Site of transcription of ribosomal RNA and intranucleolar structure in HeLa cells

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SUMMARY

Sites of transcription of ribosomal RNA in HeLa cells were visualized by electron microscopy. Cells were either incubated with Br-uridine, or permeabilized and then incubated with BrUTP, before sites containing Br-RNA were immunolabelled with gold particles. Short incubations ensured that most incorporated analogue remained at synthetic sites. Fibrillar centres were unlabelled except at their periphery; label was concentrated over certain regions of the surrounding dense fibrillar component. These results suggest that the dense fibrillar component is the site of rRNA transcription. After dispersing the granular component and the dense fibrillar component by a hypotonic treatment, removal of most chromatin and preparation of resinless sections, fibrillar centres remained fixed to a nucleoskeleton. These structural and functional features are incorporated into a model for rRNA transcription.

Key words: nucleolus, rRNA, cell nucleus, nucleoskeleton, BrUTP

INTRODUCTION

Nucleoli are the most prominent morphological structures in nuclei; they actively synthesize rRNA (for reviews, see Reeder, 1990; Hernandez-Verdun, 1991; Jordan, 1991; Schwarzacher and Wachtler, 1991; Sollner-Webb et al., 1991). Conventional electron microscopy reveals each to contain pale fibrillar centres surrounded by dense fibrillar components, which in turn are embedded in a granular component. Despite intense study, the functional organization of nucleoli remains obscure. Whilst there is general agreement that the granular component is involved in the later stages of ribosome assembly, the site of rRNA synthesis remains controversial.

Various approaches - including autoradiography, immuno-electron microscopy, and in situ hybridization - have been used to analyze structure-function relationships. For example, rDNA, rRNA, histones, RNA polymerase I, topoisomerases I and II, nucleolar transcription regulating factor (UBF or NOR 90), nucleolar snRNPs, nucleolin, protein B23 and fibrillarin - have all been localized to one or other of the compartments (for reviews see Derenzini et al., 1990; Raška et al., 1990; Scheer and Benavente, 1990; Thiry et al., 1991; Wachtler et al., 1993). However, the local concentrations detected could reflect sites where inactive components are stored after synthesis, rather than sites of activity.

In principle, sites of transcription can be labelled using the appropriate precursors; in practice, this has proved difficult. [³H]uridine is incorporated into nucleoli and can then be detected by autoradiography; however, the path-length of β-particles is hundreds of nanometers long and so does not allow precise localization of the radiation source. Moreover, the labelling times required to permit detection could allow completed transcripts to move away from their site of synthesis. The use of labelled nucleotide triphosphates is limited by their inability to cross the cell membrane and the difficulty the relevant polymerase has in using them (Jackson et al., 1993; but see Schöfer et al., 1993).

Recently, transcription sites have been immunolabelled after incorporation of BrUTP (Jackson et al., 1993; see also Wansink et al., 1993, and Dundr and Raška, 1993). Cell membranes were permeabilized with streptolysin O in a physiological buffer. In the presence of an optimal concentration of the natural precursors, such permeabilized cells continue to replicate and transcribe at essentially the rates found in vivo. Replacement of UTP with BrUTP reduces the transcription rate so that during short labelling periods few nascent chains are completed; most are still being synthesized and so remain at their site of synthesis. Subsequently, sites containing Br-RNA were immunolabelled with fluorescent tags. Fluorescent foci were seen in both nucleolar and extranucleolar regions. Active polymerases, as well as these foci, remained even when most chromatin was removed. These transcription foci are probably attached to an intermediate-filament-like skeleton seen in this material (Jackson and Cook, 1988; Hozák et al., 1993a).

We now use this approach to immunolocalize sites of rRNA transcription in HeLa cells at the ultrastructural level. We find

that the dense fibrillar components, but not the fibrillar centres, are synthetic sites.

MATERIALS AND METHODS

Labelling with Br-uridine

HeLa cells were grown for various times on coverslips in MEM + 10% foetal calf serum and Br-uridine (50 µM; Sigma), rinsed in ice-cold medium without serum and immediately fixed.

Labelling with BrUTP

Transcription sites were labelled with BrUTP as described by Jackson et al. (1993), with the following modifications. HeLa cells (grown as suspensions in S-MEM + 5% foetal calf serum) were not embedded in agarose microbeads; instead they were washed 3× in PBS, incubated with streptolysin O (Murex; 2 units/ml per 10^6 cells, 30 minutes, 0°C), unbound streptolysin removed by washing in ice-cold PBS followed by physiological buffer (PB). PB contains 22 mM Na^+, 130 mM K^+, 1 mM Mg^{2+}, 32 mM Cl^-, 100 mM acetate, 11 mM phosphate, 1 mM ATP, 1 mM dithiothreitol and 0.1 mM PMSF (pH 7.4). Transcription by RNA polymerase II was inhibited by incubating cells in PB with α-amanitin (300 µg/ml; 15 minutes, 0°C) immediately prior to permeabilization by incubation (3 minutes) at 33°C. (α-amanitin treatment inhibits extra-nucleolar incorporation but stimulates nucleolar incorporation (Jackson et al., 1993; Wansink et al., 1993).) Transcription reactions (10^6 cells/ml) were initiated by adding a 10× concentrate at 33°C to give final concentrations of 1.1 mM ATP, 0.1 mM CTP, GTP and BrUTP (Sigma), plus 2 mM $MgCl_2$. DideoxyTTP (10 µM; Boehringer) and aphidicolin (5 µg/ml; Sigma) were added to prevent incorporation into DNA; the ammonium sulphate used by Jackson et al. (1993) was omitted. Transcription reactions were conducted at 33°C to prevent any heat-induced aggregation (Jackson et al., 1988) for 1, 2 and 6 minutes for ultrastructural work and 20 minutes for light microscopy. They were stopped by adding 50 vol. ice-cold PB.

All solutions were prepared with water treated with diethyl pyrocarbonate to minimize RNase activity (Sambrook et al., 1989); human placental ribonuclease inhibitor (Amersham) was also added during incubations with antibodies (2.5 units/ml), enzymic digestion (25 units/ml) and electroelution (0.25 units/ml) and to all solution used after fixation.

Assessment of transcription rates

Transcription rates were determined using $[α$-$^{32}P]GTP$ (~3000 Ci/mmol, 69 µCi/ml; Amersham) and the above conditions as described by Jackson and Cook (1986).

