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Characterization and immunolocalization of RNA polymerase I transcription factor UBF with anti-NOR serum in protozoa, higher plant and vertebrate cells

ROSA M. RODRIGO[1], M. CARMEN RENDÓN[2], JOSÉ TORREBLANCA[1], GREGORIO GARCÍA-HERDUGO[1] and FRANCISCO J. MORENO[1,*]

[1]*Department of Cell Biology, Faculty of Biology, University of Arenida Reina Mercedes no. 6, 41012-Seville, Spain*
[2]*Department of Cell Biology and Animal Biology, Faculty of Sciences, University of Cadiz, Cadiz, Spain*

*Author for correspondence

Summary

We have used anti-NOR serum from a patient with rheumatoid arthritis, to study its reactivity on different phylogenetically separated species such as protozoa, higher plants, birds and mammals. The biochemical characteristics of the antigens detected after applying mono- and two-dimensional electrophoresis and electrophoretic transfers confirm that they correspond to the rRNA polymerase I transcription factor UBF. We have demonstrated the different molecular sizes, depending on the cell complexity, but the same neutral isoelectric points in whole cell extracts of the different species. We have also demonstrated an immunolocalization of this transcription factor to the fibrillar component in all the species studied. These results suggest a high conservation of UBF throughout evolution and the possibility of using this anti-NOR serum as a tool for the study of the structure, nucleolar organization and functional roles of the different nucleolar components.

Key words: anti-NOR serum, electrophoresis, immunoblotting, immunolabelling, nucleolar components, nucleolus, rDNA transcription, rRNA polymerase I transcription factor.

Introduction

Regulation of transcription by eukaryotic RNA polymerases requires many accessory factors that direct specific protein-DNA recognition and protein-protein interactions (Grummt, 1989). In contrast to RNA polymerases II and III, RNA polymerase I recognizes and transcribes from only one type of promoter, which directs the transcription of the tandemly arrayed genes encoding the large ribosomal RNA (rRNA) precursors that in interphase cells have been localized to the nucleolus (Jantzen et al. 1990). Each tandem repeat contains a transcribed region, which codes for the ribosomal RNA precursor, and a non-transcribed region, which codes for the non-transcribed spacer (NTS). In most vertebrates the region within 200 bp of the transcription initiation site contains the promoter that directs the synthesis of pre-ribosomal RNA (Smith et al. 1990). In mammals, this promoter consists of at least two interacting elements referred to as the core promoter element (CPE), which contains sequences essential for specific initiation, and the upstream promoter element (UPE), which enhances initiation by up to a factor of 10 to 100 in an orientation- and distance-dependent manner (Bell et al. 1988; O'Mahony and Rothblum, 1991).

Despite extensive conservation of rRNA sequences from diverse organisms, rRNA gene promoters from different species show only very limited sequence similarity and are not recognized by the transcription machinery of heterologous species unless they are closely related (Grummt, 1989; Jantzen et al. 1990; Pikaard et al. 1989). This species-specific promoter recognition appears to be mediated by one or more auxiliary transcription factors rather than by RNA polymerase I, which is functionally conserved (Bell et al. 1988; Pikaard et al. 1990). Accurate and efficient transcription initiation by vertebrate RNA polymerase I requires at least two DNA-binding proteins. One of these factors, referred to as SL1, is an essential component for reconstituting transcription, and can confer promoter specificity on a heterologous extract (Learned et al. 1985). However, SL1 does not show any detectable sequence-specific DNA-binding activity on its own, but it can form an initiation complex on the DNA template in the presence of a second factor, the upstream binding factor (UBF), that binds specifically to the UPE and CPE of the rRNA gene promoter to activate transcription in a binding site-dependent manner (Jantzen et al. 1990). Some UBF homologues have been isolated from different vertebrate species, hUBF from

human cells (Bell et al. 1988; Jantzen et al. 1990; O'Mahony and Rothblum, 1991), xUBF from *Xenopus laevis* (Pikaard et al. 1989), rUBF from rat cells (Pikaard et al. 1990; Smith et al. 1990), and it has also been purified from mouse cells (O'Mahony and Rothblum, 1991; Schnapp and Grummt, 1991). Comparison of these homologues has led to the observation that the UBF DNA-binding specificity has been strongly conserved from frogs to humans, strengthening the conclusion that UBF homologues with identical DNA-binding specificity should be found in all vertebrate cells, and suggesting that the sequence motifs in the promoters must be more conserved than is readily apparent and that the molecular cause of species-specificity must reside in some other part of the transcription machinery, not in the UBF promoter recognition (Pikaard et al. 1990).

Important tools that could facilitate further analysis of UBF would be antibodies directed against this transcription factor. In a preliminary step in this direction we have used an autoimmune serum, the anti-NOR serum, from a patient with rheumatoid arthritis, and found it to react specifically with a 92-88 kDa protein doublet with an isoelectric point of around 7.5 in extracts of whole HeLa cells; this clearly corresponds to hUBF (Rendón et al. 1992). Furthermore, after immunoelectron microscopic studies we have localized the antigen to the nucleolus, which is a highly ordered nuclear component where ribosome biogenesis occurs, involving the transcription of rRNA genes, the association of nascent transcipts with specific proteins and the multiple steps of maturation of primary transcripts leading to the formation of the pre-ribosomal subunits (Fakan, 1986; Goessens, 1984; Hernandez-Verdun, 1986; Scheer and Benavente, 1990). These different steps of ribosome biogenesis take place within morphologically well-defined components, namely the fibrillar centres and dense fibrillar component, which constitute the fibrillar component, and the granular component (Jordan, 1984). We have found immunolabelling with anti-NOR serum localized to the fibrillar component of HeLa cell nucleoli (Rendón et al. 1992), the site where ribosomal RNA transcription is believed to occur (Derenzini et al. 1990; Fakan, 1986; Goessens, 1984; Hernandez-Verdun, 1986; Jordan, 1991; Puvion-Dutilleul et al. 1991; Raska et al. 1990; Scheer and Benavente, 1990; Watchler et al. 1989).

The aim of this paper has been to increase our understanding of the activity of the above-mentioned human anti-NOR serum through the study of its reactivity with different phylogenetically separate species using protozoan, higher plant, avian and mammalian cells as sources of specific antigens. Its biochemical characteristics strongly confirm that it corresponds to the UBF rRNA transcription factor, thus suggesting that there has been high conservation of UBF throughout evolution and the possibility of using anti-NOR serum as a nucleolar marker to study the structure and nucleolar organization in these evolutionarily very distant cells.

Materials and methods

Human serum

Autoimmune serum was obtained from a patient with rheumatoid arthritis.

Material preparation

The ciliate *Histriculus similis* was mass cultured at 20 (± 1)°C in a 1l Erlenmeyer flask containing mineral water. The green alga *Chlorogonium* sp. was used to feed the protozoa.

