

retarded, phenotype in which many normal cell fate decisions are reiterated in subsequent larval stages. Examples include mutations in *lin-4* and *lin-29*. Worms with such mutations do not express certain adult cell fates, despite being sexually mature (Figure 1).

Is there conservation in heterochronic gene pathways?

An important regulator of the larval-to-adult transition in worms, *let-7*, is conserved across diverse phyla, having been identified in flies, sea urchins, humans and many other organisms. In addition, it has recently been demonstrated that the *C. elegans* heterochronic gene *lin-57* is a worm homolog of the *Drosophila* gene *hunchback* (hence its new name *hbl-1*). In the developing central nervous system of flies, *hunchback* regulates the temporal patterning of cell fate specification for neuroblasts.

But doesn't hunchback regulate spatial patterning in flies?

Hunchback is indeed best known for its role in anterior–posterior patterning in the early fly embryo; it encodes a zinc-finger transcription factor that acts to establish spatial domains of gene expression. Although downstream effectors are unknown in worms, it is possible that *hbl-1* is able to establish analogous temporal domains of gene expression.

What's the deal with microRNAs and how are they involved in the heterochronic gene pathway?

microRNAs are now appreciated as a new class of small non-coding RNAs. They are likely to act by regulating translation of target mRNAs through binding to sequences in the 3' untranslated regions with partial anti-sense complementarity. When the product of the *lin-4* heterochronic gene was found to be a small, non-coding RNA in 1993, it appeared that it might be a *C. elegans*-specific, molecular oddity. A second small non-coding RNA, *let-7*, was discovered in the heterochronic gene pathway in 2000, followed by the cloning of hundreds of small non-coding RNAs (microRNAs) in 2001 and

beyond, and it is now appreciated that the heterochronic genes *lin-4* and *let-7* are the founding members of a larger microRNA gene family.

Are microRNAs involved in other pathways?

Although the first two microRNAs identified are in the heterochronic gene pathway, there is now evidence for roles of microRNAs in cell proliferation, cell death, fat metabolism and stress resistance in flies.

Is there anything we don't know? The mechanism of action of many heterochronic genes remains to be determined. For example, *lin-14* encodes a novel nuclear protein with no identified downstream effectors to date. In addition, emerging evidence of phylogenetic conservation of heterochronic genes leads to the prospect that mechanisms to control developmental timing will be elucidated in other systems.

Where can I find out more?

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Department of Genetics, Dartmouth Medical School, Hanover, New Hampshire 03755, USA.

Primer

Nuclear substructure and dynamics

Angus I. Lamond & Judith E. Sleeman

The nucleus is the defining feature of eukaryotic cells. It contains the chromosomes and is a site of major metabolic activities, such as DNA replication, gene transcription, RNA processing and ribosome subunit maturation and assembly. The nucleus is separated from the surrounding cytoplasm by a double membrane. The outer nuclear membrane is continuous with the endoplasmic reticulum, or 'rough ER', where the translation of secreted and membrane bound proteins takes place. Thus, the nucleus serves to partition the sites of gene transcription from those of protein synthesis in eukaryotic cells.

Movement of proteins and RNA–protein complexes between the nucleus and cytoplasm occurs continually. mRNAs and newly assembled ribosomal subunits are exported from their sites of synthesis in the nucleus to the cytoplasm. Conversely, as all proteins are synthesized in the cytoplasm, nuclear factors, such as histones, transcriptional regulators and splicing factors, are selectively imported into the nucleus. Some proteins, including hnRNP proteins and transport receptors, shuttle repeatedly between the nucleus and cytoplasm. All of this nucleocytoplasmic exchange occurs via dedicated multiprotein structures located in the nuclear envelope, termed 'Nuclear Pore Complexes' (NPCs).

Like the cytoplasm, the nucleus is compartmentalized (Figure 1). As well as the chromosomes, the nucleoplasm contains numerous classes of 'nuclear bodies', including nucleoli, Cajal bodies (CBs), gems, splicing speckles and promyelocytic leukaemia (PML) bodies. However, in contrast with cytoplasmic compartments, such

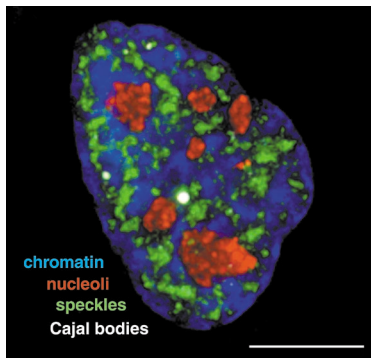


Figure 1. Fluorescence micrograph illustrating the internal compartmentalization of chromatin and nuclear bodies in mammalian cell nuclei. Scale bar = 5 μm

as mitochondria, lysosomes and the Golgi apparatus, the subnuclear compartments lack a membrane separating them from the surrounding nucleoplasm. The accumulation of nuclear factors in distinct nuclear bodies may help to generate a high local concentration of components and, thus, enhance the efficiency of reactions. Furthermore, nuclear compartmentalisation may modulate access of enzymes and receptors to their substrates, or physically separate distinct pools of mature/immature and active/inactive factors. Consequently, specific pathways and mechanisms must operate within the nucleus to control the assembly of nuclear bodies and to target factors to the proper locations.

