Structure and Function in the Nucleus

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Current evidence suggests that the nucleus has a distinct substructure, albeit one that is dynamic rather than a rigid framework. Viral infection, oncogene expression, and inherited human disorders can each cause profound and specific changes in nuclear organization. This review summarizes recent progress in understanding nuclear organization, highlighting in particular the dynamic aspects of nuclear structure.

First described by Brown in 1831, the cell nucleus is one of the best known but least understood of cellular organelles. The structure and functional organization of the nucleus remains a subject of energetic debate. At one extreme, the nucleus has been proposed to have its own nucleoskeleton and distinct organelles. At the other, it is viewed as a largely disordered, membrane-bound bag of DNA and other molecules, in which all "structures" are no more than transient complexes that form and disperse as a result of transcription, replication, and RNA processing activities in various regions of the genome. Understanding in molecular detail the organizing principles of the nucleus—including the arrangement of chromosomal DNA and how the synthesis, processing, assembly, and transport of macromolecules are coordinated and regulated—is a major goal for cell biology.

Compartments of the Interphase Nucleus

In the interphase nucleus, individual chromosomes occupy discrete patches referred to as chromosome territories (*1*), which are separated by channels called the interchromosomal domain (Fig. 1). Active genes tend to be preferentially localized to the periphery of the chromosome territories (*2*, *3*). RNA transcripts are apparently formed preferentially at the surface of the territories and are then "shed" into interchromosomal domain channels for further processing and transport. Because the volume available to factors involved in RNA transcription and processing is thereby reduced (*4*), this process may enhance the assembly of large macromolecular transcription and splicing complexes.

This model predicts that, relative to less active chromosomes, transcriptionally active chromosomes might have more surface area in contact with the channels. This has been confirmed for human X chromosomes: The volumes of the active and inactive X chromosomes were shown to be essentially identical (*5*), and the inactive X chromosome was apparently no more condensed than the active X. Instead, the inactive X, with far fewer active genes, had a much reduced surface area relative to that of its active counterpart.

Active and inactive regions are interspersed along the length of the chromosomes but are segregated from one another within the chromosome territories. When highly synchronous populations of Chinese hamster fibroblasts undergoing DNA replication were pulse-labeled for short periods with halogenated deoxyribonucleotide triphosphates (dNTPs), early-replicating (R band) DNA was found largely dispersed throughout the nuclear interior, whereas later-replicating (G band) DNA was concentrated near the nuclear periphery (*6*). A similar arrangement of the chromosome fiber was suggested by another study in which fluorescence in situ hybridization (FISH) was used to localize sequences distributed across the long arm of chromosome 2 in *Drosophila* embryo nuclei (*7*). Together, these studies suggest that within individual chromosome territories the chromatin fiber