Root meristems of *Allium cepa* L. were obtained from onion bulbs grown under standard conditions at 15°C (Cortés et al. 1982).

Primary cultures of chicken chondrocytes were obtained from the sterna of 14-day-old embryos, digested with 0.25% (w/v) trypsin, 0.2% (w/v) type IA collagenase, and 1000 units/ml type II collagenase in PBS. Cells were cultured in Ham's F-12 medium supplemented with 10% foetal calf serum and 0.002 M L-glutamine on 35- and 60-mm dishes coated with gelatin. Chondrocytes were fed every other day and 60 min before start the experiments.

PtK_1 cells were cultured in Dulbecco's medium with 0.85 g/l sodium bicarbonate supplemented with 10% foetal bovine serum (FBS), 2% glutamine, 1 mM sodium pyruvate, 100 i.u./ml penicillin-streptomycin, 1% Fungizone and 1% tylocine (antiPPLO).

TG cells were grown and maintained in cell culture flasks containing Dulbecco's medium supplemented with 10% FBS, 2 mM glutamine, 1 mM sodium pyruvate, 100 i.u./ml penicillin-streptomycin, 1% Fungizone and 1% antiPPLO.

Materials were processed for morphological and immunocytochemical electron microscopic studies. For the morphological study, cells were fixed in 1.6% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, for 60 min at 4°C, except for *Allium cepa* cells that were fixed in 4% glutaraldehyde. They were all post-fixed in 1% osmium tetroxide for 60 min at 4°C. Samples were dehydrated in acetone at progressively higher concentrations and embedded in resin following the method of Spurr (1969).

For electron microscopic immunolocalization, cells were fixed in a mixture of 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3, for 60 min at 4°C.

Cultured cells were fixed directly in the culture flasks, subsequently scraped with a rubber policeman, and pelleted by centrifugation.

Samples were washed in 0.1 M cacodylate buffer, pH 7.3, and incubated in 0.5 M NH_4Cl for 4 h at room temperature to block free aldehyde groups. They were then washed in the same buffer again.

Lowicryl K4M-embedded sections

Low-temperature embedding with the hydrophilic resin Lowicryl K4M (Chemische Werke Lowi., Waldkraiburg, FRG) was performed, with slight modifications, according to the method described by Roth (1983). Samples were dehydrated in a series of graded methanols at progressively lower temperatures. During infiltration with Lowicryl K4M at −20°C, 90% methanol was used as the dehydration agent. Lowicryl was polymerized by indirect long-wave (360 nm) UV irradiation from a 15 W Philips fluorescent lamp for 24 h.

Ultrathin sections were cut on a Reichert-Jung Ultracut E ultramicrotome, mounted on 300-mesh nickel grids and photographed in a Philips CM-10 transmission electron microscope (Servicio de Microscopía Electrónica, University of Seville).

Immunocytochemical labelling

Grids were incubated by floating them, cell sections down, on a drop of 0.1 M PBS, pH 7.4, containing 1% BSA, 0.05% Triton X-100 and 0.05% Tween 20 (PBTT), for 5 min at room temperature, and washed in PBS. The next step of the treatment was an incubation in anti-NOR serum diluted 1:100 to 1:1000 in PBS for 60 min at room temperature. After washing in PBS, the second incubation was in Protein A-gold complex prepared following Bendayan et al. (1980), diluted in PBTT until an absorbance of

0.06 (525 nm) was reached, for 60 min at room temperature (Roth et al., 1989). Grids were washed with distilled water and contrasted with 2% uranyl acetate in distilled water. To demonstrate the specificity of the labelling, controls were performed by omitting the incubation with the anti-NOR serum or by using a normal human serum during the first incubation step.

Mono-dimensional gel electrophoresis (SDS-PAGE)

Chondrocytes, PtK_1 and TG cells were trypsinized from culture flasks after attaining 75% confluence, washed in PBS and pelleted after centrifugation at 55 ***g***. Cultured *Histriculus similis* cells were also pelleted after gentle centrifugation. Extraction of proteins was performed by treating the pellets with SDS-PAGE sample buffer for 10 min at 97°C (Laemmli, 1970).

Extraction of proteins from *Allium cepa* root meristem cells was performed after treatment with 32% pectinase in citric-citrate buffer, pH 4.2, containing 1 mM PMSF. After washing in PBS, roots were pulverized in a liquid nitrogen-cooled mortar. We continued by solubilization of the proteins in SDS-sample buffer for 10 min at 97°C (Laemmli, 1970).

Electrophoresis was performed, as described by Laemmli (1970), on 7.5% to 12% SDS-polyacrylamide gels using the Mini-Protean II electrophoresis cell (Bio-Rad., Richmond, CA). A set of molecular mass standards were obtained from Bio-RAD Laboratories: myosin (200 kDa), β-galactosidase (116.25 kDa), phosphorylase B (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (42.69 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa), and lysozyme (14.4 kDa). When electrophoresis was carried out subsequently to perform transfers of proteins to nitrocellulose paper, a set of pre-stained molecular mass standards were used (Sigma Chemical Co.): α2-macroglobulin (180 kDa), β-galactosidase (116 kDa), fructose-6-phosphate kinase (84 kDa), pyruvate kinase (58 kDa), fumarase (48.5 kDa), lactic dehydrogenase (36.5 kDa), and triosephosphate isomerase (26.6 kDa).

Total proteins in gels were revealed with Coomassie blue staining.

Two-dimensional gel electrophoresis

Histriculus similis, *Allium cepa*, PtK_1 and TG cells and chondrocytes were treated as for mono-dimensional SDS-PAGE. We used the O'Farrell (1975) lysis buffer as the isoelectric focusing (IEF) sample buffer. The concentrations of proteins in the IEF sample buffer were determined with a modified Bradford assay, which allows quantitation of proteins in the presence of urea, carrier ampholytes, non-ionic detergents, and thiol compounds (Ramagli and Rodriguez, 1985). The volume of sample loaded per capillary gel tube contained a concentration of around 30 μg total proteins.

Two-dimensional polyacrylamide gel electrophoresis (2D PAGE) was performed according to O'Farrell (1975) using the Mini-Protean 2-D electrophoresis cell (Bio-Rad) as described previously (Moreno et al. 1990). The second-dimensional gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) was carried out as for mono-dimensional SDS-PAGE electrophoresis.

Total proteins in gels were revealed by using the Bio-Rad silver stain Kit.

