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# Cajal Bodies: A Long History of Discovery

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## Key Words

coilin, snRNP, stress, nucleus, disease

## Abstract

This review surveys what is known about the structure and function of the subnuclear domains called Cajal bodies (CBs). The major focus is on CBs in mammalian cells but we provide an overview of homologous CB structures in other organisms. We discuss the protein and RNA components of CBs, including factors recently found to associate in a cell cycle-dependent fashion or under specific metabolic or stress conditions. We also consider the dynamic properties of both CBs and their molecular components, based largely on recent data obtained thanks to the advent of improved in vivo detection and imaging methods. We discuss how these data contribute to an understanding of CB functions and highlight major questions that remain to be answered. Finally, we consider the interesting links that have emerged between CBs and alterations in nuclear structure apparent in a range of human pathologies, including cancer and inherited neurodegenerative diseases. We speculate on the relationship between CB function and molecular disease.

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## BACKGROUND AND INTRODUCTION

The cell nucleus has fascinated scientists for over two centuries. The efforts of both morphologists and biochemists have defined some of the basic features of nuclear structure and shown it to be the site of transcription, replication, pre-mRNA splicing, and assembly of ribosomal subunits. Nonetheless, major gaps remain in our understanding of the relationships between nuclear structure, as defined by microscopy, and molecular function, defined largely through the analysis of cell-free in vitro extracts. The advent of detailed fluorescence microscopy studies, made possible by the availability of specific antibody and antisense probes to label individual nuclear proteins and RNA-protein complexes, has helped to identify the wealth of substructure that exists within the nucleus. More recently, the development of fluorescent-protein tagging techniques has also allowed new insights into the dynamic aspects of nuclear structure in living cells. Collectively, these in vivo approaches have underlined two major concepts in relation to nuclear structure/function: (*a*) the existence and prevalence of specific subnuclear domains or organelles and (*b*) the unexpectedly dynamic behavior of these subnuclear structures and their constituents under both physiological and pathophysiological conditions. In contrast to cytoplasmic organelles, nuclear organelles are characterized by the absence of an outer membrane to separate them from the surrounding nucleoplasm. The fact that all the subnuclear domains are typically enriched for either specific nuclear proteins or RNA-protein complexes has led to the consensus that they can function as sites for coordinating complex molecular assembly and maturation pathways.

This review focuses on the dynamic properties of Cajal bodies (CBs) in mammalian cells. The first section is dedicated to a functional classification of the best characterized CB components, with special attention given to the data that suggest possible roles for these organelles. A short overview of CBs in other organisms follows. Finally, the available data suggesting a possible relationship between CBs and several human diseases are discussed and analyzed.

## CAJAL BODIES IN MAMMALIAN CELLS

CBs are ubiquitous subnuclear organelles found in both plant and animal cells. They can vary in size from less than 0.2 $\mu$m up to 2 $\mu$m or even larger, depending on cell type and species. As proposed by Gall, they are now called Cajal bodies after their initial discoverer, Ramon y Cajal, who first described them in 1903 as "nucleolar accessory bodies," based on their frequent association with the nucleolar periphery in neurons (Ramon y Cajal 1903). More than sixty years after their first description, the accessory bodies of Ramon y Cajal were rediscovered by electron microscopists and given the name coiled bodies, reflecting their typical appearance in transmission electron micrographs as a tangled ball

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of fibrillar threads (Hardin et al. 1969, Lafarga et al. 1983, Monneron & Bernhard 1969). By fluorescence microscopy in both fixed and living cells, CBs appear as bright nuclear foci, typically one to six in number, depending on the cell type (**Figure 1**). The size and number of CBs vary during the cell cycle and are maximal at the G1/S-phase (Andrade et al. 1993). Indeed, CBs disassemble during M-phase, and their subsequent reassembly is dependent upon the transcriptional status and growth rate of the cell (Carmo-Fonseca et al. 1993, Fernandez et al. 2002). Interestingly, CBs are also motile. Studies in both plant and animal cells have shown that they can make large movements during interphase, traversing the full diameter of the nucleus in some cases at rates up to $\sim 1\ \mu\text{m min}^{-1}$ (Boudonck et al. 1999, Platani et al. 2000). Individual CBs can show complex dynamic behavior, including moving together and fusing in the nucleoplasm, splitting into two daughter bodies, and reversing movement to and from nucleoli. In cases where CBs split into two smaller bodies, some components, such as fibrillarin, are seen to partition differentially between the daughter structures, whereas other components, such as coilin, are present at similar levels in both (Platani et al. 2000). This is consistent with the view that CBs represent a heterogeneous collection of related structures that can differ in their precise molecular composition and possible biological roles.

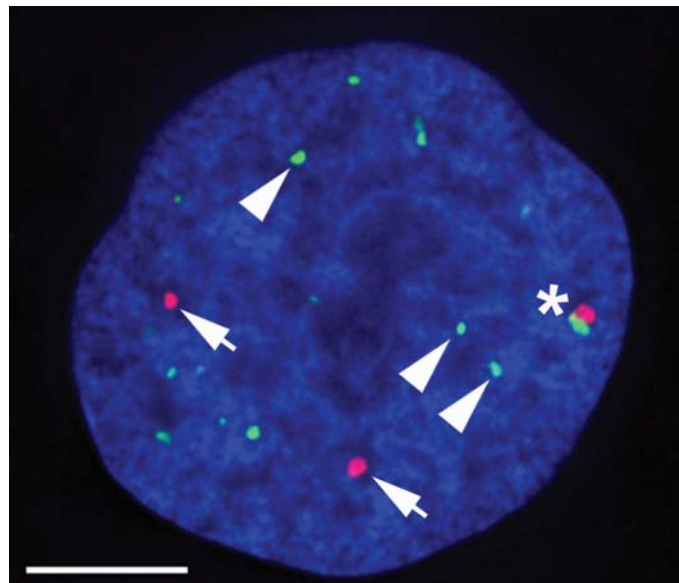

**Figure 1**

Cajal bodies and promyelocytic leukemia protein (PML) bodies in human cells. Anti-coilin antibodies label CBs (*red*), which appear as spherical, bright foci distributed throughout the nucleoplasm. PML bodies are stained in green, detected here using an anti-PML antibody. Note that some PML bodies and CBs are located in close proximity (*asterisk*). DNA is stained with DAPI (4′,6-Diamidino-2-phenylindole: blue). Size bar: 5 $\mu$m.

Much of the CB movement appears to occur via simple or constrained diffusion, although in some cases it is possible that more active processes are involved (Gorisch et al. 2004, Platani et al. 2000). Not all CBs are mobile at any one time, and in most cases they remain tethered within a confined nuclear volume, probably through interactions with specific regions of chromatin (Platani et al. 2002). This likely includes interactions with gene loci such as snRNA, snoRNA, and histone gene clusters (**Figure 2**) (Frey & Matera 1995, 2001; Gao et al. 1997; Jacobs et al. 1999; Schul et al. 1998; Shopland et al. 2001; Smith et al. 1995; Smith & Lawrence 2000). In the case of U snRNAs, Matera's group, by studying the in situ localization of repetitive arrays of U2 snRNA–coding cDNAs, has elegantly shown that their association with CBs depends upon the transcriptional status of the locus and requires the presence of nascent U snRNA transcripts and U2 snRNPs (Frey et al. 1999, Frey & Matera 2001). It is possible that CBs play a regulatory role influencing the expression of these genes. In favor of this idea is the accumulation into CBs of NPAT (nuclear protein mapped to the AT locus), a component shown to activate transcription of histone promoters (see below). However, the requirement for U2 snRNPs indicates the non-exclusive possibility that the association of CBs with specific gene loci may also be coupled to the efficient delivery of RNPs required for the processing or maturation of nascent RNA transcripts or in the recycling of RNP components involved in such processes. As well as localizing to specific gene loci, CBs can also be found

**Small nuclear RNAs (snRNAs):** a class of small RNAs, components of the snRNP particles involved in the pre-mRNA processing

**Small nucleolar RNAs (snoRNAs):** a class of small RNAs, components of the snoRNP particles involved in the modification and processing of rRNA

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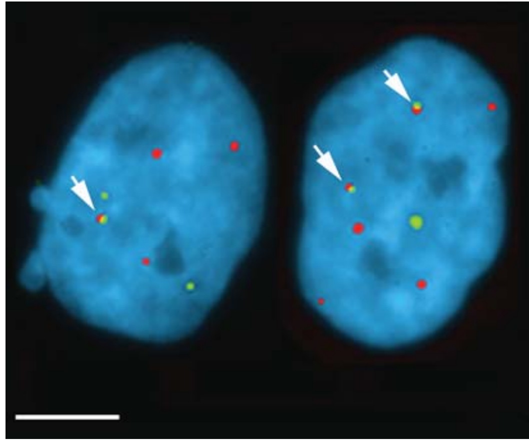

