Nuclear bodies: multifaceted subdomains of the interchromatin space

A. Gregory Matera

Higher-eukaryotic nuclei contain numerous morphologically distinct substructures that are collectively called nuclear bodies. Although the precise functions of these subdomains remain unknown, elucidation of their molecular composition has been the subject of a great deal of research in recent years. Changes in the constitution of these nuclear inclusions are associated with disease phenotypes. The wide variety of components that concentrate within these subdomains makes them a likely interface for multiple cellular processes, including transcription, RNA processing, transport, RNP assembly, protein modification, apoptosis and cell-cycle control. This review discusses the different types of nuclear bodies, with emphasis on the two most prominent subtypes – the coiled and PML bodies.

Understanding the functional organization of genes and their products in vivo requires techniques that can visualize whole cells. The complementary use of light- and electron-microscopic imaging techniques, along with a healthy dose of molecular biology, has allowed modern cell biologists to begin to build a picture of the various subdomains within the eukaryotic nucleus. Although much of this space is taken up by chromatin of varying degrees of condensation, it is now clear that the interphase nucleus has an intricate organization, with the chromosomes occupying discrete territories and the interchromatin space consisting of numerous other recognizable substructures and organelles (Table 1). For example, mammalian nuclei contain subnuclear foci of various transcription factors, hnRNP proteins, heat-shock factors, heterochromatin proteins and even elements of the cleavage and polyadenylation machinery (Fig. 1). The emerging view is that many of these subdomains are associated with specific genetic loci and that interactions between these various domains and loci are dynamic and can change in response to cellular signals.

In addition to the domains mentioned above, the nuclei of higher eukaryotes contain various structures that have been characterized morphologically and are collectively called nuclear bodies. Elucidating the molecular composition of these structures has been the subject of a great deal of research in recent years. Nuclear bodies (NBs) contain a diverse assortment of cellular components. Improper organization or maintenance of various nuclear body components is associated with disease phenotypes. This review will focus on structure-function analyses of NBs, bringing together seemingly disparate observations and illustrating their interconnections within and between various disciplines of biology and biomedical science.

Coiled bodies

Based on strictly morphological criteria, ultrastructural studies have characterized several different types of NBs. One such nuclear inclusion is called the ‘coiled’ body and can be identified in diverse species from plants to animals. These structures typically appear as a tangle of coiled, electron-dense threads roughly 0.5 μm in diameter. Although its name derives from studies in the electron microscope, the coiled body (CB) was originally identified in the light microscope around the turn of the century by Santiago Ramón y Cajal, who termed them nuclear ‘accessory’ bodies. As we approach the next century, the precise functions of these nuclear organelles are still unclear. Molecular characterization of CBs began in the early 1990s, and the number of cellular components reported to be enriched within the CB compartment continues to grow rapidly (Box 1).

CBs are highly enriched in several classes of small nuclear ribonucleoproteins (snRNPs), nucleolar and cell-cycle control proteins, as well as several basal transcription factors. However, the only epitope reported to date that can be used as an unambiguous marker for CBs is a protein called p80 coilin (Box 1). Although there is certainly a pool of this protein localized diffusely throughout the nucleoplasm (Ref. 5 and references therein), antibodies against coilin typically stain a few discrete foci per nucleus (Fig. 1). Immuno-EM studies showed that these foci correspond to CBs. Other than two ‘classical’ nuclear-localization signals (NLSs) and numerous consensus serine phosphorylation sites, coilin does not contain any other clearly recognizable peptide motifs. However, Bellini and Gall demonstrated recently that coilin can not only bind strongly to poly r(G) homopolymers and single-stranded DNA but also forms a complex with the U7 snRNP. Furthermore, immunodepletion experiments in Xenopus egg extracts and transient-transfection studies using epitope- and GFP-tagged coilin constructs suggest interactions with the other Sm snRNPs as well. Finally, Isaac et al. have reported that coilin interacts directly with Nopp 140, a nucleolar shuttling protein that is part...
of a complex that also includes a pseudouridylate synthase. Perhaps, when we explore the full range of factors that interact with CB proteins, we will begin to understand the wide variety of cellular components that concentrate in CBs.