Electrophoretic transfer and immunoblotting

Proteins were transferred electrophoretically from polyacrylamide gels to nitrocellulose paper (Towbin et al. 1979), and incubated in 3% gelatin in TBS (15 mM Tris, 200 mM NaCl) for 24 h at room temperature. After two washes of 10 min each in TBS containing 0.05% Tween-20 (TTBS), an incubation in anti-NOR serum diluted 1:100 in TTBS containing 1% gelatin was performed for 24 h at room temperature. Blots were rinsed in TTBS and incubated in peroxidase-labelled goat anti-human IgG (Boehringer Mannheim Biochemicals) diluted 1:2000 in TTBS, for 2 h at room temperature. After washing in TTBS and TBS, the reaction was visualized with chloronaphthol substrate (50 ml TBS containing 0.03 g 4-chloro-1-naphthol in 10 ml methanol and 20 μl 30% hydrogen peroxide). Colour development was stopped in distilled water and blots were allowed to air dry. Control trials were performed by omitting the incubation with the anti-NOR serum.

Photographs of gels and transfers were taken with an AGFA Copex Rapid A.H.U. film processed to 9 ASA.

Results

At the ultrastructural level, *Histriculus similis* vegetative cells showed a voluminous macronucleus consisting of heterochromatin with a reticulate distribution and interspersed euchromatin. Between the condensed chromatin threads numerous nucleoli with a rounded morphology could be observed in thin sections. These structures were composed of several moderately electron-dense small fibrillar areas, similar to the fibrillar centres of animal cell nucleoli, totally or partially surrounded by strands of dense fibrillar component, embedded in a fine granular component. They frequently showed large vacuoles or interstices, consisting of a material similar to nucleoplasm. The presence of replication bands was frequently observed in the macronuclei of these vegetative cells (Fig. 1).

When we proceeded to immunolocalization with a 1:500 anti-NOR serum dilution we observed that labelling with Protein A-gold was restricted to nucleoli. Gold particles were found mostly distributed over the fibrillar component strands. Fine granular material was devoid of labelling (Fig. 2).

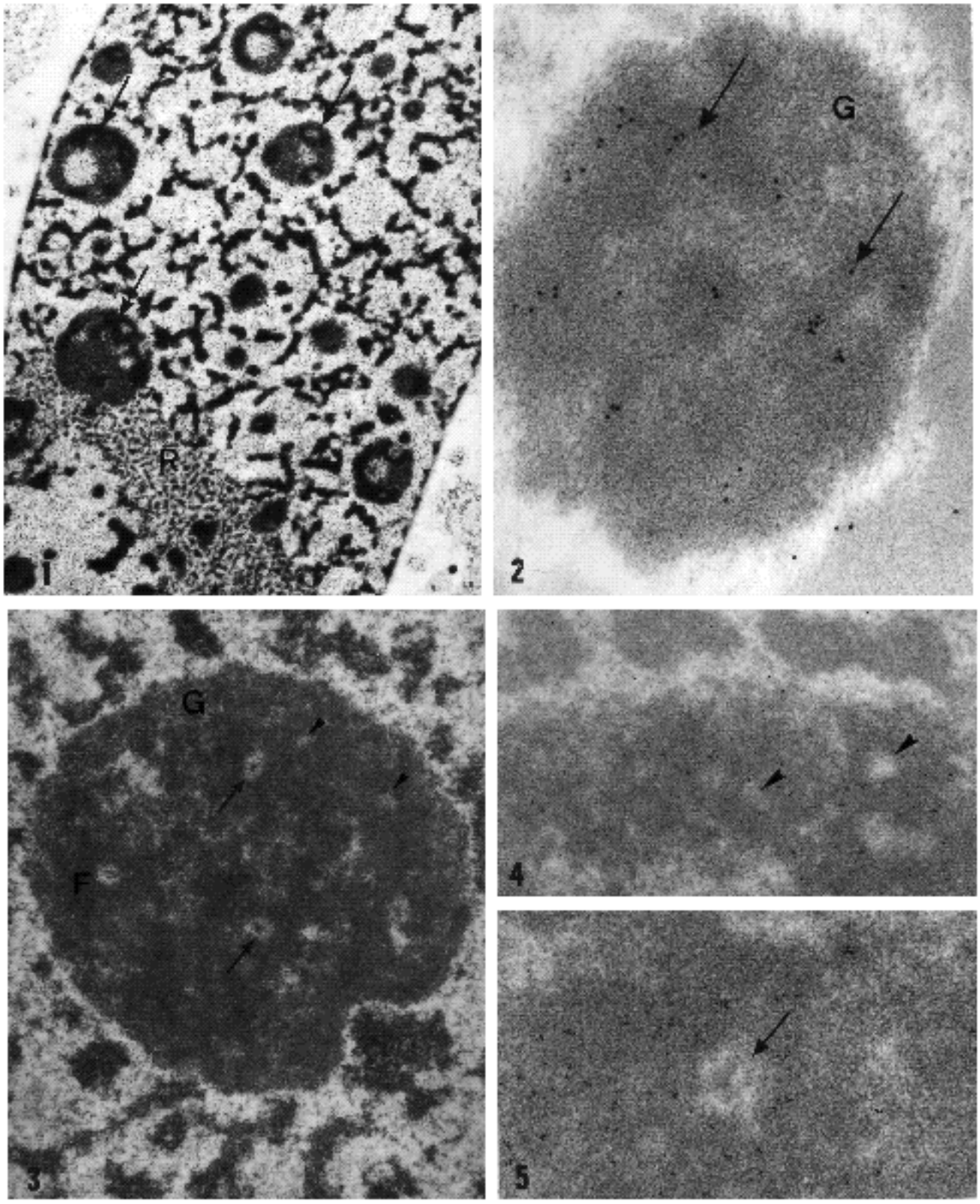
Nucleoli of *Allium cepa* were formed of a thread-like organized fibrillar component immersed in the granular component. Inside the fibrillar component, some clear areas could be observed, containing homogeneous material and/or dense inclusions. Some interstices corresponding to the nucleolar vacuoles could be found in the granular component (Fig. 3).

Immunolocalization with anti-NOR serum in *Allium cepa* showed gold particles distributed only over the nucleolar thread-like fibrillar component. Clear areas, with and without inclusions, and granular component were free of labelling (Figs 4 and 5).

Chicken chondrocytes frequently disclosed reticulate nucleoli characterized by a network of threads, or nucleolonema. Some fibrillar centres could be detected totally or partially surrounded by dense fibrillar component. Interstices usually appeared between the nucleolonema threads (Fig. 6). When we used the anti-NOR serum we found the antigen immunolocalized to the fibrillar centres and dense fibrillar component of chondrocyte nucleoli (Fig. 7).

PtK_1 nucleoli in cycling cells showed numerous small fibrillar centres partially surrounded by connected strands of dense fibrillar component, sites where we found immunolabelling with anti-NOR serum, forming a network embedded in the granular component (Figs 8 and 9).