**Figure 2**

Cajal bodies are spatially associated with U2 snRNA gene loci. Combined detection of CBs (anti-coilin antibody, *red signal* ) and U2 snRNA gene loci [fluorescence in situ hybridization (FISH), *green signal* ] in HeLa cell nuclei. DNA is visualized by DAPI staining (*blue*). Arrows indicate adjacent domains. Size bar: 10 $\mu$m. Courtesy of A.G. Matera, Case Western Reserve University, Cleveland, OH.

sometimes in close proximity to other classes of nuclear bodies. In line with this idea is the observation that CBs can occur close to cleavage bodies, nuclear structures enriched in mRNA 3′ cleavage and processing factors in human bladder carcinoma cells (Schul et al. 1996). CBs can also be found in proximity to PML bodies (**Figure 1**), which are nuclear domains whose complex and heterogeneous protein composition suggests their involvement in many different biological functions, including control of apoptosis, response to DNA damage, antiviral response, and transcriptional modulation (see below). The adjacency of CBs to cleavage bodies and PML bodies possibly reflects the existence of intranuclear trafficking pathways, supporting a continuous and dynamic exchange of components that can ultimately modulate the functional activity of the involved domains.

## CAJAL BODIES COMPONENTS

In vivo imaging studies have provided detailed information about the temporal dynamics governing the localization of CB components. Indeed, all CB components analyzed so far are dynamic, with relatively fast turnover rates, varying from seconds to minutes (Dundr et al. 2004, Sleeman et al. 2003). As our aim here is to consider the function of CBs as organelles and how this function relates to the localization of specific components, we tentatively subgroup the CB-associated nucleic acid and protein components into potentially functional categories. We believe that this criteria, although not ideal, may help to guide the interpretation of the often preliminary data available. We note that for many CB components, the biological meaning of their localization in CBs is not yet clear, and caution is needed before automatically assuming that their role in CBs must be identical to known roles that they may play in other cell locations. Furthermore, most of the CB-associated factors have been described as such on the basis of morphological evidence and immunolocalization criteria. Little or no biochemical data are available for many of them. Complementing morphological information with biochemical studies is an important future goal that will provide valuable information about targeting mechanisms, post-translational modification(s), and protein-protein interactions likely to play a role in the assembly/integrity of CBs. We consider first what is known about coilin. Interestingly, the available data about this still-enigmatic protein do not allow us to include coilin easily in any of the functional groups that are described below.

### Coilin

The human autoantigen p80 coilin was discovered by Tan and coworkers through the analysis of patient autoimmune sera (Andrade et al. 1991, Raska et al. 1991) and has been widely used as a molecular marker for CBs. In physiological conditions, CBs are represented in virtually all cells of fetal tissues (Young et al. 2001). However, not all cell types in adult tissues show CBs, although coilin is still expressed in adult tissues (Andrade et al. 1993,

Young et al. 2000). Interestingly, it is possible to induce the appearance of CBs, even in cells from adult tissues, by increasing the relative abundance of snRNPs; the snRNP concentration itself is a function of the metabolic state of the cell (Sleeman et al. 2001). Indeed, both normal and transformed cells that have high levels of gene expression and metabolic activity often show prominent CBs (Andrade et al. 1993, Carmo-Fonseca et al. 1993, Fernandez et al. 2002, Ochs et al. 1995). Thus coilin may undergo post-translational modification in a way that is controlled by developmental and metabolic stimuli and related to CB formation. A candidate modification is the phosphorylation of coilin. Hyperphosphorylation of coilin has been related to the disassembly of CBs in mitosis (Carmo-Fonseca et al. 1993) and shown to reduce its self-interacting capacity. Indeed, similar to several other components enriched in nuclear organelles, i.e., PML (Fagioli et al. 1998), self-oligomerization of coilin is strictly required for its targeting to CBs (Hebert & Matera 2000).

A mouse knockout model of coilin has been analyzed and shown to exhibit reduced viability, as coilin$^{-/-}$ animals are under-represented in litters. However, surviving coilin$^{-/-}$ animals are viable. Coilin$^{-/-}$ mouse embryonic fibroblasts (MEFs) lack normal CBs: two distinct types of remnant extranucleolar foci can be identified. One form is enriched in snoRNAs (U3), fibrillarin, and NOPP140 (Tucker et al. 2001); another form of remnant CBs accumulates snRNAs (U2 and U5) guide scaRNAs (small Cajal body–specific RNAs) (Jady et al. 2003). Interestingly none of these altered structures in coilin$^{-/-}$ MEFs accumulates either Sm proteins or the survival of motor neurons (SMN) complex, which suggests that coilin is involved either in the targeting or retention of SMN and snRNP complexes in CBs. The symmetrical dimethylation of arginine residues in coilin could play a role in this process. Treatment of cells with either periodate-oxidized adenosine (ADOX) or 5′-deoxy-5′-methylthioadenosine (MTA), which drastically reduces the amount of intracellular sDMA-modified residues, leads to the dissociation of SMN-enriched domains (gems) (see below) and Cajal bodies (Boisvert et al. 2002, Hebert et al. 2002). Additionally, the integrity and the phosphorylation state of the coilin C terminus seems to modulate the number of CBs in mouse and human cells, but this effect does not apparently change the ability of coilin to self-interact (Bohmann et al. 1995, Shpargel et al. 2003).

Coilin could also have other functions. For example, the fact that CB number and size is increased in transformed cells (Spector et al. 1992), that the coilin-containing gene locus (17q22–23) is amplified in anaplastic (but not benign or atypical) meningiomas (Buschges et al. 2002), and that coilin$^{-/-}$ mice are apparently not prone to develop spontaneous tumors, (A.G. Matera, personal communication) provide good reasons to test whether coilin is endowed with oncogenic properties and determine if its forced overexpression can influence the proliferation status and cell cycle behavior of diploid mortal cells. Because in the absence of coilin the intranuclear localization of snoRNAs and snRNAs in the remnant CBs is dissociated, a function for coilin in coupling, (spatially coordinating) the maturation of snRNPs and snoRNPs in a unique organelle could, hypothetically, support its role in cell proliferation.

**Small nuclear and nucleolar ribonucleoprotein particles (snRNPs and snoRNPs):** nuclear particles composed of a tight complex between a short RNA molecule (snRNA or snoRNA) and proteins

**Guide RNA:** small RNA molecules that, thanks to complementary base-pairing interactions, guide enzymatic activities to the sequence to be modified or processed

**Pseudouridylation:** a naturally occurring modification of many stable RNA sequences resulting from the isomerization of uridine residues to pseudouridines

## Cajal Bodies Are Linked with snRNP and snoRNP Biogenesis

**RNA/RNP components.** The biogenesis and maturation of snRNAs (and snoRNAs) is tightly linked to their subcellular localization. Newly transcribed snRNAs are exported to the cytoplasm, where they bind a set of common proteins (Sm proteins), which triggers their nuclear re-import. In the nucleus, the snRNP particles accumulate in CBs and subsequently in the interchromatin granule clusters (IGCs), which are enriched in splicing factors (Sleeman & Lamond 1999). The snRNAs present in CBs contain a

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**Spliceosome:** a high-molecular-weight multiprotein complex that catalyzes the excision of introns from pre-mRNA molecules that leads to the formation of mature mRNAs

hypermethylated 5′ cap, which indicates that they have been re-imported from the cytoplasm and do not correspond to nascent transcripts (**Figure 3*a*,*b***). In order to give rise to mature, fully functional splicing particles, the RNA component of the snRNPs undergoes several modifications, including 2′-*O*-ribose-methylation and pseudouridylation. At least some of these RNA modifications take place in CBs and are introduced by a recently discovered class of small Cajal body-specific RNAs (Darzacq et al. 2002, Jady et al. 2003). These are members of the so-called guide RNA family, which functions to align RNA modification activities with target sequences through complementary base-pairing interactions. Given that the number of guide RNAs identified to date is still far less than the number of modified nucleotides identified within spliceosomal snRNAs, it is likely that other guide RNAs will be identified. It will be interesting to see how many of these novel guide RNAs will be localized in CBs.