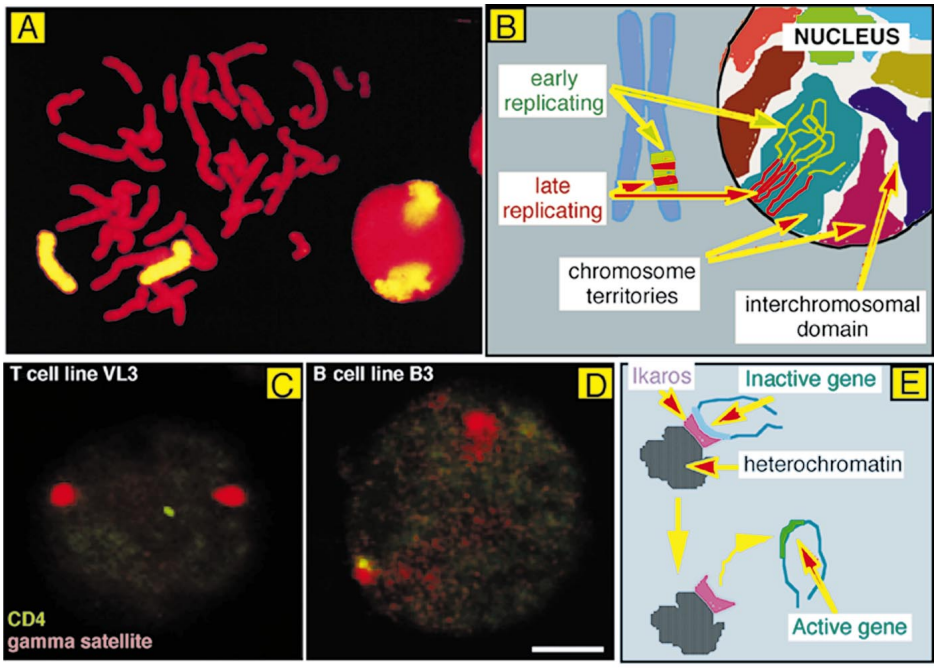

Fig. 1. (**A**) Chromosome-painting FISH performed under conditions where signal from repeated DNA sequences is suppressed (*120*). Left, metaphase spread showing labeled human chromosomes 4. Right, interphase nucleus showing the two chromosome 4 territories. (Image provided by T. Cremer.) (**B**) The organization of chromosomes in the nucleus. Each chromosome occupies a discrete territory. These territories are separated by the interchromosomal domain, in which it is believed that much RNA processing and trafficking occurs. Within individual territories, the chromatin fiber appears to be looped, with late-replicating DNA near the nuclear periphery and early-replicating DNA in the interior. (**C** and **D**) Differential localization of the CD4 locus under expressing and nonexpressing conditions. In T cell line VL3, where the CD4 gene is active (C), the gene was associated with gamma satellite in 1 of 57 nuclei. In B cell line B3, where the CD4 gene is inactive (D), the gene was associated with gamma satellite in 52 of 52 nuclei. The CD4 locus and the gamma satellite are labeled with fluorescein isothiocyanate and rhodamine, respectively. A single slice from a confocal Z-series is shown in each case. Scale bar, 2 μm. [Image provided by K. Brown and A. Fisher (*24*).] (**E**) Diagram showing how the activation or repression of the CD4 gene is correlated with movement of the gene relative to heterochromatin.

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is highly contorted, looping back and forth between the nuclear interior and the periphery (Fig. 1). Because the G and R bands are interleaved and immediately adjacent to one another in mitotic chromosomes, their segregation into different portions of the chromosome territory in interphase must involve the movement of large-scale chromosome segments within the nucleus at the completion of mitosis.

Chromosome Movements Within the Nucleus

Two recent studies using fluorescence recovery after photobleaching (FRAP) yielded different views of the nature of the nucleoplasm. In one study, microinjected fluorescent dextrans and ficolls were found to diffuse relatively unimpeded; a 500-kD protein would diffuse from one side of a 10-μm nucleus to the other in only ~7 s (*8*). Because this rapid diffusion presumably occurs within interchromosomal domain channels, they may not be filled with a mesh-like matrix of structural filaments, as suggested by previous results (*9*). Channels that end at nuclear pores might function like "tracks" along which RNAs bound for export could move (*10*).

A different result was obtained using the FRAP method when chromatin was labeled with a fluorescent dye and then bleached with a laser. No movement was observed over the next 60 to 80 min (*11*). Nonetheless, chromosomes and chromatin can move within the nucleus. Centromere movements in vivo were observed directly using the DNA binding domain of human centromere protein CENP-B (*12*) fused to green fluorescent protein (GFP) (*13*). Individual centromeres moved, albeit infrequently, over distances of a micrometer or more at 7 to 10 μm per hour.

The chromosome arms also move during interphase, as shown for a large chromosomal domain by tracking a lac repressor–GFP fusion protein bound to an artificial array containing thousands of lac operators (*14*). This movement was cell cycle–dependent; the domain moved from the nuclear interior to the periphery during the G_1 phase of the cycle, from the periphery into the interior during S phase, and back to the periphery again during G_2. These movements were accompanied by a cycle of condensation and decondensation.

Entire chromosome territories labeled with the fluorescent thymidine analog Cy3-AP3–deoxyuridine triphosphate have been tracked in living cells (*15*). Two weeks after microinjection of the tracer, the original labeled chromosomes had been replicated and segregated many times, leaving only one or a few labeled chromatids in any one cell. Four-dimensional (three-dimensional over time) movies revealed movements at three levels within these cells: (i) changes in the position of entire chromosome territories with respect to one another by distances of several micrometers over a time scale of 2 to 3 hours; (ii) movement of subchromosomal foci, 400 to 800 nm in diameter, within territories; and (iii) flexations of the chromatin fiber within the subchromosomal foci (*15*). The mechanism is unknown but may involve mechanochemical motor proteins such as chromokinesin (*16*) or nod (*17*), action of tethered RNA polymerases (*18*), localized alterations in chromosome condensation, or electrostatic gradients within the nucleus (*4*).

Why do chromosomes move? Some movements may be linked to DNA replication, consistent with the proposal that DNA replication occurs at a fixed number of sites within the nucleus, termed "replication factories" (*19*). Such sites are suggested to contain large multienzyme complexes that do not move within the nucleus; rather, chromosomes move to the factories to initiate their replication (*20*).