Immunofluorescence

After incorporation, nuclear membranes were permeabilized with Triton X-100 in PB, fixed in 3% paraformaldehyde (20 minutes, 0°C) in PB, immunolabelled using a monoclonal anti-Br-deoxyuridine antibody (3 µg/ml, 1 hour; Boehringer; this antibody cross-reacts with Br-RNA) followed by sheep anti-mouse FITC-conjugated secondary antibodies (1:500 dilution; Amersham) and photographed using Tmax 400 Kodak film and a Zeiss Axiophot.

Immunoelectron microscopy

Thin sections were prepared as follows. Labelled cells were pelleted, fixed (20 minutes, 0°C) either in 3% paraformaldehyde in Sörenson buffer (SB, 0.1 M Na/K phosphate buffer, pH 7.4) (samples incubated with Br-uridine) or in 3% paraformaldehyde plus 0.1% glutaraldehyde in PB (samples incubated with BrUTP), washed in SB (2×; 10 minutes), incubated in 0.02 M glycine in SB (10 minutes), washed in SB and dehydrated in ethanol, which was then replaced in two steps by LR White resin and polymerized (24 hours; 50°C). After cutting

90 nm sections, non-specific labelling was blocked by preincubation with 10% normal goat serum (20 minutes in PBT; PBT is PBS + 0.1% Tween-20). Next, sections were incubated with the monoclonal anti-Br-deoxyuridine antibody (1 µg/ml, 40 minutes; Boehringer) in PBT. A wide range of controls has shown that Br-RNA is labelled specifically (Jackson et al., 1993). Sections were then washed (3×; PBT), incubated with 5 nm gold-conjugated goat anti-mouse antibody (diluted 30× in PBT adjusted to pH 8.2; 30 minutes; BioCell), washed in PBT and then water. Finally, sections were stained (2 minutes) with a saturated solution of uranyl acetate in water and observed in Jeol 100CX electron microscope. The densities of gold particles over nucleolar compartments were determined using Sigmascan software (Jandel Scientific). Statistical significance was assessed using Student's t-test, or, in the case of non-normal distributions, using the non-parametric Kolmogorov-Smirnov test.

Conventional electron microscopy

Samples were also analyzed after fixation in 2.5% glutaraldehyde in SB (1 hour, 4°C), post-fixation in 1% OsO_4 (30 minutes), dehydration in an ethanol series (including a 30 minute treatment in 2% uranyl acetate in 70% ethanol), embedding in Epon and staining with uranyl acetate and lead citrate according to Reynolds.

Resinless sections

Resinless sections of isotonically and hypotonically treated cells were prepared as follows. Cells were grown in $[^3H]thymidine$ to label uniformly their DNA, washed in PBS, encapsulated (10^7 cells/ml) in 0.5% agarose beads (Jackson et al., 1988), grown for 2 hours, incubated (7 minutes, 37°C) in SB (either undiluted or diluted 30 vol. in 100 vol. water). The hypotonic buffer disperses the granular component and most of the dense fibrillar component of nucleoli to leave only fibrillar centres (Hozák et al., 1990, 1992). Until subsequent electroelution, isotonically treated cells were maintained at 0°C in PB and hypotonically treated cells in PB diluted 40 vol. in 100 vol. water. Encapsulated cells were permeabilized with 0.2% Triton X-100 (10 minutes), and washed 4×. Cells were then incubated (20 minutes; 33°C) with EcoRI (2500 units/ml) and HaeIII (500 units/ml) to cut chromatin into ~10 kb pieces and subjected to electrophoresis (4 V/cm, 3.5 hours, 4°C) in PB to remove ~90% chromatin (measured by removal of ^3H; Jackson and Cook, 1986). Samples were recovered, fixed (2.5% glutaraldehyde in PB; 20 minutes; 0°C), washed 3× in SB, post-fixed with 0.5% OsO_4 in SB (20 minutes), dehydrated through an ethanol series (including 30 minutes in 2% uranyl acetate in 70% ethanol), and ethanol replaced in three steps by n-butanol. Samples were then embedded in diethylene glycol distearate (Polysciences), resinless sections were prepared (Fey et al., 1986; Hozák et al., 1993a) and immediately observed in the electron microscope.

RESULTS

Transcription sites in non-permeabilized cells

In thin epoxy-resin sections (Fig. 1), the nucleolus appears as a granular component (gc) containing dense fibrillar components (dfc), which surround pale fibrillar centres (fc). Br-uridine was incorporated by living cells and then its distribution amongst these different compartments visualized after immuno-labelling with 5 nm gold particles. Under our conditions, significant amounts of label only become detectable in nucleoli after incubation for 10 minutes (Fig. 2A-C). As labelling for longer marks sites where transcripts accumulate, only results from short incubations are presented. LR White sections are used here because they give better immunolabelling, even though there is some loss of ultrastructural

detail. Most particles were confined to small regions within the dense fibrillar component, especially where it abuts the fibrillar centre (Fig. 2A-C). Fibrillar centres were generally unlabelled; for example, in 55 fibrillar centres in 32 sections examined in detail, only 5 were labelled with a total of 8 particles (e.g. see Fig. 2B). The granular component of the nucleolus and the rest of the nucleoplasm were slightly labelled.

Statistical analysis confirmed these impressions (Fig. 3). Particle densities over the dense fibrillar component were significantly higher than those in controls from which the anti-Br antibody had been omitted. A concentration of actinomycin D that inhibits nucleolar transcription reduced labelling over all nucleolar compartments, even though labelling over the dense fibrillar component remained significant. After incubation with Br-uridine for 30 minutes, labelling over the dense fibrillar component was roughly the same, presumably because nascent RNA is rapidly transported elsewhere; fibrillar centres remained unlabelled (Fig. 3).

If polymerization occurs at 25 nucleotides/second in vivo, a ribosomal transcript will just have time to be completed during a 10 minute labelling period, and a significant fraction of incomplete transcripts will remain at polymerization sites. In this case the results above are consistent with transcription occurring in the dense fibrillar component. However, as we can only be certain that most incorporated analogue remains at synthetic sites if the transcription rate is known, we next measured the rate of incorporation of BrUTP by permeabilized cells in vitro.

Rate of nucleolar transcription in vitro

In the presence of optimal concentrations of the natural precursors, permeabilized cells continue to elongate existing nascent RNA chains roughly at the rate found in vivo (Jackson et al., 1988). When 100 μM BrUTP replaces UTP as a precursor - using [^{32}P]GTP as a tracer - the initial rate of incorporation is ~40% of the rate found with the natural precursor (Fig. 4, curve 1; Jackson et al., 1993). As expected, 300 μg/ml α-amanitin, which inhibits extranucleolar synthesis by RNA polymerase II, reduces incorporation (Fig. 4, curve 2); most remaining synthesis is due to polymerase I, which is inhibited by actinomycin D (Fig. 4, curve 4). A combined treatment with α-amanitin and actinomycin has no further effect (Fig. 4, curve 3; the actinomycin concentration used also inhibits some polymerase II activity).