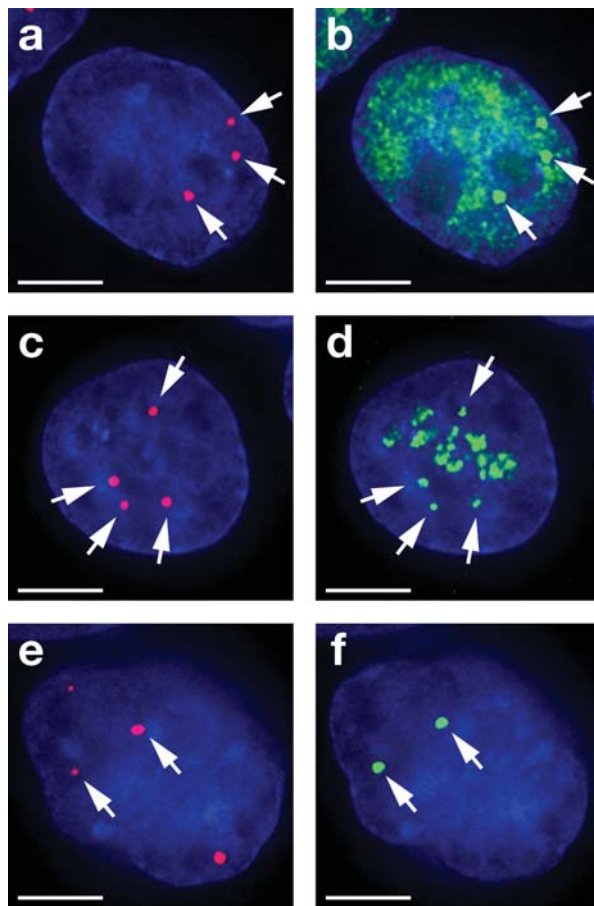


**Figure 3**

Examples of Cajal bodies components: CBs costained with anti-coilin antibodies (*red signal*: *panels a*,*c*,*e* ) and in green (*panels b*,*d*,*f* ). (*b*) Anti-fibrillarin antibody, which decorates both nucleoli and CBs; (*d*) anti-TMG CAP antibody; (*e*) anti-NPAT antibody. DNA is stained with DAPI (4′,6-Diamidino-2-phenylindole: blue). Size bar: 5 $\mu$m.

So far, the spliceosome U snRNPs (Carmo-Fonseca et al. 1992, Matera & Ward 1993), the U7 snRNP involved in histone 3′-end processing (Pillai et al. 2001), the U3, and many other snoRNPs (Boulon et al. 2004, Verheggen et al. 2002) involved in processing of pre-rRNA have been definitively shown to be enriched in CBs. Consistent with CBs having a role in snRNP maturation, overexpression of snRNP components appears to promote CB assembly (Sleeman et al. 2001). The RNA component of the telomerase RNP, the telomerase RNA (hTR), is also enriched in CBs (Jady et al. 2004, Zhu et al. 2004), and its targeting could be mediated by SMN (Bachand et al. 2002) and by the presence of sequences homologous to the CB-targeting region of scaRNAs in its 3′-terminal region (Jady et al. 2004) (see below).

NOPP140 and fibrillarin, together with coilin, are among the first CB components identified. Whereas more is known about the nucleolar functions of these proteins, their CB-associated functions are not yet clear. Fibrillarin is an essential (Newton et al. 2003) and highly conserved nucleolar and CB-associated protein (**Figure 3*c*,*d***). It acts in the nucleolus as a ribose 2′-*O*-methylase targeted to specific sites of RNA modification through its association with a guide snoRNA complementary in sequence to the RNA surrounding the modification site (Feder et al. 2003). NOPP140 is a nucleo-cytoplasmic shuttling phosphoprotein (Meier & Blobel 1992) found in both nucleoli and CBs. It interacts with the largest subunit of RNA PolI and thereby affects rDNA transcription and hence nucleolar biogenesis.

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In addition to their nucleolar functions, other properties of fibrillarin and NOPP140 link these proteins to CBs. Indeed, NOPP140 has been shown to interact directly with coilin (Isaac et al. 1998), whereas fibrillarin interacts with SMN (Jones et al. 2001, Pellizzoni et al. 2001), and both proteins bind to snoRNPs. Fibrillarin binds to the C/D box snoRNAs; NOPP140 binds to C/D and H/ACA classes of snoRNAs. This suggests that both proteins could act as chaperones for the final stages of maturation of snoRNPs en route to nucleoli. Interestingly, despite the direct interaction with SMN and coilin proteins, both fibrillarin and NOPP140 are still targeted to the remnant CBs in coilin$^{-/-}$ MEFs, which lack coilin and do not accumulate SMN. Although this targeting could be mediated by the binding to snoRNAs that still accumulate in the remnant CBs structures (Jady et al. 2003), it is also possible that the interaction of fibrillarin and NOPP140 with CBs is related to an alternative CB function that has not yet been identified.

Several proteins are involved specifically in the assembly/recycling of U snRNPcomplexes: (*a*) SART3/p110, whose localization to CBs is mediated by the binding to U6 snRNA, requires the presence of coilin and is transcription- and splicing-dependent (Stanek et al. 2003); and (*b*) the U6 snRNP-associated LSm4 and LSm8 proteins (Stanek et al. 2003) as well as the LSm 10, which is a component of U7 snRNPs (Pillai et al. 2001), are present in CBs. The SMN protein is pathogenetically linked to the spinal muscular atrophy (SMA) disease (see below). SMN is present in both the cytoplasm and nucleoplasm where it concentrates in CBs and in the nucleolus. SMN localizes to nuclear structures, called gems, located in close proximity to CBs in fetal tissues and in some cultured cell lines, hence the name meaning gemini or twin of the CBs (Liu & Dreyfuss 1996). However, in adult tissues and in both fetal and adult motoneurons, gems coincide with CBs and are not separate structures (Navascues et al. 2004). SMN plays an important role in the re-import of snRNPs into the nucleus and in the targeting of the U snRNP complexes to CBs (Narayanan et al. 2004). This last function is supported by the existence of an extensive physical and functional crosstalk between SMN and coilin. Thus coilin interacts with SMN through its C terminus (Hebert et al. 2001). Furthermore, de novo imported snRNPs localize almost exclusively to CBs that contain both coilin and SMN and not coilin alone (Sleeman et al. 2001). Moreover, the rearranged CBs in coilin$^{-/-}$ MEFs fail to accumulate both SMN and snRNPs, despite the fact that they still accumulate fibrillarin and that the latter interacts with SMN (Jones et al. 2001, Pellizzoni et al. 2001). Thus the ability of SMN to bind to fibrillarin is not sufficient to either concentrate or retain SMN in CBs in the absence of coilin and/or snRNPs.

## Cajal Bodies, Proliferation, and Cell Cycle

CBs change during the cell cycle, and their number and size are maximal at the G1/S-phase transition (Andrade et al. 1993, Fernandez et al. 2002). Furthermore, coilin$^{-/-}$ MEFs show slow growth properties (A.G. Matera, personal communication). Thus it is not entirely surprising that several CB components are functionally related to cell proliferation. Generally, their localization in CBs is increased in highly proliferating cells and, in some cases, their intranuclear accumulation depends on the mitogenic stimulation of the cells, as in the case of both the SMN-interacting ZPR1 putative transcription factor (Galcheva-Gargova et al. 1996, Gangwani et al. 2001) and of an isoform of FGF-2 (fibroblast growth factor-2; 18 kDa) (Claus et al. 2003, Joy et al. 1997). FGF-2 is a member of an expanding class of growth factors whose intranuclear targeting (ignored for years in favor of their better-characterized binding to extracellular membrane receptors) has been shown to be part of an intracrine signaling pathway, which supports their proliferation-

**Cyclin-dependent kinase (cdk):** a protein kinase whose activity fluctuates during cell cycle and that plays modulatory roles in cell cycle progression

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enhancing abilities (Stachowiak et al. 1997). The presence of FGF-2 and ZPR1 suggests, as observed for PML bodies (Matsuzaki et al. 2003), that mitogenic stimuli can directly signal to CBs.

Pigpen is a member of the TET family of RNA-processing factors (Morohoshi et al. 1998). It is enriched in bovine undifferentiated endothelial cells and retinal pigment epithelial cells and shown to associate with CBs in these cells (Alliegro & Alliegro 1996a). Nuclear microinjection of antipigpen antibodies inhibits the proliferation of endothelial cells, promoting exit from the cell cycle (Alliegro & Alliegro 2002). Pigpen shows 94% identity at the protein level with its human homologue, called TLS, which is involved in the pathogenesis of mixoid liposarcoma (see below). Similar to its human homologue, pigpen can potentially function as both a transcription factor and an RNA-binding protein (Alliegro & Alliegro 1996b).

