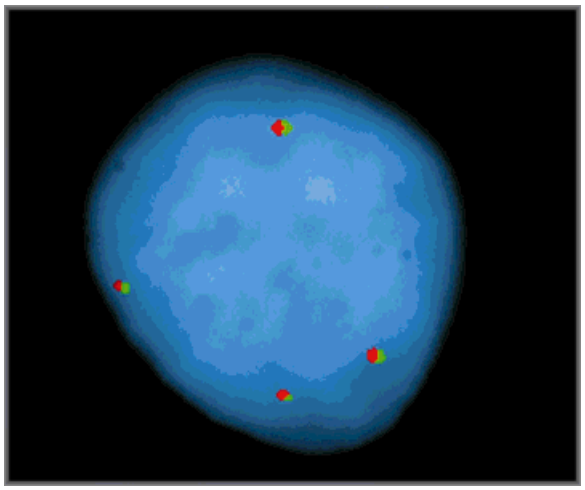

Fig. 3. Gems and coiled bodies colocalize in most interphase cells. A HeLa cell nucleus (DAPI, blue) was stained with anti-p80 coilin (coiled bodies, red) and anti-SMN (gems, green) antibodies. The four twin structures in this cell each show extensive overlap.

and Warren, 1997; Gama-Carvalho et al., 1997; Zeng et al., 1997; for a review, see Lamond and Carmo-Fonseca, 1993]. Furthermore, overexpression of the pol IIo CTD disrupts speckles (IGCs), but does not disrupt CBs. Early pulse-chase experiments with tritiated uridine in amphibian oocytes and mammalian cells revealed that spheres and CBs were neither rapidly nor efficiently labeled [Callan and Gall, 1991; Fakan and Bernhard, 1971]. Hence, CBs are probably not sites of transcription *per se*. The absence of non-snRNP splicing factors such as SC-35 and U2AF [Gama-Carvalho et al., 1997, and references therein], coupled with the lack of hnRNP proteins and poly A^+ RNA makes it unlikely that CBs are involved in splicing [Lamond and Carmo-Fonseca, 1993]. Thus, if CBs are neither sites of transcription nor centers for pre-mRNA splicing, what then is their function? Important insight into this question derives from studies in the amphibian oocyte germinal vesicle.

COILED BODIES ASSOCIATE WITH SPECIFIC DNA LOCI

In 1954, Gall described a prominent nuclear organelle within amphibian oocytes, called the sphere [see Gall and Callan, 1989, and references therein]. Spheres occur both free in the nucleoplasm and attached to specific loci, called "sphere organizers." In analogy to nucleoli, these sphere organizer regions, or SORs, were found to be adjacent to the histone genes of both frogs and newts [Gall et al., 1981; Callan et al., 1991]. Based on criteria detailed above, we now believe that spheres and coiled bodies are homologous organelles. Thus, somatic CBs might

also be expected to associate with histone gene loci. This expectation was met by Frey and Matera [1995], who showed that the histone genes on human chromosomes 1q21 and 6p21 often colocalized with CBs. However, as found in the amphibian oocyte nuclei, only a subset of CBs associated with a subset of histone genes. Intriguingly, genes encoding U1, U2, and U3 snRNAs also colocalize with CBs in this fashion [Frey and Matera, 1995; Smith et al., 1995; Gao et al., 1997]. Furthermore, no cell cycle-specific preferences for these associations have been observed [Smith et al., 1995].

It is formally possible that CBs do *not* associate with U RNA and histone genes. Rather, the *real* coiled body organizer (CBOR) sequences might simply be located nearby [Frey and Matera, 1995]. In fact, the anti-p80 coilin fluorescence pattern rarely overlaps completely with the in situ hybridization signals from the putative CBORs—most of the signals overlap only partially [Frey and Matera, 1995; Smith et al., 1995; Gao et al., 1997]. Rearrangements among the chromosomes in the various cell lines tested might therefore have translocated some CBORs to new locations, which are not likely to be located near U RNA genes. In order to exclude the possibility that CBs associate with *RNU2* loci by chance alone, our laboratory recently demonstrated that CBs colocalize with exogenous, stably transfected U2 genes (Frey et al., manuscript in preparation). Additional experiments to further define the sequence requirements for colocalization with CBs are underway, but it is clear that U RNA genes either nucleate or recruit CBs (see Coiled Body Organizers).

Why do CBs associate with a subfraction of the *RNU2* loci in a given interphase cell? Are some of the loci active while others are not? Attempts to hybridize to nascent U2 RNA using oligonucleotides that span the junction between pre-U2 and mature U2 RNA have been unsuccessful due to crosshybridization with rRNA (unpublished observations). Restriction analysis of the tandemly repeated U2 genes from human cells shows that the repeat units are indistinguishable and that concerted evolution has homogenized the U2 arrays in other primate lineages [Matera et al., 1990; Pavelitz et al., 1995]. Therefore, the individual gene loci are apparently equivalent, yet some colocalize with CBs and others do not. Further experimentation will be required to answer these questions. However, it is clear that CBs and spheres often colocalize with specific DNA loci, but what do these DNA sequences have in common?

