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Coiled Bodies and Gems: Janus or Gemini?

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Coiled bodies (CBs) were discovered around the turn of the century by Santiago Ramon y Cajal (1903), a true pioneer of cytology and neuroscience. In 1906, Cajal and Camillo Golgi shared the Nobel Prize in Physiology/Medicine for their descriptions of the architecture of the vertebrate nervous system. As an almost ancillary part of their studies, each investigator discovered a new cellular organelle: the internal reticular apparatus and the accessory body. Cajal originally termed his structure the "accessory body" because, like its larger cousin, the nucleolus, it was easily stained by silver nitrate. Golgi's organelle now bears his name, and we have since learned a great deal about its function. However, insight into the function of Cajal's organelle has been particularly opaque. In fact, it was not until the late 1960s that accessory bodies were identified in the electron microscope (Monneron and Bernhard 1969). Microscopists have since preferred the name "coiled bodies," since they appear to be composed of a tangle of coiled, electron-dense threads, ~0.5 μm in diameter. It was not until the 1990s that a molecular characterization of CBs began (Andrade et al. 1991; Raska et al. 1991; Carmo-Fonseca et al. 1992; Andrade et al. 1993; Matera and Ward 1993). Despite these discoveries, functional data on these organelles has not been forthcoming. A number of more recent studies, the focus of this review, suggest that CBs participate in the biogenesis of small nuclear ribonucleoproteins (snRNPs), the ubiquitous mediators of post-transcriptional RNA processing. It also appears that, at least in most cell types, CBs are indistinguishable from nuclear structures known as "gems" and that these Janus-faced nuclear organelles may be of central importance in the etiology of spinal muscular atrophy (SMA).

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A Diverse Assortment of Cellular Engines within Coiled Bodies

Patient autoantisera recognizing an 80-kD protein strongly and specifically label CBs (Andrade et al. 1991; Raska et al. 1991). Immunofluorescence studies reveal that coilin is a nuclear protein, localizing diffusely throughout the nucleoplasm and concentrating in a few bright foci (fig. 1). Anti-coilin antibodies stain similar structures in a wide variety of species from vertebrates to plants (Tuma et al. 1993; Beven et al. 1995; Gall et al. 1995).

CBs are dynamic structures; they disassemble during mitosis, and they reassemble in mid G_1 after nucleologenesis and the resumption of transcription (Andrade et al. 1993; Ferreira et al. 1994). Since identification of the CB signature protein, p80 coilin, the list of macromolecules that accumulate within CBs has grown steadily (for reviews see Lamond and Earnshaw 1998; Matera 1998). CBs are highly enriched in components of three major RNA processing pathways: pre-mRNA splicing, histone mRNA 3′ maturation, and pre-rRNA processing (Gall et al. 1995). Despite a lack of ongoing transcription *within* CBs, they also contain elements of the basal transcription machinery, as well as cell cycle–control proteins (Grande et al. 1997; Jordan et al. 1997; Schul et al. 1998). These observations raise the possibility that CBs may provide an interface through which these different cellular machineries interact.

The large size and molecular complexity of CBs have made it tempting to speculate on their roles, but several seemingly attractive models of CB function can now be excluded. First, experiments in a variety of cells, using tritiated uridine (Fakan and Bernhard 1971; Callan and Gall 1991) or Br-UTP incorporation (Jordan et al. 1997; Schul et al. 1998), show that CBs become labeled only slowly and inefficiently, which indicates that CBs are probably not sites of transcription per se. Second, 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 heterogeneous nuclear RNPs and poly A+ RNA, makes it unlikely that CBs are directly involved in splicing. Rather, although it seems ever more likely that CBs play a part in a number