Coiled bodies are involved in snRNP biogenesis

Several lines of evidence suggest that nascent snRNPs do not accumulate within CBs, although mature (or maturing) snRNPs are highly concentrated in them. First, pulse–chase experiments with tritiated UTP show a lag-time before CBs are labelled. Second, pulses of Bi-UTP demonstrate that transcription sites are separate from and adjacent to CBs. Third, the presence of trimethylguanosine (TMG) cap and Sm protein epitopes within CBs (Box 1) reveals that the snRNPs concentrated within are at least partially mature. As newly transcribed spliceosomal snRNAs are exported to the cytoplasm prior to Sm particle assembly and cap hypermetylation, the presence of these epitopes in CBs implies that these RNAs have completed the cytoplasmic phase of the snRNP life cycle. Furthermore, experiments in Xenopus oocyte nuclei confirmed that the U7 snRNP is not the only TMG-positive RNA species that accumulates in CBs.

Additional lines of evidence suggest that CBs are involved in downstream steps of snRNP biogenesis and trafficking. Dreyfuss and coworkers have shown that the survival motor neuron gene product, SMN, is a key player in cytoplasmic Sm particle assembly (see Box 2 for details). When mutated, this gene can cause spinal muscular atrophy (SMA) in humans. SMN protein is distributed diffusely throughout the cytoplasm but concentrates in a few discrete foci per nucleus (Fig. 1). Originally, the nuclear foci were termed ‘Gemini of coiled bodies’ or ‘gems’ because they were often adjacent to CBs. However, recent studies indicate that, in most cell lines, the nuclear foci are completely coincident with CBs at both the light- and electron-microscopic levels (Ref. 28; P. Pinol-Roma, S. Matera, A. G. Elefanty, A. Grande, M. Fakan, S. (1994) Trends Cell Biol. 4, 86–90). Nevertheless, it is intriguing to speculate that the SMN complex might mediate the initial phases of snRNP assembly and then accompany the (partially) assembled snRNPs on their journey to the nucleus (Fig. 2). Additional clues suggesting a nuclear function for SMN come from a mutational analysis of the protein in cultured cells. Overexpression of an N-terminal deletion of SMN, called SMNΔN27, results in CB-like structures that are swollen to nearly the size of nuclei. Although a functional role for the SMN complex in the nucleus has not yet been demonstrated, SMNΔN27 can also have dominant-negative effects on pre-mRNA splicing in vitro. These results suggest that, in addition to roles in snRNP assembly, SMN and the machinery within CBs might also participate in nuclear snRNP function.

Finally, perhaps the best evidence that CBs are involved in snRNP trafficking comes from studies in Xenopus oocytes. Microinjection of radiolabeled U7 snRNA shows that the injected constructs replace the endogenous U7 snRNA within CBs. Thus, there is probably a flux of snRNPs through CBs.

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<th>Name</th>
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<tr>
<td>Nuclear domain</td>
<td>Site of mRNA transcription, processing and more</td>
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<td>Speckle/IGC</td>
<td>Storage sites for splicing factors</td>
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<td>Perichromatin fiber</td>
<td>Sites of nascent transcripts</td>
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<td>Coiled body/gem</td>
<td>Involved in snRNP biogenesis?</td>
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<td>PML body</td>
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<td>Cleavage body</td>
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<td>OPT domain</td>
<td>Regiospecific foci of Oct1, PFT and transcription</td>
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<td>GATA-1 bodies</td>
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<td>P-G domain</td>
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<td>hsnRNP proteins</td>
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<td>HS/F1 foci</td>
<td>Heat-shock factor 1</td>
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More recently, Terns and colleagues have followed the fate of fluorescently tagged U3 and U8 snRNA transcripts after microinjection. They find that injected small nuclear (sno) RNAs localize transiently to CBs prior to their accumulation in nucleoli. However, snoRNA mutants that are defective in nucleolar targeting (i.e. those lacking the C/D box elements) are retained in CBs. Furthermore, Gall and coworkers have also found that, following microinjection of U1 and U2 spliceosomal snRNPs, low but detectable concentrations of RNA appear to transit through the matrix of oocyte CBs before going on to B-snurposomes and lambrush chromosomes (J. Gall, pers. commun.). Previous studies have shown that these snRNPs accumulate in CBs, but only within the surface-bound B-snurposomes and the B-like inclusions. Flexibly, mutant forms of these RNAs might fail to accumulate in the B-snurposomes and be retained in the CB matrix. Clearly, this will be an area of future investigation. Together, these results indicate that CBs play a major role in directing intranuclear snRNA sorting and might even be an obligate waystation in the biogenesis pathways of snRNPs and snoRNPs.