NPAT has been identified as a cdk2/cyclin E-binding protein (Zhao et al. 1998) and is a large (220 kDa) nucleoplasmic- and CB-associated protein (**Figure 3*e,f***). In diploid human fibroblasts, two NPAT-containing CBs can typically be identified and are found adjacent to the histone gene clusters on chromosome 6. In S-phase, two additional NPAT-containing CBs can be observed, both associated with chromosome 1. The number of NPAT-containing CBs increases in transformed, highly proliferating cells (Zhao et al. 2000). Conditional somatic knockout of the protein in HCT116 cells has revealed that its absence blocks the G1/S-phase progression, reduces the levels of histone biosynthesis, and leads to an aberrant localization of coilin when the starved cells traverse the G1/S-phase upon serum induction (Ye et al. 2003). Whether the last phenomenon is a direct consequence of the depletion of NPAT, or an indirect consequence of the cell cycle block, is not yet clear and represents an interesting question to address in the future. In HeLa cells, cyclin E and cdk2 are associated in, or at least co-targeted to, CBs during the G1/S transition (Liu et al. 2000). The role of the CB-associated cdk2/cyclin E complex represents an interesting theater of investigation. Overexpressed NPAT mutants lacking the cdk2/cyclin E phosphorylation sites are still represented in CBs (J.W. Harper, personal communication), suggesting that the phosphorylation of NPAT by the CB-associated cdk2/cyclin E complex does not represent a major targeting signal. Indeed, the CB-associated NPAT is clearly phosphorylated in S-phase, whereas the protein is associated with a subset of CBs throughout the entire cell cycle (Ma et al. 2000). However, DNA damage and overexpression of p21, events that strongly reduce the cdk2/cyclin E kinase activity, trigger dissociation of NPAT (possibly in CBs) from histone loci. This event is concomitant with the inhibition of NPAT phosphorylation and block of histone synthesis and cell cycle progression (Su et al. 2004). Therefore, although the CB-associated cdk2/cyclin E activity seems to be dispensable for the recruitment of NPAT to CBs, it could promote the interaction of NPAT with other partners, an event ultimately relevant to the control of histone gene expression and, consequently, of cell cycle progression. Future studies will clarify this issue.

A further potential connection between the CB localization of NPAT and cell cycle progression comes from the recent demonstration of a physical and functional interaction between NPAT and CBP (CREB-binding protein). NPAT interacts with CBP and the overexpression of the two partners accelerates the entry of cells into S-phase (Wang et al. 2004). CBP is a component of PML bodies that is important for cell cycle progression. However, it is still not clear whether a subset of CBP is localized to CBs in addition to PML bodies.

Cdk7/cyclin H/mat1 is a stable, trimeric complex that has been experimentally proven to be a CAK (cyclin-dependent-activating kinase) complex. It mediates the phosphorylation-dependent activation of cyclin-dependent kinases (CDKs), especially cdk2/

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cyclin A and cdk2/cyclin E, thus playing a role in regulating cell cycle progression. Association of the CAK complex with CBs has been demonstrated (Jordan et al. 1997). The role of the CB-associated CAK complex could be more intricate than it appears. Indeed, it has been shown that the presence of the mat1 subunit switches the specificity of the complex toward the p53 protein, which interacts physically with both cyclin H and mat1 (Ko et al. 1997). Conversely, p53 can negatively influence the activity of the CAK complex toward cdk2 and the PolII CTD domains (Schneider et al. 1998). p53 has been shown to accumulate in a large percentage of CBs, probably through interaction with SMN, in conditions of cell stress (Young et al. 2002). In this case, p53 itself could act as a substrate of the associated CAK complex or negatively modulate the activity of the complex toward cdk2/cyclin E. This event can potentially lead to a reduction in the rate of histone biosynthesis (i.e., by reducing the cdk2/cyclin E activity toward NPAT) and to the consequent delay of G1/S progression. Even if it is not possible to exclude that the critical functional interactions take place outside of CBs, the fact that the players are all localized in the same nuclear body is intriguing. This provides a potential conceptual framework for a CB-associated modulation of the cdk2/cyclin E complex in different environmental conditions.

## Cajal Bodies and Stress

The previously mentioned interaction of SMN and p53 and the fact that the two proteins colocalize in CBs upon proteasome inhibition and DNA damage is intriguing (Young et al. 2002). p53 is indeed an exquisite sensor of cell stress and plays important roles in cell cycle regulation, DNA repair, control of transcription, and apoptosis (Jin & Levine 2001). Although the meaning of the SMN/P53 interaction is still not clear, the fact that it takes place in a large fraction of these organelles (50–75% of CBs in HeLa cells) (P. Young & C. Lorson, personal communication) suggests that it likely represents a biologically relevant phenomenon, potentially involving CBs in the cellular response to stress. Interestingly, in addition to SMN, other CB components can interact with p53: For example, the double-strand RNA-activated kinase PKR localizes in CBs and in nucleoli (Jimenez-Garcia et al. 1993) and binds p53 in vivo and can phosphorylate it in vitro (Cuddihy et al. 1999). Furthermore, activation of the PKR (and RNAse L) pathway constitutes the major known mode of intracellular antiviral defense that leads ultimately to the block of host protein synthesis (Flodstrom-Tullberg et al. 2005). Intriguingly, it has been shown that during adenovirus infection CBs become redistributed in hundreds of coilin and fibrillarin-containing microfoci, which are dispersed throughout the nucleoplasm and, even more interestingly, that this redistribution can be recapitulated by the treatment of cells with protein synthesis inhibitors (Rebelo et al. 1996, Rodrigues et al. 1996). These similarities imply a possible involvement of PKR in the adenovirus-induced disruption of CBs. Other evidence also suggests that CBs could represent stress-responsive domains, e.g., the localization of peroxiredoxin V in CBs (Kropotov et al. 2004). As a new entry in the growing collection of Cajal bodies components, peroxiredoxin V is a conserved protein endowed with thioredoxin peroxidase activity and also localized in mitochondria and peroxisomes. Interestingly, peroxiredoxin V has been shown to counteract, at least partially, the increase in intracellular ROS (reactive oxygen species) and prevent the apoptosis induced by p53 overexpression (Zhou et al. 2000). An intriguing possibility is that its presence in CBs is related to a possible local regulation of redox potential. Thus a local redox imbalance could either activate or inactivate stress-responsive CB components, possibly including p53, and be responsible for the observed structural and physical changes of CBs in stress conditions. Ergo, many stress-related CB components seem to be directly or indirectly functionally linked to p53. This raises the interesting

**Proteasome:** a high-molecular-weight multiprotein complex that catalyzes the degradation of ubiquitinated substrates

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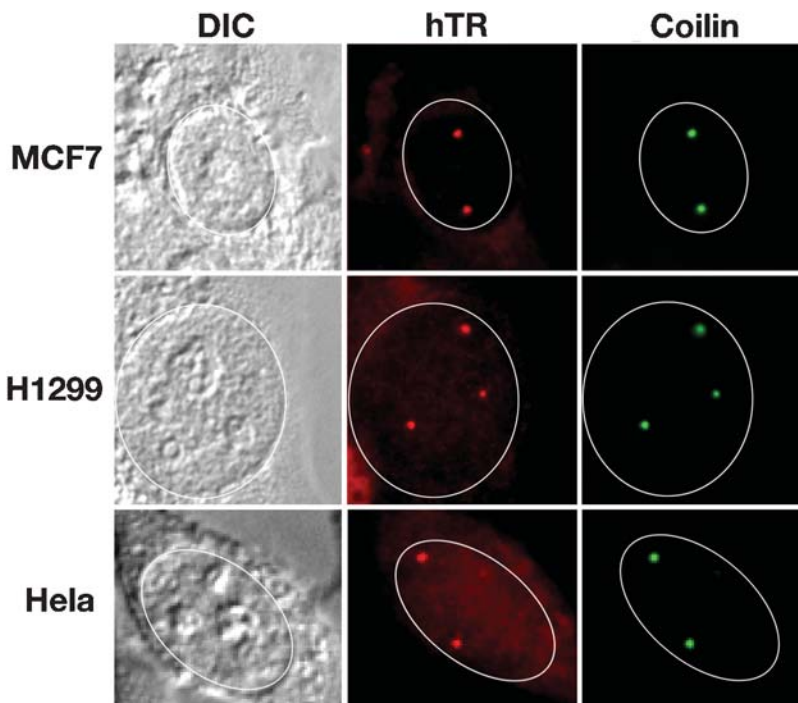


**Figure 4**

Telomerase RNA is enriched in CBs. Combined detection of coilin and telomerase RNA (FISH, *red signal*) in CBs (anti-coilin antibody, *green signal*) in three different cancer cell lines. White lines indicate the nuclear perimeter. Size bar: 10 $\mu$m. Courtesy of M. Terns, Univ. Georgia, Athens, GA.

question as to whether a CB-associated modulation of p53 takes place in stress conditions. The fact that p53 has been detected in a subset of CBs provides another clue that they represent a heterogeneous family of nuclear bodies.