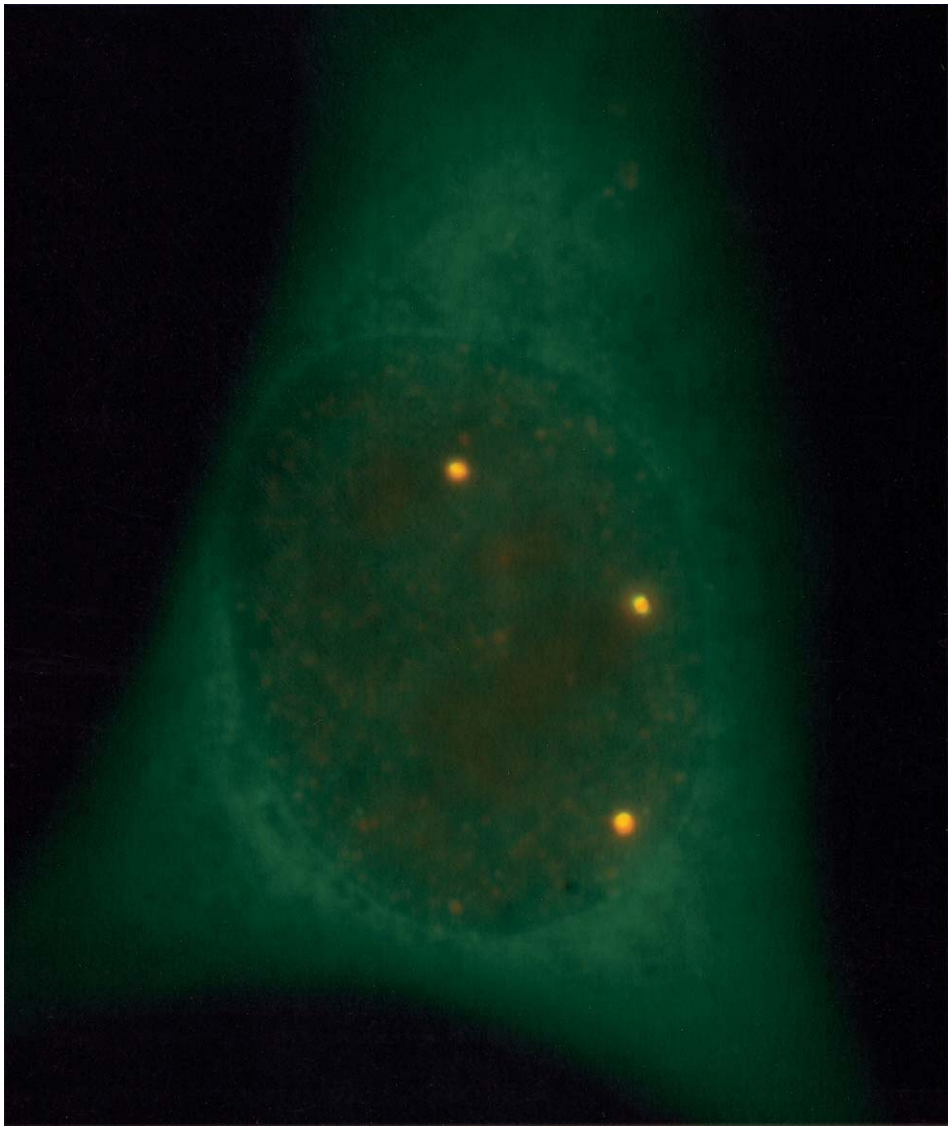

Figure 1 CBs and gems colocalize in most interphase cell types. HeLa-ATCC cells were fixed without detergent preextraction and then stained with anti-p80 coilin (coiled bodies are red) and anti-SMN (gems are green) antibodies. The twin structures in this cell type show complete overlap, as revealed by the yellow signal. Another strain of HeLa (HeLa-PV; Liu et al. 1996, 1997) often displays separate SMN and/or coilin foci (see text). In addition to the nuclear gem/CB staining, the SMN antibody localizes to the cytoplasm.

of cellular functions, the high concentration of snRNPs suggests that they participate in snRNP maturation.