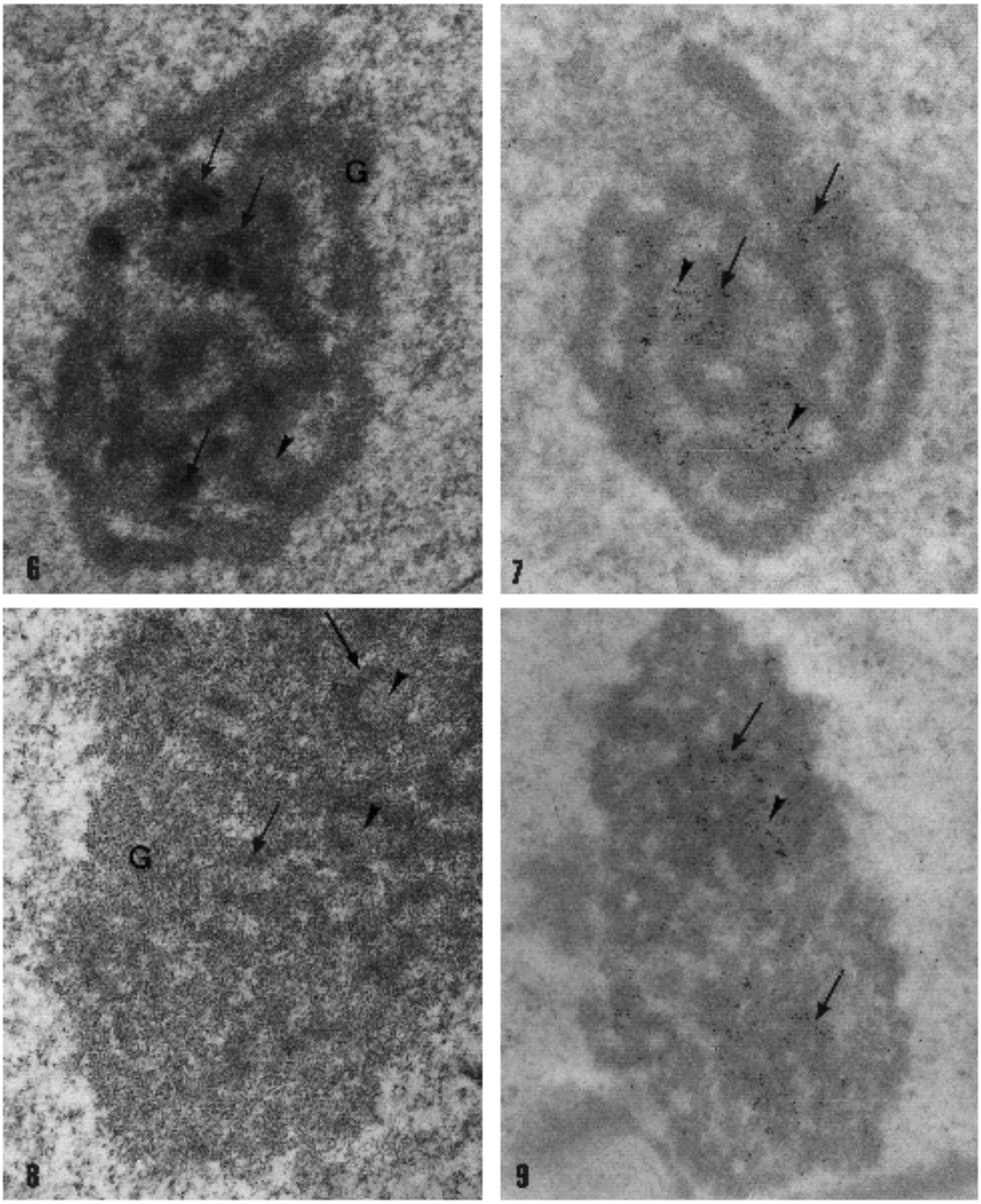
At the ultrastructural level TG cells disclosed nucleoli with a compact morphology, formed by fibrillar centres that were found to be at least partly surrounded by dense



Figs 1-5. For legends see p. 1058

fibrillar component, and the granular component (Fig. 10).

The precise localization of antigens obtained with anti-NOR serum was detected over the fibrillar centres and dense fibrillar component of TG cell nucleoli (Figs 11 and 12). The granular component, chromatin, nucleoplasm and



Figs 6-9. For legends see p. 1058

cytoplasm were devoid of labelling. We did not observe gold particles in any of the controls performed.

To characterize the nucleolar antigens recognized by the anti-NOR serum we carried out mono-dimensional gel electrophoresis of whole cell extracts obtained from the different species studied, followed by electrophoretic transfer.

Fig. 1. Portion of a *Histriculus similis* nucleus with numerous rounded nucleoli formed by fibrillar component (arrows) and granular component. Replication band (R). Material fixed in 1.6% glutaraldehyde, postfixed in 1% osmium tetroxide and embedded in Spurr resin. ×15500.
Fig. 2. Immunolabelling with anti-NOR serum localized to the fibrillar component strands of a *Histriculus similis* nucleolus fixed in 4% paraformaldehyde - 0.1% glutaraldehyde and embedded in Lowicryl K4M. Granular component (G). ×95700.
Fig. 3. *Allium cepa* root meristem cell nucleolus formed by a thread-like fibrillar component (F) embedded in the granular component (G). Clear areas with inclusions (arrows) and without inclusions (arrowheads) can be observed. Roots fixed in 4% glutaraldehyde, postfixed in 1% osmium tetroxide and embedded in Spurr resin. ×18850.
Figs 4 and 5. The localization of gold particles after anti-NOR serum was found over the fibrillar component threads of *Allium cepa* nucleoli fixed in 4% paraformaldehyde - 0.1% glutaraldehyde and embedded in Lowicryl K4M. Clear areas without inclusions (arrowheads), with inclusions (arrow), granular component and heterochromatin are devoid of labelling. Fig. 4. ×23900. Fig. 5. ×46300.
Fig. 6. Reticulate nucleolus of a chicken chondrocyte disclosing fibrillar centres (arrowhead) surrounded by dense fibrillar component (arrows). Granular component (G). Fixation in 1.6% glutaraldehyde, postfixation in 1% osmium tetroxide and embedding in Spurr resin. ×47500.
Fig. 7. Immunolabelling with anti-NOR serum over the fibrillar centres (arrowheads) and dense fibrillar component (arrows) in a chicken chondrocyte nucleolus fixed in 4% paraformaldehyde - 0.1% glutaraldehyde and embedded in Lowicryl K4M. ×47000.
Fig. 8. Nucleolus of a Ptk_1 cell constituted of small fibrillar centres (arrowheads) surrounded by strands of dense fibrillar component (arrows). Granular component (G). Material fixed in 1.6% glutaraldehyde, postfixed in 1% osmium tetroxide and embedded in Spurr resin. ×33000.
Fig. 9. Immunolocalization of antigens with anti-NOR serum over fibrillar centres (arrowhead) and dense fibrillar component (arrows) in a Ptk_1 nucleolus fixed in 4% paraformaldehyde - 0.1% glutaraldehyde and embedded in Lowicryl K4M. ×41600.
Fig. 10. Compact nucleolus of a TG cell showing two fibrillar centres surrounded by dense fibrillar component, and immersed in granular component. Cell fixed in 1.6% glutaraldehyde, postfixed in 1% osmium tetroxide and embedded in Spurr resin. ×45500.
Figs 11 and 12. Immunolabelling with anti-NOR serum in TG cells fixed in 4% paraformaldehyde - 0.1% glutaraldehyde and embedded in Lowicryl K4M.
Fig. 11. Gold particles are localized to the fibrillar centres and dense fibrillar component. Granular component and nucleoplasm are devoid of labelling. ×46750.
Fig. 12. Antigens immunolocalized inside a fibrillar centre and over the portions of dense fibrillar component surrounding it. ×121300.