## Cajal Bodies and Aging

Telomerase is an RNP reverse transcriptase that uses an RNA template to synthesize telomeric DNA repeats at the ends of the chromosomes. The enzymatic complex is minimally composed of an internal RNA template (hTR) and a catalytic protein subunit (hTERT). In normal, untransformed cells, the telomeres progressively shorten at each cell division. This contributes to genomic instability (Hande et al. 1999). The progressive shortening of telomeres is an important aspect of the cellular senescence program, because older cells with shorter telomeres will undergo apoptosis and thus be prevented, in physiological conditions, from accumulating mutations and chromosomal aberrations over time. In cancer, this mechanism of regulation is subverted and there is generally an increase in telomerase activity (Hahn & Meyerson 2001). Consequently, cancer cells do not undergo the physiological shortening of telomeres, which contributes to their immortalization (Satyanarayana et al. 2004). Recent FISH studies from two independent groups revealed that the hTR component is enriched in CBs, especially during the S-phase of the cell cycle (**Figure 4**). Localization of hTR requires the presence of a conserved scaRNA motif in its sequence (Jady et al. 2004) and the expression (endogenous or ectopic) of the catalytic subunit hTERT (Zhu et al. 2004). We note that these data support a possible role for CBs either in the maturation of the telomerase RNA component and/or in the assembly of the active RNP complex. However, several observations warrant further investigation. For example, except for the presence of the hypermethylated 5′ cap structure, no known modifications have been found

in mature hTR that could be specifically attributed to its residency in CBs (Jady et al. 2004). Despite the high frequency of colocalization of hTR with CBs, little or no colocalization/adjacency has been reported between CBs and telomeres. This raises the possibility that CBs could act as storage sites and deliver components of the telomerase complex when needed, but it would be expected that a transient colocalization of CBs with telomeres, at least during S-phase, should be observed; so far this has not been reported. Furthermore, the localization of hTR in CBs seems to be specific for cancer cells. In primary cell lines, even in the case of overexpression of hTERT, an event that readily triggers hTR accumulation in their transformed counterparts, the localization of telomerase RNA in CBs is generally not observed (Zhu et al. 2004). One simple explanation could be that CBs are not evident in some primary cell lines (Carmo-Fonseca et al. 1993, Spector et al. 1992). However, it is also possible that in mortal diploid cells, stimuli other than hTERT overexpression could be required for hTR accumulation in CBs. Alternatively, the cancer cell-specific enrichment of hTR in CBs could reflect the high degree of spatial disregulation of nuclear organization in transformed cells (Wong et al. 2002). Notably, the SMN protein, which interacts directly with coilin, binds to components of the telomerase complex and SMN-containing immunoprecipitates show telomerase activity in vitro (Bachand et al. 2002). In coilin$^{-/-}$ MEFs, the residual CB-like structures fail to recruit SMN, although it is not clear whether they can accumulate hTR. Based on what is known about the role of telomerase in aging, we predict that the coilin knockout mice might show signs of premature aging or that the derived coilin$^{-/-}$ MEFs would be more prone to undergo replicative senescence in vitro owing to a dysfunctional telomerase complex. So far there is no clear evidence indicating that this is the case, but it will be interesting to test this prediction in the future.

## Cajal Bodies and the Transcription Factor Link

Despite the fact that no nascent RNA has been shown to localize in CBs (Cmarko et al. 1999, Raska 1995), several CB-associated factors either have transcriptional activation domains (**Table 1**) or are involved in active transcription processes, and the CBs themselves can be surrounded by transcription sites (Jordan et al. 1997). The reason why these transcription-related factors are associated with CBs is puzzling. Little is known about either the mechanism of action or the target genes for these factors, except in few cases, such as the factors involved in the transcriptional activation of snRNA genes and ELL/EAF1.

**Transcriptional activation domain (TAD):** a protein domain that mediates the interaction with the basal transcription machineries, thus promoting an increase in the transcriptional rate of target genes

The 45 kDa-subunit of the snRNA gene-specific transcription factor PTF$\gamma$ has been found enriched in domains partially overlapping with CBs, along with a subset of components of the RNA polymerase II complex, i.e.,TBP, TFIIH, TFIIF, and the hypophosphorylated form of RNA PolII. The hyperphosphorylated form of PolII is instead enriched in dots localized at the periphery of CBs (Schul et al. 1998). ELL is an RNA polymerase II (PolII) transcription elongation factor that interacts with the transcription factor EAF1 (ELL associated factor-1). Both ELL and EAF1 are components of CBs (Polak et al. 2003). Interestingly, ELL has been shown to interact with p53 and to inhibit both sequence-specific transactivation and sequence-independent *trans*-repression by p53 (Shinobu et al. 1999). It is possible that CBs could function as storage sites for transcription factors that could be mobilized when needed to meet the transcriptional requirements of adjacent loci (histone or snRNA loci). However, considering that only a subset of CBs are associated with snRNA and histone loci in steady-state conditions, we consider another possibility, namely that CBs could be involved in the transport of components to chromatin loci during specific conditions, such as stress responses, to promote or

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**TABLE 1 Schematic summary of CB components described in this review**

| | Endogenous | Over-expressed | Morphological evidence | Biochemical interaction | Coilin dep targeting | CB-targeting signal | TAD |
|---|---|---|---|---|---|---|---|
| Coilin | • | • | • | pos (SMN) | | • | |
| Fibrillarin | • | • | • | pos (SMN) | No | | |
| NOPP140 | • | | • | pos (coilin) | No | | • |
| SART3/p110 | • | | • | | Yes | • | |
| Lsm proteins | • | • | • | | Yes | | |
| Sm proteins | • | • | • | pos (SMN) | Yes | | |
| SMN | • | • | • | pos (coilin) | Yes | • | |
| ZPR1 | • | | • | pos (SMN) | | | • |
| FGF-2 | • | | • | | | | |
| Pigpen | • | | • | | | | • |
| NPAT | • | • | • | | | | • |
| Cdk2/cyclin E | • | | • | | | | |
| Cdk7/ cyclinH/mat1 | • | | • | | | | |
| TFIIH | • | | • | | | | • |
| EAF1 (EAF2) | • | | • | Neg | | | • |
| ELL | • | • | • | Neg | | | |
| PTF$\gamma$ | • | | • | | | | • |
| p53 | • | | • | pos (SMN) | | | • |
| Peroxiredoxin | • | | • | | | | • |
| PKR | • | | • | | | | |
| Profilin I | • | | • | Neg | | | |
| Ataxin-1 | | • | • | pos (coilin) | | | |
| FRG1 | | • | • | | | | |
| hTERT | | • | • | | | | |

TAD, transcriptional activation domain; pos, biochemical data available and interaction demonstrated; Neg, biochemical data available, interaction not demonstrated; Empty space, no data available at present.

enhance the transcription of responsive genes. CBs are known to be motile structures that can undergo dramatic changes in number, size, shape, and intranuclear distribution. Although most of the movements of CBs appear to involve either simple or anomalous diffusion (Platani et al. 2000, 2002), it is still possible that some CB movement may involve forms of active transport. In this regard, it is interesting that profilin I has been localized in CBs. Profilin is an essential and conserved protein whose historically described localization is cytoplasmic and whose functions are linked to the modulation of the nonmuscle actin polymerization, achieved through the formation of a tightly controlled complex with G-actin (Witke 2004). Interestingly, the localization of profilin I in CBs requires active transcription (Skare et al. 2003). Profilin I can possibly bind to nuclear actin and thus influence both the ATP-dependent intranuclear movements and the assembly of transcription complexes, events that require actin (Bettinger et al. 2004). Although a previous study did not observe a change in CB movement after treating cells with actin-depolymerizing agents (Platani et al. 2002), the recent finding that CBs are enriched in actin and that the actin content of CBs is dynamically altered by adenovirus infection adds

interest to this hypothesis (Gedge et al. 2005). Future studies are needed to assess whether a complex of profilin I and G-actin exists in CBs and whether the CB-associated pool of actin is related to their intranuclear movements.