Coiled bodies associate with specific genetic loci

Long before the cloning of p80 coilin homologues in human and frog and the subsequent realization that amphibian ‘sphere organelles’ and mammalian CBs were analogous structures, spheres had been
known to associate with specific chromosomal loci. The frequent presence of CBs at the histone gene loci on amphibian lampbrush chromosomes thus became one of the many ‘landmarks’ cytologists used for chromosomal identification. Interestingly, the chromatin loops corresponding to the amphibian histone gene clusters often seemed to be ‘plastered over the surfaces’ of the adjacent CBs. Parallel studies in mammalian nuclei have since revealed that the preferential association of CBs with histone genes is conserved in both human and murine cells.

Histone loci are not the only somatic genes that associate with CBs. Interestingly, gene clusters encoding the U1, U2 and U3 snRNAs colocalize with CBs in interphase human cells. However, clustering within the genome does not appear to be a requirement for CB association as several single-copy snRNA loci show a statistical preference for CB colocalization. One theme that links these various genes together is that they all encode small RNAs involved in RNA processing or, in the case of the histone genes, intronless mRNA precursors that are processed by factors known to accumulate in CBs.

Although the biological basis for the observed associations between CBs and genes remains uncertain, there has been some progress towards understanding the mechanism through which these interactions take place. Using stably transfected cell lines, Frey et al. showed that artificial tandem arrays of U2 snRNA genes colocalize with CBs and that the frequency of the colocalization depends directly on the transcriptional activity of the array. Association was abolished by promoter mutations or when polymerase II was globally inhibited. Strikingly, the U2 coding region was required for the association as replacement constructs driving expression of a heterologous sequence did not colocalize with CBs. Thus, association of U2 genes with CBs is mediated by the nascent snRNA transcripts themselves (or perhaps in complex with the polymerase) and not by DNA or DNA-bound proteins. These experiments suggest that CBs might facilitate communication between the snRNP assembly machinery and the transcriptional apparatus of snRNA genes. Additional findings that CBs can interact simultaneously with multiple chromosomal loci raised the possibility that these organelles might also play a role in coordinate feedback regulation (see below).

Based on staining with antibodies against the TMG cap, mature spliceosomal snRNPs localize within three different nuclear compartments: coiled bodies, interchromatin granule clusters (also called ‘speckles’) and perichromatin fibrils (Table 1). However, in any given cell, there are many speckles and only a few CBs. What limits the number of CBs and how are they generated? One attractive hypothesis is that transcription of the various CB-associated genes nucleates CBs – just as rDNA transcription is thought to mediate formation of nucleoli. However, if CBs only form near the associated genes, then it is difficult to explain why ‘free’ CBs

