

Nucleolus

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The nucleolus is a nuclear substructure where the genes for three of the four ribosomal RNAs are transcribed and where ribosomal subunits are assembled.

Introduction

Most eukaryotic cells contain one or more prominent regions within the nucleus called nucleoli. They have a substantially different refractive index from the rest of the nucleus and thus are clearly seen by methods such as phase-contrast or differential-interference contrast microscopy (Figure 1a). They also stain differently from the rest of the

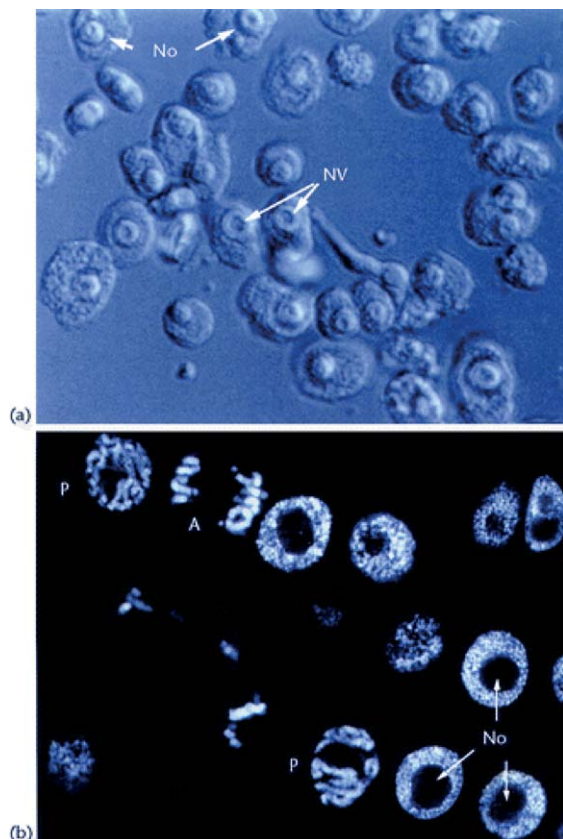


Figure 1 The nucleolus as visualized by optical microscopy. (a) Isolated tobacco nuclei visualized by differential-interference contrast microscopy. The nucleoli are clearly seen as prominent bodies inside each nucleus (No). Within many nucleoli, nucleolar vacuoles or cavities can be seen (NV). (b) Pea root tissue stained with the DNA dye DAPI and imaged by confocal microscopy. The nuclear chromatin is brightly stained, whereas the nucleoli are visible as dark unstained regions within the nuclei (No). The nucleolus begins to break down during prophase (P), and disappears during mitosis. A cell at anaphase (A) is present in this micrograph.

Advanced article

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nucleus with a variety of nucleic acid stains (Figure 1b). Nucleoli vary in size in different cells, from 1 μm diameter or less in small cells such as yeast cells, to 10 μm or more in large cells such as pea and wheat root cells. It is now known that nucleoli are the sites of transcription of the ribosomal ribonucleic acid (rRNA) genes – multiple tandem copies of the sequences encoding three of the four RNA species present in ribosomes – and of the biosynthesis of the large and small preribosomal subunits. These multiple tandem copies of the rRNA genes are present at one or more chromosomal sites called nucleolar organizer regions (NORs). The NORs are the sites on the metaphase chromosomes where nucleoli become organized when the postmitotic cell reinitiates transcription as it enters interphase. They are usually visible as secondary constrictions or narrowing of the metaphase chromosomes, and there is a tendency for the NORs to be close to telomeres on the chromosome arms. (The major, primary constrictions are the chromosome centromeres.) The number of NORs varies among species, with little apparent reason. **See also:** Cell structure; Ribosomal RNA

Cells require a huge number of ribosomes; it has been estimated that some cells contain several million. This represents an enormous investment in biosynthetic activity and an immense flux of material into and out of the nucleus. An active cell might divide in less than 24 h and thus producing enough ribosomes for each daughter cell means synthesizing more than a hundred each second. This is the fundamental reason why so many copies of the genes are needed – a single gene copy could not possibly be transcribed fast enough. Even so, each active rRNA gene is loaded with many RNA polymerase molecules, and may complete one or more transcripts each second. Each ribosome subunit must be exported from the nucleus through a nuclear pore, and again this means that each nuclear pore is passing out subunits at a rate of one or more per second. (One estimate put the export of ribosomal subunits as high as 40 per second per pore in exponentially growing yeast cells.) Furthermore, besides the rRNAs, each ribosome

contains many different proteins, which are made in the cytoplasm and are imported through the nuclear pores. The flux of material in and out of the nucleus required to supply the cell with ribosomes may well outweigh the transport of all other proteins and messenger RNAs. As a result, the efficiency of ribosome biosynthesis is likely to be of great importance to an organism's fitness. Subtle factors that increase this efficiency may well have evolutionary advantages, while being difficult to quantify in a laboratory setting.