Assuming that ~12,000 polymerase I molecules are active in each cell (see Jackson et al., 1993) under our conditions, nascent chains are elongated by ~250 and ~500 nucleotides in 6 and 20 minutes (i.e. the longest labelling periods used for electron and light microscopy, respectively). As nascent rRNA is ~15,000 nucleotides long, this means that during the labelling times used below, the majority of label (i.e. ~98% after 6 minutes and ~96% after 20 minutes) will be contained in chains that are still being synthesized and so have not had time to move away from their site of synthesis.

Transcription sites in permeabilized cells

Sites of incorporation were first visualized by light microscopy; 300 μg/ml α-amanitin was used to inhibit extranucleolar incorporation. In most cells, label was concentrated in 15-25 nucleolar foci, which were often clustered like beads on a string (Fig. 5; see also Haaf et al, 1991; Jackson et al., 1993). The number of these foci roughly equals the number of fibrillar centres and associated dense fibrillar components seen in HeLa cells by fluorescence microscopy (e.g. see Hozák et al., 1992).

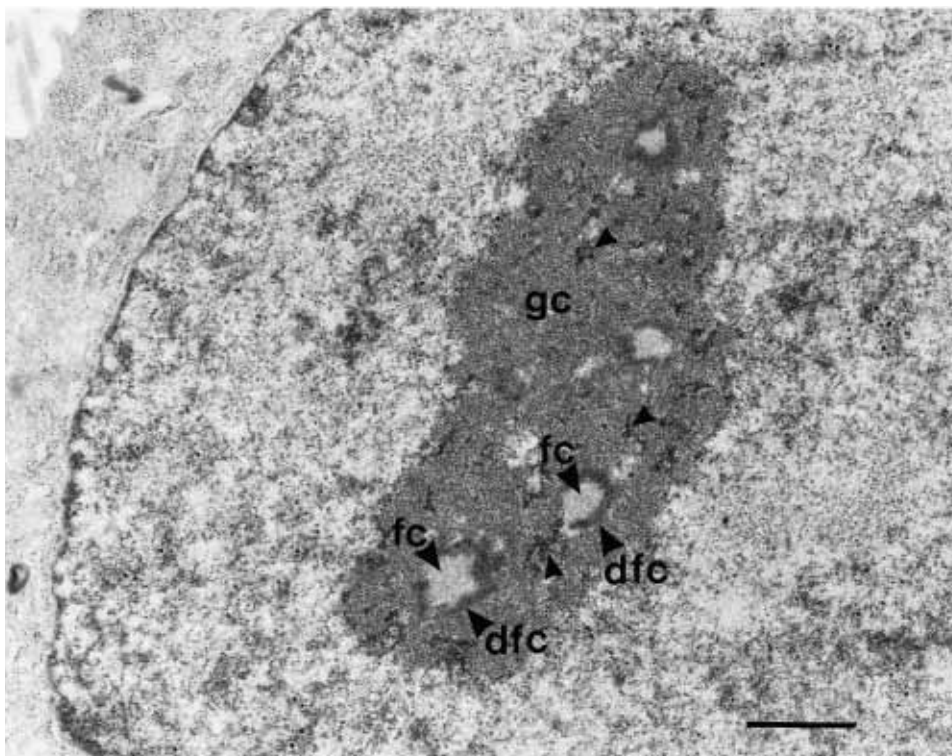We next visualized these incorporation sites by electron

Fig. 1. Electron micrograph of epoxy resin section of part of a nucleus with a nucleolus. Fibrillar centres (fc), dense fibrillar component (dfc), granular component (gc), intranucleolar chromatin (small arrowheads). Bar, 1 μm.

microscopy. First, we confirmed that the excellent ultrastructure seen in epoxy resins was retained after lysis and transcription in vitro (compare Figs 1 and 6). Although less structure is retained after embedding in the LR White resin used for immunolabelling, different nucleolar compartments could still be distinguished (Figs 7-9).

After 1 min incubation with BrUTP in vitro, a few gold particles can be found over ~20% nucleoli; the majority lie over the dense fibrillar component, especially at its internal and external borders; almost none localize over the granular component (Fig. 7). After 2 minutes, ~40% nucleoli become labelled but the pattern is the same (Fig. 8). After 6 minutes, ~90% nucleoli were labelled (Fig. 9). Actinomycin D (0.5 µg/ml) eliminated almost all this labelling (not shown). Gold particles were frequently concentrated in small regions over the dense fibrillar component, especially at its internal and external borders. If particles lying within 10 nm of the dense fibrillar component were excluded, essentially none lay over fibrillar centres. There was slight labelling over the granular component, but even so particle densities were 15 times less than over the dense fibrillar component. The dense fibrillar component was also immunolabelled using an antibody against 116 kDa protein - a marker of this component (Masson et al., 1990) and could be doubly labelled with 5 and 10 nm particles, marking incorporated BrUMP and this protein, respectively (not shown). These results provide clear evidence that the dense fibrillar component - not the fibrillar centre - is the site of synthesis.

