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Localization of the RNA polymerase I transcription factor hUBF during the cell cycle

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SUMMARY

Autoantibodies directed against nucleoli that recognized a doublet of 97-94 kDa in HeLa nuclear protein extracts were identified. The two polypeptides bound equal amounts of antibody, and each was recognized by antibodies affinity purified using the other polypeptide. These antigens were localized in the secondary constriction of PtK_1 cells, i.e. the nucleolar organizer regions (NORs) where ribosomal genes accumulate. They were observed in human cells in the same sites as the NOR-silver-stained proteins. The molecular mass of the antigens, their characteristics in Western blotting and their localization in nucleoli and NORs during mitosis are consistent with them being RNA polymerase I transcriptional factor, UBF. This identification was confirmed on Western blotted proteins by their identical labelling patterns, using these autoantibodies and an anti-mUBF antibody that had been previously described. We obtained definitive evidence that these autoantibodies recognize UBF by the strong positive labelling of purified hUBF (1 to 4 ng). During interphase, these autoantibodies directed against UBF labelled in a folded filament pattern as small beads that may correspond to individual transcriptional units. In electron microscopy, the antibodies were observed in the dense fibrillar component (DFC) of the nucleoli and at the periphery of the fibrillar centers (FCs). At the end of G_2 phase, transcription inactivation was concomitant with the gathering of UBF at mitotic NORs. UBF was not equally distributed between NORs in human cells: some NORs scored negative (2 to 4) and the intensity of labelling of positive NORs (6 to 8) differed. In confocal microscopy, 3-dimensional analysis of mitosis indicated that UBF remained associated with NORs during all mitotic stages and that there was equal partition of UBF between the daughter cells. The relationship between proteins associated with the NORs and ribosomal gene transcription is discussed.

Key words: UBF, RNA polymerase I transcription factor, confocal laser scanning microscopy, electron microscopy, mitosis, nucleolus, autoimmune sera, NOR

INTRODUCTION

Transcription of ribosomal RNA genes (rDNA) involves several factors besides RNA polymerase I (for review see Reeder, 1990; Sollner-Webb and Mougey, 1991). For accurate in vitro initiation by RNA polymerase I at least two factors, UBF (upstream binding factor) and SL1 proteins, are required (Bell et al., 1988). The exact roles of these two factors in the initiation of transcription are not completely clarified, but it has been established that both are required to activate the promoter regions of the rDNA (Bell et al., 1989). Recognition and binding to the rDNA promoter are carried out by UBF, whereas activation requires SL1 acting in conjunction with UBF to trigger transcription (Learned et al., 1986).

SDS/PAGE analysis of UBF from rDNA transcriptional complexes from four different vertebrate species reveals two bands (Bell et al., 1988; Jantzen et al., 1990; O'Mahony and Rothblum, 1991; Pikaard et al., 1990). Purified human UBF is composed of two closely related polypeptides (97-94 kDa) that are both required for the UBF DNA binding to the rDNA upstream control element and the core promoter of HeLa cells (Bell et al., 1988; Jantzen et al., 1990). The two polypeptides are immunologically related and they have similar reactivity with antibodies (Bell et al., 1988; Jantzen et al., 1990).

Most of the information concerning UBF and its relationship with rDNA transcription comes from reconstituted in vitro transcription systems and deoxyribonuclease (DNase) I footprinting experiments. However, the role of UBF in cells has not been investigated: only its localization in HeLa cell nucleoli and mitotic nucleolar organizer regions (NORs) is known (Chan et al., 1991; Jantzen et al., 1990; Rendon et al., 1992). An analysis of the distribution of UBF in cells with different ribosomal transcriptional activities would help in our understanding of the relationship between UBF and rDNA transcription. We therefore investigated the distribution of UBF in human cells at var-

ious stages during the cell cycle, so as to answer two questions. (i) Does distribution of UBF vary through the cell cycle, as rDNA transcription is successively activated and inactivated? and (ii) is there an even distribution of UBF between the rDNA in the 5 NOR-bearing chromosomal pairs (i.e. 13, 14, 15, 21 and 22), which are believed not to have equivalent transcriptional activity (see for review Babu and Verma, 1985)?

We used anti-UBF sera screened from human autoantibodies directed against nucleoli during interphase and against NORs during mitosis. This UBF affinity was established using a specific antibody (Voit et al., 1992) and purified hUBF (Jantzen et al., 1990). These sera are similar to those called NOR-90 autoantibodies (Rendon et al., 1992; Rodriguez-Sanchez et al., 1987), which have been recently characterized as anti-UBF antibodies by screening of cDNA library and sequencing (Chan et al., 1991).

We found that UBF is distributed during interphase into small beads that could correspond to individual transcriptional units. UBF antibodies were observed in the dense fibrillar component (DFC) of the nucleoli and at the periphery of the fibrillar centers (FCs). At the end of G_2 phase, the transcription switch off was concomitant with the gathering of UBF at mitotic NORs. We also noted that the amount of UBF varied between positive NORs and that some NORs were negative. We showed by 3-dimensional analysis of mitosis that UBF remained associated with NORs during all mitotic stages and that there was equal partition of UBF molecules between the daughter cells.

MATERIALS AND METHODS

Autoimmune sera

We selected nine autoimmune sera that labelled both nucleoli during interphase and a few chromosomes during mitosis. Four of these sera, S14, B15, D16 and A17, were found to react with a doublet in Western blots of nuclear antigens. We verified that these sera did not contain any additional associated autoantibodies against DNA, nRNP, Sm, La/SS-B, Scl 70, Jo1, PCNA and Ro/SS-A using standards and conditions previously described (Masson et al., 1990). These sera contained only nucleolar autoantibodies that were characterized as immunoglobulin G. Solubilization of the antigens was checked on rat liver cryosections. However, as antigens may be modified by incubation with the buffers, we were not able to obtain conclusive data on the association of these antigens with DNA and RNA. This indicated that the preservation of the antigens needs special attention.

Immunoblotting

Actively growing HeLa cells were washed in culture medium without serum. They were lysed at 4°C in TKM buffer (10 mM Tris-HCl, pH 7.4, 10 mM KCl, 3 mM $MgCl_2$), containing 1 mM PMSF, 10 μg/ml aprotinin, 1 μg/ml pepstatin and 1 μg/ml leupeptin. Lysis was stopped when the nuclei appeared free of cytoplasmic components as assessed by phase-contrast microscopy. The nuclear pellets were then washed twice with 0.25 M sucrose in TKM buffer and the protein concentration of each sample was evaluated using the BCA Protein Assay Reagent (Pierce Chem. Co.). Laemmli sample buffer was added (Laemmli, 1970) and the samples were sonicated, boiled for 5 min and centrifuged for 30 s.