rDNA Organization

In prokaryotes such as *Escherichia coli*, there are three ribosomal RNAs (16S, 23S and 5S), which are organized as a single transcription unit. In all eukaryotes studied so far, the organization of the ribosomal RNA genes is recognizably similar to that of prokaryotes, but with major differences: the size of the small subunit RNA (s-rRNA) has increased from 16S to 18S, and that of the large subunit (l-rRNA) has increased from 23S to 28S; a new small 5.8S rRNA has become interspersed between the s-rRNA and the l-rRNA; and the 5S rRNA has become separated from the other RNAs in a different transcription unit (Hadjilov, 1985). The former transcription unit is generally simply referred to as the rRNA gene or the ribosomal DNA (rDNA). The separation of the 5S genes is intriguing and so far unexplained. In *Saccharomyces cerevisiae* and some

other lower eukaryotes, the 5S gene is still contained within a single rDNA repeat unit along with the other rRNA genes, but is transcribed in the opposite direction from the other DNA strand. In most eukaryotes, the 5S genes have become completely separated from the rDNA repeats at entirely different chromosomal sites and are located within the nucleoplasm rather than the nucleolus. 5S genes are transcribed by a different RNA polymerase from rRNA genes (RNA polymerase III rather than RNA polymerase I). The resulting 5S RNA molecules must be imported into the nucleolus and their transcription must be coordinated with the transcription of the rDNA. **See also:** Eukaryotic ribosomes: assembly; Phylogeny based on 16s rRNA/DNA; Translational components in prokaryotes: genetics and regulation

There are generally more repeats of the 5S sequences than that of the rDNA, but in both cases there are huge variations in the number of repeats in different species. The human genome contains about 100 rDNA copies per haploid set, which is a relatively small number, in common with other mammals. Many other species, including most plants, have several thousand copies. Very closely related species or different lines within a species can differ greatly in rDNA copy number, and there can be differences between individuals, or even between cells in a single organism. In some cells, notably oocytes, a requirement for many more ribosomes than in the other cells in the organism is met by amplifying the rDNA with many extrachromosomal copies (Hadjilov, 1985). **See also:** Genome organization: human

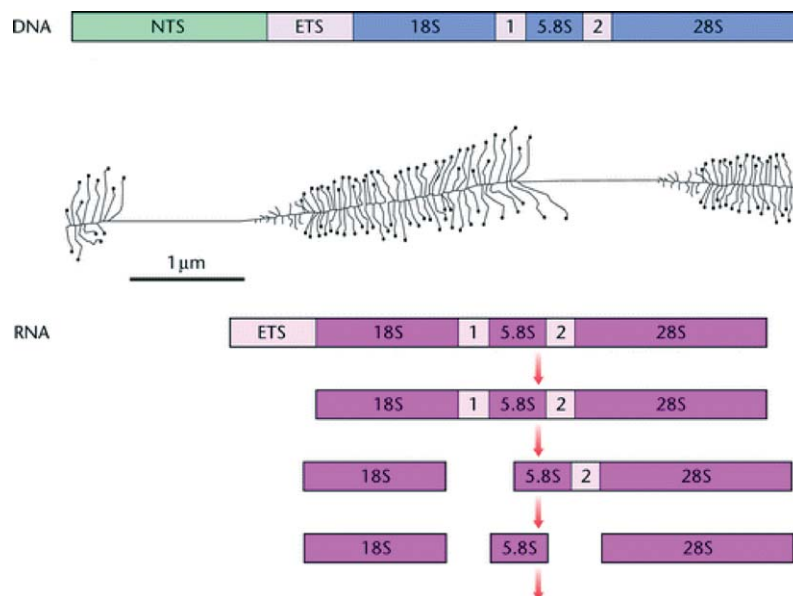


Figure 2 Miller spread of ribosomal DNA (rDNA) transcription units ('Christmas trees'), redrawn from an original micrograph. A diagram of the organization of a single repeat unit of the rDNA is shown above. A diagram of the initial pre-rRNA 45S transcript, and of its processing pathway to three of the mature rRNAs is shown below. NTS, nontranscribed spacer; ETS, external transcribed spacer; 1, internal transcribed spacer 1; 2, internal transcribed spacer 2; the three mature rRNAs encoded by the rDNA are 18S, 5.8S and 28S.

The overall structure of the rDNA repeat is conserved among all eukaryotes (Figure 2) (Hadjiolov, 1985). The individual transcription units are separated by an intergenic spacer, which is generally untranscribed, and which is often referred to as the nontranscribed spacer (NTS). The rRNA-containing region is transcribed to give a precursor, the 45S pre-rRNA, which is processed in a series of post-transcriptional modifications to the mature rRNA species (Figure 2). The pre-rRNA contains a relatively long 5' leader sequence – the 5' external transcribed spacer or 5' ETS (often simply called the ETS) – followed by the 18S s-rRNA. Two internal transcribed spacers, ITS1 and ITS2, flank the 5.8S rRNA, followed by the 28S 1-rRNA, and finally a very short 3' external transcribed spacer (3' ETS). Although the rRNA regions themselves are well conserved right across the phylogenetic range, the spacer regions are highly divergent, so that there is often little homology even between quite closely related species. The size of the NTS varies from between 2 and 3 kb in most plants and *S. cerevisiae*, to 20–30 kb in vertebrates. The transcribed spacers are larger in vertebrates than in other species (particularly the ETS in mammals, and ITS1 and ITS2 in birds). **See also:** RNA editing

