Nucleolus

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Advanced article

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The nucleolus is a nuclear substructure where the genes for three of the four ribosomal ribonucleic acids (RNAs) are transcribed and where ribosomal subunits are assembled. Although the nucleolus has been intensively studied for many years, recent progress has been very rapid. We are beginning to understand how the biochemical processes carried out in the nucleolus relate to the observable structure. There is much emerging evidence that the nucleolus is also involved in many other roles, particularly in the biogenesis of RNA-containing complexes, in stress sensing and in the control of cellular activity and proliferation. Recent observations of nucleolar proteins in living cells have shown that the nucleolus and its components are highly dynamic and that the observed structure is a steady state result of the dynamic diffusion of proteins and other macromolecules throughout the nucleoplasm and nucleolus and their relative residence times in the various locations.

Introduction

Most eukaryotic cells contain one or more prominent regions within the nucleus called nucleoli. The nucleolus was first described by Fontana (1781), who noted its occurrence in the slime of an eel, and the name 'nucleolus' was coined by Valentin (1839) to denote the 'nucleus within a nucleus'. Nucleoli have a substantially different refractive index from the rest of the nucleus and thus are clearly seen by optical microscopy (**Figure 1a**). They also stain differently from the rest of the nucleus with various 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 organiser regions (NORs). The NORs are the sites on the metaphase chromosomes where nucleoli become organised 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 synthesising of the order of a hundred each second. This is the fundamental reason why so many copies of the genes are needed – a single gene copy could not 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

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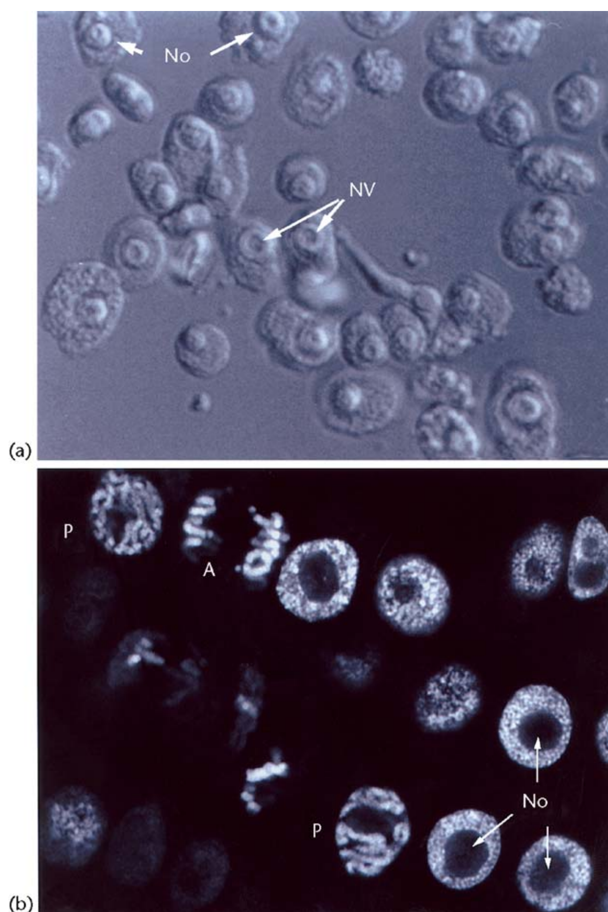

Figure 1 The nucleolus as visualised by optical microscopy. (a) Isolated tobacco nuclei visualised by differential-interference contrast microscopy. The nucleoli are clearly seen as prominent bodies inside each nucleus (No). Within many nucleoli, nucleolar vacuoles or cavities can be seen (NV). (b) Pea root tissue stained with the DNA dye 4′,6′-diamidino-2-phenylindole (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.