A Role for CBs in snRNP Biogenesis

There is little doubt that small ribonucleoprotein particles play central roles in pre-mRNA splicing, pre-rRNA processing, histone mRNA 3′ end maturation, and pre-tRNA processing. Because CBs are enriched in components that carry out at least the first three of these pathways, these structures might represent supply centers for the various factors required for transcription and processing of nearby genes and gene products. Viewed in this light, nucleoplasmic CBs may play a role analogous to nucleolar fibrillar centers (Hozák 1995 and references therein). Recent evidence demonstrating that CBs associate with snRNA genes in interphase human cells is consistent with this idea (Frey and Matera 1995; Smith et al. 1995; Gao et al. 1997). However, although CBs often colocalize with specific snRNA genes, they do not appear to contain nascent transcripts. Rather, epitopes present on mature snRNPs (e.g., Sm proteins and trimethylguanosine [TMG] cap structures) are highly enriched within CBs. After transcription, most of the splic-

ing or "Sm" class snRNAs are exported to the cytoplasm. Assembly into snRNP particles, cap hypermethylation, and 3′ end trimming takes place in the cytoplasm, followed by import back into the nucleus (Mattaj et al. 1993). The paths taken by newly assembled snRNPs once they reenter the nucleus are unknown. However, at least a fraction of them transit through CBs.

In effect, mature (or nearly mature) snRNP particles return to CBs that are located next to the genes that spawned them. This salmonlike behavior of snRNPs returning to the sites of their synthesis provides the cell with a plausible means to effect feedback regulation and gene dosage compensation (Frey and Matera 1995; Matera 1998). Other hints that CBs and snRNP import may be coupled come from in vitro experiments in *Xenopus* egg extract. Addition of demembranated sperm to amphibian egg extracts results in formation of typical pronuclei (Lohka and Masui 1983). Bauer and Gall (1997) have used this procedure to demonstrate that extracts immunodepleted of coilin still form CB-like structures. However, the CBs thus formed do not contain Sm snRNPs (Bauer and Gall 1997). Additional links between CBs and Sm snRNP trafficking come from Lamond and coworkers, who showed that coilin's phosphorylation state is important for its localization (Lyon et al. 1997; Sleeman et al., in press). Inhibition of Ser/Thr dephosphorylation or 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). On the surface, it would seem that Sm snRNPs and nucleoli make strange bedfellows, yet the nucleolus may well play a central role in intranuclear trafficking of other RNAs and proteins besides ribosomal ones. Additional evidence implicating CBs in snRNP biogenesis comes from the discovery that CBs have twins.

Spinal Muscular Atrophy: The snRNP Connection

Gemini of coiled bodies, or gems, are nuclear structures that have size and shape similar to those of CBs but that do not contain snRNPs (Liu and Dreyfuss 1996). Instead, gems contain high concentrations of the survival motor neuron protein, SMN. The *SMN* gene is duplicated on human chromosome 5q13 (Lefebvre et al. 1995) but is an essential, single-copy locus in mice (Schrank et al. 1997). Deletion of the telomeric copy of the human gene (*SMN1*) leads to an autosomal recessive disorder, SMA, in which spinal motor neurons degenerate, causing progressive paralysis and muscular atrophy (Lefebvre et al. 1995). SMA is the most common genetic cause of infant mortality (Crawford and Pardo 1996). There is a strong inverse correlation between the severity of the disease and the SMN protein level (Coovert et al. 1997; Lefebvre et al. 1997). The predicted protein products of the duplicate human SMN genes are identical except for several silent codon changes (Burghes 1997; Melki 1997). However, most transcripts of the centromeric copy of SMN (called *SMN2*) are spliced to generate an isoform of the protein that fails to self-assemble (Lorson et al. 1998 and references therein). It is possible that the oligomerization domain encoded by exons 6 and 7 is required for proper association of SMN with other components of the snRNP biogenesis pathway (Lorson et al. 1998). Moreover, these oligomerization-defective SMN proteins may somehow impair gem formation. Indeed, cells derived from patients with the most severe forms of the disease display fewer gems than do those from less severely affected patients (Coovert et al. 1997; Lefebvre et al. 1997).

SMN protein is localized throughout the cytoplasm, but its nuclear staining is restricted to gems (fig. 1). SMN directly interacts with several snRNP core factors, including Sm proteins (Liu et al. 1997). These polypeptides form a complex, along with the SMN interacting protein 1 (SIP1), that is >300 kD (Liu et al. 1997). SIP1 and SMN colocalize in the nucleus and the cytoplasm (Liu et al. 1997). Most important, 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 homolog of a yeast protein, called Brr1p, that is also involved in snRNP particle assembly (Noble and Guthrie 1996; Liu et al. 1997).