CBS ARE NOT snRNP NUCLEOLI, BUT MAY BE snRNP FIBRILLAR CENTERS

One theme that links the aforementioned U RNA and histone genes is that they all encode nonpolyadenylated, polymerase II transcripts that do not contain introns and have conserved 3′-terminal stem-loop structures. Furthermore, these RNAs are each transcribed from TATA-less promoters and are either clustered (histone and U3) or tandemly repeated (U1 and U2) in the human genome [Frey and Matera, 1995; Gao et al., 1997]. One question that arises is whether or not CBs might associate with more than one DNA locus at a time. Given that there are at least five different CBOR loci reported to date and the relatively limited number of CBs per cell, it seems likely that the observed association rates would require that at least some CBs might be shared by more than one locus. Smith et al. [1995] suggested that U1 and U2 loci (in HeLa cells at least) associate in a nonrandom fashion with CBs. While the human U1 and U2 gene clusters are located on different chromosomes (1p36.1 and 17q21.3, respectively), U3 genes are located at 17p11.2. These observations seemingly indicate that CBs are a kind of "snRNP nucleolus," where snRNA genes on disparate chromosomes get together to perform snRNA transcription [Matera and Ward, 1993; Frey and Matera, 1995].

However, the analogy to the nucleolus is incomplete, since several lines of evidence suggest that CBs are not sites of transcription *per se*. Pulse-chase studies with tritiated uridine show a significant time lag before accumulation of label [e.g., Fakan and Bernhard 1971; Callan and Gall, 1991] and the presence of mature snRNP epitopes within CBs also suggests that transcription takes place elsewhere. Setting aside for the moment possible roles that might be played by having mature snRNPs accumulate within CBs, why might snRNA *genes* be localized around them? One reason that springs to mind is that CBs may be a kind of supply center for the various factors required for transcription of CBOR genes. Thus CBs might not be snRNP nucleoli, but in many ways they are analogous to fibrillar centers (FCs). Current models for the role of FCs within the nucleolus [Hozák, 1995, and references therein] depict FCs as roughly spherical structures, with rRNA transcription taking place on its surface (i.e., at

the interface between the FC and the dense fibrillar component). Interestingly, Callan et al. [1991] describe the chromatin loops adjacent to the sphere organizer loci (i.e., histone genes) in *Xenopus* as being "plastered over the surfaces of the attached sphere organelles." Thus, in many respects CBs may be a kind of snRNP fibrillar center.

GEMINI OF COILED BODIES

Recently, exciting work from the Dreyfuss laboratory has shown that CBs have twins. Gemini of coiled bodies, or gems, are nuclear structures of similar size and shape to CBs, but do not contain snRNPs [Liu and Dreyfuss, 1996]. Instead, gems contain high concentrations of the survivor of motor neurons protein, SMN. The *SMN* gene is an essential, single-copy locus in mice [Schrank et al., 1997], but is duplicated in humans [Lefebvre et al., 1995]. Deletion of the telomeric copy of the human gene leads to the autosomal recessive disorder called spinal muscular atrophy (SMA), characterized by loss of spinal motor neurons, progressive paralysis, and muscular atrophy [Lefebvre et al., 1995]. There is excellent correlation between the severity of the disease and the SMN protein level [Coovert et al., 1997; Lefebvre et al., 1997].

SMN protein is localized throughout the cytoplasm, but its nuclear staining is restricted to the gems. As implied by their name, Gemini bodies are most often found in tight association with CBs (Fig. 3). SMN interacts directly with several snRNP core factors, including Sm proteins B, D1-3, and E [Liu et al., 1997]. These polypeptides form a complex, along with SMN interacting protein 1 (SIP1), that is greater than 300 kD [Liu et al., 1997]. SIP1 and SMN colocalize in the nucleus and the cytoplasm [Liu et al., 1997]. Most importantly, SIP1 has been shown to play an essential role in spliceosomal snRNP biogenesis [Fischer et al., 1997]. When injected into the cytoplasm of *Xenopus* oocytes, anti-SIP1 antibodies inhibit Sm core particle assembly and transport [Fischer et al., 1997]. SIP1 is thought to be the mammalian homologue of a yeast protein, called Brr1p, that is also involved in Sm particle assembly [Noble and Guthrie, 1996; Liu et al., 1997].

Although the precise function of SMN remains unclear, the protein contains two distinct domains through which it can bind SIP1 and the Sm proteins, respectively [Liu et al., 1997]. These observations provide a new link for SMN and SIP1 to the SMA phenotype and suggest that defects in snRNP biogenesis are responsible for the disease [Fischer et al., 1997]. It is interesting that a mutation in a housekeeping gene can have such a tissue-specific phenotype. The tissue that shows the most significant alteration of SMN expression is the spinal cord [Coovert et al., 1997]. In light of the lethal phenotype in the *Smn* knockout mice [Schrank et al., 1997], it seems likely that the reason that human cells survive is because they have two copies of the gene. Most cell types can get by with only one copy, but loss of SMN from the gems in motor neurons has a catastrophic effect. Detailed analyses of the elements controlling expression of the two SMN genes will be required in the future in order to explain this effect.