Chromosome organization

Much of the nuclear volume is occupied by the genome, which is packaged into separate chromosomes. Chromosomes are built up through folding and compaction of the DNA–histone complexes. The nuclear DNA–protein complex is termed ‘chromatin’ and contains a host of additional DNA binding and regulatory factors. Nucleosomes are twisted into a helical 10nm fibre, which is further folded into a 30nm fibre and perhaps into even higher order structures. This high degree of folding is required in order to fit the large quantity of genomic DNA within the volume of the nucleus. For example, a typical human fibroblast cell must accommodate $\sim 6 \times 10^9$ base pairs

of DNA within a nuclear volume of $\sim 500 \mu\text{m}^3$.

In the nucleoplasm, chromosomes are organized into large scale domains called ‘chromosome territories’. Each chromosome territory occupies a predominantly distinct space in the nucleus, hence the different chromosomes are not extensively intermingled, even during interphase when they are in their most decondensed state. Nonetheless, each chromosome can include regions with different levels of compaction, with the most condensed regions usually referred to as ‘heterochromatin’ and the less condensed regions termed ‘euchromatin’. Individual chromosomes can extend between the interior of the nucleus and the nuclear periphery. Due to the looping and unfolding of chromosomes, multiple, non-adjacent segments can localize close together at the nuclear interior or periphery. In general, condensed heterochromatin domains tend to cluster preferentially at the nuclear periphery and around nucleoli, whereas the less condensed euchromatin is located within the nuclear interior. Heterochromatin regions repress gene expression and tend to replicate later in S-phase than euchromatin. Active genes can be located both on the surface of and within chromosome territories. However, there is growing evidence that the nuclear location of genes can, at least in some cases, affect their expression level. Pulse-labelling experiments have shown that transcription by RNA polymerase II takes place at several thousands of separate chromosomal loci that are spread throughout the nucleoplasm rather than in restricted or specialized regions of the nucleus. Most splicing events for intron containing transcripts are thought to take place co-transcriptionally, probably while the mRNAs are still tethered to the site of their transcription.

The nucleolus

Nucleoli are nuclear bodies that form around the tandemly repeated clusters of ribosomal RNA (rRNA) genes. These loci are

termed ‘nucleolar organizer regions’ (NORs). The major function of the nucleolus is to synthesize rRNA and assemble ribosomal subunits. The rRNA genes are transcribed by RNA polymerase I as a large pre-rRNA precursor that is subsequently cleaved to generate the separate 5.8S, 18S and 28S rRNAs found in ribosomes. Each of these rRNAs is also post-transcriptionally modified at both base and sugar residues and assembled with ribosomal proteins to form the large and small ribosome subunits. These are then exported from the nucleolus to the cytoplasm to take part in mRNA translation. The nucleolus, therefore, contains a host of factors responsible for site-specific cleavage and modification reactions during rRNA maturation. It also contains RNA helicases and chaperones that facilitate the correct folding of rRNA and protein assembly during the generation of ribosomal subunits. Recent findings show that factors not obviously connected with ribosome subunit biogenesis, including viral proteins, cell cycle regulators and polyadenylated mRNAs, are also present in nucleoli. This suggests that the nucleolus may have additional biological functions that are still not clearly defined.

When the structure of the nucleolus is viewed in the electron microscope, at least three morphological regions can be distinguished: the fibrillar centres (FCs), which are surrounded by the dense fibrillar component (DFC), and the granular component (GC), which constitutes the remainder of the nucleolus. Separate steps during the maturation of pre-rRNA into ribosomal subunits can be correlated with nucleolar structures. Pulse-labelling studies indicate that rRNA gene transcription occurs either in FCs, or at the FC/DFC boundary, although in some cases transcribed genes may extend into the DFC. Consistent with this model, more FCs are detected in nucleoli when the level of transcription by RNA polymerase I increases. As judged by the concentration of processing factors in this region, much of the

cleavage and modification of rRNAs occurs in the DFC, whereas later steps during protein assembly on the ribosomal subunits occur in the GC.