## CAJAL BODIES IN OTHER ORGANISMS

CB homologues, or similar structures, have been reported in many types of organisms apart from mammalian cells. It is beyond the scope of this review to provide detailed information about the structure and function of CB-like structures in other organisms. However, we do consider some features of nonmammalian CBs to illustrate what could be common principles of nuclear structure and function that have been conserved in eukaryotic cells, for example, the need to coordinate the temporal maturation of nuclear functional complexes with their spatial localization.

***Xenopus* Cajal bodies.** The homologue of human coilin in *Xenopus* is called SPH-1. It is a component of the so-called sphere organelle (Wu et al. 1994), which is a large structure (>10 $\mu$m in diameter) present in variable numbers (50–100) in the germinal vesicle of *Xenopus* oocytes (**Figure 5**). The sphere organelles have been the object of extensive study mainly by Gall's group, often anticipating results that later have been confirmed in mammalian CBs. For example, studies in *Xenopus* provided the first demonstration that U7 snRNAs are concentrated in sphere organelles (Wu & Gall 1993). As in human CBs, *Xenopus* sphere organelles contain, inter alia, coilin (SPH-1), fibrillarin, NOPP140, snoRNAs, and histone mRNA 3′-end-processing factors, such as U7 snRNA and SLBP1. For this reason, Gall proposed that sphere organelles be given the name Cajal bodies.

Despite these striking similarities between mammalian CBs and *Xenopus* CBs/sphere organelles, it is important to note that there are also apparent differences between these structures. First of all, *Xenopus* CBs are morphologically distinct with respect to mammalian CBs, being composed of a spherical structure with an internal matrix and often associated with specialized small, rounded substructures enriched in snRNPs, called B snurposomes (**Figure 5**). Interestingly, *Xenopus* CBs contain components of all three types of RNA polymerase complexes and also factors involved in the cleavage and polyadenylation of mRNA transcripts, such as symplekin, cstf 77, and cpsf100 (Hofmann et al. 2002). In mammalian cells, not all of the components of polymerase complexes have been observed to date in CBs, and cleavage and polyadenylation factors, such as cstf 64 and cpsf 100, have been localized instead in cleavage bodies, which are domains sometimes found adjacent to CBs (Schul et al. 1996). Another interesting difference between *Xenopus* and mammalian CBs is represented by the lack of a spatial association of the sphere organelles with the U1- and U2-snRNA gene loci (Abbott et al. 1999). Interestingly, cleavage bodies do not associate with U1- and U2-snRNA gene loci adjacent to CBs (Schul et al. 1999). This similarity raises the possibility that sphere organelles represent prototypical CBs whose components, which in *Xenopus* are grouped in a single type of organelle, have been redistributed, during evolution, to multiple specialized structures: CBs and cleavage bodies. An alternative possibility is that *Xenopus* CBs could instead

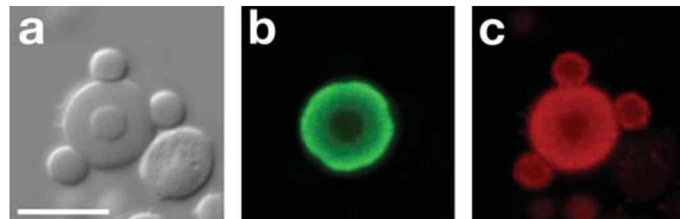


**Figure 5**

*Xenopus* CBs. Micrographs representing a (*a*) DIC image of a *Xenopus* CB surrounded by three snurposomes; (*b*) staining of a *Xenopus* CB with an anti-coilin antibody (*green signal*); (*c*) staining (anti-TMG CAP antibody, *red signal*) of TMG-capped RNA-containing structures: the TMG cap is a CB-associated antigen in both amphibian and human cells. Size bar:10 $\mu$m. Courtesy of J.G. Gall, Carnegie Institution of Washington, Baltimore, MD.

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constitute a specific subset of the CBs observed in mammalian cells, possibly specialized for the unique requirements of the amphibian oocyte.

**Cajal body-like structures in *Drosophila*.** The characterization of a *Drosophila* CB (similar to the situation in yeast and *Caenorhabditis elegans*) has been limited by the absence of an obvious coilin homologue in these organisms. Surprisingly, the use of an antibody raised against a C-terminal portion of recombinant human coilin (Andrade et al. 1993) has allowed the visualization of CB-like structures in *Drosophila* neurons (Yannoni & White 1997). It is possible that other CB proteins, perhaps sharing conserved structural motifs with coilin, can functionally substitute for the role played by coilin in mammalian CBs. The presence of coilin-like factors in CB-homologous structures in invertebrate species clearly underlines the evolutionary conservation of this nuclear body.

**Cajal bodies in plants.** CBs have been widely observed in plant cells, and all plant species studied so far have CBs (Shaw & Brown 2004). The advent of suitable probes for detecting plant CBs and of advanced tools for live-cell-imaging studies has shown that, in common with the human CBs, tobacco and *Arabidopsis* CBs are heterogeneous, dynamic structures. They can move through the nucleoplasm, fuse with each other, and move in and out of the nucleolus (Boudonck et al. 1999). Their number changes during plant cell differentiation and during the cell cycle (Boudonck et al. 1998) and, interestingly, with the metabolic activity of the cell (Acevedo et al. 2002). Plant CBs accumulate snRNPs that, strikingly, can be recognized by antibodies produced against their human counterparts, e.g., U2B″ (Beven et al. 1995). The presence of common epitopes and the ability to accumulate similar subsets of U snRNAs clearly suggest that plant and human CBs are related structures that can play similar roles in plant and animal cells.

**The yeast nucleolar body.** In budding yeast grown on solid media, it is possible to observe an intranucleolar structure, called, for this reason, the nucleolar body (NB). The yeast NB accumulates both mature and precursor forms of U3 snoRNAs and is enriched in TGS-1, a conserved methyltransferase that catalyzes the formation of the 5′ terminal tri-methyl-CAP structure, characteristic of most sno- and snRNAs (**Figure 6**) (Verheggen et al. 2002). The same components are enriched in mammalian CBs. Furthermore, mammalian CBs can also be observed within nucleoli under a variety of circumstances. For example, intranucleolar CBs have been reported in human breast carcinoma cells (Ochs et al. 1994) and also in liver cells of hibernating dormice (Malatesta et al. 1994). In addition, treatment of HeLa cells with the phosphatase inhibitor, okadaic acid, or transient expression of a single serine to aspartate mutant of coilin promotes the formation of CB-like structures within nucleoli that contain coilin and snRNPs (Bohmann et al. 1995, Lyon et al. 1997, Sleeman et al. 1998).

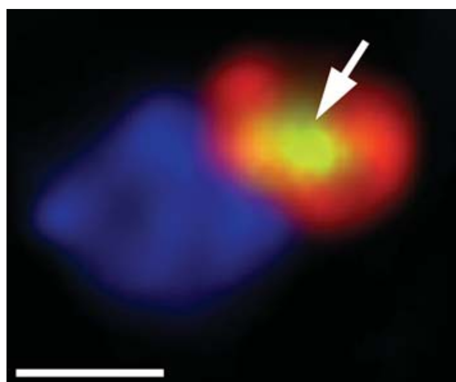

**Figure 6**
The yeast nucleolar body (NB). A TGS1-GFP fusion protein (*green signal*) is selectively enriched in the yeast NB, a CB-like structure located within the nucleolus (*arrow*). Staining for U3 snoRNA (FISH, *red signal*) is concentrated in the NB as well as in other regions of the nucleolus. The DNA is stained with DAPI (*blue signal*). Size bar: 1 $\mu$m. Courtesy of E. Bertrand, Montpellier, France.



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## LINKS TO DISEASE

There are increasing examples where the detailed organization of the cell nucleus is altered in specific pathologies. Whether these effects are direct or indirect, they likely reflect the disruption of cellular function contributing to the disease state and may provide useful clinical markers as well as possible new insights into the underlying pathogenic mechanisms. In the following discussion we consider examples of human diseases that affect nuclear structure and CB organization.