The proteins were separated by electrophoresis in 1-dimensional 12% to 8% SDS-polyacrylamide gels in Protean II cells (Bio-Rad Laboratories). Size standards from 200 to 14 kDa, purchased from Bio-Rad, were included in each gel. The polypeptides were electrotransferred to reinforced cellulose nitrate membranes (BA-S 83, Schleicher & Schuell), which were then cut into strips. For general staining of the proteins, we used the Protogold kit (BioCell Research Laboratories, Cardiff, UK) as recommended by the supplier.

The strips were blocked by incubation for 1 h in PBS containing 5% dried skimmed milk and 0.05% Tween 20. They were then incubated with the sera for 2 h in the same buffer. The membranes were then washed three times and incubated for 1 h in the presence of HRP-labelled second antibody (dilution 1/3,000). After several rounds of washing, the HRP activity was detected with the enhanced chemiluminescence kit (Amersham) and recorded on Fuji X-ray films.

The antibodies were affinity purified using nuclear proteins, following the strategy of Olmsted (1981). 8% SDS-PAGE was used for a better resolution of the polypeptides of high molecular mass. Briefly, antibodies were eluted with 3 M KSCN and the suspension was dialysed under vacuum to a final volume of 200 μl (Masson et al., 1990).

The anti-mUBF antibody kindly provided by R. Voit and I. Grummt has been described (Voit et al., 1992). It was used (dilution 1/1000) in parallel with S14 serum in adjacent strips.

Affinity-purified hUBF was generously provided by L. Comai and R. Tjian (Comai et al., 1992).

Cells and immunofluorescence labelling

Human cells (HeLa, TG and HEp-2) were cultured in Eagle's minimum essential medium (MEM) (Flow Laboratories) supplemented with 10% fetal calf serum. PtK_1 cells were cultured in MEM containing 0.85 g/l $NaHCO_3$ (Flow Laboratories).

For immunofluorescence labelling, cells were grown as monolayers on glass slides. Glass slides were previously treated with 5% KOH-methanol for 12 h, rinsed in water, treated with 0.01 M HCl for 1 h, washed in distilled water and ethanol, and sterilized. Cell monolayers were rinsed in phosphate-buffered saline (PBS) and fixed with 3% formaldehyde in PBS containing 1% Triton X-100 for 20 min. Cells were incubated with serum for 45 min and the antibodies revealed by FITC-conjugated rabbit anti-human IgG (dilution 1/200) or rhodamine-conjugated goat anti-human IgG (dilution 1/50). DNA was visualized with DAPI or propidium iodide. All preparations were mounted with an antifading solution (Citifluor, London).

PtK_1 chromosome spreading for immunolocalization was carried out according to Merry et al. (1985). Before harvesting, cells were blocked in mitosis by incubation in 0.4 μg/ml of colchicine for 2 h.

Confocal laser scanning microscopy (CLSM)

Confocal laser scanning microscopy was performed using a BioRad MRC-600, mounted on an Optiphot II Nikon microscope equipped with a 60× objective (Plan Apo; NA 1.4). An argon ion laser adjusted to a wavelength of 488 nm was used for the fluorescein signal, and a helium-neon ion laser adjusted to 543 nm for propidium iodide. The emitted light was separated by a dichroic mirror (DR565) and a (540DF30) long-pass filter was placed in front of the photomultiplier to collect the fluorescein emission. For each optical section, double fluorescence images were acquired in two passes: fluorescein first, propidium iodide second. The emitted signal was digitalized by "photon counting" in order to increase the signal-to-noise ratio, each section was scanned 30 times and for phase-contrast the signal was treated by Kalman filter (average of 8 images). The pinhole of the confocal system was adjusted to allow a field depth of about 0.5 μm, corresponding to the increment between two adjacent sections. A

focal series of up 16 sections apart was collected for each specimen and then processed to produce single composite images (extended focus), combining a high spatial resolution with an increased depth of field. Furthermore, the extended focus of fluorescence signals could be superimposed onto the phase-contrast image for structure correlation (Shotton, 1989). Color pictures from screen images were taken on Fujichrome 100 with an Olympus OM-135 mm camera.

Silver staining of Ag-NOR proteins for light microscopy

The selective silver staining method for the Ag-NOR proteins was adaptated from Howell and Black (1980). The preparations were washed for 5 min in 70% ethanol and then in deionized water. The slides were then covered with a freshly made staining solution prepared by mixing 2 volumes of solution A and 1 volume of solution B: (A) 0.5 g/ml of silver nitrate (Merck) in deionized water; (B) 1 mg/ml of gelatin dissolved in 1% (v/v) formic acid. The slides were incubated for 15-20 min at room temperature. After staining, they were vigorously washed in distilled water, dehydrated in ethanol and mounted in ethanol-Eukitt.

Immunoelectron microscopy

Human TG cells were fixed with phosphate-buffered 4% paraformaldehyde for 1 h. Free aldehyde groups were blocked by incubation in 0.5 M NH_4Cl for 2 h and the cell pellets were embedded at low temperature in Lowicryl K4M. HeLa cells were also processed by EM cryotechniques for monolayers as recently described (Hernandez-Verdun et al., 1991).

Affinity-purified antibodies were used for immunolabelling. Thin sections of the pellets were floated overnight on sera (dilution 1/30), washed several times and incubated for 1 h with 10 nm anti-human IgG-gold particles (dilution 1/20). The control assays were carried out with a pool of normal human sera. Cell structures were contrasted with uranyl acetate.