How Heterochromatin Can Regulate Gene Expression

Heterochromatin contains relatively few genes, replicates during late S phase, is enriched in specific nuclear proteins, and can suppress the transcriptional activity of active genes that are translocated adjacent to it (*21*). When genes that normally reside in euchromatin are translocated near to centromeres or telomeres, transcription is decreased. This "position effect" is driven by heterochromatin proteins, for which more than 50 candidates have so far been reported in *Drosophila* alone.

Two recent studies on the *Brown*^{dominant} (Bw^D) mutant in *Drosophila* show that gene silencing by heterochromatin can involve the movement of the silenced gene from one area of the nucleus to another. Bw^D has an insertion of ~2 Mb of AAGAG satellite DNA into the *Brown* locus, located in euchromatin near the end of the long arm of chromosome 2 (*22*). In Bw^D/+ flies, the expression of the wild-type allele is only ~2% of normal. FISH analysis revealed that Bw^D and Bw^+ preferentially associate with one another and with centromeric heterochromatin of chromosome 2 (*23*), thereby bringing the Bw^+ gene into proximity with the centromere and transcriptionally inactivating it. This somatic pairing of homologs, which drives the inactivation of Bw^+ expression, is not observed in mammals. However, recent evidence suggests that a similar "recruitment" model for gene inactivation by heterochromatin may also be valid in mammals.

Ikaros is a transcriptional regulator essential for lymphoid development. When six transcriptionally active and inactive genes were localized in mouse pre-B and mature B cell lines, the inactive genes were invariably recruited to sites of Ikaros localization at centromeric heterochromatin (*24*). In contrast, active genes were localized elsewhere in the nucleoplasm (Fig. 1). These results provide a striking correlation between gene position within the nucleus and transcriptional activity, and they suggest that Ikaros may transcriptionally inactivate genes by "recruiting" them from permissive loci in the nucleoplasm to a zone of transcriptional suppression near centromeric heterochromatin. Together, these results in *Drosophila* and mouse cells lend strong support to the notion that gene silencing by heterochromatin involves the movement of chromosomal loci to a functionally discrete nuclear compartment in which gene transcription is actively suppressed.

The Polycomb Group (PcG) Domain: A Euchromatin Silencing Domain?

Heterochromatin is thought to contain large protein complexes that propagate laterally along the chromatin fiber and silence genes with which they come into contact (*25*). This mechanism can explain the silencing of genes that are transposed near to the telomeres of yeast chromosomes (*21*), which requires the formation of a complex between the silent information regulators SIR3 and SIR4, repressor activator protein RAP1, and core histones (*26*).

The best known heterochromatin protein in higher eukaryotes, HP1 (heterochromatin protein 1), was identified in a screen for monoclonal antibodies that stained *Drosophila* polytene chromosomes (*27*). HP1 shares an ~50–amino acid NH_2-terminal sequence motif, the chromo domain, with *polycomb*, an important regulatory gene that functions in the stable repression of homeotic genes during *Drosophila* development (*28*). A second chromo domain–like motif, the chromoshadow domain, located near the HP1 COOH-terminus (*29*), appears to be a key element in the assembly of multiprotein complexes in heterochromatin.

Emerging evidence suggests that HP1 is a structural adapter whose role is to assemble macromolecular complexes in chromatin. HP1 interacts with multiple nuclear proteins, including itself (*30*); the lamin B receptor, an integral protein of the inner nuclear envelope (*31*); BRG1/SNF2b, a component of the SWI/SNF complex that

collaborates with nuclear hormone receptors in transcriptional activation (*30*); nuclear receptor cofactor TIF1-α, a protein that interacts with the ligand-dependent activation domain of steroid hormone receptors (*30*); βLAP, a leucine amino peptidase (*30*); a RAD54-like presumed helicase (*30*); the product of the *Su(var)3-7* gene, a heterochromatin protein of unknown function (*32*); Orc1 and Orc2, two members of the origin recognition complex, a hexamer that binds to origins of replication and is essential for the initiation of DNA replication (*33*); and SP100, a major component of PML nuclear bodies (see below) (*34*). Targeting of HP1 to reporter genes inactivates transcription (*30*, *34*). The role of most of these interactions is unknown, but localization of heterochromatin at the inner surface of the nuclear envelope may, at least in part, be explained by the lamin B receptor–HP1 interaction (*31*).