The expansion of a CAG triplet in an otherwise wild-type protein is a pathological hallmark of a class of hereditary neurodegenerative disorders including at least 14 known syndromes (Costa Lima & Pimentel 2004): for example, DentatoRubral-PallidoLuysian Atrophy (DRPLA), Machado-Joseph disease (MJD), and Spino-Cerebellar-Ataxia type 1 (SCA-1). Severe neuromuscular defects associated with cognitive and behavioral deficits in the DRPLA are a common trait in long-term affected patients. The CAG triplet diseases are characterized by the intranuclear accumulation of neurotoxic cleavage products of the mutant proteins and by a selective loss of neuronal subpopulations (Goti et al. 2004). One of the proposed mechanisms responsible for this phenomenon is the transcriptional deregulation of key survival/antiapoptotic pathways. In DRPLA and MJD the cleavage products of the mutated atrophin-1 and ataxin-3 proteins, respectively, accumulate in membrane-free, spherical aggregates composed of electron-dense material with an amorphous or fibrillar morphology. Interestingly, such structures show striking spatial relationships with CBs in neurons of affected patients. EM analysis has revealed that CBs are either in direct contact with the aggregates or connected to them through filamentous structures (Yamada et al. 2001). The fact that structurally different proteins, such as atrophin-1 and ataxin-3, show similar spatial relationships with CBs strongly suggests that the presence of poly-Q stretches plays a role in this phenomenon. The biological mechanism and significance of such relationships is not clear. It has been shown that poly-Q protein aggregates are capable of trapping poly-Q-containing transcription factors, even though it is not generally accepted whether this event is directly responsible for transcriptional deregulation. The transient association of transcription factors with CBs could be an explanation for their location adjacent to the poly-Q-containing aggregates. Alternatively, it is also possible that CBs are attracted to the aggregates because of their ability to supply stress-responsive factors. This is in line with the emerging idea that the formation of poly-Q-containing aggregates could represent a cellular stress response mechanism (Arrasate et al. 2004).

A recent report has provided evidence for a physical association between ataxin-1, the protein mutated in SCA-1 (Klement et al. 1998), and coilin. When both proteins are overexpressed, ataxin-1 interacts with and colocalizes with coilin in the ataxin-1-positive inclusions (**Figure 7**) (Hong et al. 2003), and the strength of interaction is not apparently influenced by the number of the ataxin-1 CAG repeats. Thus the interaction between ataxin-1 and coilin does not seem to take place within CBs (Skinner et al. 1997). However, because nucleoplasmatic coilin exchanges quickly and continuously with the CB-associated pool, it cannot be excluded that ataxin-1 can be

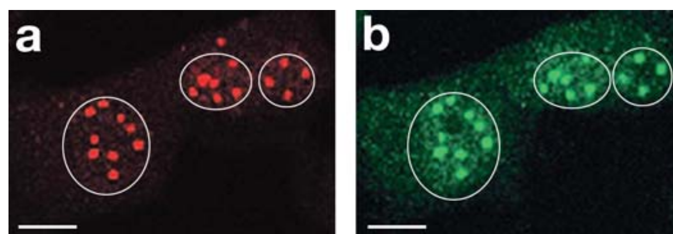


**Figure 7**

Exogenously expressed ataxin-1 and coilin colocalize. When both coilin (*a*) (*red signal*) and ataxin-1(*b*) (*green signal*) are exogenously overexpressed, they colocalize in ataxin-1-positive inclusions. White lines indicate the nuclear perimeter. Size bar: 10 $\mu$m. Courtesy of S. Kang, Korea University, Seoul.

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**Nuclear speckles:** irregularly shaped nuclear domains that likely represent storage and assembly sites for most splicing factors

transiently associated and functionally connected to CBs via its interaction with coilin. In line with this idea, it is noteworthy that ataxin-1 nuclear inclusions recently have been shown to break down upon transcriptional inhibition and to contain RNA-transport factors (Irwin et al. 2005), both features common to CBs (Boulon et al. 2004).

Spinal Muscular Atrophy (SMA) is the most common genetically determined neurodegenerative disease in children in the United States (Pearn 1980). It is caused by either deletion or loss-of-function mutations of the telomeric copy of the duplicated SMN genes that lead, inter alia, to a reduction in the nucleoplasmic levels of SMN (Lefebvre et al. 1997). The clinical manifestations are characterized by a progressive and profound atrophy of the voluntary muscles of the limbs and trunk, which can be variable, depending on the residual levels of protein expression. All the different SMA subtypes are characterized by a loss of motor neurons of the spinal cord. Without forgetting that a pathogenic role could be played by cytoplasmic defects in the axonal transport of RNAs induced by the SMN deficiency (Zhang et al. 2003), it is also possible that CBs play a role in the SMA scenario. It is of note that in SMA there is a defect in the intranuclear targeting of SMN associated with loss of discrete SMN and coilin-containing foci (Frugier et al. 2000): thus less SMN is available for the interaction with CB components. Furthermore, an RNA-binding protein, BRUNOL3, found upregulated in muscle biopsies from SMA patients, has been shown to interact and colocalize with SMN in gems in a motoneuronal cell line (Anderson et al. 2004). Notably, in motoneurons, gems coincide with CBs. On the other hand, the fact that coilin$^{-/-}$ mice apparently do not develop clear phenotypical signs of the SMA disease argues against a dramatic involvement of CBs in the pathogenesis of SMA. Additional data characterizing the localization of BRUNOL3 in CBs will be needed to define the requirement for CBs in the SMN-BRUNOL3 interaction/function. Another interesting experiment would be to cross coilin$^{-/-}$ mice and SMN$^{-/+}$ mice [SMN$^{-/-}$ mice are not viable (Schrank et al. 1997)] in order to unravel any possible contribution of coilin to SMN function, for example, at an embryonal stage.

Recently FRG1, a protein encoded by a candidate gene for the FSHD (facio scapulo humeral distrophy), when overexpressed, has been found to localize to CBs, in addition to nucleoli and ICGs (van Koningsbruggen et al. 2004). The function of FRG1 is still unknown. However it is intriguing to note that it represents another example of a growing group of proteins whose involvement suggests a link between RNA metabolism, neuromuscular disorders, and nuclear organization.

Both the nuclear localization of coilin and the morphology of CBs are altered in cells expressing an MLL-ELL fusion protein, which is the hallmark of a rare form of leukemia (Polak et al. 2003). As previously mentioned, the transcription factor ELL and its associated proteins, EAF-1 and EAF-2, are CB components, and ELL negatively modulates p53 function. Recent work has shown that the leukemic fusion protein is even more powerful than its physiological counterpart in inhibiting p53, by displacing its binding to P300 (Wiederschain et al. 2003).

The TLS/CHOP fusion protein is the product of a translocation found in more than 90% of mixoid and round cell liposarcomas t(12;16) (Aman et al. 1992). The fusion protein has powerful transforming activity and retains the N-terminal region of TLS, which appears essential for its oncogenic properties, and the entire sequence of CHOP, a stress-induced transcription factor. TLS is a member of the TET family of RNA-processing factors (Morohoshi et al. 1998). Thus the fusion protein can potentially affect both RNA processing and transcription processes. An FP-tagged TLS/CHOP fusion protein is localized to discrete nuclear structures that coincide with nuclear speckles (SC35 domains). Interestingly, a fraction of TLS/CHOP-containing domains overlaps

with CBs (Goransson et al. 2002). The localization of TLS/CHOP in splicing speckles is temperature sensitive and is lost when cells are exposed to 25°C or lower before fixation. This would indicate the possibility that TLS/CHOP is subject to intranuclear trafficking, involving movements between both CBs and splicing speckles. Furthermore, the bovine homologue of TLS, called Pigpen, is a CB component (Alliegro & Alliegro 1996a), and our preliminary observations indicate that endogenous TLS is present in CBs (M. Cioce, unpublished results). It is possible that the overlap between TLS/CHOP-containing domains and CBs could be supported by the binding of the TLS wild-type protein and its oncogenic counterpart to a common interactor or protein complex, likely mediated by the N-terminal portion of TLS. It is worth noting that the targeting of a fusion protein to nuclear domains that contain one or both of its physiological counterparts, has been proven to represent an aspect of oncogenesis. For example, the leukemia-specific PML/RAR$\alpha$