Structure of the Nucleolus and rDNA Transcription

Miller spreads

Actively transcribing rRNA genes were first visualized by Miller and colleagues in the 1960s (Miller and Beatty, 1969). They lysed cells with a simple detergent treatment and spread out active rDNA on to electron microscope grids. The classic pictures that they obtained are now in many textbooks. In the best images, the line of a single DNA molecule can be clearly seen, with many polymerase molecules attached to successive transcribed regions (typically 50–100 polymerases for each transcription unit – see Figure 2). Nascent RNA strands can be seen emanating from each polymerase, the length of the strands increasing for polymerases further along the gene. Often 'knobs' are seen at the end of the strands, corresponding to the binding of proteins and the formation of folded pre-rRNP particles. Because of their characteristic appearance, these rDNA transcription complexes have been called 'Christmas trees'. Despite these beautiful and readily interpretable images, it is only recently that this view of the rDNA transcription complexes has begun to be reconciled with the images of nucleoli obtained by thin-section electron microscopy (EM) of intact cells.

Optical microscopy

Various different structural approaches using both electron and optical microscopy have been used to determine

the functional organization of transcription and the subsequent stages of ribosome biogenesis within the nucleolus.

When nuclei are stained by fluorescent dyes that bind strongly and specifically to DNA, the nuclear chromatin is brightly stained, whereas the nucleoli are generally almost unstained (Figure 1b). Detailed three-dimensional microscopy using a sensitive camera and image processing or confocal microscopy sometimes shows faint traces of fluorescence within the nucleolus, which must correspond to rDNA. This demonstrates that, although the nucleolus probably contains the highest concentration of active genes in the nucleus, active genes are decondensed and spread out. The brightly stained chromatin that is revealed by the fluorescent DNA dyes must mostly represent the condensed, inactive DNA in the nucleus. **See also:** Eukaryotic ribosomes: assembly

Electron microscopy

Although difficult to interpret at the molecular level, standard thin-section electron micrographs of most nucleoli show recognizable substructures (Figure 3). Many nucleoli contain small, lightly staining regions, typically a fraction of a micrometre across, which often have a fibrillar appearance. They have been termed fibrillar centres (FCs). Often, particularly in the mammalian nucleoli that have been studied, the FCs are surrounded by a more densely staining fibrillar material, called the dense fibrillar component (DFC). The rest of the nucleolar volume is filled with closely packed particles, assumed to be preribosomal particles. This region has been termed the granular component (GC). Nucleoli, particularly in plants, sometimes have a central clear region, often called the nucleolar vacuole or cavity (Figure 3d and Figure 1a). Condensed chromatin is often seen at the periphery of nucleoli, and sometimes within the different nucleolar regions. In some plant cells, there are large FCs containing subregions of condensed chromatin, which have been called heterogeneous FCs. In the yeast *S. cerevisiae*, the nucleolus is much less distinct than in higher eukaryotes. It consists of a crescent-shaped region of the nucleus, appressed to the nuclear membrane. Granular and fibrillar substructures are visible by thin-section EM, but it is difficult to correlate the features seen directly with those in higher eukaryotic nucleoli. **See also:** Electron microscopy

Features resembling these defined regions have been identified in the nucleoli of most species and cell types, but there is considerable variability, and the components can be difficult to distinguish. It should also be kept in mind that the structures seen are the result of rather unspecific staining reactions with the various heavy-metal salts used. Since it has not yet been possible to identify supramolecular complexes directly by their structure in thin sections of most nucleoli, the features seen have been classified only on the basis of density of heavy-metal staining and texture (granular or fibrous). Although such features probably correspond broadly to the