RESULTS

Characterization of anti-UBF antibodies

The four sera used in this study (S14, B15, D16 and A17) recognized two bands in Western blots of nuclear proteins. The molecular mass of this doublet in HeLa nuclear extracts was 97-94 kDa (Fig. 1A, lane b). The relative intensity of the two bands of the doublet was constant up to a dilution of 1 in 200 with serum S14 and 1 in 2000 for the others. The 97 kDa affinity-purified antibodies recognized, with identical intensity, the two bands of the doublet (Fig. 1A, lane c), as did the 94 kDa affinity-purified antibodies (Fig. 1A, lane d). The molecular mass of the doublet is identical to that of the RNA polymerase I transcription factor, UBF, described in the same cells by Jantzen et al. (1990). Moreover, the similar proportions of the two bands of the doublet and their immunological cross-reactions are in agreement with this identification. This is also in accordance with the identification of NOR-90 autoantibodies as anti-UBF antibodies (Chan et al., 1991). We proved that the 97-94 kDa doublet in HeLa cell extracts corresponds to the position of UBF by labelling with a specific anti-mUBF, already characterized (Voit et al., 1992). The pattern of labelling was identical using anti-UBF and serum S14 in parallel in adjacent strips (Fig. 1B). Finally, we tested the autoimmune sera on purified hUBF. As observed in dot blots (Fig. 1C), the autoantibodies possess a high affinity for purified hUBF; we found a positive reaction between 1 and 4 ng of protein as illustrated (Fig. 1C) but this is not the minimum amount that can be detected in dot blots using the autoimmune sera. Thus: (i) the autoantibodies recognized a doublet of 97-94 kDa in HeLa nuclear protein extracts; (ii) the same doublet has been identified as UBF, using a specific anti-mUBF antibody, already characterized (Voit et al., 1992); (iii) the autoantibodies reacted with purified hUBF. We can conclude therefore that they are directed against RNA polymerase I transcription factor, UBF.

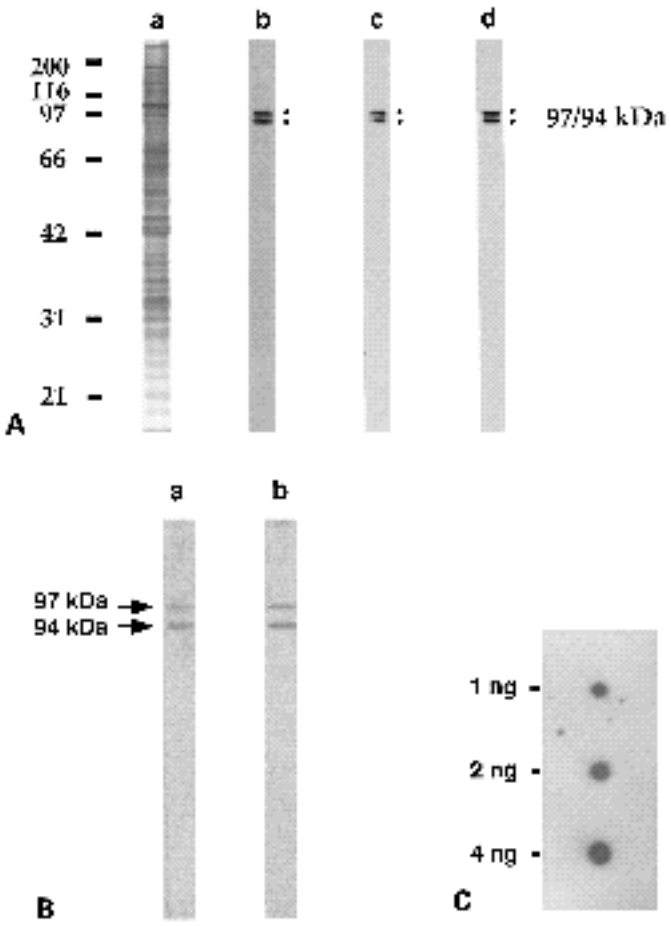

Fig. 1. Identification of the antigens by Western blots and dot blots. (A) Proteins revealed on Western blot (SDS-10% PAGE) by general Protogold staining or by serum S14; 20 μg of HeLa nuclear proteins extracts were loaded per lane. Among the numerous nuclear proteins revealed by Protogold (lane a), two polypeptides of 97-94 kDa (filled circles) are recognized by serum S14 (lane b). Antibodies immunopurified against the 97 kDa polypeptides revealed the two bands of the doublet with identical intensities (lane c). Similarly, antibodies immunopurified against the 94 kDa polypeptides revealed both bands of the doublet with identical intensities (lane d). (B) Proteins revealed on Western blot (SDS-7% PAGE) by an anti-mUBF antibody, already characterized (lane a), and serum S14 (lane b). The 97-94 kDa doublet specific for UBF is identical with the doublet revealed by serum S14 using adjacent strips. (C) Purified hUBF dot blots probed with serum S14. The three amounts tested (1, 2 and 4 ng) were strongly positive.