THE SALMON THEORY

Taken together with the fact that a complex containing SMN is involved in spliceosomal snRNP biogenesis, the proximity of gems to CBs is fertile ground for speculation on CB functions. At least three lines of evidence suggest that the snRNAs within CBs are not nascent ones. First, pulse-chase experiments (described above) with tritiated uridine show a significant time lag before CBs become labeled. Similarly, pulses of Br-UTP show that CBs are adjacent to and separate from transcription sites [Jordan et al., 1997; Schul et al., 1998]. Second, the presence of TMG cap, Sm, and U2B" epitopes within CBs demonstrates that at least some of the RNAs are mature. Third, nuclear-injected U1 RNA does not associate with the SMN/SIP1 complex; only after export to the cytoplasm does the injected U1 associate directly with SMN/SIP1 [Fischer et al., 1997]. Presumably, the interaction between U RNAs and the SMN complex is initiated in the cytoplasm. Subsequent dissociation of the snRNA from the complex takes place in the nucleus, plausibly in the CB/gem structures [Fischer et al., 1997]. The scenario outlined above depicts gems and CBs as being intimately involved in snRNA metabolism.

If the snRNAs within CBs are mature ones, why are they located near intronless snRNA and histone genes? The presence of U7 RNA within CBs might explain their proximity to histone genes. However, the association of CBs and snRNA genes is not coincidental; ectopically expressed U2 genes function as CBORs [Frey et al., unpublished communication]. Thus, after export to the cytoplasm, at least a subfrac-

tion of the mature snRNAs return to the sites of their synthesis, their "spawning waters" if you will. This salmon-like behavior of snRNAs suggests a mechanism for feedback regulation of snRNA transcription [Frey and Matera, 1995]. Several corollaries arise from this hypothesis (see Fig. 4).

1. If snRNAs *are* autogenously regulated, then CBs might also participate in dosage compensation. For example, human U2 genes are organized in tandem arrays of between 5 and 30 repeat units per *RNU2* allele [Pavelitz et al., 1995]. Yet despite this >5-fold variation in gene copy number, the amount of mature U2 snRNP remains essentially constant [Bailey et al., 1995]. Similarly, overexpression of marked U1 genes results in downregulation of the endogenous genes [Mangin et al., 1985].
2. If CBs are involved in dosage compensation, there must exist specific mechanisms to recognize individual snRNPs. It seems unlikely that feedback circuits would rely upon levels of common snRNP proteins to regulate a diverse set of snRNAs. Indeed, the regulatory events responsible for dosage compensation of U1 RNA do not seem to affect the other snRNPs [Mangin et al., 1985]. Wu et al. [1996] showed that the amount of U7 snRNP within CBs (spheres) was saturable. Import of radiolabeled U7 snRNA constructs into CBs replaced the endogenous U7 [Wu et al., 1996]. Hence, there are a fixed number of U7 binding sites within a CB. Assuming that