The nucleolus is a dynamic structure that undergoes cycles of assembly and disassembly during each cell cycle. Following mitosis in telophase nuclei, assembly of nucleoli at NORs is dependent on the production of rRNA transcripts. Subsequently, the nucleoli are stable as long as transcription continues and can be readily isolated as intact structures *in vitro*. Despite this structural stability, recent photobleaching studies show that many nucleolar proteins are not permanently anchored within the nucleolus, but rather show a continual and rapid turnover.

Cajal bodies

The Cajal body (also known as 'coiled body' or CB), is related to the nucleolus, but does not contain rRNA or rRNA genes. Instead, it may act to deliver newly assembled processing factors to nucleoli. It is now named after its original discoverer, Ramon y Cajal, who termed it the 'nucleolar accessory body' as it is often found in association with the nucleolar periphery. Direct visualization of Cajal bodies by time-lapse imaging in cells expressing green fluorescent protein-tagged Cajal body factors shows that they can move to and from the nucleolar periphery. Cajal bodies are also found to associate with specific gene loci, including histone and snRNA genes, but unlike nucleoli they appear not to assemble around defined DNA sequences. They contain a small fraction (usually 1–5%) of the nuclear pool of small nuclear RNPs (snRNPs) that are subunits of spliceosomes. Small nucleolar RNPs (snoRNPs) that modify and cleave pre-rRNA also accumulate in Cajal bodies.

A major function of the Cajal body appears to lie in the maturation of nuclear RNP complexes, including snRNPs and snoRNPs. Newly assembled snRNPs and snoRNPs will accumulate transiently in Cajal bodies, before they later concentrate in either nuclear

speckles, or nucleoli, respectively (Figure 2). The snRNAs and snoRNAs are themselves, like rRNA, extensively modified after transcription. Cajal bodies have been found to contain specific small guide RNAs, termed small Cajal body RNAs (scaRNAs), that target sites for modification on snRNAs via complementary scaRNA/snRNA base pairing. Also consistent with them having a role in RNP maturation, rather than being sites for pre-mRNA splicing, Cajal bodies contain snRNPs but not the other splicing proteins that act in the spliceosome. Like nucleoli, Cajal bodies are suggested to have more than one function and it is possible that they can also modulate gene expression at loci to which they bind.

Most Cajal bodies also contain the survival of motor neurons (SMN) protein. Loss of this protein leads to spinal muscular atrophy (SMA), a fatal inherited degenerative disease in humans. The SMN protein is also localized in the cytoplasm, where it coordinates the assembly of many RNA–protein complexes, including splicing snRNPs. In some transformed cell lines and primary fetal tissues, the SMN protein localizes to another form of nuclear body, called 'gem', rather than to Cajal bodies. The name gem indicates 'Gemini' or 'twin' of the Cajal body, because the two structures are often next to one another and similar in size. In most primary cells and transformed cell lines they appear to be the same structure and it is not clear why they are separate in some cells.

Splicing speckles

Speckles are irregularly shaped structures of varied size that are found in the interchromatin region of the nucleoplasm. In the electron microscope they appear as clusters of interchromatin granules. Speckles are enriched in splicing factors, including both the snRNPs that also are found in Cajal bodies and additional non-snRNP protein splicing factors. Speckles are often located close to actively transcribed genes, but are unlikely to be direct sites of splicing, although this issue remains controversial. It is widely

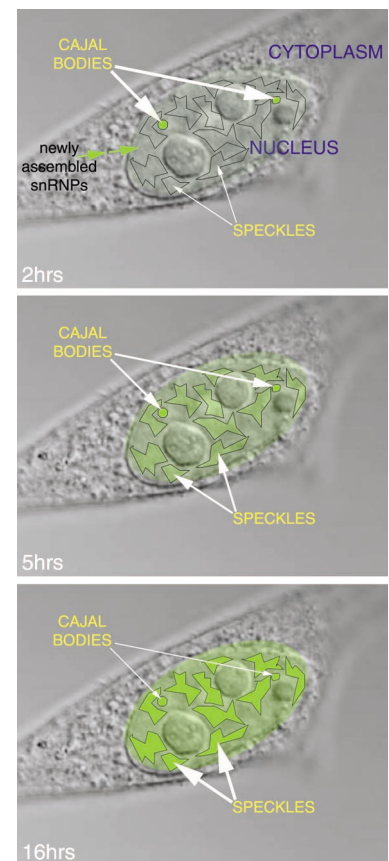


Figure 2. Nuclear trafficking of newly imported snRNPs illustrated in cartoon micrographs.

The GFP-labeled snRNPs accumulate first in Cajal bodies and only later in their steady state speckled and diffuse nucleoplasmic distribution. (For further details see: Sleeman, J.E. and Lamond, A.I. Newly assembled snRNPs associate with coiled bodies before speckles, suggesting a nuclear snRNP maturation pathway. *Curr. Biol.* 9, 1065–1074.)

thought that speckles instead act as reservoirs that supply factors for the splicing of nascent pre-mRNA at nearby genes. Consistent with this view, when transcription is inhibited, and hence splicing is blocked, all the snRNP and protein splicing factors accumulate in enlarged speckles. However, speckles do also contain polyadenylated RNA species and it is still not clear whether these are structural components of the speckles or mRNAs destined for export to the cytoplasm.