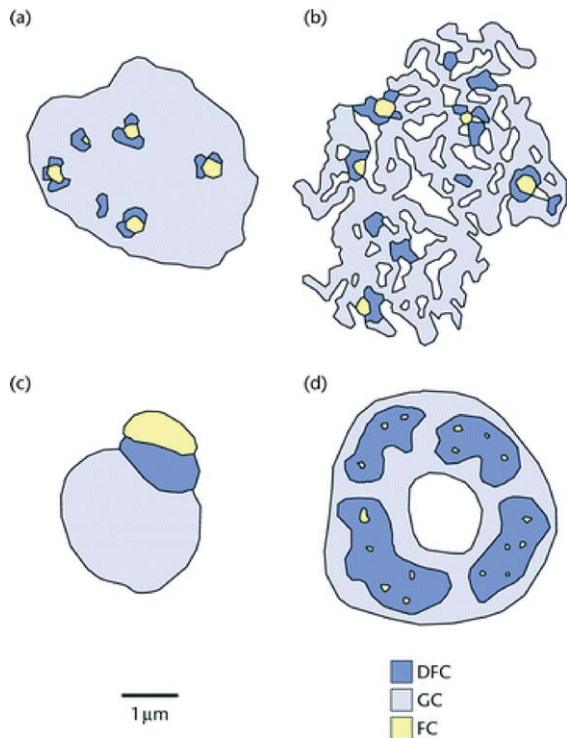


Figure 3 Diagrams of nucleolar ultrastructure seen by conventional electron microscopy. (a)–(c) Nucleoli from a mammalian cell culture under different growth conditions. (a) Three well-differentiated types of structure can be seen; lightly staining fibrillar centres (yellow), surrounded by regions of dense fibrillar component (darker blue). Most of the volume of the nucleolus is filled with particles – the granular component (lighter blue). (b) In a cell undergoing rapid growth and division, the nucleolus is often irregular and reticulated or stranded in appearance. (c) In arrested or inactive cells, or cells where transcription has been inhibited by drug treatment, the three components can become segregated into large blocks. (d) In a typical plant cell nucleolus, the DFC occupies a much larger proportion of the nucleolus, and can often only be distinguished from the granular component by a different texture. Fibrillar centres are usually small and dispersed throughout the DFC, and there is often a central nucleolar cavity or vacuole.

presence of different proteins and other components in different regions of the nucleolus, it seems inherently unlikely that such general features will correlate in detail with the different steps in ribosome biosynthesis.

Specific labelling

Specific labelling methods – fluorescence methods at the optical level and immunogold methods at the EM level – have been used extensively to define the functional organization of the nucleolus in more detail. It has been shown by immunogold EM that the FCs in mammalian cells contain concentrations of RNA polymerase I (Scheer and Rose, 1984). However, it is not clear how much of the polymerase at these sites is active; RNA polymerase I is also seen concentrated at the NORs during mitosis, when no transcription occurs. **See also:** Chromosome mechanics

Fluorescence *in situ* hybridization coupled with three-dimensional confocal microscopy has been used to show the arrangement of the rDNA within nucleoli. In mammalian nucleoli, this technique typically shows an arrangement that has been likened to ‘beads on a string’ – a number of small, bright foci connected by regions of fainter labelling. In plant nucleoli, the labelling pattern is more complex; there are usually large, bright knobs of labelling at the nucleolar periphery, which correspond to many inactive, condensed copies of the rDNA. Within the plant nucleolus are many faint foci of labelling interspersed with a number of brighter foci. The brighter internal foci probably also correspond to multiple condensed rDNA copies, probably largely inactive, and they may represent the chromatin within the heterogeneous fibrillar centres (see above).

However, to determine where the active transcription units are it is necessary to use a method to detect the nascent RNA itself. The factors necessary for rDNA transcription – RNA polymerase I and other proteins, or rRNA genes themselves – are certainly present in both active and inactive forms and locations. Nucleolar transcription has been visualized in both plants and animals directly by incorporating the labelled RNA precursor bromouridine triphosphate (BrUTP), and subsequently detecting it with antibodies (Hozak *et al.*, 1994). Comparison of *in situ* fluorescence and BrUTP results has shown that in plant cells the fainter rDNA foci present correspond to transcription sites. High-resolution EM immunogold labelling on serial sections has shown that the majority of sites, of which there may be several hundred in an active cell, represent single copies of the gene, dispersed through the region of the nucleolus corresponding to the DFC. The detailed immunogold labelling patterns suggested that each transcription unit was in the form of a condensed ‘Christmas tree’ about 300 nm in length (Gonzalez-Melendi *et al.*, 2001). In mammalian cells, the transcription sites revealed by BrUTP labelling are also seen as distinct foci within the DFC region, or at the border between the DFC and FC regions. In human HeLa cells, detailed EM immunogold labelling showed clusters of labelling within the DFC, which were interpreted as sections through compacted transcription units (Koberna *et al.*, 2002), in good agreement with the plant results. EM analysis of the nucleoli of grasshopper oocytes, which contain many amplified, extrachromosomal copies of the rDNA, has also shown structures very reminiscent of condensed Christmas trees – lines of particles packed around a central axis, each line a maximum of approximately 0.4 μm in length and about 0.1 μm in diameter (Scheer *et al.*, 1997). **See also:** DNA synthesis: autoradiography and BrdU staining; Fluorescence *in situ* hybridization