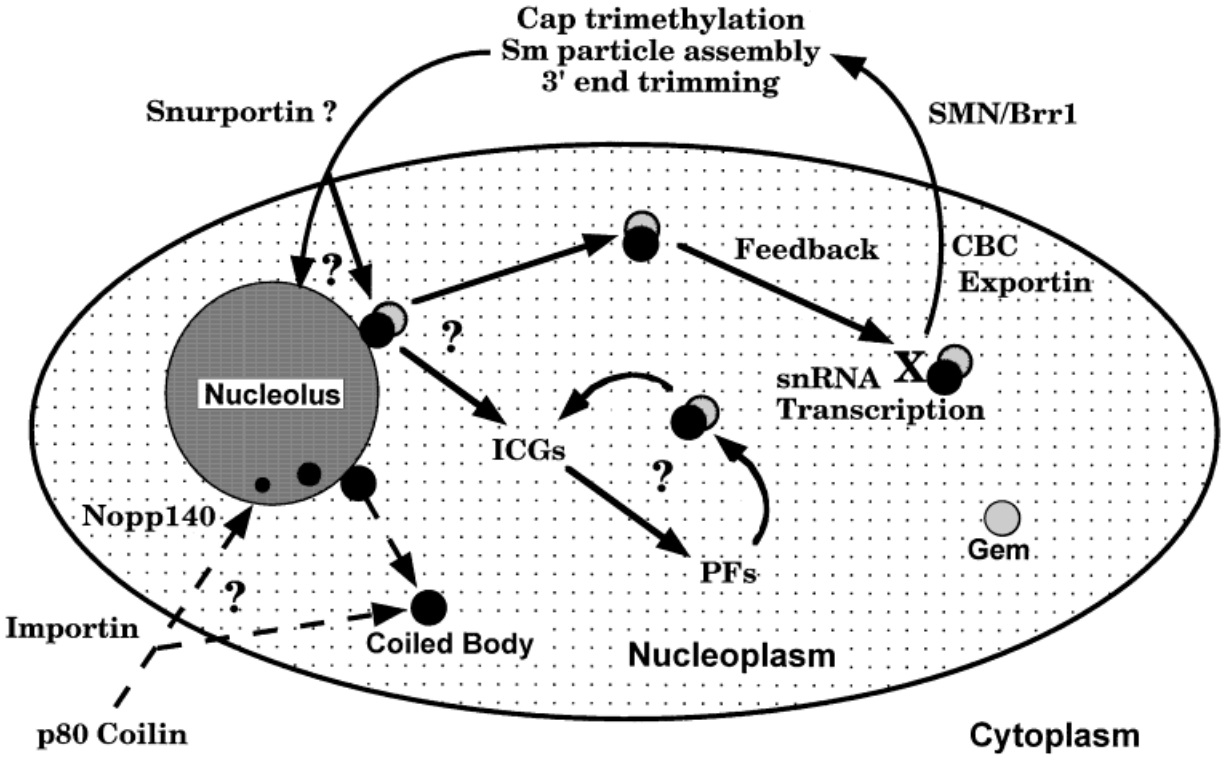

Fig. 4. The "salmon theory" of snRNP biogenesis. Solid lines mark possible traffic patterns for snRNAs, while dashed lines those of p80 coilin. Small nuclear RNA transcription takes place in the nucleoplasm ("X"), often adjacent to CBs and gems. Newly synthesized spliceosomal snRNAs are then exported to the cytoplasm, mediated by the cap binding complex (CBC) and exportin, where they undergo cap trimethylation and Sm core assembly. Monoparticle snRNP assembly involves the activities of SMN and Brr1(SIP1). Nuclear re-entry is then likely to be mediated through a putative snRNA-specific cargo carrier (snurportin?). Next, snRNPs either traffic through the nucleoplasm or nucleoli before at least a subfraction of them accumulate in CBs. The majority of the snRNPs eventually accumulate in the interchromatin granule clusters (IGCs) and perichromatin fibrils (PFs). Recycling of snRNPs from sites of pre-mRNA splicing in the PFs to storage sites in IGCs might also proceed through the CB/gem structures (see text). At least a subset of the mature snRNPs make their way into CBs that are adjacent to snRNA genes. This salmon-like behavior of snRNAs completes the putative autogenous feedback loop. Regulation of gene expression (including dosage compensation) would then be accomplished by other factors that accumulate in CBs. Import of p80 coilin via the importin-NLS pathway may also interact with Nopp140 for its putative trip through the nucleolus and targeting to the CB. Another alternative is that p80 coilin traffics directly to nucleoplasmic CBs (illustrated by black circles; gems are in light gray).

this result obtains for the other snRNAs, CBs might plausibly function as intracellular "snRNA gauges." In this case, it is important to recognize that snRNAs would act as "nuclear messengers," carrying feedback from the cytoplasmic snRNP assembly machinery directly into the nucleus. Moreover, recognition of specific snRNAs within CBs predicts the existence of receptor molecules.

3. Upon inhibition of transcription using actinomycin D or α-amanitin, CBs are still detectable, but snRNAs no longer concentrate within them [Carmo-Fonseca et al., 1992]. These and other observations [e.g., Wu et al., 1996] indicate that there is a flux of snRNPs through the CB/gem organelles. If snRNPs *do* flow through these structures, then defects that block the flow should result in their enlargement. The exact routes taken by Sm snRNPs after nuclear re-entry remain to be established (Fig. 4). Some snRNPs might be required to pass through CBs prior to localization in the speckled domains. This requirement may also hold for recycled spliceosomal components after splicing has been completed (Fig. 4). It seems plausible that components of the machinery that perform this job in the cytoplasm (SMN, SIP1, etc.) may also carry out this function in the nucleus (i.e., in gems). To date, no mutations in coilin have been identified that cause large quantities of snRNPs to accumulate in CBs. However, a dominant negative coilin point mutation (S202D) does cause a fraction of the Sm snRNPs and p80 coilin to accumulate in the nucleolus [Lyon et al., 1997], prompting speculation that Sm snRNPs and coilin may traffic through the nucleolus on their way to the nucleoplasm (Fig. 4). Additional dominant negative effects resulting from overexpression of coilin deletion variants have also been observed [Bohmann et al., 1995b].

In sum, the "salmon theory" predicts that mature snRNPs act to regulate their own expression by returning to CBs adjacent to the sites of their synthesis. Whether all or only a fraction of the RNAs traffic through CBs remains to be determined. The fact that components within gems, twin structures to CBs, are involved in snRNP biogenesis strengthens this hypothesis. Furthermore, it suggests that snRNAs can act as "nuclear messengers" and that specific mechanisms must exist to recognize and regulate individual snRNA levels. The model does not specify whether snRNAs travel through the nucleoplasm independently from CBs or if pre-loaded CBs are recruited to specific DNA loci. Additionally, the feedback regulation model does not exclude other putative functions for CBs such as assembly, recycling, transport, or modification of snRNPs.