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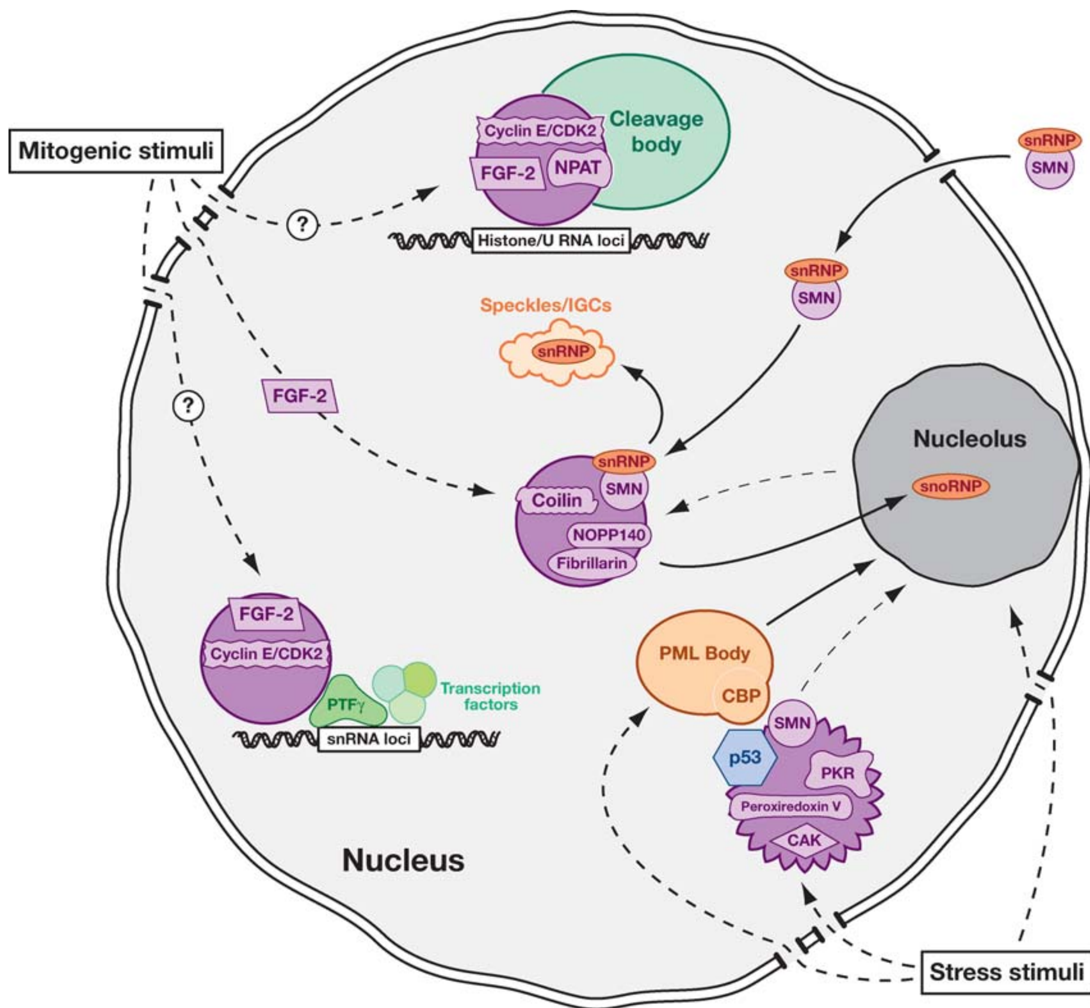


**Figure 8**

Schematic model illustrating a range of possible functions of CBs discussed in this review. TF: transcription factors, CB: Cajal body.

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fusion protein is targeted to PML bodies, leading to their apparent disruption and redistribution in hundreds of nucleoplasmic tiny dots (Dyck et al. 1994). Even more interesting, PML/RARα triggers deacetylation and degradation of p53, which is a component of PML bodies (Insinga et al. 2004), thus contrasting with the stabilizing functions of the wild-type PML protein toward p53 (Bernardi et al. 2004). P53 mutations are rare in leukemia. However, the example of MLL/ELL and of PML/RARα shows that the p53 tumor suppressor protein function can be inactivated in other ways. A possible interference of the MLL/ELL, TLS/CHOP, and, possibly, another TLS-containing leukemic fusion protein, TLS/ERG (Ichikawa et al. 1994) with the function(s) of CB-associated p53, represents an intriguing, if speculative, possibility.

## NEW PERSPECTIVES

We have reviewed here recent data about the composition and the behavior of CBs. A significant volume of evidence clearly implicates CBs as having a role in both snRNP and snoRNP maturation, processing of histone mRNA 3′ ends and, probably, the modulation of the expression of snRNA and histone genes. In addition, the presence of new entries in the ever-growing list of CB components warrants further investigation. Indeed, the presence of the telomerase RNA and hTERT in CBs suggests a possible relationship between CBs and cellular aging. The presence of peroxiredoxin V and p53-interacting proteins (SMN, PKR) and of p53 itself in stress conditions opens the possibility that CBs could also be involved in cellular stress response pathways. The fact that the expression of neoplastic fusion proteins can specifically affect the structure of CBs represents an appealing invitation to investigate possible relationships between these conserved nuclear bodies and cancer (**Figure 8**). We look forward to future studies that will shed more light on the function of these old, but always surprising, multifaceted organelles.

### SUMMARY POINTS

1. Cajal Bodies are heterogeneous, motile nuclear domains. The number of CBs change during the cell cycle and is influenced by the proliferative and metabolic status of the cell.
2. CBs play a role in the maturation of snRNPs and snoRNPs. CBs are enriched in small guide RNAs that direct the modification of snRNA, a step in the maturation of the spliceosomal snRNP particles.
3. The observation that some components accumulate in CBs preferentially upon mitogenic stimulation of the cells and the presence of both NPAT and of the cdk2/cyclin E complex support a role for CBs in integrating several cell proliferation-related phenomena, such as cell cycle progression and histone biosynthesis.
4. The presence of p53 in CBs during stress conditions and the existence among the CB components of stress-related proteins support a possible involvement of these organelles in the cellular response to stress.
5. CBs of cancer cells are enriched in the human telomerase RNA (hTR) and possibly play a role in the maturation of hTR. This suggests a possible involvement of CBs in cellular replicative senescence.

6. CB-like structures are conserved among mammalian, amphibian, insect, yeast, and plant cells. The fact that these structures accumulate common components (e.g., snRNPs) reveals the existence of evolutionarily conserved functions.
7. The hallmark of an expanding class of neurodegenerative diseases is the accumulation of mutated proteins in intranuclear aggregates, containing abnormally long polyglutamine stretches. CBs are often spatially adjacent to these aggregates. Among the mutated proteins, ataxin-1 interacts with coilin when overexpressed.
8. Expression of the leukemia-specific MLL/ELL fusion protein alters the intranuclear distribution of coilin and the integrity of CBs.

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## GLOSSARY

Cyclin-dependent kinase (cdk): a protein kinase whose activity depends on the presence of a class of regulatory subunits called cyclins. Cyclins are proteins whose levels of expression fluctuate periodically during the cell cycle. The phosphorylation of target proteins by the cyclin/cdk complexes plays a modulatory role in cell cycle progression

Guide RNA: small RNA molecules that, thanks to complementary base-pairing interactions, guide enzymatic activities to the sequence to be modified or processed

Nuclear speckles: irregularly shaped nuclear domains, typically 20–50 in number per cell nucleus. The consensus is that these domains are storage and assembly sites for most splicing factors

Proteasome: a high-molecular-weight multiprotein complex that catalyzes the degradation of ubiquitinated substrates. Inhibition of the proteasome results in the accumulation of unprocessed substrates and causes cell stress

Pseudouridylation: a naturally occurring modification of many stable RNA sequences resulting from the isomerization of uridine residues to pseudouridines, achieved through the cleavage and reattachment of the base to the sugar

Small nuclear and nucleolar ribonucleoprotein particles (snRNPs and snoRNPs): nuclear particles composed of a tight complex between a short RNA molecule (snRNA or snoRNA) and proteins, involved either in the processing of pre-mRNA (snRNPs) or in the modification and processing of rRNA (snoRNPs)

Small nuclear RNAs (snRNAs): a class of small RNAs, components of the snRNP particles involved in pre-mRNA processing that form base-pairing interactions with the pre-mRNA substrate.

Small nucleolar RNAs (snoRNAs): a class of small RNAs, components of the snoRNP particles involved in the modification and processing of rRNA. SnoRNAs function by coupling the enzymatic activities of the snoRNP complexes to the target sequences, achieved through base-pairing interactions with the substrate RNA

Spliceosome: a high-molecular-weight multiprotein complex that catalyzes the excision of introns from pre-mRNA molecules that leads to the formation of mature mRNAs. It is composed of five snRNPs and many additional protein-splicing factors

Transcriptional activation domain (TAD): a protein domain in a transcription factor responsible for the interaction with the basal transcription machinery that results in an increased transcriptional rate of the target gene

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