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 (mRNAs). 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. **See also**: Ribosome Structure and Shape; RNA Intracellular Transport

rDNA Organisation

In prokaryotes such as *Escherichia coli*, there are three rRNAs (16S, 23S and 5S), which are organised as a single transcription unit. In all eukaryotes studied so far, the organisation of the rRNA genes is recognisably 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 (Hadjiolov, 1985). The former transcription unit is generally simply referred to as the rRNA gene or the ribosomal deoxyribonucleic acid (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 of Ribosomes

There are generally more repeats of the 5S sequences than of the rDNA, but in both cases, there are huge variations in the number of repeats in different species. The human genome contains approximately 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 (Hadjiolov, 1985). **See also**: Genome Organization: Human

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 posttranscriptional 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 l-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

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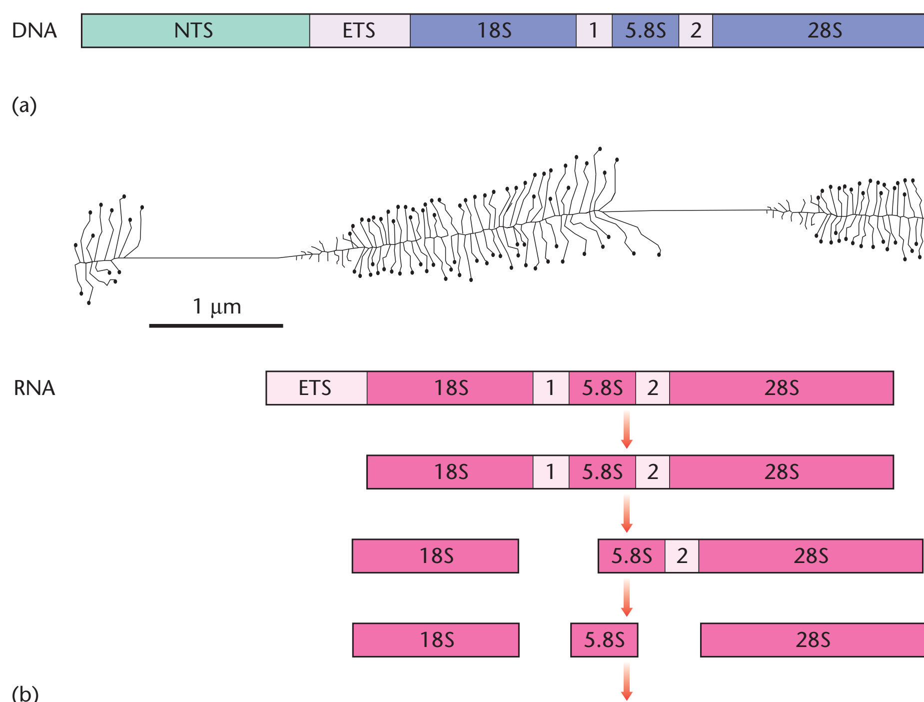

Figure 2 Miller spread of ribosomal DNA (rDNA) transcription units ('Christmas trees'), redrawn from an original micrograph. (a) Diagram of the organisation of a single repeat unit of the rDNA. (b) Diagram of the initial pre-rRNA 45S transcript and of its processing pathway to three of the mature rRNAs.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

highly divergent, so that there is often little homology even between quite closely related species. The size of the NTS varies between 2 and 3 kb in most plants and *S. cerevisiae* and in vertebrates between 20 and 30 kb. 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 visualised 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 organisation 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 recognisable 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

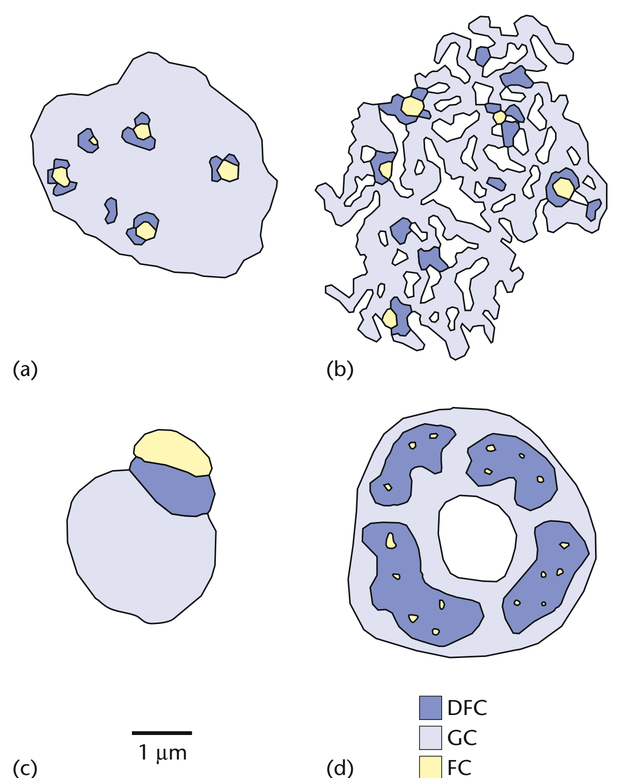

Figure 3 Diagrams of nucleolar ultrastructure seen by conventional electron microscopy. (a)–(c) Nucleoli from a mammalian cell culture under different growth conditions. (a) Three well-differentiated types of structure can be seen; lightly staining fibrillar centres (yellow), surrounded by regions of 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.