COILED BODY ORGANIZERS

Two models of CB formation have been proposed: a DNA-directed model and a self-assembly model [Roth, 1995]. In the former model, the DNA provides the positional information, while in the latter CBs are generated when the molecular components of CBs reach a high enough local concentration to cause them to aggregate. I would like to propose two alternative models that are mechanistically similar, but differ substantially in their conceptual details.

CBOR Nucleation Model

The association of CBs with specific DNA loci suggests that CB formation may be either DNA-directed or RNA-directed. That is, the presence of the requisite DNA or RNA sequences at the CBOR loci would nucleate CBs. The DNA-directed scenario is the simplest; CB components would bind to distinct CBOR sequences and nucleate CBs. An alternative is that CB formation requires production of RNA and that CBs form as a *result* of transcription. In this scenario, only actively transcribed CBOR loci would generate CBs. This latter hypothesis is attrractive, since it is analogous to rDNA and nucleoli [Karpen et al., 1988]. However, as discussed above, one important difference is that CBs are merely adjacent to sites of transcription and do not incorporate labeled UTP. Thus, if CB formation is RNA-dependent, it would have to be a post-transcriptional event.

Nucleolar-Genesis Model

If CBs only form near CBORs, then how do we explain why CBs are also found free in the nucleoplasm? One "fly in the ointment" of the CBOR nucleation model is that CBs can also form in nuclei assembled from *Xenopus* egg extract using lambda DNA (i.e., in the absence of frog DNA) [Bauer et al., 1994]. Thus, at least in some circumstances, CBs can assemble independently. It seems clear that there *are* free spheres within spreads of amphibian oocyte

nuclei; however, we don't really know if any of the CBs in mammalian interphase nuclei are unattached. Additional multicolor analyses with multiple CBOR loci will be required to address this question. However, assuming for the moment that some of the CBs within somatic nuclei are not associated with CBOR loci, how do we explain their existence? It is possible that CBs are not nucleated by CBORs, but instead are recruited. Recruitment of pre-formed CBs to CBOR loci could also be accomplished by DNA- or RNA-directed mechanisms similar to those described above. In this version of the salmon theory, CBs would shuttle mature snRNPs from the sites of CB formation to the CBORs (Fig. 4). The reverse situation (with CBOR loci being recruited to pre-existing CB locations) is also possible, but not considered here. Given the presence of nucleolar epitopes such as fibrillarin and Nopp140 within CBs, their most likely nucleation site is the nucleolus. Additional support for this idea comes from numerous electron micrographs showing a close association of CBs with the nucleolar periphery as well as the work of Lyon et al. [1997], who showed that coilin and Sm epitopes are intranucleolar in the presence of either a phosphatase-insensitive coilin mutant or the Ser/Thr phosphatase inhibitor, okadaic acid.

Within each of the models, we can ask the following question: Does the organization within the genome and/or the transcriptional activity of CBORs affect the rate of CB association? The answers to these questions are within our grasp; however, we have very little understanding of the biogenesis of gems and thus are now only beginning to formulate hypotheses regarding those organelles. Is gem formation concomitant with that of CBs? Do they also colocalize with specific DNA sequences? What role, if any, does p80 coilin play in the pathogenesis of SMA? Are the centromeric and telomeric isoforms of SMN protein localized differentially in cells and/or tissues?

PROSPECTUS

Future research on gems and coiled bodies will necessarily touch on new areas of biology. CBs and gems are dynamic organelles that assemble and disassemble with each cell cycle and likely undergo other changes during each phase. The discovery of cell-cycle control machinery within CBs also opens up new directions for research. We know that coilin's phosphorylation state is important for its subcellular localization. What kinases are responsible for this phosphorylation? Most of the experiments to date only show a snapshot of the cell; experiments directed towards solving the riddle of CB motility will require a temporal component. Perhaps with the aid of green fluorescent protein constructs expressed in living cells, investigators will begin to address some of these concerns. These and other questions will fuel further inquiry into the structure and function of these fascinating nuclear organelles.

ACKNOWLEDGMENTS

I thank M. Frey for providing the image in Figure 3, as well as the entire lab for helpful dialogue about snRNAs, CBORs, and salmon. I also thank E. Chan and G. Dreyfuss for antibodies against p80 coilin and SMN, respectively. I am particularly grateful to G. Dreyfuss, J. Gall, A. Lamond, I. Mattaj, A. Weiner, and R. van Driel for stimulating discussions and helpful advice. This work was supported by NIH grant GM53034 as well as by a Junior Faculty Research Award from the American Cancer Society (JFRA-570).

REFERENCES

Andrade LEC, Chan EKL, Raska I, Peebles CL, Roos G, Tan EM (1991): Human autoantibody to a novel protein of the nuclear coiled body: Immunological characterization and cDNA cloning of p80 coilin. J Exp Med 173:1407–1419.