We found two polypeptides which, respectively, migrated at 92 and 88 kDa immunoblotted with anti-NOR serum in TG and PtK_1 cells and chicken chondrocytes. A similar protein doublet reacted in *Histriculus similis* cells, but with a molecular mass of 58-56 kDa, which comigrated with a single polypeptide in whole *Allium cepa* root meristem cell extracts. Corresponding Coomassie-stained gels of cell extracts showing the full complement of extracted proteins and a negative control assay in which anti-NOR was excluded together confirmed the blotting immunospecificity (Fig. 13).

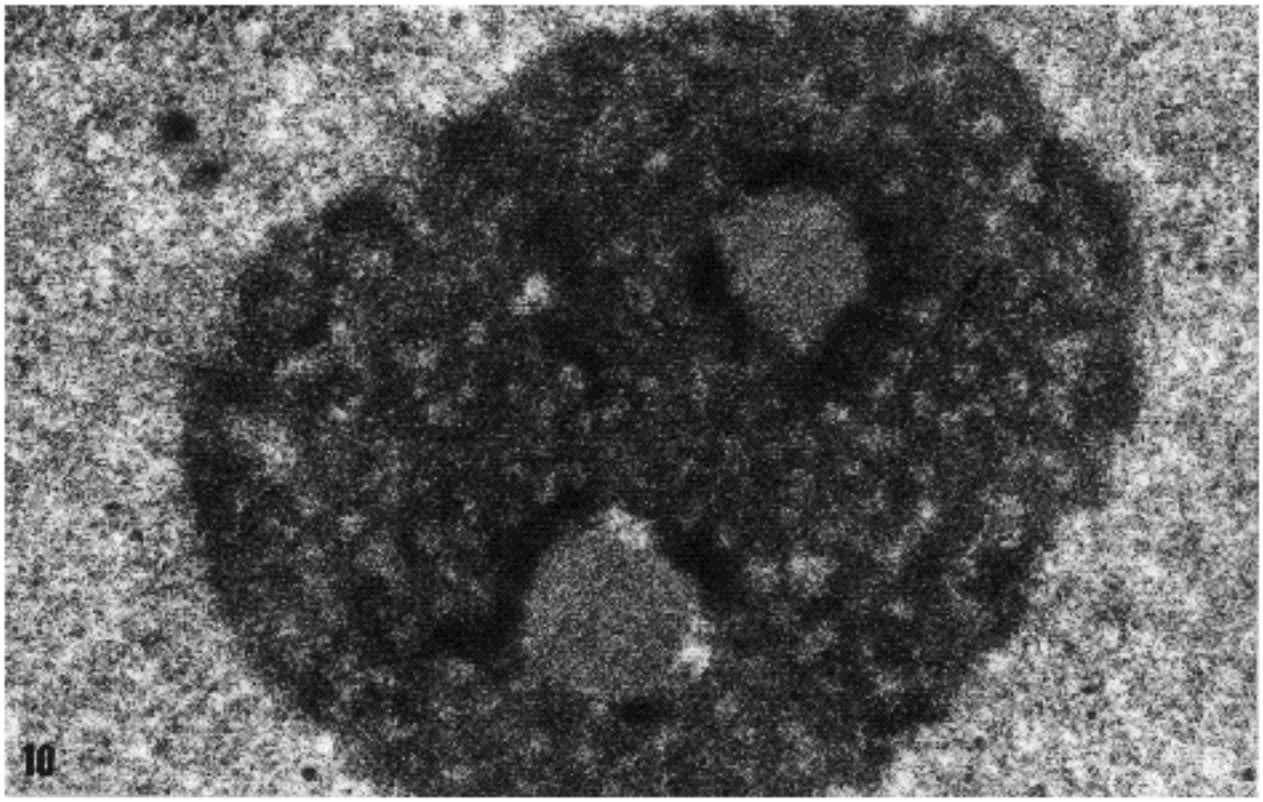
In order to characterize further the polypeptides revealed by the anti-NOR serum we carried out two-dimensional electrophoresis followed by electrophoretic transfers of whole cell extracts of the different species studied. We found, after immunoblotting with anti-NOR serum, specific antigens with approximate molecular masses of 92 kDa and isoelectric points of around 7 in TG cells, PtK_1 cells and chondrocytes. In *Allium cepa* root meristem cell extracts, three polypeptides with molecular masses of around 58 kDa immunoblotted with anti-NOR serum, with isoelectric points of approximately 7 (Figs 14-17).