Models for rRNA transcription units

The molecular organization of the transcription units must overcome some severe topological problems in the

arrangement of the genes, attached polymerase molecules and nascent transcripts. Miller spreads of rDNA transcription units show structures several micrometres in length, whereas there is now good experimental evidence that, *in vivo*, transcription units are a fraction of a micrometre in size. Thus, the native structure must be highly condensed and closely packed compared to the spread preparations. There are two fundamental physical mechanisms by which one could envisage transcription occurring: in the first, a polymerase would attach at the beginning of the gene and then travel along the gene as it catalysed the growth of the RNA transcript; the second idea is that the polymerase could be fixed, and the gene, on a mobile loop of DNA, could pass through the polymerase. In either case, there must be relative rotation of the DNA and the polymerase during transcription, since DNA is a helix. In the former mechanism, the polymerase would have to rotate around the fixed gene, carrying the nascent RNA with it. In the latter mechanism, the polymerase and transcript would be stationary, and the gene would rotate as it moved through the polymerase. In either case, topoisomerases would be necessary to relieve torsional stresses in the DNA. For rDNA transcription, the high loading of 50–100 polymerase molecules on to the gene would cause more severe packing problems. In one model of rDNA transcription in animal nucleoli, it has been suggested that many polymerase molecules aggregate to form what are seen as FCs, and that several genes are wound around this mass. The polymerases are envisaged as moving, snake-like, around the outside of the polymerase aggregate, while nascent transcripts are spun off into the surrounding space (Hozak *et al.*, 1994). **See also:** RNA polymerases: subunits and functional domains; Topoisomerases

There is no direct experimental evidence at the moment to decide whether the DNA moves through the polymerase or the polymerase tracks along the DNA; either hypothesis would fit the structural data outlined above. However, it is clear that rDNA transcription units are not necessarily associated with FCs, although they may be in some species and cell types. In particular, in plants the transcription units are widely dispersed into single-gene units within the extensive DFC. In animal cells, such as HeLa cells, the transcription units are more densely packed into the smaller volume of DFC, and the DFC regions are often adjacent to FCs. It is probable that a large part of the structural organization of the nucleolus is built up around the scaffolding provided by the transcribed genes, and elaborated by the biochemical events of ribosome biosynthesis (Melese and Xue, 1995). Nevertheless, nucleoli from different cell types and different species show clear and consistent differences in organization, despite the fact that they all carry out essentially the same biochemical processes, and we currently have little idea of why this is the case.

One clear difference between plant and vertebrate rDNA is the size and structure of the intergenic spacer regions (NTS). Besides containing upstream transcriptional enhancer sequences, these regions include various repetitive sequences, and may well be important in modulating the expression of the neighbouring coding regions, or in determining the larger-scale organization of transcription units, or both. They may be involved in anchoring the genes to protein structures within the nucleolus in a manner similar to scaffold attachment regions of nuclear, polymerase II-transcribed genes. Thus, differences in the NTS regions in different organisms may give rise to different supramolecular arrangements of the rDNA transcription complexes. **See also:** Genes: definition and structure; RNA polymerase II holoenzyme and transcription factors

Posttranscriptional Steps

After transcription of the 45S pre-rRNA, it is cleaved in a number of stages to mature 18S, 5.8S and 28S rRNAs (**Figure 2**). The cleavage pathway has been studied in some detail, particularly in yeast. The first cleavage removes the leader 5' ETS sequence. In yeast and mammals, successive portions of the ETS are removed in at least two separate steps. This cleavage occurs immediately after transcription, and may even begin before completion of transcription. This is followed by cleavages in ITS1, which produce 18S rRNA, and then cleavages to give 5.8S and 28S rRNAs. The initial endonucleolytic cleavages at defined sites are followed by exonucleolytic trimming to the correct ends for the mature rRNAs. The different processing steps are organized into successive enveloping layers surrounding the transcription sites (**Figure 4**). Thus, an *in situ* probe, which detects the ETS sequence on the pre-rRNA, labels nucleolar regions surrounding the transcription sites. In plant nucleoli this includes most, but not quite all, of the DFC. In mammalian nucleoli, individual probes to the different parts of the ETS show that the two steps of cleavage themselves occur in successive zones around the transcription sites. A probe to the ITS1, the next sequence to be excised, in turn labels the nucleolar zone surrounding the ETS cleavage zone; in plant nucleoli, this corresponds to the granular component (**Figure 4**). This shows that ITS1 excision, and all the subsequent events of ribosome biogenesis occur in this region of the nucleolus. Thus, the functional organization of the nucleolus can be regarded as layers enveloping the transcribing genes in a series corresponding to the temporal series of biochemical pre-rRNA processing steps (Beven *et al.*, 1996). Each individual transcript must move outward from the transcription site, undergoing different modifications as it travels away from the gene. **See also:** rRNA structure; RNA editing; RNA editing: evolutionary implications