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. As 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 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 organisation 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, much of the polymerase at these sites is probably inactive; RNA polymerase I is also seen concentrated at the NORs during mitosis, when no transcription occurs. **See also**: Chromosome Mechanics

Fluorescence *in situ* hybridisation 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 that 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 FCs (see discussion earlier).

However, to determine where the active transcription units are, it is necessary to use a method to detect the

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nascent RNA itself. The factors necessary for rDNA transcription – RNA polymerase I and other proteins, and rRNA genes themselves – are certainly present in both active and inactive forms and locations. Nucleolar transcription has been visualised in both plants and animals directly by incorporating the labelled RNA precursor bromouridine triphosphate (BrUTP), and subsequently detecting it with antibodies (Dundr and Raska, 1993). 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' approximately 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 the FC regions. In human HeLa (Henrietta Lacks) 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 approximately 0.1 μm in diameter (Scheer *et al*., 1997). **See also**: Labelling of Cells Engaged in DNA Synthesis: Autoradiography and BrdU Staining; Fluorescence *in situ* Hybridization

Models for rRNA transcription units

The molecular organisation 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, because 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 earlier. 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 organisation 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 organisation, despite the fact that they all carry out essentially the same biochemical processes, and we currently have little idea 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 organisation 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

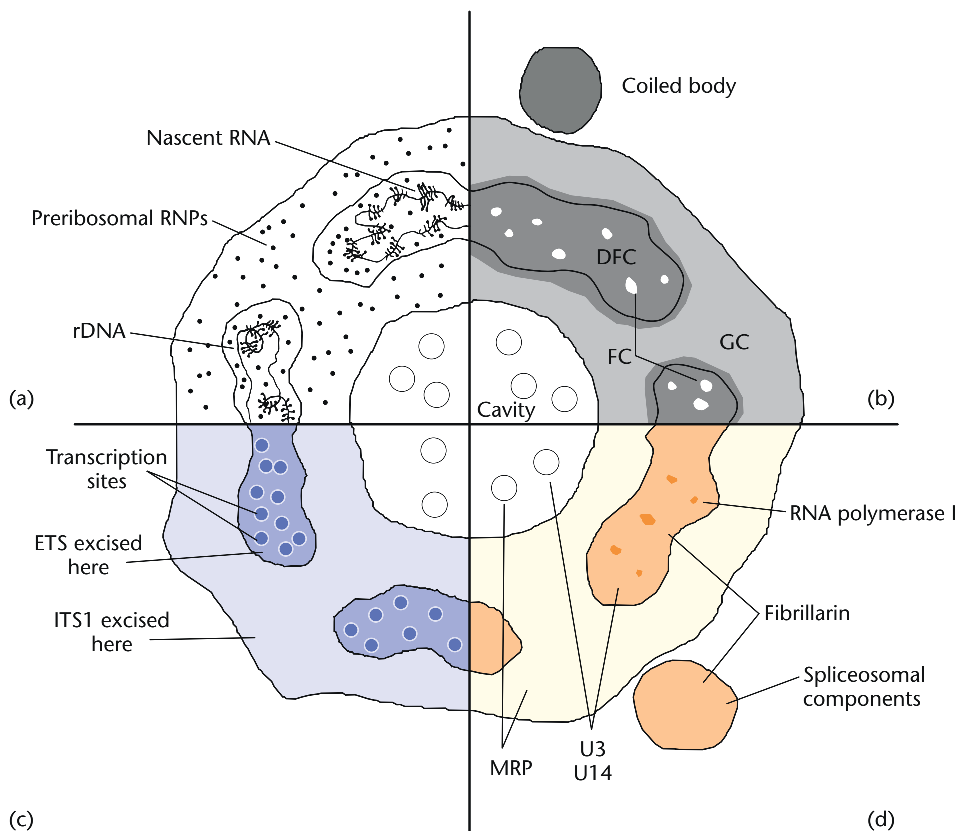

Figure 4 Different views of nucleolar organisation. A typical plant nucleolus is illustrated diagrammatically. To give a common reference structure, an outline corresponding to the nucleolar region labelled by a probe to the external transcribed spacer (ETS) portion of the pre-rRNA is shown. This corresponds to most, but not quite all, of the DFC. (a) Possible model for the organisation 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 and GC, granular component. (c) Organisation of transcription sites and zones of transcript processing. ITS1, internal transcribed spacer 1. (d) Localisation of some nucleolar proteins and small nucleolar RNAs.

(**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 organised 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 GC (**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 organisation 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

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 characterised

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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 localised to the DFC. This is consistent with their role in early cleavage events and the layered model of the nucleolus described earlier. 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 discussion later) 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 characterised, except MRP, can be categorised 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. A recently discovered class of small RNA – scaRNAs – is specific to Cajal Bodies; some scaRNAs contain both a box C/D and a box H/ACA motif (Darzacq *et al*., 2002). 