The Polycomb protein is also involved in gene silencing, although it binds exclusively to sites in euchromatin (*35*). Four cloned human Polycomb group members (*36*) can interact with one another and colocalize in 10 to 20 nuclear domains of unknown function, termed PcG domains. These are distinct from PML nuclear bodies, interchromatin granule speckles, and centromeric heterochromatin (see below). PcG domains may represent silencing compartments for the inactivation of specific genes in euchromatin. Because a dominant negative Polycomb mutant specifically derepresses c-*myc* expression in human and rat cell lines, it will be interesting to see whether the c-*myc* gene resides in or near PcG domains when in its basal state.

The Nucleolus

The nucleolus is formed around the ribosomal DNA (rDNA) repeats, which cluster at chromosomal loci called nucleolar organizers, and is the factory in which 28S, 18S, and 5.8S ribosomal RNAs (rRNAs) are transcribed, processed, and assembled into ribosome subunits (Fig. 2). Nucleolus formation is both transcription- and cell cycle–dependent: In most eukaryotic cells, the entire structure breaks down and reforms during each mitotic cycle. Thus, the nucleolus is a dynamic structure that forms in response to the requirement for new ribosome synthesis.

Within the nucleolar factory, the rRNA is extensively modified during ribosome biogenesis in a process involving a series of specific nucleolytic cleavages as well as base modifications to introduce ~100 2′-*O*-methyl ribose and ~90 pseudouridine residues per molecule (*37*). Small nucleolar ribonucleoproteins (snoRNPs) have been shown to act as "guide RNAs" during rRNA maturation (*38*), targeting sites of 2′-*O*-methyl ribose and pseudouridine formation [D box and ACA snoRNPs, respectively (*39*)] and directing nucleolytic cleavage of the rRNA precursor [U3, U8, U14, and U22 snoRNPs (*38*)]. Small nucleolar RNAs can be engineered to target 2′-*O*-methylation to regions of RNA that are normally unmodified (*40*). This approach should have important applications for studying how RNA modification influences RNA function.

In addition to its major role in ribosome production, the nucleolus may also be involved in messenger RNA (mRNA) export or degradation. This was first suggested by the observation that inactivating nucleoli by ultraviolet irradiation prevented the export of nonribosomal RNAs in mammalian cells (*41*). Although bulk polyadenylate [poly(A)] mRNA does not localize in nucleoli in higher eukaryotes, specific transcripts, including MyoD and N-myc, have been detected in nucleoli (*42*). Furthermore, interference with mRNA transport by mutation of the *mtr1-1* and *mtr2-1* genes of *Saccharomyces cerevisiae* (*43*) or severe heat shock in *S. pombe* (*44*) results in the accumulation of poly(A) RNA in the nucleolus.

The existence of the newly described perinucleolar compartment (PNC) in 10 to 50% of cells (depending on cell type) also raises the possibility of further functions associated with nucleoli. The PNC contains several RNA polymerase III transcripts and the hnRNPI protein (also called PTB) (*45*). Targeting of hnRNPI to the PNC requires the presence of specific RNA binding domains (*46*). The function of the PNC is unclear at present. As discussed below, the nucleolus also interacts with coiled bodies (see Fig. 2). Thus, despite the long history of nucleolar research, the nucleolus may still have some secrets left to divulge.

Speckles, snRNPs, and Punctate Patterns

Many nuclear factors localize either partly or completely in distinct "bodies" or subnuclear compartments that produce a punctate staining pattern when analyzed by indirect immunofluorescence (Fig. 3). Interest in these subnuclear bodies has been rekindled by recent discoveries that some of them contain factors involved in the processing and transcription of RNA and that in several cases both subnuclear bodies and the factors they contain are intimately linked with human disease.

The U1, U2, U4/U6, and U5 small nuclear ribonucleoproteins (snRNPs) are nucleoplasmic RNA-protein complexes that function as subunits of the spliceosome, which removes introns from nuclear mRNA precursors. Antibodies that stain snRNPs show punctate ("speckled") labeling against a more diffuse background of nucleoplasmic staining (*47*). The diffuse staining is largely attributable to snRNPs interacting with