**FIGURE 1**

Mammalian cells contain many different subnuclear foci. Examples of two proteins that display such foci are shown in panels (a) and (b). In addition to a more general nucleoplasmic distribution, immunofluorescence with antibodies against heterogeneous nuclear ribonucleoprotein F (hnRNPF; a) and the 64kDa subunit of cleavage stimulation factor (CstF-64; b, green) often reveals bright foci (arrows). In some cases, these inclusions can overlap with other structures, such as coiled bodies (b, orange) in a cell-cycle-dependent fashion (Table 1). Most coiled bodies (c, green) and promyelocytic leukaemia (PML) bodies (d, red) do not colocalize in interphase HeLa cells, although one or two foci can often be seen to overlap (arrows). SMN protein (d) localizes diffusely throughout the cytoplasm but also colocalizes with PML bodies in nuclear-coiled bodies (see text). Coiled bodies (e, red) associate with specific chromosomal loci. Nuclei from two different experiments are shown; probes from the mouse histone gene clusters (e, green) on chromosomes 3 (left) and 13 (right) associate preferentially (arrows) with coiled bodies (e, red) in interphase 3T3 cells. In (f), antibodies against the trimethylguanosine cap display the so-called ‘speckled’ immunofluorescence pattern. The bright patches correspond to interchromatin granule clusters (IGCs), while the diffuse nucleoplasmic staining corresponds to the perichromatin fibrils (PFs, see text for details).
et al. have shown recently using time-lapse microscopy that CBs are so plentiful in the nucleoplasm. Alternatively, CBs might assemble independently and then be recruited to specific chromosomal loci. The presence of nuclear colloid such as fibrillarin and Nopp 140 in CBs, along with numerous electron micrographs showing a close apposition of the two organelles, suggests that the nucleolus is the most likely CB nucleation site. Notably, inhibition of coilin and Sm snRNPs–GFP fusion protein as a CB marker, Boudonck et al. have shown recently using time-lapse microscopy that CBs in plant cells are motile. These investigators observed movements of CBs in the nucleolus or the nucleoplasm, as well as events involving CB fusion or coalescence. As mentioned above, CBs are often found adjacent to nucleoli or attached physically to them as though in the process of emergence or fusion. Most of the movements appeared to be towards the nucleolus; there was no evidence of nuclear CBs emerging into the nucleolus. Although it is possible that CBs can move independently, we cannot rule out the possibility that CBs might simply be tethered to chromatin, which is also capable of translational movement. Thus, the biogenesis of coiled bodies remains an open, but intriguing, question.

A plurifunctional organelle?

Although CBs are now clearly implicated in snRNA biogenesis, several lines of evidence suggest that, like their larger cousin the nucleolus, CBs might be multifunctional organelles. For example, if CBs and gems are simply involved in snRNA biogenesis, why do they contain basal transcription and cell-cycle factors? Why do they associate with snRNAs and histone genes? One model is that, in dosage compensation, the feedback circuits cannot rely upon the levels of common snRNP proteins (e.g. Sm antigens) to regulate a diverse collection of U snRNAs because U snRNAs within any given class (Sm, fibrillarin, H/ACA) can vary in abundance by orders of magnitude. Thus, it seems likely that regulatory mechanisms that determine the concentration of individual snRNAs or snRNPs by interacting with the nascent RNAs will be different from receptor molecules that bind together the diverse array of CB components into a characteristic nuclear organelle. The components listed above were reported in the literature to be enriched in nuclear bodies. Many of these epitopes are also localized in other cellular compartments and some of them are likely erroneous assignments owing to antibody cross-reactivities. Some components with multiple names or subunits are in parentheses.

References


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\section*{reviews}

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\textbf{BOX 2 - SMA: THE SNMP CONNECTION}

Spinal muscular atrophy (SMA) is a recessive-lethal condition resulting from mutations in the survival motor neurone 1 gene, SMN1. This common neurodegenerative disorder has a carrier frequency of 1 in 50 and is the most common genetic cause of childhood mortality. SMN1 has been mapped to human chromosome 5q13 (Fig. 1), but the genomic organization of the surrounding region is complex. There are actually two closely related SMN genes, located within a 500 kb inverted duplication. There are at least four different transcription units within each inverted repeat. Deletions in the telomeric, SMN1 side of the repeat cause SMA, whereas similar mutations in the centromeric portion, containing SMN2, have no phenotypic effect. Despite the fact that the coding regions of the two SMN genes differ by only five silent codon changes, the genes are not functionally redundant, owing to an expression preference for slightly different isoforms. The SMN protein is part of a large (~300 kDa) complex containing both 'SMN interacting proteins' (SIP), SP2, SP3, etc.) and Sm 'core' proteins (B, D1–3, E–G) and plays an important role in the assembly of small ribonucleoprotein particles in the cytoplasm. One intriguing question is why a defect in a general cellular function can have such a tissue-specific phenotype. Motor neurones, despite their proliferative quiescence, are very transcriptionally active. Although far from proven, the general speculation is that these tissues are particularly sensitive to perturbations in snRNP biogenesis and regeneration.