Hidden intranucleolar structure

Thick resinless sections are especially suitable for visualizing nucleoskeletal elements (Capco et al., 1984); however, the
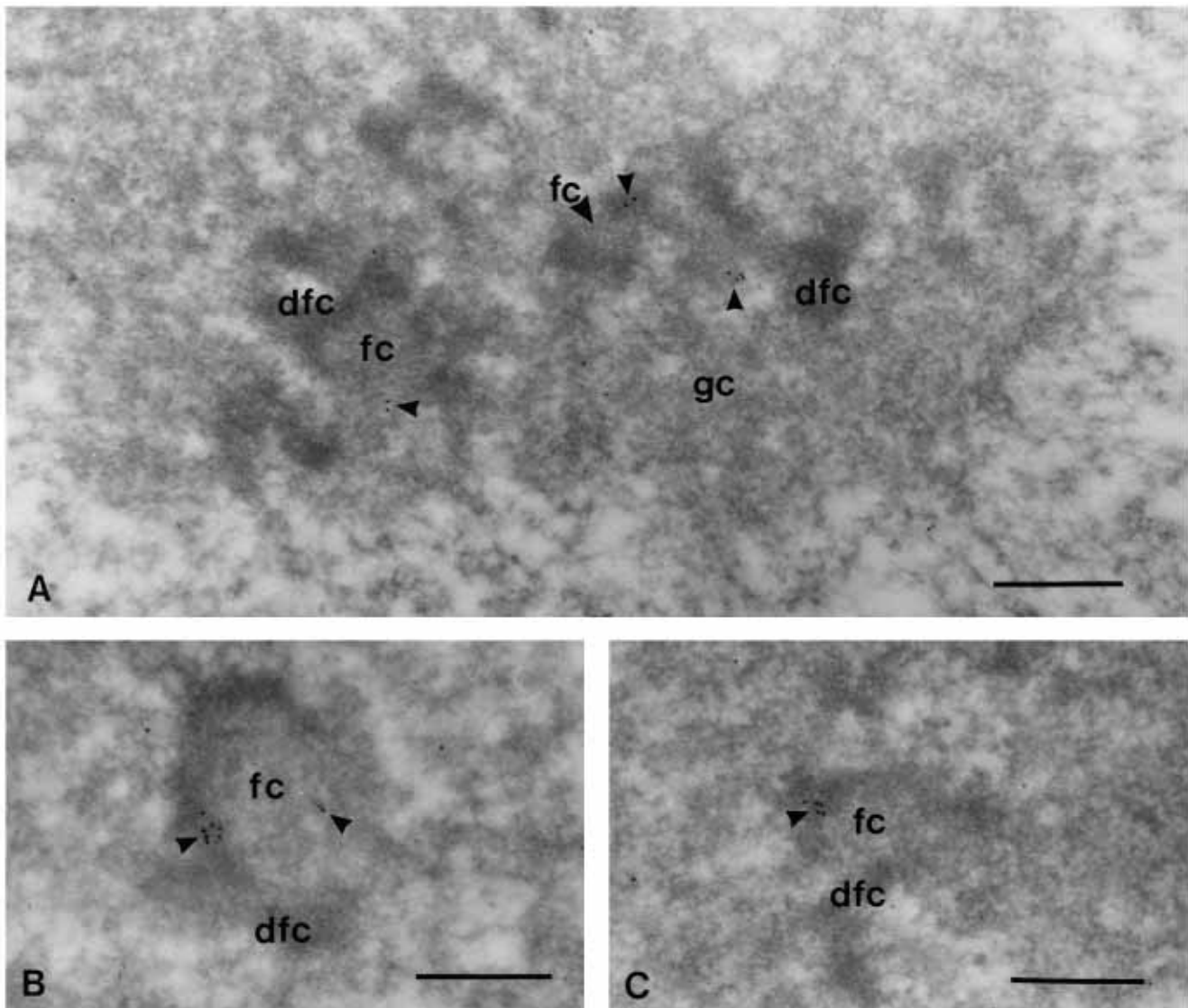

Fig. 2. Electron micrographs of sites where Br-uridine has been incorporated by living cells. Cells were incubated (10 minutes) with Br-uridine, LR White sections prepared and sites containing Br-RNA immunolabelled with 5 nm gold particles. (A-C) Typical labelling patterns. Gold particles (arrowheads) are frequently clustered over the dense fibrillar component (dfc); exceptionally, fibrillar centres (fc) are labelled with 1 or 2 particles as in Fig. 2B (right arrowhead). Granular component (gc). Bars: (A), 0.5 µm; (B,C), 0.2 µm.

relationship of nucleoli with any skeleton is obscured in such sections by the surrounding chromatin and granular component. Therefore most of these were removed. HeLa cells were encapsulated in a protective coat of agarose and the granular component was dispersed by hypotonic treatment (Hozák et al., 1992). After permeabilization, resinless sections were prepared; even though the granular component and most of the dense fibrillar component have dispersed, chromatin still obscures the fibrillar centres (Fig. 10A). Therefore chromatin was removed by first fragmenting chromatin with *Eco*RI and *Hae*III and then removing any detached fragments electrophoretically (Fig. 10B). Now a nucleoskeleton consisting of core filaments and more diffuse components can be seen concentrated in the nucleolar region. Fibrillar centres lie at nodes in this network, suggesting that they constitute a core around which the nucleolus is built. Elements of the skeleton radiate from such nodes to the lamina.

DISCUSSION

The approach

Sites of rRNA synthesis within nucleoli were visualized by incubating permeabilized HeLa cells with BrUTP in a physiological buffer; then sites containing nascent Br-RNA were indirectly immunolabelled with gold particles. This approach has several advantages. First, the transcription rate can be reduced in vitro so that most of the incorporated analogue remains at the polymerization site, with little being transferred to distant processing sites. Thus, after the longest labelling period used for electron microscopy (i.e. 6 minutes), nascent ribosomal transcripts are elongated by ~250 nucleotides. As the primary rRNA transcript is much longer than this (i.e. ~15,000
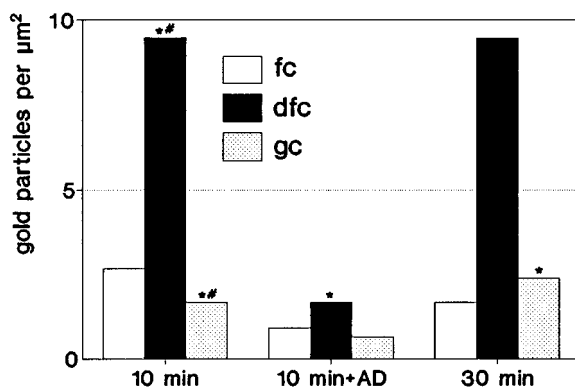

Fig. 4. Transcription rates of permeabilized cells. Cells were permeabilized, incubated with BrUTP plus [^{32}P]GTP and the rate of incorporation of radio-label into acid-insoluble material determined. Reactions were conducted: (1) without any inhibitors; or in the presence of (2) 300 μg/ml α-amanitin; (3) 300 μg/ml α-amanitin and 0.2 μg/ml actinomycin D; or (4) 0.2 μg/ml actinomycin D.

nucleotides), only ~2% of transcripts will be completed during incubation and so able to move away from their site of synthesis. Second, the use of physiological conditions and retention, under optimal conditions, of most transcriptional activity of the living cell up to fixation provides some assurance that structures analyzed are not generated artifactually. Third, immunogold labelling coupled with electron microscopy provides sufficient resolution to distinguish where label is relative to the different nucleolar compartments. Fourth, brominated precursors are incorporated after short pulses both in vivo and in vitro into the same places (Figs 2, 7-9).