Andrade LEC, Tan EM, Chan EKL (1993): Immunocytochemical analysis of the coiled body in the cell cycle and during cell proliferation. Proc Natl Acad Sci USA 90:1947–1951.

Antoniou M, Carmo-Fonseca M, Ferreira J, Lamond A (1993): Nuclear organization of splicing snRNPs during differentiation of murine erythroleukemia cells in vitro. J Cell Biol 123:1055–1068.

Bailey A, Li Z, Pavelitz T, Weiner A (1995): Adenovirus type 12-induced fragility of the human RNU2 locus requires U2 small nuclear RNA transcriptional regulatory elements. Mol Cell Biol 15:6246–6255.

Bauer DW, Murphy C, Wu ZW, Wu CHH, Gall JG (1994): In vitro assembly of coiled bodies in Xenopus egg extract. Mol Biol Cell 5:633–644.

Berezney R (1991): Visualizing DNA replication sites in the cell nucleus. Semin Cell Biol 2:103–115.

Beven AF, Simpson GG, Brown JWS, Shaw PJ (1995): The organization of spliceosomal components in the nuclei of higher plants. J Cell Sci 108:509–518.

Bohmann K, Ferreira J, Santama N, Weis K, Lamond A (1995a): Molecular analysis of the coiled body. J Cell Sci 19:107–113.

Bohmann K, Ferreira J, Lamond A (1995b): Mutational analysis of p80 coilin indicates a functional interaction

between coiled bodies and the nucleolus. J Cell Biol 131:817–831.

Brasch K, Ochs RL (1992): Nuclear bodies (NBs): A newly "rediscovered" organelle. Exp Cell Res 202:211–223.

Callan HG, Gall JG (1991): Association of RNA with the B and C snurposomes of Xenopus oocyte nuclei. Chromosoma 101:69–82.

Callan HG, Gall JG, Murphy C (1991): Histone genes are located at the sphere loci of Xenopus lampbrush chromosomes. Chromosoma 101:245–251.

Carmo-Fonseca M, Tollervey D, Pepperkok R, Barabino SML, Merdes A, Brunner C, Zamore PD, Green MR, Hurt E, Lamond AI (1991): Mammalian nuclei contain foci which are highly enriched in components of the pre-mRNA splicing machinery. EMBO J 10:195–206.

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

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

Chan EKL, Takano S, Andrade LEC, Hamel JC, Matera AG (1994): Structure, expression and chromosomal localization of the human p80-coilin gene. Nucleic Acids Res 22:4462–4469.

Coovert D, Le T, McAndrew P, Strasswimmer J, Crawford T, Mendell J, Coulson S, Androphy E, Prior T, Burghes A (1997): The survival motor neuron protein in spinal muscular atrophy. Hum Mol Genet 6:1205–1214.

Cremer T, Kurz A, Zirbel R, Dietzel S, Rinke B, Schrock E, Speicher MR, Mathieu U, Jauch A, Emmerich P, Scherthan H, Ried T, Cremer C, Lichter P (1993): Role of chromosome territories in the functional compartmentalization of the cell nucleus. Cold Spring Harbor Symp Quant Biol 58:777–792.

de Jong L, Grande M, Mattern K, Schul W, van Driel R (1996): Nuclear domains involved in RNA synthesis, RNA processing and replication. Crit Rev Eukaryot Gene Expr 6:215–246.

Dingwall C, Laskey R (1991): Nuclear targeting sequences: A consensus? Trends Biochem Sci 16:478–481.

Du L, Warren S (1997): A functional interaction between the carboxy-terminal domain of RNA polymerase II and pre-mRNA splicing. J Cell Biol 136:5–18.

Elefanty A, Antoniou M, Custodio N, Carmo-Fonseca M, Grosveld F (1996): GATA transcription factors associate with a novel class of nuclear bodies in erythroblasts and megakaryocytes. EMBO J 15:319–333.

Elicieri GL, Ryerse JS (1984): Detection of intranuclear clusters of Sm antigens with monoclonal anti-Sm antibodies by immunoelectron microscopy. J Cell Physiol 121:449–451.

Fakan S (1994): Perichromatin fibrils are in situ forms of nascent transcripts. Trends Cell Biol 4:86–90.

Fakan S, Bernhard W (1971): Localisation of rapidly and slowly labelled nuclear RNA as visualized by high resolution autoradiography. Exp Cell Res 67:129–141.

Fakan S, Leser G, Martin TE (1984): Ultrastructural distribution of nuclear ribonucleoproteins as visualized by immunocytochemistry on thin sections. J Cell Biol 98:358–363.

Ferreira J, Carmo-Fonseca M, Lamond A (1994): Differential interaction of splicing snRNPs with coiled bodies and interchromatin granules during mitosis and assembly of daughter cell nuclei. J Cell Biol 126:11–23.

Fischer U, Liu Q, Dreyfuss G (1997): The SMN-SIP1 complex has an essential role in spliceosomal snRNP biogenesis. Cell 90:1023–1029.