\begin{figure}[h]
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\caption{SMA crucial gene}
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Gene & Description & Reference \\
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SP2, SP3 & 'Core' proteins (B, D1–3, E–G) & Pham, J. (1980) Lancet 1, 919–922. \\
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\begin{itemize}
\item Candidates for such receptors are the so-called modification 'guide' RNAs since they have the potential to basepair with specific RNAs and their protein constituents are known to localize in CBs. Curiously, although CBs are typically defined by the presence of p60-colin, the protein might not be essential for CB formation.
\item Ring-shaped and other 'simple' nuclear bodies comprise a class of substructures that are morphologically distinct from CBs. The signature protein within one type of simple nuclear body is the promyelocytic leukaemia protein, PML. (Fig. 1). Known variously as nuclear dots, PODs (PML onconpigic domains), ND10 (nuclear domain 10), Kp bodies and PML bodies (the term used hereafter), these structures are also approximately 0.5 μm in diameter and comprise a dense ring that contains PML, surrounding a central core that does not. PML bodies contain a number of other interesting components, the most prominent of which are the retinoblastoma protein RB, Sp100 and PICSU/SUMO-1 (Box 1). Like the CBs discussed above, PML bodies may well be plurifunctional; these organelles are thought to be involved in various aspects of transcriptional regulation and as targets of viral infection. As these topics have been reviewed excellently, they will not be considered fully here. Instead, this article will focus on recently reported functional aspects of the PML protein that provide insight into disease pathogenesis. PML bodies first gained prominence after the discovery that they are disrupted in patients bearing a recurrent translocation (t15;17;q22;q21), which leads to acute promyelocytic leukaemia (APL). This translocation, involving genes encoding PML and the retinoic acid receptor α (RARα), creates a fusion protein that not only fails to localize within PML bodies in patient-derived cell lines but has dominant effects on the localization of wild-type PML. Interestingly, treatment of both cell lines and patients with retinoic acid results in relocalization of PML and cancer remission, respectively.
\item PML goes apoptotic
\begin{itemize}
\item Patients with APL typically display bone marrow abnormalities caused by a block in differentiation of the normal promyelocytes and leading to their replacement by abnormal cells. As mentioned above, treatment with retinoids can overcome this differentiation block. However, previous studies have demonstrated that APL is not simply caused by mutation of the gene encoding RARα but involves the PML-derived portion of the fusion protein as well. Although disruption of PML bodies is a common feature in the etiology of APL, recent studies suggest that disorganization of PML protein is not an essential component of the differentiation block that results in neoplasia. Studies of several rare leukaemias resulting from translocations of RARαs with partners other than PML reveal that the distribution of PML is unaltered in these patient-derived cells. Thus, since the differentiation block is not simply due to delocalization of PML from the nuclear bodies, perhaps some other crucial role for PML is compromised in the APL cells.
\item PML protein appears to function as a negative growth regulator and a tumour suppressor, but the mechanisms through which PML functions to suppress tumours are not well understood. Recently, knockout studies have shown that Pml−/− mice are viable but are more susceptible to tumours and viral
infections. Further recent studies reveal that one role for PML might be in mediating programmed cell death. Treatment of cells and cell lines with a number of exogenous cellular signals, including cytokines and gamma rays, leads to an upregulation of PML, an increase in the size of PML bodies and results in apoptosis (Fig. 3). The PML–RAR fusion protein is thought to block the action of wild-type PML, thus conferring a survival advantage to APL cells and leading to leukaemia.