rRNA polymerization takes place in the dense fibrillar component

Nucleoli are the site of rRNA synthesis and typically contain a number of fibrillar centres surrounded and interconnected by the dense fibrillar component, which - in turn - is embedded in a granular component. Our results clearly point to the dense fibrillar component as the site of rRNA transcription. Labelling

Fig. 3. Preferential labelling of the dense fibrillar component. Cells were incubated (10 or 30 minutes) with Br-uridine, sections like those illustrated in Fig. 2 prepared and densities of gold particles lying over the fibrillar centre (fc), dense fibrillar component (dfc) and granular component (gc) measured. In one case, actinomycin D (AD; 0.2 μg/ml) was present 5 minutes prior to, and during, incubation with Br-uridine. Student's *t*-test was used to evaluate whether labelling was statistically significant, except for the non-normal distribution over fibrillar centres, where the non-parametric Kolmogorov-Smirnov test was used. *,#: significant (*P*<0.001; *n*=32) compared with controls where primary antibody was omitted or with actinomycin-treated sample, respectively. Other differences were insignificant at *P*<0.01.
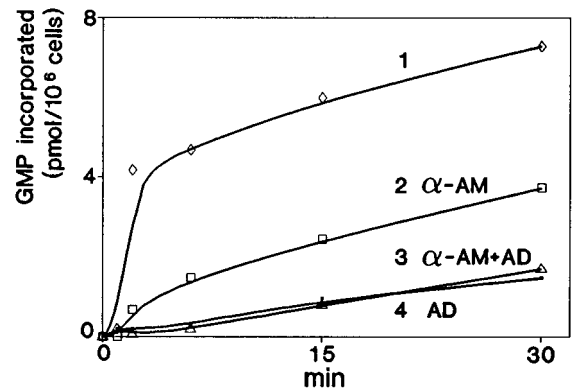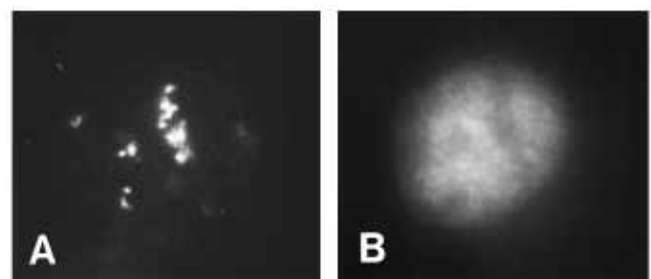

Fig. 5. Immunofluorescence detection of Br-RNA after incorporation of BrUTP in vitro. Permeabilized cells were incubated for 20 minutes with BrUTP and sites containing incorporated analogue immunolabelled; 300 μg/ml α-amanitin was present 15 minutes prior to, and during, incorporation. (A) ~15 nucleolar foci are seen with very little extranucleolar fluorescence. (B) DAPI-fluorescence of (A); nucleoli appear dark. Bar, 5 μm.

was not spread uniformly throughout the dense fibrillar component, but concentrated locally. This is consistent with the known distribution of template (Derenzini et al., 1987; Wachtler et al., 1989; Puvion-Dutilleul et al., 1991; Wachtler et al., 1992; Hozák et al., 1993b; Jiménez-García et al., 1993; Derenzini et al., 1993; but see Thiry et al., 1991; Thiry, 1992; Puvion-Dutilleul et al., 1992) and with the localization of UBF - a transcription factor of RNA polymerase I (Roussel et al.,
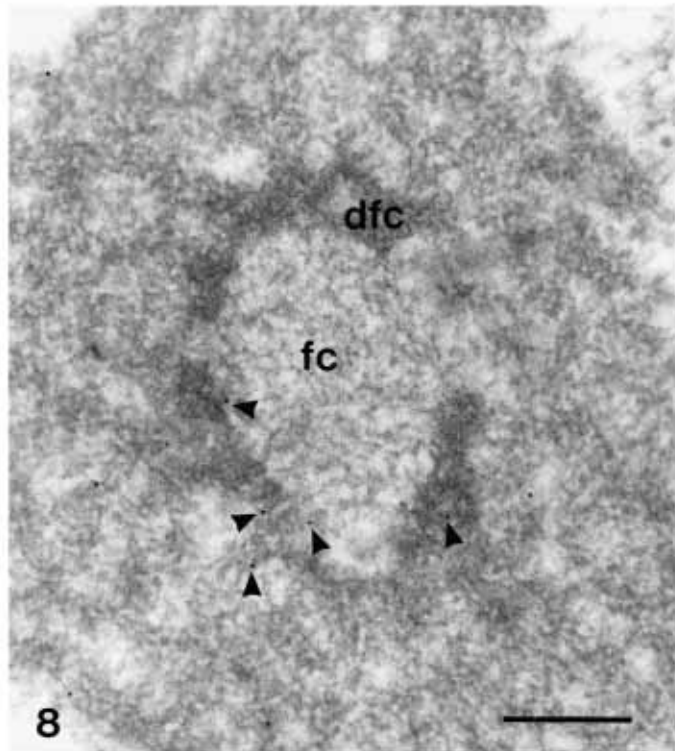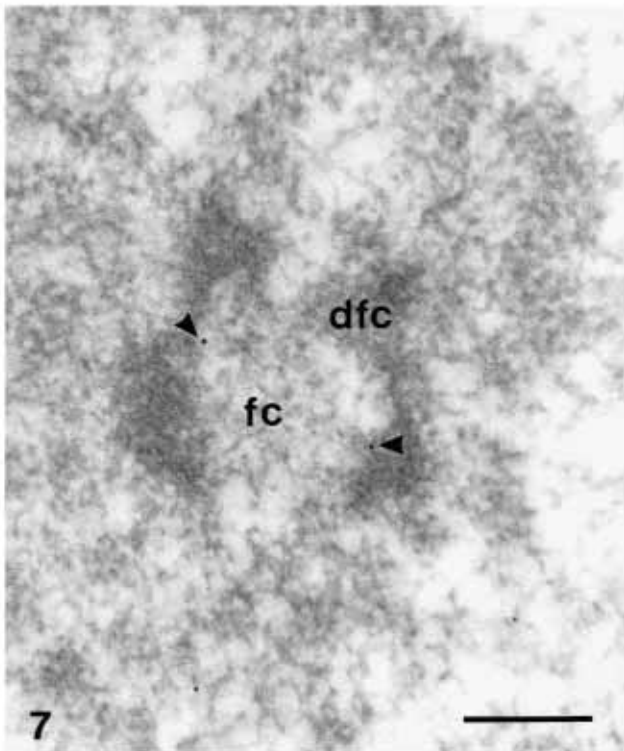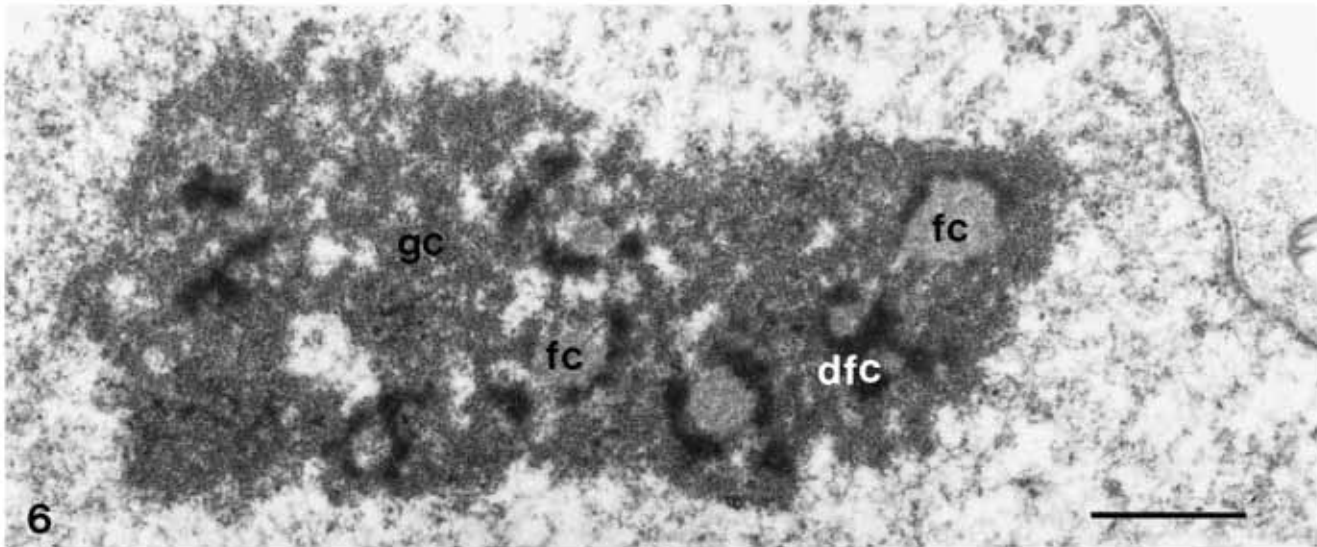