Frey MR, Matera AG (1995): Coiled bodies contain U7 small nuclear RNA and associate with specific DNA sequences in interphase cells. Proc Natl Acad Sci USA 92:5915–5919.

Gall JG, Callan HG (1989): The sphere organelle contains small nuclear ribonucleoproteins. Proc Natl Acad Sci USA 86:6635–6639.

Gall JG, Stephenson EC, Erba HP, Diaz MO, Barsacchi-Pilone G (1981): Histone genes are located at the sphere loci of newt lampbrush chromosomes. Chromosoma 84: 159–171.

Gall JG, Tsvetkov A, Wu Z, Murphy C (1995): Is the sphere organelle/coiled body a universal nuclear component? Dev Genet 16:25–35.

Gama-Carvalho M, Krauss R, Chiang L, Valcarcel J, Green M, Carmo-Fonseca M (1997): Targeting of U2AF65 to sites of active splicing in the nucleus. J Cell Biol 137:975–987.

Gao L, Frey MR, Matera AG (1997): Human genes encoding U3 snRNA associate with coiled bodies in interphase cells and are clustered on chromosome 17p11.2 in a complex inverted repeat structure. Nucleic Acids Res 25:4740–4747.

Ghetti A, Pinol-Roma S, Michael WM, Morandi C, Dreyfuss G (1992): hnRNP I, the polypyrimidine tract-binding protein: distinct nuclear localization and association with hnRNAs. Nucleic Acids Res 20:3671–3678.

Görlich D (1997): Nuclear protein import. Curr Opin Cell Biol 9:412–419.

Grande M, van der Kraan I, de Jong L, van Driel R (1997): Nuclear distribution of transcription factors in relation to sites of transcription and RNA polymerase II. J Cell Sci 110:1781–1791.

Hoeijmakers J, Egly J, Vermeulen W (1996): TFIIH: A key component in multiple DNA transactions. Curr Opin Genet Dev 6:26–33.

Hozák P (1995): Catching RNA polymerase I in Flagranti: Ribosomal genes are transcribed in the dense fibrillar component of the nucleolus. Exp Cell Res 216:285–289.

Hozák P, Jackson D, Cook P (1994): Replication factories and nuclear bodies: The ultrastructural characterization of replication sites during the cell cycle. J Cell Sci 107: 2191–2202.

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

Huang S, Deerinck TJ, Ellisman MH, Spector DL (1994): In vivo analysis of the stability and transport of nuclear poly(A)$^+$ RNA. J Cell Biol 126:877–899.

Iborra FJ, Pombo A, Jackson DA, Cook PR (1996): Active RNA polymerases are localized within discrete transcription "factories" in human nuclei. J Cell Sci 109:1427–1436.

Izaurralde E, Mattaj IW (1992): Transport of RNA between nucleus and cytoplasm. Sem in Cell Biol 3:279–288.

Jiménez-García LF, Segura-Valdez MdL, Ochs RL, Spector DL (1994): Nucleologenesis: U3 snRNA-containing prenucleolar bodies move to sites of active pre-rRNA transcription after mitosis. Mol Biol Cell 5:955–966.

Jordan P, Cunha C, Carmo-Fonseca M (1997): The cdk7-cyclin H-MAT1 complex associated with TFIIH is localized in coiled bodies. Mol Biol Cell 8:1207–1217.

Karpen GH, Schaefer JE, Laird CD (1988): A *Drosophila* rRNA gene located in euchromatin is active in transcription and nucleolus formation. Genes Dev 2:1745–1763.

Lamond AI, Carmo-Fonseca M (1993): The coiled body. Trends Cell Biol 3:198–204.

Lefebvre S, Burglen L, Reboullet S, Clermont O, Burlet P, Viollet L, Benichou B, Cruaud C, Millasseau P, Zeviani M, Le Pasilier D, Frezal J, Cohen D, Weissenbach J, Munnich A, Melki J (1995): Identification and characterization of a spinal muscular atrophy-determining gene. Cell 80:155–165.

Lefebvre S, Burlet P, Liu Q, Bertrandy S, Clermont O, Munnich A, Dreyfuss G, Melki J (1997): Correlation between severity and SMN protein level in spinal muscular atrophy. Nat Genet 16:265–269.

Liu Q, Dreyfuss G (1996): A novel nuclear structure containing the survival of motor neurons protein. EMBO J 15:3555–3565.

Liu Q, Fischer U, Wang F, Dreyfuss G (1997): The spinal muscular atrophy disease gene product, SMN, and its associated protein SIP1 are in a complex with spliceosomal snRNP proteins. Cell 90:1013–1021.

Lyon C, Bohmann K, Sleeman J, Lamond A (1997): Inhibition of protein dephosphorylation results in the accumulation of splicing snRNPs and coiled bodies within the nucleolus. Exp Cell Res 230:84–93.

Malatesta M, Zancanaro C, Martin T, Chan E, Amalric FLR, Vogel P, Fakan S (1994): Is the coiled body involved in nucleolar functions? Exp Cell Res 211:415–419.