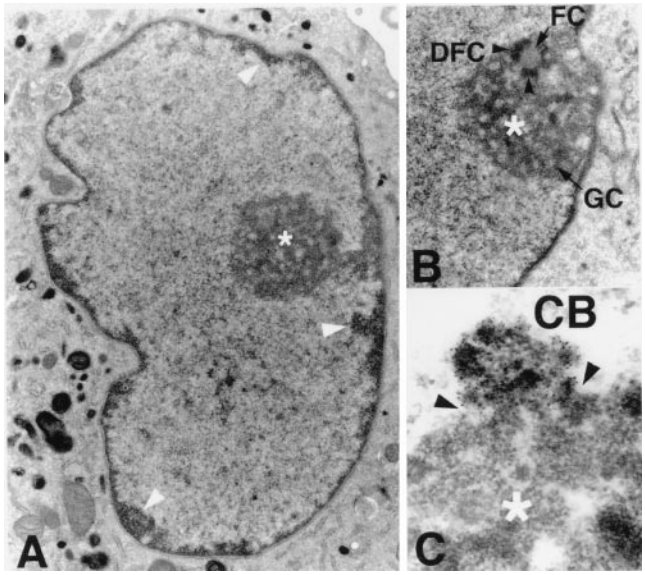

Fig. 2. Electron micrographs showing (**A**) a cross section of a mammalian cell nucleus, (**B**) higher magnification view of the nucleolus, and (**C**) a coiled body (CB) attached to the nucleolar periphery. The nucleolus (asterisk in each panel) is differentiated into the fibrillar center (FC), dense fibrillar component (DFC), and granular component (GC), although there is heterogeneity in nucleolar morphology between different cell types and under different growth conditions and nucleoli also frequently contain heterochromatin and nucleoplasmic vacuoles (*121*). The consensus view is that the FCs contain rDNA, RNA polymerase I, and associated transcription factors, with transcription of rDNA occurring largely at the boundary between the FC and DFC. Nascent rRNA transcripts appear in the DFC and are processed there. Some processing steps may also occur in the GC, together with the assembly of the rRNA into ribosomal subunits. Arrowheads in (A) indicate peripheral heterochromatin. In (C), arrowheads point to fibers connecting the coiled body with the nucleolus. This section has been immunogold-labeled with an antibody to p80 coilin.

nascent RNA (*48*) and disappears when transcription is inhibited. The speckled pattern results from the association of snRNPs with several structures previously visualized by electron microscopy: clusters of dense particles that contain aggregates of snRNPs and other protein splicing factors (interchromatin granules), found in the spaces between more densely staining regions of chromatin (*47*); interchromatin granule–associated zones that flank interchromatin granules and contain U1, but not the other splicing snRNPs (*49*); perichromatin fibrils that are closely associated with active chromatin and contain newly transcribed mRNA precursors and processed mRNA (*50*); and coiled bodies that are fibrillar structures, discussed below (*51*).

We use the term "speckles" specifically to denote the interchromatin granule–related clusters of snRNP staining, and not the overall punctate pattern that includes diffuse nucleoplasmic staining and coiled bodies. The importance of speckles is still being debated; the speckled pattern may be exaggerated by antibody labeling conditions (*52*) or as a result of threshold effects during image analysis (*53*). Nonetheless, interchromatin granule speckles are genuine nuclear structures that can be visualized directly in the electron microscope. Speckles disperse when cells enter mitosis, but snRNPs and protein splicing factors reform into speckle-like structures during telophase, before their reimport into daughter nuclei (*54, 55*). Thus, snRNP speckles can occur in the absence of DNA and transcription.

Cells showing high transcription exhibit more widespread nucleoplasmic localization of RNA processing factors and less speckled staining, whereas reduced transcription is often accompanied by increased speckled staining (*56*). The mRNA from some highly transcribed genes is enriched near speckles (*57, 58*), suggesting a possible role in mRNA transcription or maturation. However, speckles are not major sites of transcription, which occurs in thousands of foci throughout the nucleoplasm (*3, 59–61*). Speckles do not incorporate either tritiated uridine or bromouridine triphosphate (Br-UTP) during pulse-labeling experiments and lack detectable DNA (*3, 50, 59–61*), but they do contain poly(A) RNA [detected with poly(dT) or poly(U) oligonucleotide probes (*62*)]. Speckles may thus be involved in mRNA export (*63*), although it is still unproven whether their poly(A) RNA can be chased to the cytoplasm when transcription is blocked (*58, 64, 65*). An alternative scenario is that speckles function either as depots supplying splicing factors to active gene loci, or way stations accumulating snRNPs bound either to partially spliced pre-mRNA or to excised introns after release of mRNA from the spliceosome (*53, 66*). Some or all of the speckle-associated poly(A) RNA could be structural or aberrant, rather than mRNA bound for export.