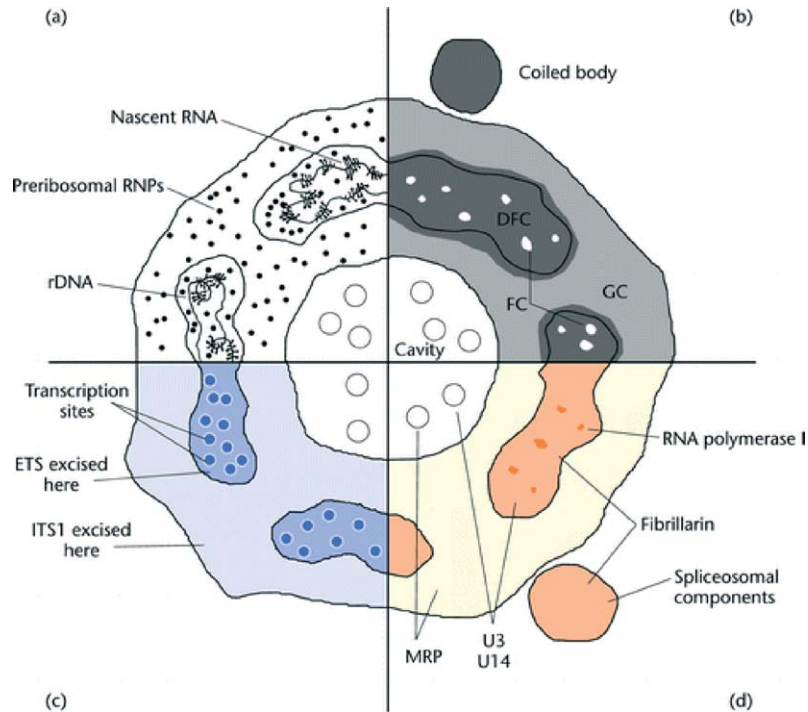


Figure 4 Different views of nucleolar organization. A typical plant nucleolus is illustrated diagrammatically. To give a common reference structure, an outline corresponding to the nucleolar region labelled by a probe to the external transcribed spacer (ETS) portion of the pre-rRNA is shown. This corresponds to most, but not quite all, of the DFC. (a) Possible model for the organization of rDNA transcription units within the nucleolus. RNP, ribonuclear particle. (b) Nucleolar structure seen by conventional thin-section EM. DFC, dense fibrillar component; FC, fibrillar centre; GC, granular component. (c) Organization of transcription sites and zones of transcript processing. ITS1, internal transcribed spacer 1. (d) Localization of some nucleolar proteins and small nucleolar RNAs.

Small Nucleolar RNAs

Various small nucleolar RNAs or snoRNAs, for example U3 and U14, are needed for the cleavage steps, each snoRNA being required at a specific step or steps. This is similar to the requirement for small nuclear RNAs (snRNAs) in mRNA splicing. Specific nucleolar proteins must also be required for these processing stages. The nucleolar protein fibrillarin has been well characterized biochemically, and associates with many snoRNAs. However, defining the precise role of the various nucleolar proteins has proved difficult, and is still a subject of intense research. Both U3 and U14, like fibrillarin, are localized to the DFC. This is consistent with their role in early cleavage events and the layered model of the nucleolus described above. Another snoRNA called MRP has been shown to be involved in ITS1 cleavage, and, again in confirmation of the layered model, has been shown in plants at least to be located in the GC (Figure 4). Several snoRNAs, along with some spliceosomal components, and Cajal bodies (see below) have also been seen in the nucleolar cavity. The significance of this is uncertain, but it may suggest a role for this part of the nucleolus in transport or processing of

these components. **See also:** mRNA splicing: role of snRNAs; snoRNAs: biogenesis, structure and function; Spliceosomal machinery

In addition to the snoRNAs that have been shown to play a role in pre-rRNA cleavage, many other snoRNAs have been discovered. All snoRNAs so far characterized, except MRP, can be categorized into two classes – box C/D and box H/ACA – according to conserved sequence elements and the way they are assumed to fold into defined secondary structures. It has been shown that the function of most snoRNAs is to guide the enzymes that catalyse posttranscriptional modifications of specific rRNA bases to the correct sites (Brown *et al.*, 2003; Kiss, 2002). The most common base modifications of rRNAs are 2'-O-ribose methylation and uridine isomerization to pseudouridine. There are about 100 modifications of each kind in most higher eukaryote rRNAs. Box C/D snoRNAs are the guides for the methylations, and box H/ACA for the pseudouridylation. There is now good evidence that fibrillarin is the methylase that is guided by the box C/D snoRNAs. The cognate pseudouridylylase for the box H/ACA snoRNAs is thought to be *cbf5p* in yeast (Dyskerin in higher eukaryotes). It was originally thought that all these base

modifications occur cotranscriptionally, but localization of the guide snoRNAs suggests that some of them may occur later. The role of the modified bases can now be analysed by deletion of specific snoRNAs to abolish individual modifications, but so far it is not clear what their purpose is; they are presumed to modulate or improve the efficiency of ribosomal activity or biosynthesis.