Mangin M, Ares MJ, Weiner A (1985): U1 small nuclear RNA genes are subject to dosage compensation in mouse cells. Science 229:272–275.

Matera AG, Ward DC (1993): Nucleoplasmic organization of small nuclear ribonucleoproteins in cultured human cells. J Cell Biol 121:715–727.

Matera AG, Weiner AM, Schmid CW (1990): Structure and evolution of the U2 small nuclear RNA multigene family in primates: Gene amplification under natural selection? Mol Cell Biol 10:5876–5882.

Matera AG, Tycowski KT, Steitz JA, Ward DC (1994): Organization of small nucleolar ribonucleoproteins (snoRNPs) by flourescence in situ hybridization and immunocytochemistry. Mol Biol Cell 5:1289–1299.

Meier UT, Blobel G (1992): Nopp140 shuttles on tracks between nucleolus and cytoplasm. Cell 70:127–138.

Meier UT, Blobel G (1994): NAP57, a mammalian nucleolar protein with a putative homolog in yeast and bacteria. J Cell Biol 127:1505–1514.

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

Noble S, Guthrie C (1996): Transcriptional pulse-chase analysis reveals a role for a novel snRNP-associated protein in the manufacture of spliceosomal snRNPs. EMBO J 15:4368–4379.

Ochs R, Stein TJ, Tan E (1994): Coiled bodies in the nucleolus of breast cancer cells. J Cell Sci 107:385–399.

O'Keefe RT, Mayeda A, Sadowski CL, Krainer AR, Spector DL (1994): Disruption of pre-mRNA splicing in vivo results in reorganization of splicing factors. J Cell Biol 124:249–260.

Pavelitz T, Rushé L, Matera AG, Scharf J, Weiner AM (1995): Concerted evolution of the tandem array encoding primate U2 small nuclear RNA occurs without changing the cytological context of the RNU2 locus. EMBO J 14: 169–177.

Pinol-Roma S, Adam SA, Do Choi Y, Dreyfuss G (1989): Ultraviolet-induced cross-linking of RNA to proteins in vivo. Methods Enzymol 180:410–418.

Ramon y Cajal SR (1903): Un sencillo metodo de coloracion selectiva del reticulo protoplasmico y sus efectos en los diversos organos nerviosos de vertebrados y invertebrados. Trab Lab Invest Biol (Madrid) 2:129–221.

Raska I, Andrade LEC, Ochs RL, Chan EKL, Chang C-M, Roos G, Tan EM (1991): Immunological and ultrastructural studies of the nuclear coiled body with autoimmune antibodies. Exp Cell Res 195:27–37.

Roth MB (1995): Spheres, coiled bodies and nuclear bodies. Curr Opin Cell Biol 7:325–328.

Schrank B, Gotz R, Gunnersen J, Ure J, Toyka K, Smith A, Sendtner M (1997): Inactivation of the survival motor neuron gene, a candidate gene for human spinal muscular atrophy, leads to massive cell death in early mouse embryos. Proc Natl Acad Sci USA 94:9920–9925.

Schul W, van Driel R, de Jong L (1998): Coiled bodies and U2 snRNA genes adjacent to coiled bodies are enriched in factors required for snRNA transcription. Mol Biol Cell (in press).

Smith K, Carter K, Johnson CV, Lawrence JB (1995): U2 and U1 snRNA gene loci associate with coiled bodies. J Cell Biochem 59:473–485.

Tuma RS, Stolk JA, Roth MB (1993): Identification and characterization of a sphere organelle protein. J Cell Biol 122:767–773.

van Gool A, van der Horst G, Citterio E, Hoeijmakers J (1997): Cockayne syndrome: Defective repair of transcription? EMBO J 16:4155–4162.

van Steensel B, Brink M, van der Meulen K, van Binnendijk E, Wansink D, de Jong L, de Kloet E, van Driel R (1995): Localization of the glucocorticoid receptor in discrete clusters in the cell nucleus. J Cell Sci 108:3003–3011.

Weis K, Mattaj I, Lamond A (1995): Identification of hSRP1 alpha as a functional receptor for nuclear localization sequences. Science 268:1049–1053.

Wu C-HH, Gall JG (1993): U7 small nuclear RNA in C snurposomes of the Xenopus germinal vesicle. Proc Natl Acad Sci USA 90:6257–6259.

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

Wu Z, Murphy C, Gall JG (1994): Human p80-coilin is targeted to sphere organelles in the amphibian germinal vesicle. Mol Biol Cell 5:1119–1127.

Wu C, Murphy C, Gall J (1996): The Sm binding site targets U7 snRNA to coiled bodies (spheres) of amphibian oocytes. RNA 2:811–823.

Yannoni Y, White K (1997): Association of the neuron-specific RNA binding domain-containing protein ELAV with the coiled body in Drosophila neurons. Chromosoma 105:332–341.

Zeng C, Kim E, Warren S, Berget S (1997): Dynamic relocation of transcription and splicing factors dependent on transcription activity. EMBO J 16:1401–1412.