The localization of splicing factors is dynamic and involves trafficking between nuclear substructures. An elegant demonstration of this dynamic organization was recently provided by visualizing the splicing factor ASF (or SF2) fused to GFP in living cells (*67*). It seems likely that mRNA precursors are transcribed and processed at active gene loci dispersed throughout the nucleoplasm and that snRNPs and other RNA processing factors cycle between these transcription sites and interchromatin granule speckles. Such cycling may be regulated by protein phosphorylation (*66*), because perturbation of both kinase and phosphatase activities can cause changes in the degree of punctate staining shown by splicing factors (*68*). It remains to be established whether the interchromatin granule speckles simply store inactive factors or participate more actively in one or more steps connected with mRNA maturation and transport.

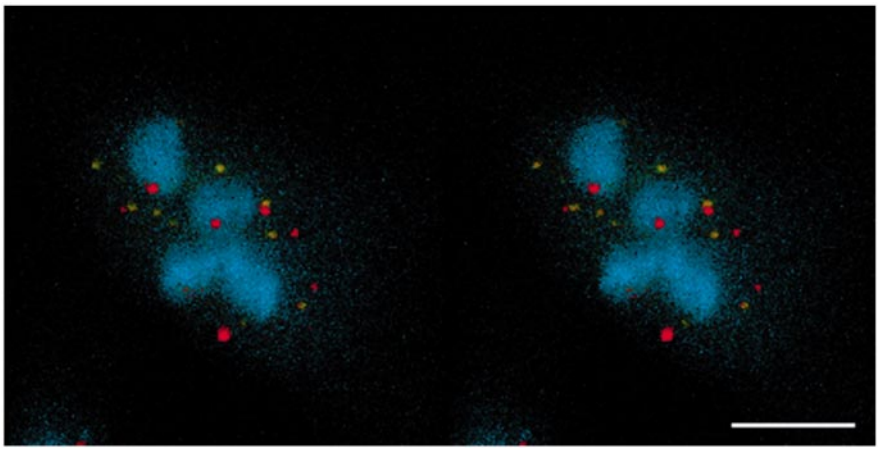

Fig. 3. Stereo view of a triple-labeled HeLa cell nucleus showing the localization of coiled bodies (green, GFP-coilin), PML bodies (red, autoimmune serum recognizing SP100), and nucleoli (blue, anti-fibrillarin). Scale bar, 5 μm.

Coiled Bodies and Gems

Coiled bodies appear in the electron microscope as a ball of tangled threads 0.15 to 1.5 μm in diameter (*69*). They disassemble during mitosis and reform during G_1 phase after transcription is reinitiated (*55, 70*). The coiled body is a highly dynamic structure that may play a role in snRNP transport, maturation, or both. Coiled bodies contain spliceosomal snRNPs (*71, 72*) as well as several nucleolar antigens, including fibrillarin, NOPP140, and U3 snoRNP (*73–75*) and a human autoantigen called p80 coilin (*76*). Coilin is highly enriched in coiled bodies (although it is also present in a diffuse nucleoplasmic pool) and has been widely used as a marker for the coiled body. Coiled bodies vary in number from 1 to 10 or more; they are most prominent in rapidly growing cells (*77*), and their protein composition may differ slightly in different cell types (*78*).

Amphibian oocytes contain nuclear bodies, originally called sphere organelles, that have recently been described as resembling coiled bodies. Like coiled bodies in somatic cells, they contain splicing snRNPs and a protein, called SPH-1, with significant homology to p80 coilin (*79*). They accumulate human p80 coilin when it is expressed in *Xenopus* oocytes (*80*) and can associate with histone gene loci, possibly acting to supply U7 snRNP to these sites (*81*). Spheres (or *Xenopus* coiled bodies) can be assembled in vitro from *Xenopus* egg extracts (*82*). Depletion studies show that sphere-like bodies can assemble without SPH-1, although SPH-1 and Sm proteins may need to interact to allow their mutual assembly into this structure (*83*).

The function of coiled bodies is still unknown. They might be involved in regulating snRNA gene expression; in some cell types, a striking spatial relation exists between gene loci encoding certain U snRNAs and a subset of nucleoplasmic coiled bodies (*72, 84*). However, coiled bodies are probably not sites of coordinated snRNA transcription, processing, and assembly, because they lack nascent RNA and because snRNP particle assembly from snRNA and Sm proteins occurs in the cytoplasm (*85*). Coiled bodies do not contain DNA (*86*), and they are unlikely to be sites of pre-mRNA transcription or splicing because they lack protein splicing factors such as SC-35, nascent pre-mRNA, or poly(A) mRNA (*3, 50, 59–61, 64, 73, 74*). Nor are

they likely to be storage sites for inactive snRNPs, because when transcription and splicing are blocked, coiled bodies disperse and snRNPs concentrate instead in large interchromatin granule speckles. Coiled bodies may, however, play a role in histone mRNA 3′ processing, because they contain the U7 snRNP that cleaves the 3′ terminus of histone pre-mRNA, and a subset of coiled bodies are found adjacent to histone gene loci in some cells (*81, 87*).