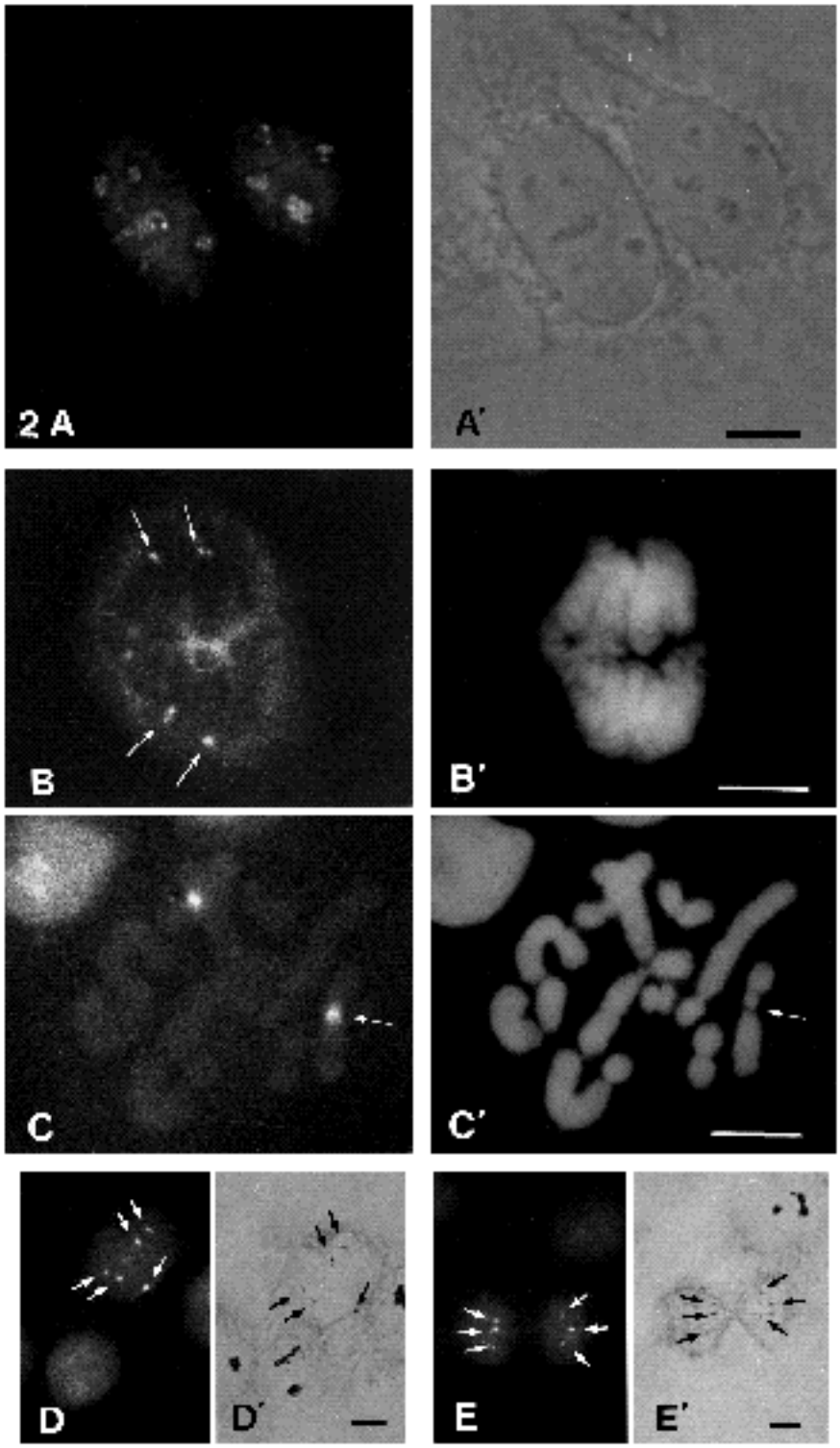

Fig. 2. Localization of UBF by light microscopy. (A, A′) Interphasic HEp-2 cells labelled with serum S14. The immunofluorescence (A) is observed in the nucleoli visible by phase contrast (A′). Bar, 10 μm. (B, B′) Anaphase PtK_1 cell labelled by serum S14. Two positive signals (arrows) are observed in each daughter cell (B) in association with the chromosomes revealed by DAPI (B′). Bar, 10 μm. (C, C′) Labelling by serum S14 of mitotic PtK_1 chromosomes after spreading. Two positive signals are observed (C) in the secondary constriction of the chromosomes visualized after DAPI (arrows) (C′). Bar, 10 μm. (D, D′) Successive detection of UBF by serum S14 and of the Ag-NOR proteins in prometaphase HEp-2 cell. The intense fluorescent spots (arrows; D) exhibit the same distribution as the Ag-NOR positive sites (arrows; D′). Bar, 10 μm. (E, E′) as D, D′ in an anaphase HEp-2 cell. Bar, 10 μm.

As these sera are specific for UBF, their affinity for nucleolar components that correspond to NORs was checked. In HEp-2 nucleoli, the labelling formed chain-like structures, which appeared in the most central region of the nucleolar body in light microscopy (Fig. 2A, A′). These central regions of the nucleoli are known to correspond to interphasic NORs (see for review Hernandez-Verdun, 1986). During mitosis UBF recognized by the sera remained associated with a few chromosomes. This labelling appeared as intense spots. In PtK_1 cells known to possess two NORs, we found two intense spots in association with the chromosomes. In anaphase, the two spots were symetrically distributed in positions that would be predicted for the NOR at this stage, i.e. close to the centromeres (Fig. 2B, B′). On PtK_1 spread chromosomes, the spots were asso-

ciated with the secondary constriction (Fig. 2C, C′). This is precisely the location of the NORs in the PtK_1 caryotype. In the human cell lines, HeLa, TG and HEp-2, there were several spots visible at different foci in association with the chromosomes. We identified the NORs of the chromosomes as the target of the antibodies by successive localization in the same cells of UBF and Ag-NOR proteins. The most intense fluorescent spots were superimposed on the silver-stained NORs at each stage of mitosis (Fig. 2D, D′, E, E′). This observation clearly identifies the NORs, since the Ag-NOR proteins are specific markers for the NORs (for review see Derenzini and Ploton, 1991).

Localization of UBF during interphase by EM immunolabelling

We used the immunopurified antibodies directed against UBF to probe actively growing human cells. In these cells, ribosomal gene transcription is active from telophase until G_2 phase.

Bound antibodies were localized by electron microscopy using anti-human IgG bound to gold particles. We observed a large number of nucleoli in thin sections and found the labelling confined to the fibrillar components of nucleoli (DFC and FC) and absent from the granular component (GC) and other nuclear organelles (Fig. 3A). In the fibrillar nucleolar component we found a preferential accumulation in DFC (see the lower DFC in Fig. 3B) and also in FC (see upper FC of the same nucleolus Fig. 3B**)**. The periphery of FC was generally more intensely labelled than its central region (Fig. 3C). The same distribution was observed in samples that were processed by EM cryomethods (Fig. 3D). The samples were quick-frozen in liquid helium, cryosubstituted and cryoembedded at −60°C (the best conditions known to preserve the fragile antigens) (Hernandez-Verdun et al., 1991). As shown in Fig. 3D, the labelling is superimposed on fibrils at the periphery of FC and in DFC.

Distribution of UBF during the cell cycle observed by CLSM

During interphase the nucleolar labelling formed a chain-like structure composed of beads (Fig. 4A, A′). The number of beads per nucleolus varied in the different cells and in nucleoli of the same cell. The number of beads was evaluated in 37 HEp-2 cells chosen at random and the average number was 45 beads per cell. By confocal reconstruction it is clear that the beads were distributed regularly, forming a folded filament. In some nucleoli, we also observed an intense spot in addition to the chain-like filament. In human peripheral lymphocytes, a central positive spot was observed in resting nucleoli. This observation indicates that the distribution of UBF depends on the transcriptional activity of the ribosomal genes during interphase.

rDNA transcription is inactivated at the end of G_2 phase when the chromatin condenses. Consequently, the nucleolar structures disassemble, with the exception of some nucleolar proteins, which remain associated with the NORs. Phase-contrast observation of human cell monolayers could not be used for the identification of cells in late G_2 phase because the nuclear shape is maintained, the nucleolar structures are still visible and the condensation of the chromatin is not detectable (Fig. 4B). However, in these cells, the condensation of the chromatin into mitotic chromosomes was clearly visible in optical sections recorded by CLSM using nucleic acid-specific fluorochrome (Fig. 4B″). At this stage the serum S14 formed discrete spots of variable intensity (Fig. 4B′, B‴) localized in the nucleolar structures visible by phase-contrast (Fig. 4B). These images showed the redistribution of UBF as rDNA transcription was inhibited.

During prophase in human cells, UBF remained associated with several chromosomes but the amount per chromosome varied.