Fig. 6. Nucleolar morphology is retained after transcription in vitro. Part of the sample used for Fig. 8 was embedded in Epon so that nucleolar morphology could be compared with that found in vivo (Fig. 1). Fibrillar centres (fc), dense fibrillar component (dfc), granular component (gc). Bar, 1 μm.

Fig. 7. Immunolocalization of Br-RNA after incorporation of BrUTP by permeabilized cells for 1 minute. Cells were permeabilized, incubated with BrUTP, LR White sections prepared and Br-RNA immunolabelled with gold particles. The electron micrograph shows there is no labelling inside fibrillar centres (fc); what few particles (arrowheads) there are lie over the dense fibrillar component (dfc) and its border with the fibrillar centre. Bar, 0.2 μm.

Fig. 8. As Fig. 7, but incorporation for 2 minutes. Overall labelling is higher than after 1 minute labelling, but the pattern is similar; no particles are over the fibrillar centre (fc) but 5 particles (arrowheads) are over the periphery of the dense fibrillar component (dfc). Bar, 0.2 μm.

1993). The concentration of label at the borders of the dense fibrillar component to a greater extent than its interior suggests that transcription is concentrated at the borders but might also reflect differences in accessibility to immunolabel.

The role of the fibrillar centre

If rRNA synthesis does not take place in the fibrillar centre, what is its role? Even after incubation in vivo for 30 minutes

when labelling in the granular component is rising, there is no significant labelling of fibrillar centres, suggesting they are not on the processing pathway. Several observations show that essential components are stored there, including RNA polymerase I and topoisomerase I (Scheer and Rose, 1984; for review, see Derenzini et al., 1990; Wachtler et al., 1993). After removing the granular component, most of the dense fibrillar component and chromatin, fibrillar centres remain at nodes in
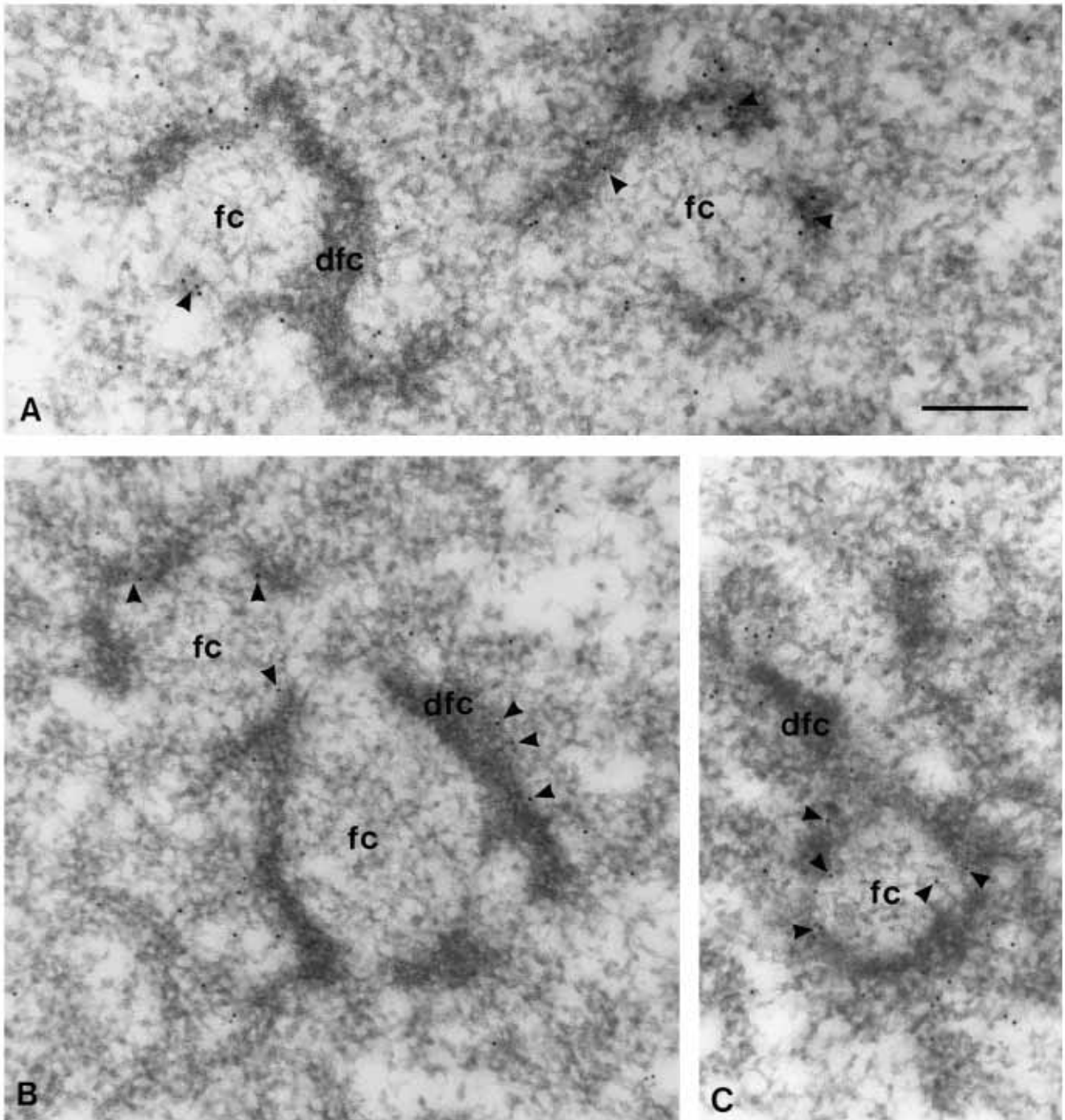