Speckles are dynamic structures that disassemble as cells enter mitosis and reform in daughter nuclei. Furthermore, they show peripheral movements and changes in shape during

interphase. The exchange rate for speckle components during interphase is high, with splicing factors moving rapidly between speckles and sites of gene expression. For example, kinetic modeling studies on the splicing factor ASF/SF2 indicate that it has a mean residence time in speckles of ~50 seconds. This cycling behaviour appears to be regulated by reversible protein phosphorylation. Speckles contain multiple protein kinases, such as Clk/STY and hPRP4, as well as forms of protein phosphatase I. It is likely that speckles act as stores for inactive splicing factors, but they may also have other roles, such as participating in the active recycling of spliceosome components and/or RNA transport.

Other nuclear bodies

In addition to the nucleoli, Cajal bodies and speckles discussed above, numerous other classes of nuclear bodies have been identified and are currently being studied. For example, PML bodies are named after their component PML, which is a RING finger protein that was first identified through its role in the dominant PML-RAR α oncoprotein. This is formed through chromosome translocations that fuse the PML and RAR α genes, resulting in the disease Acute Promyelocytic Leukemia (APL). Cells from APL patients show a disrupted micropunctate pattern in place of the usual 10-20 PML bodies. They also show mislocalization of several nuclear proteins, including the steroid receptor RXR. Treatment of APL patients with retinoic acid can cause a reversal of the leukemia and a concomitant restoration of the normal PML body pattern. This establishes a clear link between a molecular disease and subnuclear organization.

It is still not clear which primary role PML bodies play. It is possible that they have a role in anti-viral responses. As is true for all forms of nuclear bodies studied so far, PML and other PML body components exist in a diffuse nucleoplasmic pool as well as within the bodies. Several components of the PML body are conjugated to the post-

translational protein modifier SUMO-1 specifically when they are localized in PML bodies and not in the diffuse pool. It is possible that a range of reversible protein modifications, including SUMOylation and phosphorylation, may act to modulate binding interactions that determine the distribution of different factors between the nucleoplasmic and nuclear body pools.

A recently described nuclear body is the 'clastosome', which is enriched in 26S proteasome components, ubiquitin and proteasome substrates. Clastosomes appear specifically during high levels of proteasome activity and, conversely, disappear when cells are exposed to proteasome inhibitors. Therefore, clastosomes may act as major nuclear sites for localizing ubiquitin-mediated protein degradation.

Paraspeckles are another distinct class of nuclear bodies. They resemble splicing speckles but do not contain snRNPs or protein splicing factors. Instead, they contain a group of RNA binding proteins that cycle continually between nucleoli and paraspeckles. This cycling is transcription-dependent, and in the absence of RNA polymerase II transcription the paraspeckle components p54/nrb, PSP1 and PSP2 accumulate at a cap-like structure on the periphery of the nucleolus.

Dynamics in the nucleus

Many aspects of nuclear organization exhibit dynamic properties. Both nuclear bodies and individual chromatin regions can move through the nucleoplasm. Certain nuclear bodies are able to assemble and disassemble from soluble components in response to the metabolic demands of the cell. The cycling behaviour of nuclear factors and the rapid turnover of nuclear body components also underlines the importance of measuring the dynamic properties of proteins and RNPs when interpreting both localization patterns and their possible functional roles. Static fluorescence or electron microscopy images usually indicate

steady state accumulations of nuclear factors, but can fail to reveal sites where factors localize transiently. Recent work has also shown that some nuclear proteins and RNPs participate in directional trafficking pathways, moving in a temporal sequence between distinct classes of nuclear bodies. For example, the nucleolar snoRNP protein NHPX/p15.5kDa transiently accumulates in splicing speckles before adopting a steady state concentration in nucleoli and Cajal bodies.

Our understanding of the dynamic organization of the cell nucleus is expanding rapidly and this is facilitated by the increasing range of new methods to study the composition, structure and movement of nuclear bodies and their components. In the case of nucleoli, NPCs, speckles and Cajal bodies, they can be purified and their protein components can be identified using mass spectrometry. The ability to label nuclear structures *in vivo* through the expression of fluorescent protein-tagged factors also now allows many dynamic parameters to be measured directly in living cells using photobleaching techniques. Further developments in quantitative fluorescence microscopy and quantitative proteomics will help to develop a more robust model of how the nucleus is organized and should inform our view as to how nuclear structure influences cellular function.

Further reading

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