During metaphase, the positive chromosomes presented a polarized distribution forming four or five bands perpendicular to the metaphasic plate (Fig. 5A, A′, A″). Serial optical sectioning of labelled cells in metaphase distinguished between spots of different intensity, indicating variable amounts of UBF in the different chromosome pairs (Fig. 5B). In most cases there were four intense spots and three or four spots of medium intensity. The spots of medium intensity were distributed in pairs separated by a distance similar to the chromosome thickness. These symmetrical spots of medium intensity probably corresponded to the labelling of two chromatids. For intense spots distinguishing between the two chromatids was not possible because of the signal intensity. This interpretation suggests that only three or four pairs of NOR-bearing chromosomes are associated with UBF in HEp-2 cells. Chromosomes bearing intense spots and those bearing medium spots were segregated (Fig. 5A″). The schematic representation of UBF indicates a clustering of homologous chromosomes on the metaphasic plate (Fig. 5A″).

During anaphase, UBF was equally partitioned between the two chromosomal sets and their distribution was symmetrical (Fig. 6A, A′). At this stage, there is only one chromatid per chromosome and as postulated above there were only single small spots on each chromosome (Fig. 6B). The 3-dimensional analysis of the distribution of UBF as illustrated in CLSM optical sections demonstrated that there are six active NORs per anaphase cell that possess either large amounts of antigens (two intense spots), or moderate amounts (four medium spots), and one pair with a small amount of antigen (two weak spots) that is difficult to detect (Fig. 6A′, B). We can conclude that one pair of NOR-bearing chromosomes, having no detectable amount of UBF, is not likely to be active in rDNA transcription in HEp-2 cells.

During telophase, the same number of spots and the same fluorescence intensities were observed, indicating that rDNA transcription activation is not correlated with a detectable increase in the amount of UBF.

DISCUSSION

UBF and ribosomal genes

UBF has sequence-specific DNA binding activity and this property has been used for its purification (Kadonaga and Tjian, 1986). UBF binds to upstream regions of the rDNA promoter and also interacts with the core promoter domain (Bell et al., 1988; Jantzen et al., 1990). This DNA-binding property is conserved among different species including

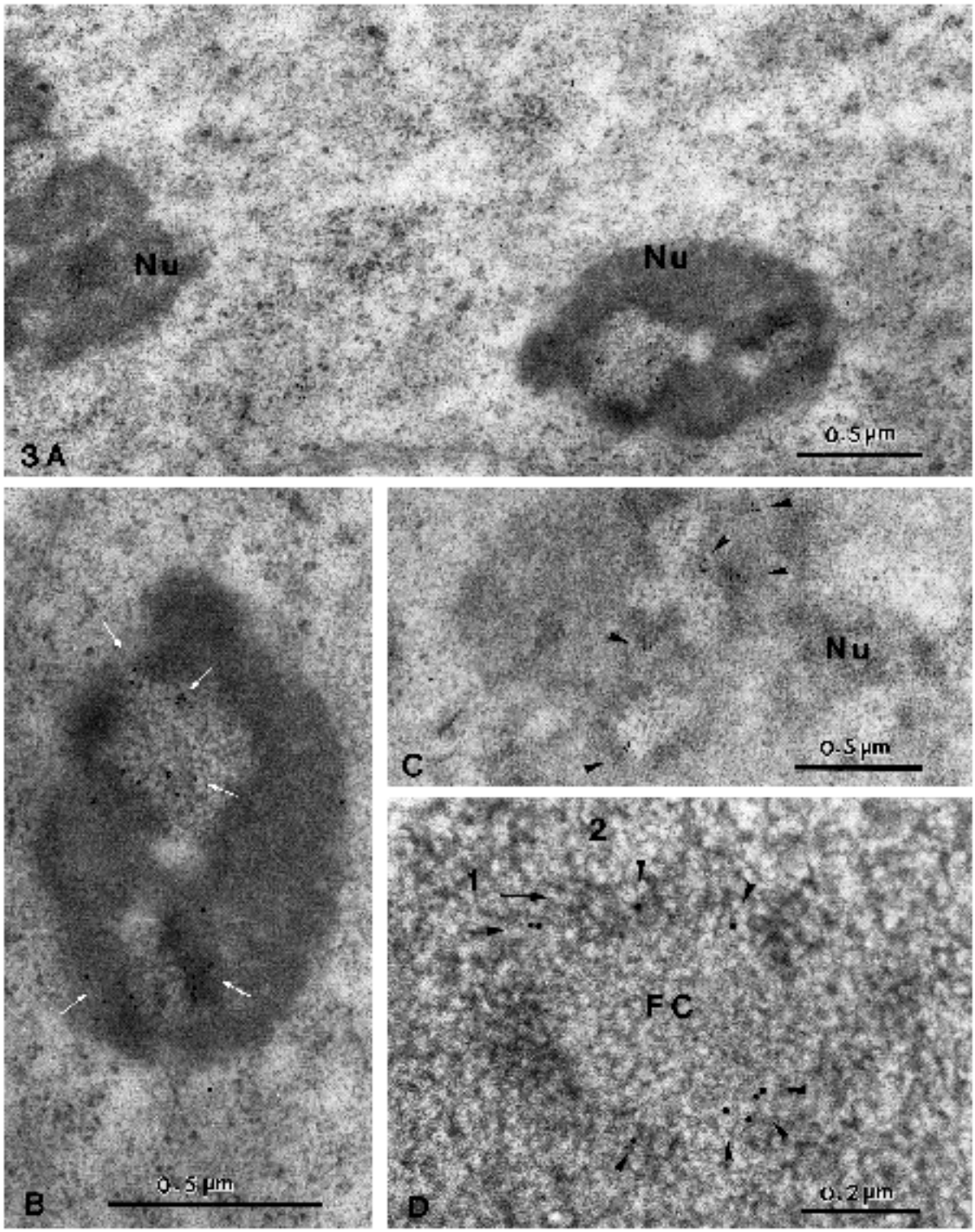

Fig. 3. Localization of UBF by electron microscopy. (A) Detection of UBF in human TG cells fixed with paraformaldehyde and embedded in Lowicryl K4M. Immunopurified antibodies from serum B15 labelled the nucleoli (Nu). No labelling was observed outside the nucleoli. Bar, 0.5 µm. (B) Detail of the nucleolus observed in A. The gold particles are visible in the FC and the DFC (arrows). Bar, 0.5 µm. (C) Same preparation as in A. The gold particles (arrowheads) are observed over the DFC and at the periphery of the FC. Bar, 0.5 µm. (D) Detection of UBF in human HeLa cells fixed with liquid helium, cryosubstituted and cryoembedded in Lowicryl K11M. Serum S14 labelled (arrowheads) mainly the DFC (1) and fibrils in the FC. No labelling is observed in the GC (2). Bar, 0.2 µm.