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 isomerisation to pseudouridine. There are approximately 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 pseudouridylations. Fibrillarin is the methylase that is guided by the box C/D snoRNAs, and the cognate pseudouridylate synthase for the box H/ACA snoRNAs is Cbf5p in yeast (dyskerin in higher eukaryotes). It was originally thought that all these base modifications occur cotranscriptionally, but localisation 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 U22 snoRNA host gene (UHG), 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, many of which are also present in other cellular locations. 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 approximately a dozen protein subunits. Initiation of transcription requires the coordinated activity of a series of transcriptional activators and binding factors, which are now being characterised in detail. The nucleolus also contains both active and inactive chromatin, which may carry a nucleolus-specific complement of chromatin-associated proteins (McKeown and Shaw, 2009). 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 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 localisation sequences (NLS) of other nuclear proteins. It is possible that these proteins are involved in nucleolar/cytoplasmic transport processes. **See also**: Chaperones, Chaperonin and Heat-Shock Proteins; Eukaryotic Ribosomes: Assembly; Nuclear-Cytoplasmic Transport; Protein–RNA Interactions; Ribosomal Proteins in Eukaryotes

Methods for the analysis of complex protein mixtures by mass spectrometry have now been applied to the nucleolus and ribosome biogenesis. For example, mass spectrometric studies of purified human nucleoli (e.g. Andersen *et al*., 2005) and of plant (*Arabidopsis*) nucleoli (Pendle *et al*., 2005) have been published, in each case identifying several hundred proteins as nucleolar components. In other studies (e.g. Tschochner and Hurt, 2003), preribosomal particles have been purified by immunopurification of specific tagged proteins and analysed 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 the function of the nucleolus.

Many nucleolar proteins, including fibrillarin, have also been found in subnuclear bodies called Cajal bodies

(formerly called coiled bodies). These bodies, which were originally called nuclear 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 is clear that they are involved in processing and assembly of nucleolar and spliceosomal components. In particular, calbindins (CBs) have been shown to be involved in the assembly of the U4/U6/U5 tri-snRNP and of the U4/U6 di-snRNP, as well as in the maturation of the U2 snRNP. CBs are also involved in small interfering RNA (siRNA) pathways, at least in plants (Pontes and Pikaard, 2008).

Formation and dynamics of the nucleolus

Prokaryotes synthesise 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 organise a normal nucleolus. On the contrary, 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. As the supply of ribosomes is such a huge investment for a cell and so can 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

Dynamic studies of the nucleus and nucleolus have shown that almost all proteins diffuse rapidly throughout the nucleus and nucleolus (e.g. Misteli, 2001), and the mean residence time of most nucleolar proteins in the nucleolus is a few tens of seconds. This realisation has led to a reassessment of the nature of nucleolar structure. In the current model, a subset of proteins bind to the rDNA, forming a more or less stable core on which complex sets of interacting complexes and dynamic processes are built. The nucleolus can thus be viewed as a 'super-complex', most of whose components are in continuous exchange with the surrounding nucleoplasm. The steady state composition of the nucleolus, and to a large extent the existence of the nucleolus as an identifiable entity, is the result of the fact that the residence time of non-nucleolar proteins that do not find interacting partners in the nucleolus is at least an order of magnitude shorter than that of the nucleolar proteins.