Fig. 9. As Fig. 7, but incorporation for 6 minutes. Most particles (some indicated by arrowheads) in three typical sections (A-C) lie over the periphery of the dense fibrillar component (dfc) with no significant labelling in the middle of the fibrillar centres (fc) or in the granular component. Bar, 0.2 μm.

the nucleoskeletal network (Fig. 10B); they also remain associated with rDNA when the nucleolus is disassembled during mitosis (e.g. see Schwarzacher et al., 1978; Hernandez-Verdun and Derenzini, 1983; Scheer and Rose, 1984). This suggests an additional structural role for the fibrillar centres, perhaps a central component around which the nucleolus is built after mitosis, nucleating the formation of the network that connects the nucleolus with the lamina. Once established, this network could integrate individual fibrillar centres into nucleoli, as well as whole nucleoli into the rest of the nucleus.

A working model of the nucleolus

Fig. 11 illustrates our current working model for the nucleolus based on these, and earlier, results. The nucleolus is built around fibrillar centres, which store the polymerases, topoisomerases and other proteins required for the synthesis of
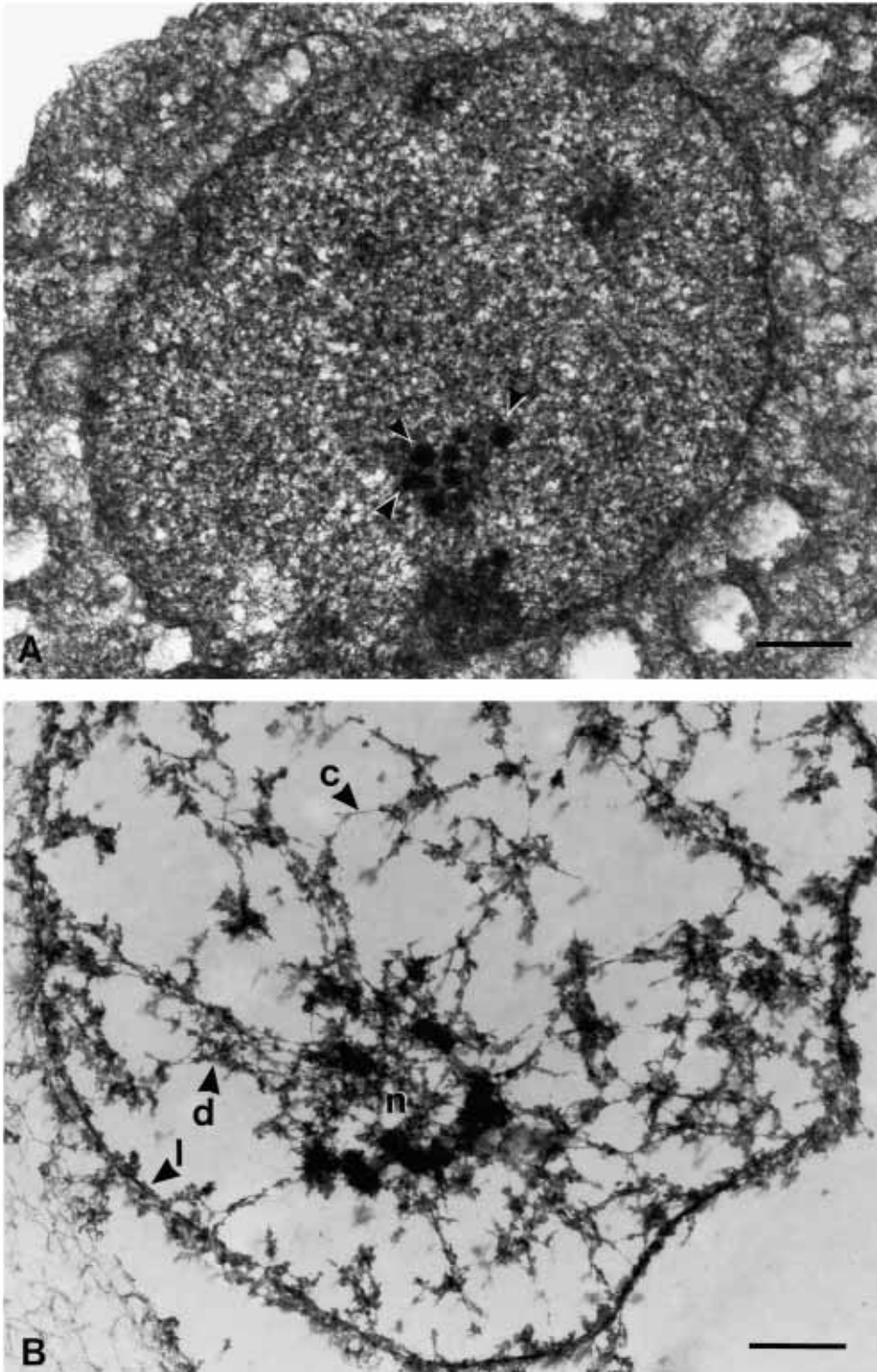