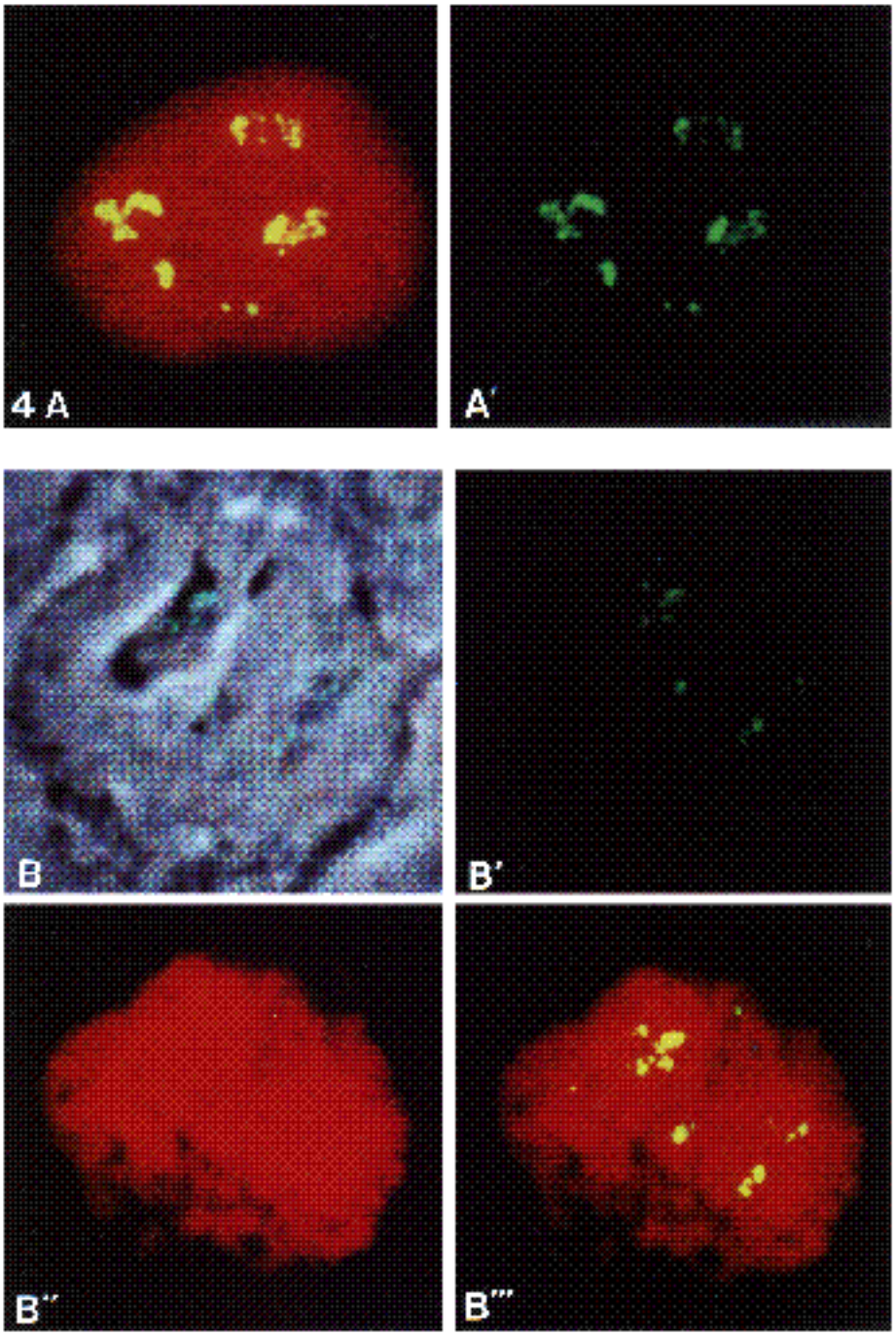

Fig. 4. Localization of UBF by confocal microscopy. (A, A′) Interphase HEp-2 cell labelled with serum S14. Extended focus of the labelling observed in four nucleolus. Three of them form chain-like structures. (B, B′, B″, B‴) Transition from G_2 to prophase in a HEp-2 cell labelled with serum S14. (B) Combined images from optical sections of the labelling and phase-contrast image. Superimposition demonstrates the localization of the labelling in the nucleolar structures. (B′) Extended focus of the immunolabelling with serum S14 recorded on 6 serial sections 0.8 μm apart. (B″) Extended focus of the distribution of DNA labelled with propidium iodide recorded on 6 serial sections 0.8 μm apart. Chromosome condensation is visible in this cell. (B‴) Combined images from optical sections of immunolabelling and of the DNA.

man (Jantzen et al., 1990), mouse (Pikaard et al., 1990), rat (O'Mahony and Rothblum, 1991) and *Xenopus* (Pikaard et al., 1989). Therefore, it was proposed that UBF activates rDNA transcription in a binding site-dependent manner (Jantzen et al., 1990) and recognizes in different species the same DNA sequence elements for specific RNA polymerase I transcription factors. Anti-UBF antibodies recognized in situ antigens in human cell lines as well as in PtK_1 cells derived from a marsupial. Most probably, UBF recognizes specific sequences of the rDNA promoter that are conserved in all vertebrates.

In vitro, direct binding of transcription factors to DNA is rapid. It appears that UBF is the first factor to bind to rDNA, and this binding permits the fixation of the SL1 transcription complex as well as the positioning of rRNA polymerase I. RNA polymerase I is not capable of binding to rDNA in the absence of transcription factors. UBF appears to be present at approximately 50,000 copies per cell as compared to approximately 200 copies of rDNA per haploid genome in HeLa cells (Bell et al., 1988). However, we have no indication of the percentage of UBF molecules that bind in vivo to the rDNA or whether there is a pool of inactive UBF in cells (as is the case for RNA polymerase I). The present data on UBF distribution during the cell cycle indicate that the majority of the molecules are bound in the