The transcription of snoRNAs is itself interesting. Although the more abundant snoRNAs are transcribed from their own promoters, in vertebrates and yeast many snoRNAs are encoded within introns of other genes, often but not always ribosomal or nucleolar protein genes. An extreme example is the vertebrate UHG gene, which carries a series of introns encoding several snoRNAs, but has no open reading frame; it is therefore transcribed purely for its introns. After mRNA splicing, the introns are then further processed to produce the mature snoRNAs. In contrast, many plant snoRNAs are transcribed as polycistronic precursors, each containing several different snoRNA sequences. These precursors are imported into the nucleolus and cleaved there into the individual snoRNAs. **See also:** RNA synthesis

Nucleolar Proteins and Cajal Bodies

The nucleolus contains many different proteins, some of which are also present in other cellular locations, others of which are specific to the nucleolus. They include the ribosomal proteins, the proteins involved in transcription of pre-rRNA and its subsequent processing, and many other enzymes such as methylases, topoisomerases, nucleases, kinases and phosphatases. rDNA transcription is catalysed by RNA polymerase I, a complex of about a dozen protein subunits. Initiation of transcription requires the coordinated activity of a series of transcriptional activators and binding factors, which are now being characterized in detail. Two of the most abundant nucleolar proteins are nucleolin and fibrillarin. Fibrillarin, now known to be the box C/D associated methyl transferase, is located in the DFC, from which it derived its name (the yeast homologue is Nop1p). Nucleolin has been implicated in several different stages of ribosome biogenesis, and has been located in both DFC and GC. Different studies have suggested roles in transcriptional activation, in ribosome maturation and as a helicase. Thus, it may have several different functions, or may act as a chaperonin-like factor in facilitating RNA folding and RNA–protein interactions in ribosome maturation. Several nucleolar proteins, including nucleolin, have been shown to shuttle back and forth between the nucleus and the cytoplasm, and some, again including nucleolin, have been shown to bind to the nuclear localization sequences (NLS) of other nuclear proteins. It is possible that these proteins are involved in nucleolar/cytoplasmic transport processes. **See also:** Chaperones, chaperonins

and heat shock proteins; Eukaryotic ribosomes: assembly; Nuclear–cytoplasmic transport; Protein–RNA interactions

New methods for the analysis of complex protein mixtures by mass spectrometry are now being applied to the nucleolus and ribosome biogenesis. In one recent study, nucleoli were purified from human culture cells and analysed by mass spectrometry (Andersen *et al.*, 2002). Over 400 proteins were identified, including both known nucleolar and ribosomal proteins, proteins of unknown function and proteins not previously recognized as nucleolar proteins. In other studies (e.g. Bassler *et al.*, 2001), pre-ribosomal particles have been purified by tandem affinity (TAP) tagging of specific proteins, and analysis by mass spectrometry. This type of study promises to complete the catalogue of nucleolar proteins that will be necessary for a full description of the structure and function of the nucleolus.

Many nucleolar proteins, including fibrillarin, have been found in subnuclear bodies called Cajal bodies (formerly called coiled bodies). These bodies, which were originally called nucleolar accessory bodies, are seen within the nucleus, often associated within the nucleolus at the nucleolar periphery, or even inside it or in the nucleolar cavity. They also contain components of the mRNA splicing apparatus, such as the snRNAs U2 and U6 and associated proteins. Cajal bodies have been shown to be dynamic subnuclear structures, moving within the nucleus, fusing and budding (Boudonck *et al.*, 1999). Their function is still being investigated, but it seems likely that they are involved in processing or recycling of nucleolar and spliceosomal components.

Formation of the nucleolus

Prokaryotes synthesize ribosomes in much the same way as eukaryotes, so why do eukaryotes invariably have a nucleolus whereas prokaryotes do not? One possible explanation is that the formation of a distinct nucleolus is a consequence of having multiple tandem repeats of the rDNA. In support of this idea, mutants of *S. cerevisiae* have been constructed that lack chromosomal rDNA repeats, and instead transcribe rDNA from a plasmid using RNA polymerase II. These mutants make functional ribosomes but fail to organize a normal nucleolus. On the other hand, the nuclei of certain cells, such as oocytes, contain amplified extrachromosomal rDNA copies, and these are located and transcribed by RNA polymerase I in the nucleolus. Thus, the formation of a nucleolus requires copies of rDNA, whether in tandem arrays on chromosomes or not, to be transcribed by RNA polymerase I. The best current hypothesis for the existence of rDNA in tandem repeats, and thus of a distinct nucleolus, is that this has the effect of concentrating all the factors necessary for ribosome biosynthesis into a restricted nuclear compartment. This may increase the overall efficiency of the various

biochemical processes. Since the supply of ribosomes is such a huge investment for a cell and can so easily become a limitation to cell growth, any increase in the efficiency of ribosome biosynthesis is likely to have a significant selective advantage. **See also:** Bacterial ribosomes: assembly; Eukaryotic ribosomes: assembly