A key unexplained feature of coiled bodies is their intimate relationship with the nucleolus. In neuronal cells, which undergo extensive transcription and splicing, coiled bodies [originally called "nucleolar accessory bodies" (*88*)] are often very large (>1 μm in diameter), and electron microscopy studies have shown fibers connecting coiled bodies to the nucleolar periphery (Fig. 2). Coiled bodies have also occasionally been observed within nucleoli of mammalian cells (*89*). The interaction between coiled bodies and nucleoli may be regulated by phosphorylation (*90*). This morphological association of coiled bodies and nucleoli appears to be of genuine functional importance. When transcription is inhibited, nucleoplasmic coiled bodies disappear and p80 coilin forms "caps" on the nucleolar periphery (*71, 72*). The Wilms tumor protein WT1 also relocates into the same perinucleolar caps when transcription is inhibited, which suggests that it may play some role connected with coiled bodies (*91*). Finally, transient expression of a truncated p80 mutant can disrupt both coiled bodies and nucleoli (but not interchromatin granule speckles) (*92*).

Why should snRNPs and coiled bodies interact with the nucleolus? Trafficking of snRNPs to the nucleolus may be important for snRNP maturation, because snRNAs receive many of the same modifications as rRNA, including 2-*O*-methylation and pseudouridine formation (*51, 90*). If the nucleolar activities that modify rRNA also modify snRNAs, coiled bodies may then act as nuclear transport or sorting structures.

Coiled body–like structures called "gems" (gemini of coiled body) (*93*) have recently been shown to be paired frequently with coiled bodies. Sometimes the two structures overlap, and both are similarly affected by cell growth temperature and transcription inhibitors (*93, 94*). Gems contain the SMN (survival of motor neurons) protein, encoded by the gene responsible for a severe inherited form of human muscular wasting disease, spinal muscular atrophy (*93, 95*). The SMN protein interacts with the Sm class of snRNP proteins and (through a separate binding site) with a cellular protein called SIP1 (*96*). The SMN-SIP1 complex plays an essential role in cytoplasmic snRNP biogenesis (*97*). Mammalian SIP1 shows significant homology to an *S. cerevisiae* gene called *Brr1*, which, when mutated, impairs snRNP biogenesis (*98*). Thus, defects in spliceosomal snRNP assembly may be involved in spinal muscular atrophy, and an intranuclear snRNP trafficking pathway may involve interactions between gems and coiled bodies.

PML Nuclear Bodies

PML nuclear bodies [also known as PODs (PML oncogenic domains), Kr bodies, and ND10 (nuclear domain 10) (*99, 100*)] are nuclear domains that are specifically disrupted in human acute promyelocytic leukemia (APL) cells. A typical mammalian nucleus has ~10 to 20 PML bodies, which vary in size from ~0.3 to 1 μm and are tightly associated with the nuclear matrix. The role of PML bodies is unknown. They are not major sites of transcription, they lack snRNPs and protein splicing factors, and they contain little or no replicating DNA during S phase (*101, 102*). Their name derives from their most intensively studied protein component, PML, which is a RING-finger motif protein that was discovered through analysis of the dominant oncogene responsible for APL. The characteristic t(15;17) chromosome translocation in APL cells fuses cellular PML to the retinoic acid receptor alpha (RARα) gene to form the oncoprotein PML-RARα (*103*).

PML bodies in APL cells are disrupted and replaced by a micropunctate pattern in which PML, PML-RARα, and the steroid receptor RXR ectopically associate in many small nucleoplasmic foci (*100, 101, 104*). Few of these microfoci incorporate Br-UTP, which suggests that their major effect on gene expression may be to repress transcription rather than activate it; however, it is possible that they stimulate transcription at some loci. Remarkably, retinoic acid and arsenic trioxide, both of which are used in clinical treatment of APL patients (*105, 106*), induce reformation of PML bodies and trigger degradation of PML-RARα in APL cells in culture (*100, 101, 104, 105, 107*). This points to an intimate relationship between nuclear organization in APL blasts and the malignant phenotype.