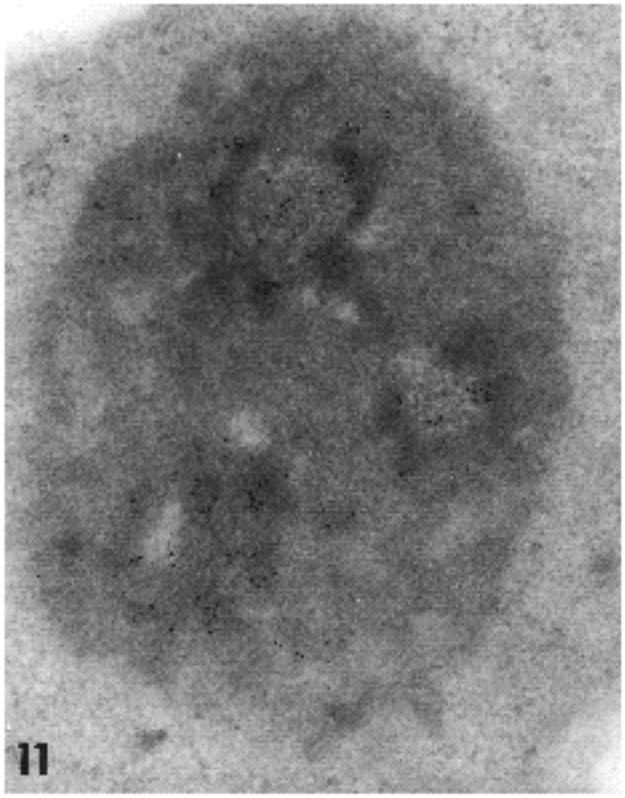
Discussion

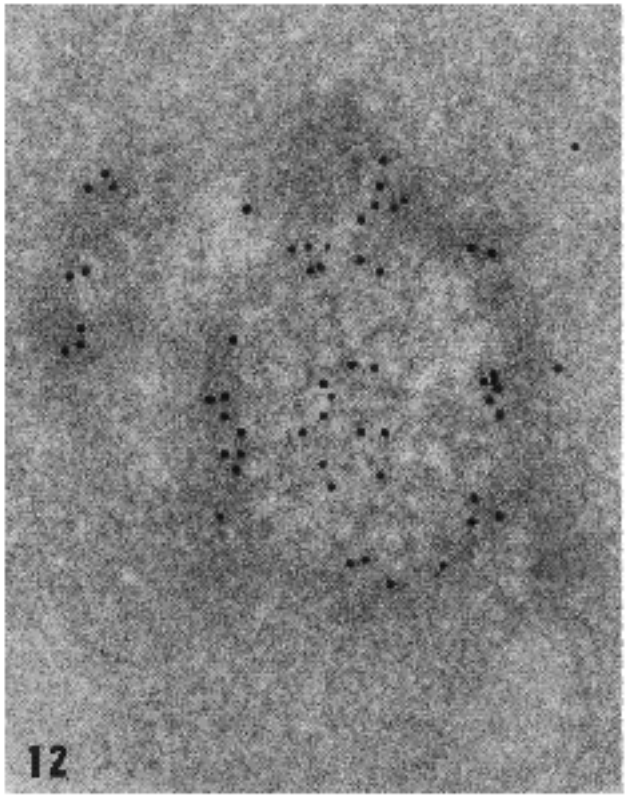
The nucleolus is a domain of the interphase cell nucleus where ribosomal RNA (rRNA) synthesis takes place and where several non-histone proteins are found (Goessens, 1984; Hernandez-Verdun, 1986; Jordan, 1991). It is generally agreed that transcription of rDNA genes and the first steps of rRNA processing occur at the fibrillar component of the nucleolus. Interestingly, by using autoantibodies from different patients with autoimmune diseases, different proteins have been localized to the fibrillar component, such as RNA polymerase I (Reimer et al. 1987), DNA topoisomerase I (Shero et al. 1986), nucleolin (Minota et al. 1991) and fibrillarin (Ochs et al. 1985; Reimer et al. 1987). Other autoimmune sera have also been shown to react against specific antigens localized to the fibrillar centres or dense fibrillar component of nucleoli from different cell types (Hernandez-Verdun et al. 1988, 1991; Masson et al. 1990; Rodriguez-Sanchez et al. 1987). The finding that some of these proteins have been conserved during evolution indicates that they might participate in some fundamental function (Hernandez-Verdun, 1991).

In a previous study, we used an autoimmune serum from a patient with rheumatoid arthritis and detected a protein doublet at 92-88 kDa in whole HeLa cell extracts, which correspond to hUBF, and we immunolocalized it to the fibrillar component of the nucleolus of HeLa cells (Rendón et al. 1992). Some authors have shown that, despite the extensive sequence divergence of polymerase I promoters, there exists an evolutionarily strong conservation of the primary UBF DNA-binding specificity in vertebrates (Pikaard et al. 1990). This UBF transcription factor has been shown to consist of two polypeptides with apparent molecular masses of 97-94 kDa in mammals (Bell et al. 1988; Jantzen et al. 1990; O'Mahony and Rothblum, 1991; Pikaard et al. 1990; Smith et al. 1990; Schnapp and Grummt, 1991) and 85-82 kDa in frogs (Pikaard et al. 1989).

We have extended these studies to different cell types in mammals (TG and PtK_1), birds (chicken chondrocytes), higher plants (*Allium cepa* root meristem cells) and protozoa (*Histriculus similis*). After mono-dimensional electrophoresis of whole cell proteins, electrotransfer to nitrocellulose and immunoblotting with anti-NOR serum, we have detected specific antigens with molecular masses of 92 and 88 kDa in TG cells, PtK_1 cells and chicken chondrocytes, while two polypeptides at 58 and 56 kDa have been localized in *Histriculus similis* and a single polypep-







tide at 58 kDa in *Allium cepa* root meristem cells. After two-dimensional electrophoresis these antigens showed isoelectric points of around 7, thus confirming the results of our previous study with HeLa cells (Rendón et al. 1992).

Notwithstanding the divergence in the apparent molecular masses of the antigens specifically detected in whole cell extracts, we have observed that immunolocalization with anti-NOR serum always remains restricted to the