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, whereas others are distributed around the periphery of the mitotic chromosomes. Polymerase I transcription is halted at mitosis and is restarted at telophase/G_1 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 then disappear as new nucleoli are formed. 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; Boisvert *et al*., 2007 for reviews). 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; 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-transfer RNAs (tRNAs) undergo initial processing in the nucleolus, and some snRNAs are methylated in the nucleolus. There is also good evidence that telomerase RNA as well as its associated protein factor (TERT) are localised in the nucleolus, at least in human culture cells, either during telomerase biogenesis or sequestered to this compartment as part of its regulation (Raska *et al*., 2006). There is now also good evidence that many microRNAs (miRNAs) and their precursors are found in the nucleolus in mammals (Politz *et al*., 2009) and that the nucleolus and

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CBs are involved in both miRNA and siRNA silencing pathways in plants (Pontes and Pikaard, 2008).

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, because in higher plants and animals, the nucleolus is completely disassembled during mitosis. Recent studies have also localised 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.

Nucleolar morphology has long been one of the key factors used in tumour grading, partly because of the relation between nucleolar morphology and cell proliferation. The occurrence of the perinucleolar compartment provides a further guide in prognosis. This structure is as yet poorly understood, but is associated with aggressive behaviour of breast cancer cells, and may be useful to monitor clinical progression of this disease (Kopp and Huang, 2005). Other links between the nucleolus and cancer are mediated by the onco-suppressor proteins pRb (retinoblastoma protein) and p53, both of which are concentrated in the nucleolus.

The p53 transcription factor is a major mediator of cellular stress responses in mammalian cells. Elevated nucleoplasmic levels of p53 inhibit cell growth or lead to apoptotic cell death and more than 50% of human cancers have impaired p53 function. One of the major control pathways of p53 level is the degradative pathway involving MDM2, which functions as an E3 ubiquitin ligase, ubiquitinating p53 and targeting it for degradation by the proteasome. A major regulator of MDM2 is ARF (so called because it is encoded in an alternative reading frame of p16INK4a, a regulator of pRb). ARF is localised to the nucleolus, but under cellular stress conditions can relocalise to nuclear bodies where it is associated with MDM2 and p53, suggesting a nucleolar link to cancer and stress response (David-Pfeuty and Nouvian-Dooghe, 2002). It has been suggested that the structure of the nucleolus itself is the stress sensor, because stresses that activate p53 also cause nucleolar disruption or breakdown, which seems to be upstream of the p53 response (Rubbi and Milner, 2003). **See also**: P53 and Cell Death

Nucleostemin is predominantly localised to the nucleolus and shuttles between the nucleolus and nucleoplasm. It appears to play a role in controlling cell growth and proliferation and is preferentially expressed in neural stem cells, embryonic stem cells and a number of cancer cell lines (Tsai and McKay, 2002; Tsai and Meng, 2009). It is concentrated in specific RNA-deficient subdomains within the granular component, suggesting a more complex structure and different roles for this nucleolar component (Politz *et al*., 2005).

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 organisation. In plants, it is clear that purified nucleoli contain a large spectrum of mRNA species and that, remarkably, mis-spliced mRNAs are actually concentrated in the nucleolus (Kim *et al*., 2009). This confirms earlier observations by mass spectrometry of many mRNA-associated proteins with the nucleolus (Pendle *et al*., 2005). Most of the mis-spliced RNAs would be predicted to be targets of the nonsense-mediated degradation (NMD) pathway, and indeed, UPF3 and UPF2, proteins required for NMD, were clearly also concentrated in the nucleolus (Kim *et al*., 2009). Thus, it is likely 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 organisation of the nucleolus. In many respects, the nucleolus is a good system for analysing and understanding the way gene transcription is organised; 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 characterised. 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 processes 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 organised, 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 organised. 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 organisation? The fact that prokaryotes, and specific yeast mutants, can make ribosomes without any apparent nucleolus-like structures, and also the variability of nucleolar structure itself, suggests that an organisation 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 have shown that the nucleus and its component subdomains are highly dynamic (e.g. Dundr *et al*., 2002; Kaiser *et al*., 2008; Hager *et al*., 2009). Most nucleolar proteins are 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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