During mitosis, transcription ceases and the nucleolus disassembles. The GC is lost first, followed by the DFC, with different proteins leaving the nucleolus in a more or less defined progression. RNA polymerase I and many of the other proteins involved in transcription remain associated with the inactive rDNA at the NORs of the mitotic chromosomes; this is probably the reason why NORs appear as chromosome constrictions. Some nucleolar proteins are dispersed through the mitotic cell, while others are distributed around the periphery of the mitotic chromosomes. Polymerase I transcription is halted at mitosis and is restarted at telophase/G₁ by a cycle of dephosphorylation and rephosphorylation of one or more transcription factors. Unprocessed pre-rRNA transcripts persist through mitosis, showing that transcript processing is also halted. Reformation of the nucleolus takes place in two stages. First, small bodies called prenucleolar bodies (PNBs) are formed late during mitosis. These aggregate around the NORs as transcription is reinitiated, and then fuse to form new nucleoli. In cells containing several active NORs, each forms a nucleolus, and these smaller nucleoli often fuse together as interphase progresses. If transcription is inhibited, or if no NOR is present, for example in an aneuploid cell, no nucleolus is formed and the PNBs persist as separate bodies. PNBs have been shown to contain various nucleolar components including fibrillarin, nucleolin and pre-rRNA, and some at least of the snoRNAs. **See also:** Cell cycle; Cell cycle: regulation by cyclins; Mitosis

Other Nucleolar Functions

The nucleolus has been implicated in a number of processes other than ribosome biogenesis (see Olson *et al.* (2002) for a recent review). For example there is good evidence for the preassembly of the signal recognition particle (SRP) in the nucleolus. The function of the SRP is to anchor ribosomes to the endoplasmic reticulum; hence, perhaps it is not too surprising to find a link between SRP biogenesis and ribosome biogenesis. Several other RNA species pass through the nucleolus in their biosynthesis; pre-tRNAs undergo initial processing in the nucleolus, and some snRNAs are methylated in the nucleolus.

More surprisingly, the nucleolus seems to be involved in cell cycle regulation, at least in yeast. Exit from mitosis in yeast requires the protein phosphatase Cdc14p. This protein is sequestered in the nucleolus during most of the cell cycle, but is released at anaphase, whereupon it promotes the degradation of a cyclin. This protein is part of a nucleolar complex that has been called the RENT complex

(Regulator of Nucleolar silencing and Telophase exit), which also includes the telomeric silencing protein Sir2p. This mechanism is likely to be restricted to lower eukaryotes, since in higher plants and animals the nucleolus is completely disassembled during mitosis. Recent studies have also localized the mammalian homologues of Sir2p to the nucleolus. The mammalian Sir2p homologues have been shown to deacetylate both histones and the tumour-suppressor protein p53. Whether nucleolar sequestration of Sir2p in mammals is a factor in the regulation of p53 remains to be determined.

There is evidence that the nucleolus may be involved in the export not only of ribosomal subunits but also of mRNAs. Early experiments in the 1960s showed that ultraviolet irradiation of the nucleolus inhibited mRNA production. More recently, yeast mutant studies have shown that some defects in mRNA export can cause changes in nucleolar organization. Furthermore, it is known that some of the most abundant NLS-binding proteins are primarily found in the nucleolus, and that these proteins can shuttle between the nucleolus and the cytoplasm. Thus, it is possible that the nucleolus is involved in the export of some mRNAs, or alternatively plays a role in mRNA surveillance and nonsense-mediated decay processes.

Conclusion

Much progress has been made in understanding the functional organization of the nucleolus. In many respects, the nucleolus is a good system for analysing and understanding the way gene transcription is organized; it is a well-defined site at which many copies of a single-gene sequence are transcribed, and we have a fairly detailed description of the biochemical processes occurring. However, the formation of ribosomes is a complex process, and involves interactions between a large number of different components, many of which remain to be characterized. Even when we have a complete inventory of all the proteins and other molecules involved, the analysis of ribosome formation and maturation and the determination of the part each of the components plays in the process will require a great deal of painstaking work.

We still have little real understanding of the reasons for the characteristic structure of the nucleolus. It is clearly highly organized, as is becoming apparent for the rest of the nucleus. However, as with the rest of the nucleus, we still have only a limited understanding of the principles on which the nucleolus is organized. Is it, as one review has suggested, 'an organelle formed by the act of building a ribosome' (Melese and Xue, 1995), or are there specific 'nucleolar skeleton' components that are responsible for determining its organization? The fact that prokaryotes, and specific yeast mutants, can make ribosomes without any apparent nucleolus-like structures suggests that an

organization that inevitably follows from ribosome biosynthesis is not the whole explanation.

Finally, we have very little idea of how the production, import and export of the various nucleolar and ribosomal components are coordinated. Knowledge about nuclear/cytoplasmic transport is increasing rapidly, particularly about the role of the nuclear pore complex. New microscopical approaches in living cells, particularly using green fluorescent protein (GFP) fusions to specific proteins, are now being applied to nuclear and nucleolar processes, and are showing that the nucleus and its component subdomains are highly dynamic (e.g. Dundr *et al.*, 2002). Most nucleolar proteins are likely to be in a constant flux in and out of the nucleolus, with many of them visiting other nuclear locations such as Cajal bodies. In the next few years, we can expect both a better definition of the components and functions of the nucleolus and of the dynamics of the biochemical processes that take place in this structure. **See also:** Nuclear protein import: methods; RNA intracellular transport

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