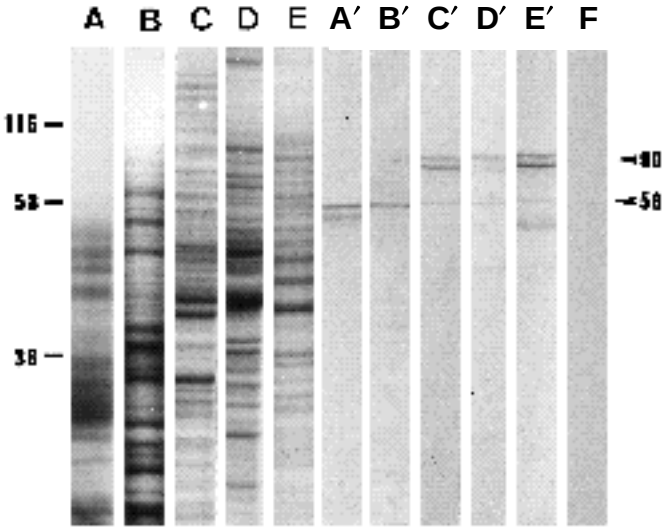
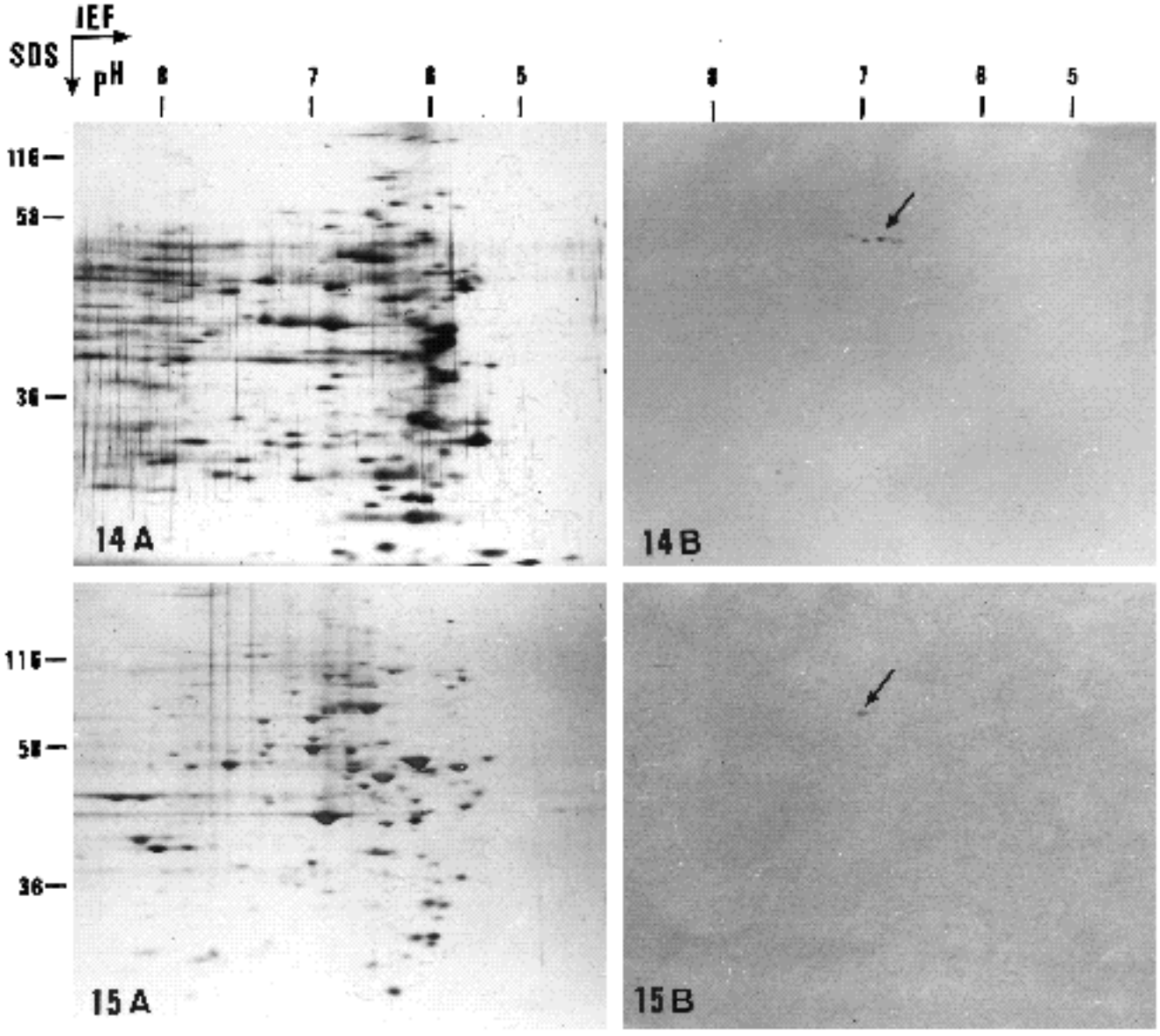
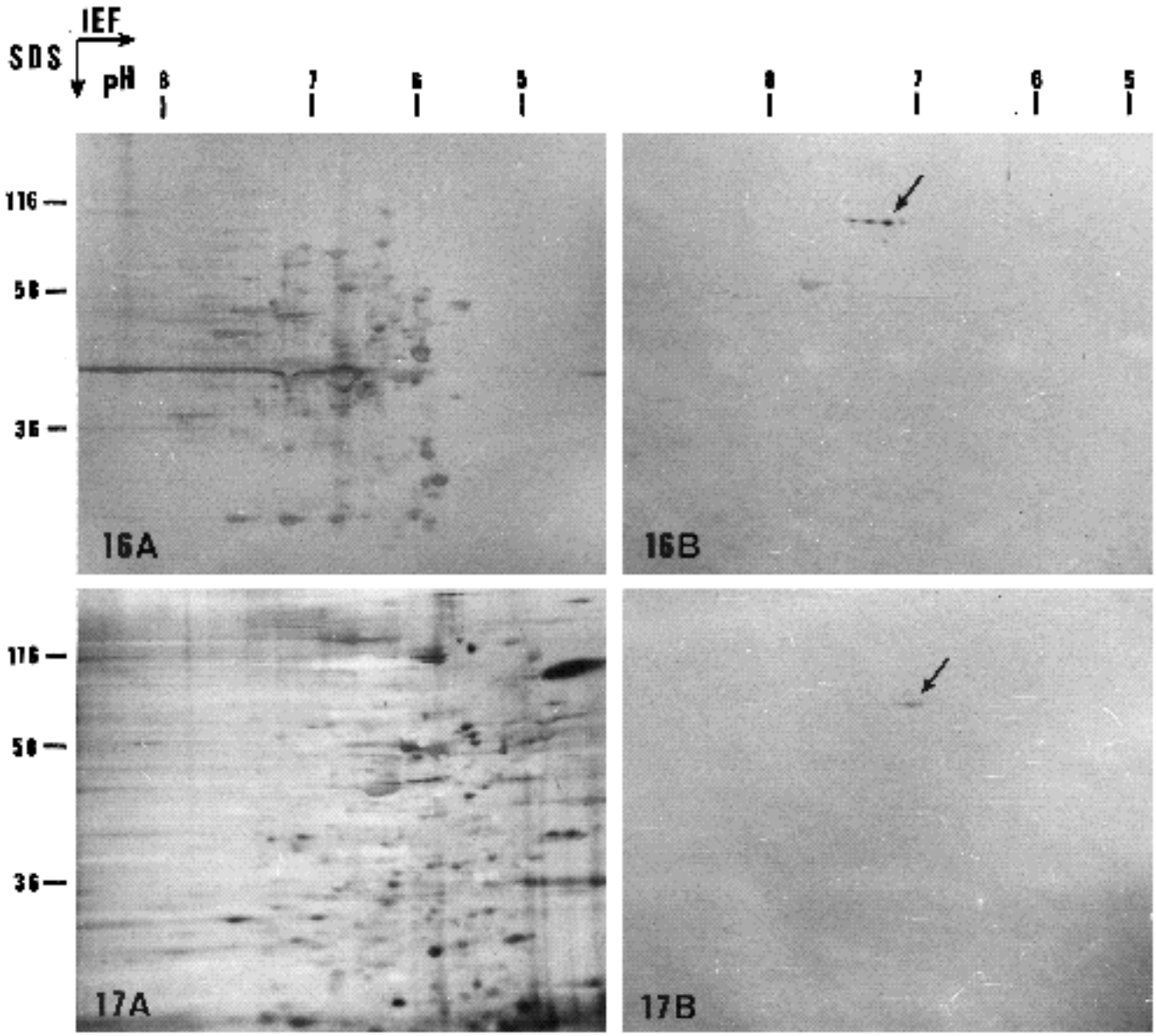


Fig. 13. Coomassie blue staining of 12% PAGE-separated proteins present in whole cell extracts of (A) *Histriculus similis*, (B) *Allium cepa*, (C) chicken chondrocytes, (D) PtK_1 and (E) TG and the respective characterization by immunoblotting of the antigen recognized upon probing with 1:100 diluted anti-NOR serum. Two polypeptides, 58 and 56 kDa, are revealed in *Histriculus similis* (A′), a single protein at 58 kDa in *Allium cepa* (B′), and a protein doublet of 92 and 88 kDa in chicken chondrocytes (C′), PtK_1 (D′) and TG cells (E′). (F) The corresponding control assay in which anti-NOR serum was omitted.