Interestingly, treatment of APL cells with arsenic compounds also causes apoptosis and can be attributed to the degradation of PML–RAR fusion proteins (Fig. 3). Moreover, cell lines derived from Pml-knockout mice also display cell-cycle abnormalities, with an increased proportion of cells in S phase. Thus, PML bodies might play a role in cell-cycle regulation.

Notably, PML interacts with Rb and ISG20, a putative negative regulator of cell division. Covalent modification of PML body components

The missing link between transcriptional activation, cell-cycle control and apoptosis might be a small, ubiquitin-like molecule called SUMO-1. Among the targets of this protein are RanGAP1, Sp100 and PML – the latter two of which are enriched in PML bodies. However, unlike the ubiquitin system, SUMO-1-modified target proteins typically are not degraded. Furthermore, only nuclear forms of PML and Sp100 are covalently modified by SUMO-1, and the modification site for PML maps to two lysine residues within the NLS.

It is possible that SUMO-1 modification targets PML to nuclear bodies; it is also conceivable that modification takes place within the nuclear bodies. Given that PML shuttles between the nucleus and the cytoplasm, it is tempting to speculate that, upon export to the cytoplasm, the SUMO-1-modified protein might be unable to re-enter the nucleus. The presence of modified protein in the cytoplasm could conceivably trigger cytoplasmic (mitochondrial?) apoptotic enzymes. Regulated binding of nuclear import/export factors is an emerging paradigm in intracellular protein localization (e.g. see Refs 62 and 63) and could play a role in APL pathogenesis.

Although PML–RARs is also modified efficiently by SUMO-1 in vitro, overexpression of PML–RARs disrupts normal PML bodies, and the fusion protein

FIGURE 2
A model for trafficking of Sm small nuclear ribonucleoprotein particles (snRNPs) in mammalian cells. (a) Small RNPs accumulate in various intracellular compartments: perichromatin fibrils (PFs), interchromatin granule clusters (IGCs), coiled bodies (CBs), nucleoli and the cytoplasm. In the model, multiple equilibria govern the partitioning of snRNPs within and between the various compartments. As described in the text, CBs appear to be involved in trafficking of newly assembled snRNPs. Monoparticle snRNP assembly occurs in the cytoplasm, followed by nuclear import. In the model, the newly imported snRNPs traverse the nucleolus and proceed to CBs before accumulating in IGCs and PFs. CBs can also interact with snRNA and histone gene loci, providing an opportunity for feedback (positive or negative) regulation. The possible mobility of these organelles is depicted by a dashed line. (b) Flow chart of the snRNP life cycle. Coiled bodies might occupy a nexus point between less related snRNP assembly activities: biogenesis of new snRNPs and regeneration of ‘used’ splicing factors. Although consistent with experimental observations, trafficking of snRNPs through nucleolus and IGCs on both the ‘outbound’ and ‘inbound’ legs of the recycling and biogenesis cycles is completely hypothetical.
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Promyelocyte

Mature cell

Arsenic

Degradation

PML

RARα

Reticin acid

Apoptosis

γ Irradiation

IFNs

Fas

Ceramide

TNF

Role of promyelocytic leukaemia protein (PML) in apoptosis and acute promyelocytic leukaemia (adapted from Ref. 52). Upstream cellular signals such as tumour-necrosis factor (TNF), γ irradiation, interferons (IFNs), Fas receptor or ceramide all act to upregulate PML and induce apoptosis. PML–RARα inhibits the downstream action of PML, leading to a block in apoptosis and differentiation of promyelocytes. Treatment with retinoid acid or arsenic compounds causes the fusion protein to be degraded and alleviates the block.