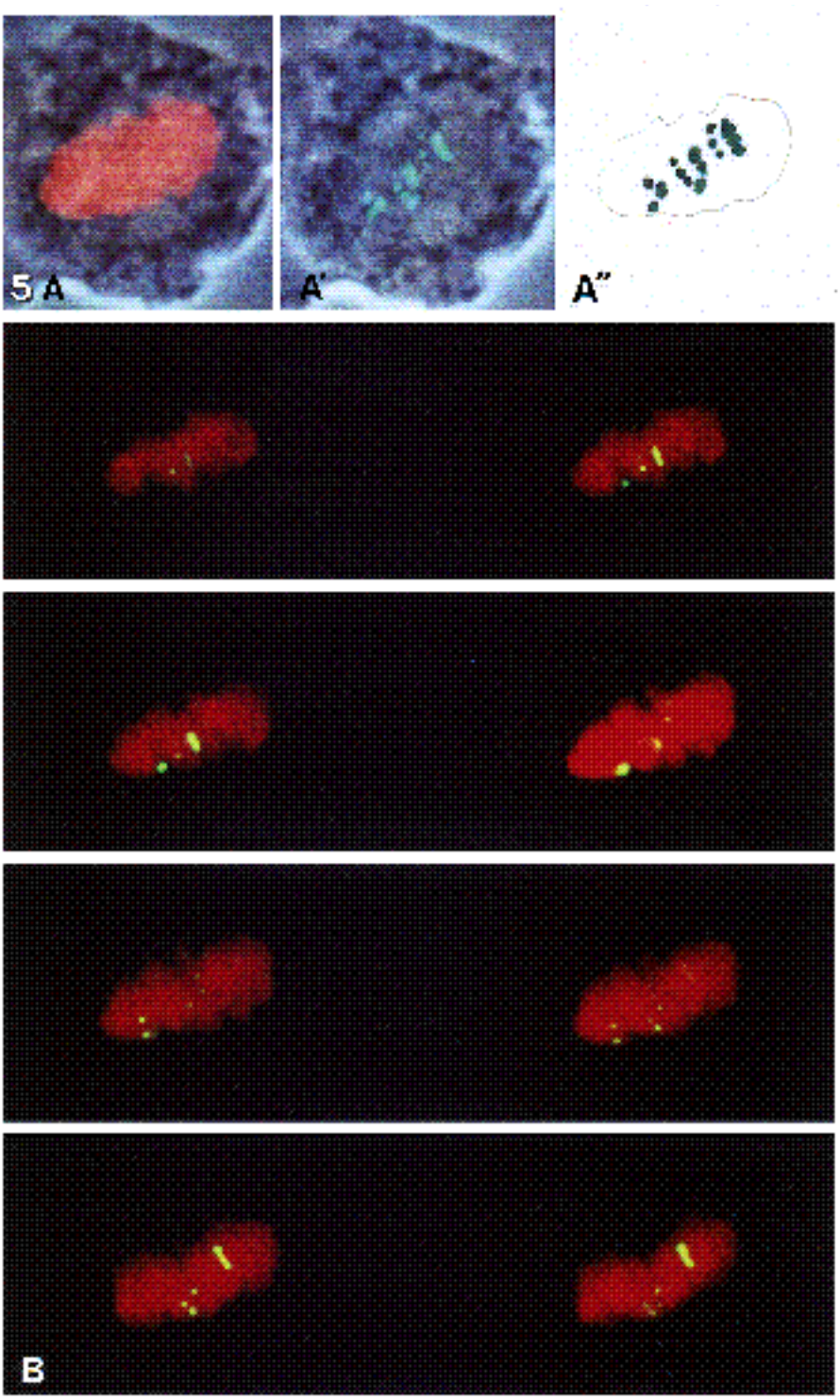

Fig. 5. Localization of UBF in metaphase by confocal microscopy. (A, A′, A″) Metaphase HEp-2 cell. (A) Combined images from optical sections of the DNA labelled with propidium iodide and phase-contrast image. The superimposition shows the position of the metaphasic plate. (A′) Combined images from optical sections shown in B of immunolabelling with serum S14, and the phase-contrast image. The positive spots are aligned on the metaphasic plate. (A″) Schematic presentation of the labelling indicating the position of spots of different intensity. (B) Serial confocal optical sections, 0.8 μm apart, of the immunofluorescence labelling through the metaphase plate. Immunolabelling and DNA fluorescence were recorded simultaneously.

nucleolus and in the mitotic NORs, since there was no other diffuse or discrete labelling by the sera.

UBF during active transcription in situ

rDNA is transcribed from late telophase to G_2 phase but only a limited number of the rDNA sequences are simultaneously transcribed. A recent in situ estimation suggested that an average of one hundred ribosomal transcriptional units are simultaneously transcribed in human cells (Haaf et al., 1991). This value was obtained by dissociating nucleolar structures by drug treatment and then identifying the transcriptional units by localization of RNA polymerase I. The labelling was characterized by positive spots called beads or granules arranged as a necklace-like structure. Each bead of the necklace is supposed to correspond to the labelling of one transcriptional unit (Scheer et al., 1984). Using anti-UBF antibodies the number of beads estimated per cell was close to that obtained with anti-RNA poly-