One intriguing question is how a defect in a general cellular function such as snRNP biogenesis can have such a tissue-specific effect. Clearly the facts that mice have only a single copy of the gene (DiDonato et al. 1997) and humans have two copies (Lefebvre et al. 1995) offer some clues to the pathogenesis in humans. In this regard, gene conversion events within the SMN-inverted duplication can create alleles of the gene that are particularly telling (Campbell et al. 1997). A combination of two severe-SMA alleles results in type I SMA, whereas other combinations are less severe (Burghes 1997). It is well known that neural tissues also express a wide variety of alternatively spliced messages. Thus, neurons may be particularly sensitive to perturbations in snRNP biogenesis. In the absence of the telomeric *SMN1* gene, the fact that the centromeric *SMN2* gene tends to produce transcripts lacking the SMN oligomerization domain (Lorson et al. 1998) is prima facie evidence that the protein products from the two genes are not equivalent. Furthermore, motor neuron survival plausibly depends not only on expression of SMN protein but on its ability to assemble. The original identification of CBs was in neurons; indeed, some stages of neuronal development are

accompanied by a burst in overall transcriptional activity and coilin production (Santama et al. 1996). This increase in transcription would therefore require large quantities of snRNPs. Thus, the emerging view is that gem formation (and presumably snRNP assembly) is impaired in SMA patients by deletion or mutation of the *SMN1* gene.

Janus versus Gemini

As implied by their name, Gemini bodies are most often found in tight association with CBs. But are they really separate entities? The answer to this question awaits confirmation in the electron microscope; however, as shown in figure 1, antibodies against p80 coilin (red) and SMN (green) stain structures that are often indistinguishable in the light microscope. We screened several different human cell lines and found that, in most of them, CBs and gems were invariably associated (authors' unpublished observations), even when incubated at low temperatures (Liu and Dreyfuss 1996). This finding agrees with published findings that show that SMN and coilin have very similar localization patterns in neurons, including nucleolar cap staining (Francis et al. 1998). For example, there appears to be good concordance if one compares the coilin staining in "stage 5" neurons (Santama et al. 1996) with the nucleolar SMN staining noted above (Francis et al. 1998). Curiously, two different strains of HeLa cells displayed two different staining patterns: HeLa strain PV (a gift of G. Dreyfuss) indeed displayed distinct SMN and coilin foci (data not shown), whereas HeLa strain ATCC (fig. 1) did not. Other human cell lines (e.g., HT-1080) fail to show the separation phenotype, suggesting that, in most tissues, CBs are inseparable from gems.

CBs, as judged by the presence of p80 coilin, seemingly contain all the various CB components. However, evidence that CB-like structures can be formed in *Xenopus* egg extracts depleted of coilin (Bauer and Gall 1997) may provide insight into the possible identity of CBs and gems. The nuclear bodies thus formed are devoid of coilin and Sm snRNPs. Whether or not these CBs contain SMN is an open question. Perhaps the structures detected in HeLa-PV cells, which contain SMN but lack both coilin and splicing snRNPs, are similar to Bauer and Gall's in vitro CBs. Alternatively, if gems are indeed separate structures, perhaps they associate with different chromosomal loci that have been scrambled in the two aneuploid HeLa strains. The onus is upon the electron microscopists to answer this question definitively, but it may well be that CBs and gems are just two different faces of the same structure.

The Janus hypothesis raises some interesting questions. Not only would the unity of gems and CBs strengthen putative roles for the organelle in snRNP biogenesis but would suggest that defects in other parts of the pathway (e.g., in the gene for p80 coilin) could also have neurodegenerative phenotypes. Although our laboratory is currently using both genetic and cell culture systems to address this question, it is clear that additional structural studies are in order. For example, do other CB components besides SMN and SIP1 localize in coilin-negative CBs (i.e., gems)? Are there factors other than SMN and SIP1 that fail to localize in CBs from SMA patient–derived cells? These and other questions should fuel investigation of the structure and function of CBs well into the next century.

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