Fig. 10. Nucleolar structure in hypotonically treated cells. HeLa cells were encapsulated in agarose beads, regrown, subjected to a short hypotonic treatment, permeabilized and electron micrographs of resinless sections (~500 nm) taken. Bars, 1 μm. (A) Dense chromatin fills the nucleus and the granular component of the nucleolus has almost completely dispersed to leave a few fibrillar centres (arrowheads) and remnants of the dense fibrillar component. (B) As (A) but after permeabilization chromatin was cut with *Eco*RI plus *Hae*III and any detached fragments electroeluted. A network of core filaments (c) and more diffuse components (d) radiates from the nucleolar region (n) to the lamina (l); the network is concentrated in the nucleolar region where fibrillar centres remain attached.
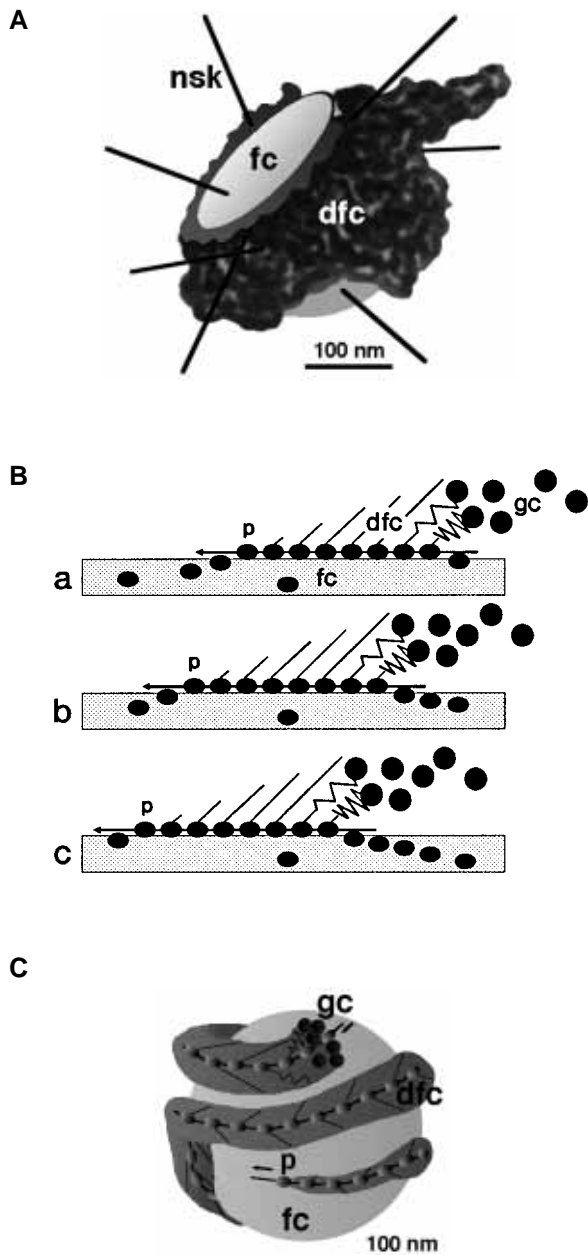
A

B

C

Fig. 11. A working model for nucleolar transcription. We have assumed there are 6 active cistrons (5 μm long and packed with 100 active polymerases) on each fibrillar centre (see Haaf et al., 1991; Jackson et al., 1993). fc, fibrillar centre. dfc, dense fibrillar component. gc, granular component. nsk, nucleoskeleton. Elipses, polymerases; p, promoter; angled lines, transcripts. (A) A section drawn roughly to scale through a fibrillar centre covered by the dense fibrillar component. (B) Three snapshots (a-c) of the template (horizontal line) sliding (arrow) through fixed polymerases as transcripts are extruded and supercoils are removed by flanking topoisomerases (not shown; see Dickinson et al., 1990). On termination, polymerases are recycled through the store in the fibrillar centre (stippled rectangle). (C) A view of one of the ~6 transcription units as it slides (arrows) over the surface of the fibrillar centre, drawn roughly to scale. As the promoter advances, polymerases initiate and then extrude nascent rRNA whilst at the 3′ end nascent rRNA in the dense fibrillar component condenses into the granular component (spheres).

primary transcripts (Fig. 11A). The dense fibrillar component surrounds the fibrillar centre and is the site where rRNA is polymerized (Figs 7-9), as suggested by Goessens and Lepoint (1979). Nascent transcripts are then converted into the granular component where they complete their maturation, ultimately to be transported along the skeleton to the cytoplasm (see also Meier and Blobel, 1992).

Although most models for transcription involve polymerases that track along the template, this seems unlikely. First, both polymerase and attached transcripts would have to rotate around the template once for every 10 bp transcribed; such rotation through the very dense fibrillar component - or any other part of the nucleolus - seems improbable (Cook, 1989; Cook et al., 1993). Second, if the chromatin fibre were looped by attachment to a skeleton - perhaps through a matrix attached region or MAR - and if active polymerases tracked along the fibre, fragmentation with restriction enzymes should allow polymerases to electroelute from nuclei; however, polymerizing activity remains after elution, implying that active polymerases attach the template to the underlying structure (Dickinson et al., 1990). Therefore we imagine that the template - and not the polymerase - moves, pulled through fixed polymerization sites as nascent transcripts are extruded (Fig. 11B). Then templates travel end-on through the dense fibrillar component. According to this view, the template is attached through active polymerases to the underlying structure (i.e. the fibrillar centre and the skeleton).

This template movement means that the dense fibrillar component apparently moves over the surface of the fibrillar centre; one end advances whilst the other is converted into the granular component (Fig. 11C). When the promoter emerges from one polymerase at the leading edge of the dense fibrillar component, it engages another polymerase on the surface of the fibrillar centre, which is then incorporated into the dense fibrillar component. So within the limits allowed by its dense surroundings, the promoter performs a random walk from one polymerase to another on the surface of the fibrillar centre. Meanwhile, at the other end of the cistron, full-length transcripts mature into granular components and newly inactive enzymes are re-cycled through the fibrillar centre.

This model can be reconciled with the beautiful images of spreads of ribosomal DNA (for a review, see Miller, 1981) if the hypotonic treatment used disperses the granular component and strips transcription units from the surface of the fibrillar centre much as in Fig. 10 (see also Hozák et al., 1992), before spreading produces the well known 'Christmas-trees'.

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