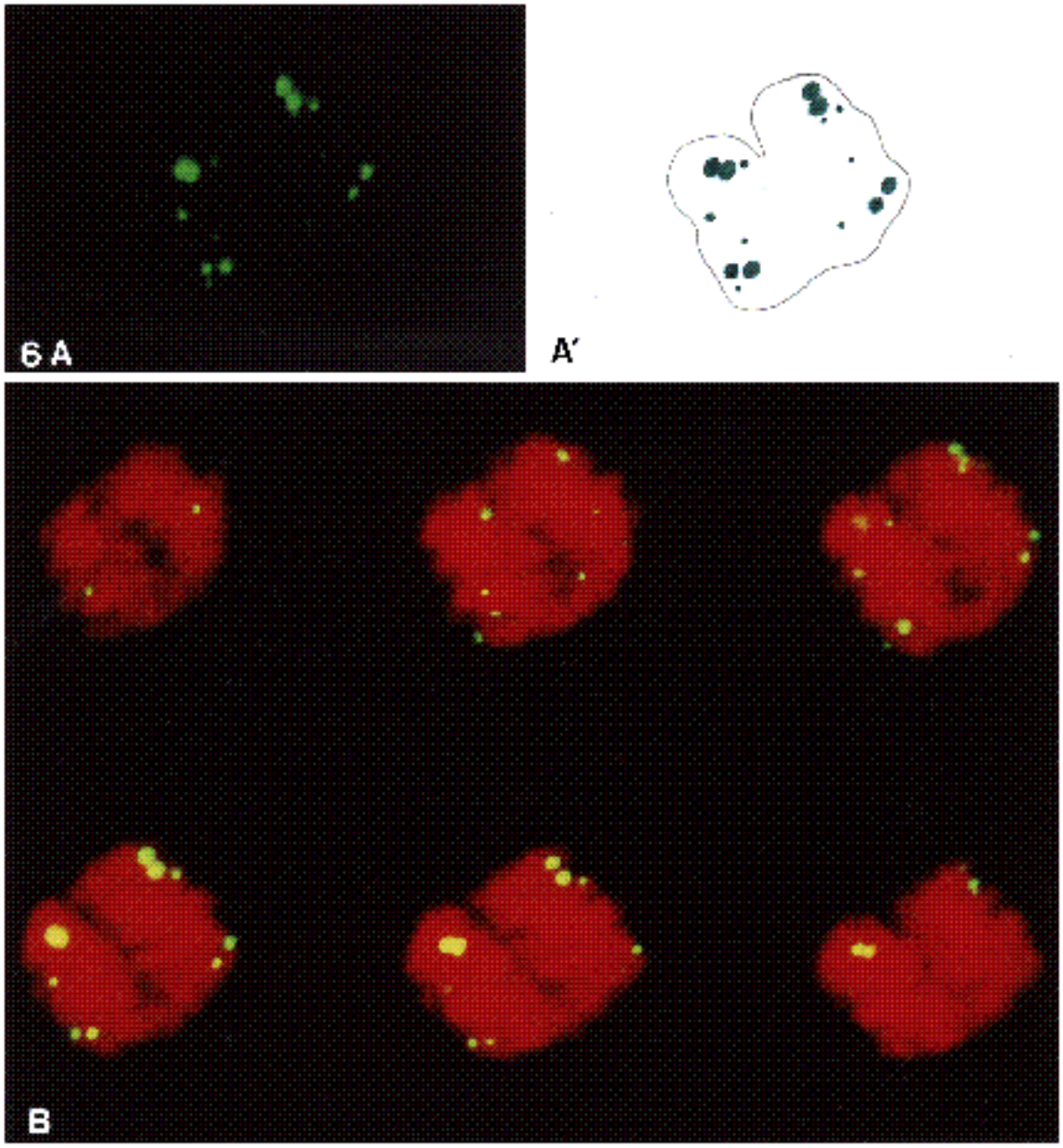

Fig. 6. Localization of UBF in confocal microscopy in anaphase. (A) Extended focus of the immunolabelling observed in a HEp-2 anaphase cell, from serial optical sections presented in B. The positions and the sizes of the positive spots appear symmetrical. (A′) Schematic presentation of the labelling indicating the position of spots of different intensity. The spots are observed as pairs of similar signal intensity probably corresponding to chromosome pairs. (B) Six serial optical sections, 0.8 μm apart, in which the immunolabelling and the DNA fluorescence were recorded simultaneously. The association of the labelling with the chromosome is clearly visible. Note the different sizes of the spots.

merase I antibodies without drug treatment, i.e. in intact nucleoli (Haaf et al., 1991). Thus anti-UBF antibodies may label each ribosomal transcriptional unit, and therefore be able to identify the rDNA corresponding to the promotor sequences in undissociated rDNA. If this hypothesis is correct the chain-like organization of the UBF labelling suggests that in intact nucleoli rDNA forms a folded chain-like structure corresponding to the tandemly arranged transcribed genes.

In immunoelectron microscopy, we observed anti-UBF antibodies in the dense fibrillar component (DFC) of the nucleolus and the fibrillar component (FC), mostly at its periphery. These components have both been proposed to be sites of rDNA transcription (for recent review see Jordan, 1991). However, the precise localization of transcription in these two nucleolar components is still being debated: FC, at the periphery of FC, or in DFC have been suggested (for review see Fischer et al., 1991; Jordan, 1991; Schwarzacher and Wachtler, 1991; Thiry and Goessens, 1992). The localization of UBF in DFC and at the periphery of FC indicates that the rDNA promotor sequences are found in these sites during active rDNA transcription. On the contrary, if

transcription was sequestered in FC, UBF should accumulate in FC but not in DFC. In this case, DFC would exclusively contain primary transcripts and associated proteins.

UBF during mitosis

When the cells enter mitosis the pattern of distribution of UBF changes rapidly. Concomitantly with the early chromosome condensation in late G_2, UBF molecules gather, forming a few intense spots in the remnant of nucleolar structure and the chain-like distribution of UBF is no longer visible. The gathering of UBF is most probably correlated with the stopping of rDNA transcription that takes place at that time and is similar to the distribution of UBF observed in mature lymphocytes. It is tempting to propose that UBF gathers in the NORs. The localization of the Ag-NOR proteins, which are markers of the mitotic NORs (for review see Babu and Verma, 1985; Hernandez-Verdun, 1991), was therefore determined. UBF was indeed found in the major Ag-NOR sites at various stages of mitosis in human cells. Therefore, even in absence of rDNA transcription UBF can be found associated with the chromosomal regions corresponding to ribosomal genes.

It is noticeable that in PtK_1 cells the two NORs exhibit similar amounts of UBF, whereas the different NORs of human cells possess different amounts of UBF. The amount of UBF may reflect the number of genes in the NORs or their abilility to fix UBF. The latter hypothesis seems more probable, since the sensitivity of the technique permits the detection of UBF associated with one transcriptional unit (above) we can expect to detect the signal from a few genes.

In human cells, rDNA is distributed among ten NOR-bearing chromosomes. Only six or eight are associated with UBF. Therefore it is possible that the roles of the different NORs depend on differential binding of transcriptional factors; this difference differentiating, at least in part, active from inactive NORs.

The differential binding of transcriptional factors seems to be inheritable, since UBF is equally partitioned between daughter cells. Moreover, the strictly symmetrical distribution of the labelling in anaphase indicates that there is conservation of the amount of UBF in NORs corresponding to the same chromosome pairs.

Characterization of the NORs

NORs contain rDNA associated with specific proteins. Proteins associated with NORs have been detected using antibodies and by Ag-NOR staining. In the latter case, the proteins stained are currently being characterized (Hozak et al., 1992; Roussel et al., 1992). Ag-NOR staining (Babu and Verma, 1985) and immunodetection of UBF (this work) indicate that the amount of NOR-associated protein varies between NORs in human cells, and this variation correlates with NOR activity during interphase. The presence of nucleolar proteins such as RNA polymerase I and nucleolin has also been reported in active NORs (Gas et al., 1985; and for review see Fischer et al., 1991). Thus, proteins directly involved in transcription appear to remain bound or associated with rDNA during mitosis. However, the presence of proteins associated with ribosomal genes does not mean that they are active or bound to the genes. The presence of the proteins required for transcription, including UBF and RNA polymerase I, close to the rDNA during mitosis may allow the rapid switch-on of rDNA transcription in telophase: activation would only require the addition of one factor or modification of NOR-associated proteins.

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