FIGURE 3

Nuclear bodies and molecular disease: future challenges

Upon discovery of a new protein, it has virtually become standard laboratory practice to ask questions about its subcellular localization. Thus, the number of proteins that concentrate in nuclear foci seems to be increasing at a rate some might find alarming. It is important, therefore, that we find common paradigms to compare and contrast these various structures and thus design better experimental hypotheses. Arguably, the structures described in detail in this review, CBs and PML bodies, are the two best studied. What lessons have we learned? PML and coiled bodies are each associated with genetic diseases. Both CBs (gels) and PML bodies are either diminished in number or completely disaggregated in patient-derived cells. The structures might even be functionally or spatially compartmentalized. As mentioned above, components of amphibian oocyte CBs and mammalian PML bodies occupy discrete domains within the organelles that suggest roles in molecular transport or sorting. There are also hints that the molecules trafficking through these NBs are somehow modified during their visit. For example, PML and Sp100, and others might be targeted by SUMO-1. Various aspects of snRNP maturation (e.g. 2′-O-methylation, pseudouridylation, higher-order complex assembly and/or reassembly) might take place in CBs. Alternatively, nuclear bodies might represent sites of storage or sequestration of various nuclear components – a kind of molecular warehouse.

It is interesting to note that PML bodies have no known homologues in the amphibian oocyte nucleus. The only ‘free-living’ organelles within the frog oocyte are the extrachromosomal nucleoli, CBs (spheres) and B-snurpsomes. Where are the PML bodies and the other nuclear foci? Most likely, they are attached to the lambrush chromosomes, and, if so, where are they attached? Thus, while at least some of the associated loci (e.g. snRNA and histone genes) have been defined for CBs, the genomic targets for PML bodies remain to be identified. Furthermore, once associated with the chromosomes, what kinds of cellular processes do these organelles direct? These are questions for the future.

Finally, it seems clear that the associations among and between CBs, PML bodies and chromosomes will be regulated by cellular signals. Defining these interactions and signals will therefore be important keys to understanding the functions of these fascinating nuclear organelles. Thus, as we move into the next millennium, nuclear bodies should continue to provide new insights into the functional organization of the nucleus and provide much food for thought as cell biologists and geneticists begin to unravel the metabolic processes that take place within these domains.

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RENT control in the nucleolus

The most prominent type of subdomain in the nucleolus, not touched on by Greg Matera in the review above but described in considerable detail elsewhere, is the nucleolus. This is of course best known for its role in ribosome synthesis, but three recent papers have characterized two new roles for a complex that resides in cellular regulation.

Ribosome synthesis, but three recent papers have characterized two new roles for a complex that resides in cellular regulation.

In anaphase, however, like Cdc14p, Sir2p seems to be released from the RENT complex in late anaphase, even though Net1p remains associated with the rDNA. This suggests that there is an overall change in rDNA and the RENT complex structure in anaphase. Though Net1p remains associated with the rDNA, this suggests that there is an overall change in rDNA.

There may be other changes during anaphase due to the high level of Net1p, which binds to the cell-cycle-regulatory phosphatase Cdc14p and localizes it to the nucleolus during G1 and S phases. In late anaphase, Cdc14p dissociates from RENT, disperses through the cell and triggers exit from mitosis in a process dependent on the dephosphorylation of Swi5p, Sic1p and Cdh1p. This is the first example of the use of nucleolar localization as a regulatory mechanism and could be a paradigm for cell-cycle regulation like the localization to the cytoplasm of cyclins (e.g. see Ref. 5).

Another protein in the RENT complex that binds to Net1p is the silencing mediator Sir2p. During interphase, Net1p and Sir2p colocalize at the nucleolus, and Net1p is required for Sir2p rDNA silencing function. However, like Cdc14p, Sir2p seems to be released from the RENT complex in late anaphase, even though Net1p remains associated with the rDNA. This suggests that there is an overall change in rDNA and the RENT complex structure in anaphase.

Thus, characterization of these interactions has revealed two new roles for a nucleolar complex and also hinted that there might be a general change in nucleolar structure-function as cells exit anaphase. More generally, it has highlighted that the nucleolus is not merely a ribosome factory but in fact has diverse roles in cellular regulation.

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