In addition to PML protein, PML bodies contain the autoantigen SP100 (*108*) and may also contain the LIM-motif protein NDP52 (*109*), although this has been disputed (*110*). All three proteins are up-regulated upon interferon treatment (*109, 111*). Several viral proteins also associate with PML bodies; infection with herpes simplex virus–type 1 (HSV-1), adenovirus, and human cytomegalovirus disrupts PML bodies, which suggests that they may play some role in cellular antiviral defense (*112*). The HSV-1 immediate early gene product Vmw110 (also called ICP0) associates with PML bodies at an early stage of HSV-1 infection. This protein strongly activates the transcription of many genes in transient transfection assays, and mutations in the *Vmw110* gene severely reduce the ability of HSV-1 to mount a lytic infection (*113*). Vmw110 interacts with a cellular protein called HAUSP, which is a member of the ubiquitin-specific protease family (*114*). In uninfected cells, HAUSP shows a diffuse nucleoplasmic localization, excluding nucleoli, and also labels punctate structures, some of which colocalize with a subset of PML bodies. After HSV-1 infection, HAUSP concentrates prominently in PML bodies, presumably targeted through its interaction with Vmw110.

The PML protein exists in two forms: in a "free" form that is dispersed throughout the nucleoplasm, and as a conjugate with the ubiquitin-like protein SUMO1 [small ubiquitin-like modifier, also called PIC1 (*115*)] (*107, 116*). The PML-SUMO1 conjugate is exclusively localized to PML bodies, suggesting that linkage of PML to SUMO1 may either stabilize or promote the assembly of PML bodies. Consistent with this idea, treatment of cultured cells with arsenic trioxide promotes multi-SUMO1 conjugates to PML and concomitantly enhances PML body formation (*107*). SUMO1 conjugation is reversible and may be regulated by protein phosphorylation.

There is a recent precedent for SUMO1 conjugation promoting association of a soluble protein with a nuclear structure. The protein RanGAP1 is a cytoplasmic guanosine triphosphatase (GTPase)–activating factor for the small GTPase Ran that functions in protein transport through the nuclear pore complex. Although cytoplasmic RanGAP1 is unmodified, a fraction of RanGAP1 that is conjugated to SUMO1 is specifically localized to the nuclear pore complex, where it interacts with the nuclear pore protein RanBP2 (or Nup358) (*117*). SUMO1 is linked to RanGAP1 by a specific lysine residue (Lys^{526}), and the SUMO-conjugated form of RanGAP1 remains stably associated with the nuclear pore complexes during multiple cycles of protein import. For both RanGAP1 and PML, conjugation to SUMO1 appears to direct protein localization rather than degradation. Posttranslational conjugation with SUMO1, and perhaps with other members of the ubiquitin-related protein family, may

thus be a general mechanism for directing protein assembly into specific nuclear structures.

Perspectives

The development of new microscopy techniques and methodologies for analyzing chromosomes and the localization of nuclear factors has helped to stimulate a resurgence of interest in studying the functional organization of the nucleus. For example, chromosome-painting FISH (Fig. 1) has revolutionized clinical cytogenetics by facilitating the mapping of chromosome breakpoints and rearrangements in metaphase chromosome spreads (*118*). Single mRNA transcripts can also now be detected in situ with the use of oligodeoxynucleotide probes containing five fluorochromes per molecule, hence allowing quantitative analysis of transcription from individual gene loci (*119*). However, we still have only a rudimentary knowledge of how chromosomes are organized, or how the metabolic activities that take place within the nucleus relate to the substructures that are revealed by fluorescence and electron microscopy. A major question is to what extent nuclear substructure arises transiently as a consequence of activities such as transcription and replication, and to what extent it reflects the assembly of dedicated "factories" at specific sites in the nucleus (which in turn impose constraints on the location of replication, transcription, and RNA processing events).

The nucleus is a highly dynamic organelle in which the assembly of compartments in response to metabolic requirements of the cell may be a general feature, because many factors appear able to equilibrate between a free nucleoplasmic pool and assembly into large structures. Although nuclear bodies may have more similarity to cytoplasmic structures such as the mitotic spindle and Golgi apparatus than to long-lived organelles such as mitochondria, this does not lessen the potential importance to the cell of using large organizing structures to bring together molecules of related function that will consequently enhance the efficiency of nuclear processes. The currently available data lend support to the view that a nucleus is far from a randomly arranged bag of molecules, but rather functions as an integrated and highly ordered machine, albeit one with a high degree of built-in structural flexibility. We are confident that future studies will illuminate the basic principles underlying nuclear organization and will increase our understanding of how disruptions of this organization contribute to human disease.

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