Figs 14 and 15. Characterization of the antigens by means of two-dimensional electrophoresis using 12% SDS-PAGE, followed by electrophoretic transfer of whole cell extracts in *Allium cepa* root meristem cells (Fig. 14) and chicken chondrocytes (Fig. 15). (A) Pattern of total proteins stained with silver. (B) Immunoblotting with a 1:100 dilution of anti-NOR serum. The antigens with isoelectric points of around 7 (arrows) can be observed.





Figs 16 and 17. Characterization of the antigens by means of two-dimensional electrophoresis using 12% SDS-PAGE, followed by electrophoretic transfer of whole extracts of PtK_1 cells (Fig. 16) and TG cells (Fig. 17). (A) Pattern of total proteins stained with silver. (B) Immunoblotting with a 1:100 dilution of anti-NOR serum. The antigens can be observed disclosing isoelectric points of around 7 (arrows).

nucleolar fibrillar component of the different species studied, suggesting a uniformity in the functional role of this protein. The persistence of the antigen at the fibrillar component after treatment with the rRNA synthesis inhibitor AMD, and the existence of labelling at the NORs during mitosis (Rendón et al. 1992) demonstrate that this protein remains joined to the rDNA under situations of low transcriptional activity. These data have been very recently corroborated by O'Mahony et al. (1992), who have shown that the level of expression of UBF does not change in response to serum deprivation in CHO cells. However, they have demonstrated that the degree of phosphorylation of serine residues of this phosphoprotein is reduced following serum deprivation and that, after refeeding, this level increases dramatically. These authors have also observed that under the conditions of serum starvation, the subcellular distribution of UBF shifts from being predominantly nucleolar to a dispersed nucleolar/nuclear/cytoplasmic distribution and, after refeeding, it returns again to being immunolocalized to the nucleolus. This translocation from the nucleolus to the nucleoplasm, which originated after serum starvation, also occurs with the nucleolar phosphoprotein B23 or NO38 (Busch et al. 1987). Moreover, O'Mahony et al. (1992) suggest that the phosphorylation state of UBF may affect its transactivation properties and that there also is a possibility that the phosphorylation of UBF is necessary for its nucleolar localization. Besides that, this DNA-binding protein contains DNA-binding domains homologous to HMG

nuclear proteins and a hyperacidic tail that could mediate the interaction with SL1 transcription factor (Jantzen et al. 1990). It is believed that in the cell most of the RNA polymerase I transcription factors remain associated as a multiprotein complex (Schnapp and Grummt, 1991).

Taking all these data together, and considering that the immunolocalization of hUBF to NORs during mitosis has allowed other authors to suggest that this crucial protein is probably never disengaged from rDNA even when rRNA synthesis is minimal (Chan et al. 1991), we are led to believe that our anti-NOR serum just possibly detects the phosphorylated UBF that remains joined to the rDNA, as we have not found any labelling outside the fibrillar component of the nucleolus in interphase cells under the conditions of decreased transcriptional activity, or outside the NOR in mitosis (Rendón et al. 1992). If this hypothesis is confirmed, quantification of the immunolabelling with anti-NOR serum could be used as a method to determine the level of transcriptional activity.

The strong conservation of UBF throughout evolution, which permits it to be specifically detected with our anti-NOR serum, allows us to observe a different nucleolar distribution of this transcription factor in the different species studied. In animal cells disclosing nucleoli where fibrillar centres and dense fibrillar component appear well defined, such as in compact nucleoli of TG cells, the UBF transcription factor is clearly located to both the fibrillar centres and dense fibrillar component. In nucleoli with a reticulate structure, like those in chicken chondrocytes and PtK_1 cells, immunolocalization is distributed over the fibrillar centres and the dense fibrillar component strands. In plant cells, however, only nucleoli of the compact type have been described. In these nucleoli, a nucleolonema can appear, but it is structurally different from that of animal reticulated nucleoli (Deltour and Motte, 1990). In *Allium cepa* root meristem cell nucleoli, we have observed the UBF transcription factor immunolocalized only to the nucleolonema threads, where the active ribosomal genes have been proposed to be located (Deltour and Motte, 1990; Moreno et al. 1989a,b, 1990). This result confirms our previous studies on higher plant nucleoli and suggests that there is no evidence of the existence of fibrillar centres with a rDNA content, forming part of the fibrillar component's structural organization. Finally, in protozoa, UBF appeared distributed throughout the fibrillar component strands after immunolocalization with our anti-NOR serum.

This seemingly variable nucleolar localization of the UBF transcription factor, over the fibrillar centres, the dense fibrillar component or the nucleolonema strands, together with the different fibrillar component structural organization, can be regarded as a consequence of different stages of transcriptional activity, as has been proposed recently, in a manner consistent with our data, by Schwarzacher and Wachtler (1991).

We have immunolocalized the UBF transcription factor to the fibrillar component, to both the fibrillar centres and to the dense fibrillar component of nucleoli in the different cell types studied, sites where rDNA transcription is believed to occur. By means of different approaches carried out in different cell types and species in order to locate rDNA transcription, controversial results have been obtained by several authors. In this way, RNA polymerase I has been found either in the fibrillar centres (Scheer and Rose, 1984) or in both the fibrillar centres and the dense fibrillar component, the amount in the latter being low and depending on the cell type (Raska et al. 1989). Similar results have also been found for topoisomerase I, an enzyme required for proper transcription of rRNA genes, as it has been immunolocalized to the fibrillar centres (Rose et al. 1988) and is also enriched in dense fibrillar component (Raska et al. 1989). Another approach has been made by means of in situ hybridization using probes corresponding to different locations along the ribosomal genes; rDNA being detected either in the fibrillar centres (Puvion-Dutilleul et al. 1991; Thiry and Thiry-Blaise, 1989) or in the dense fibrillar component (Gosh and Paweletz, 1990; Wachtler et al. 1989). It has been proposed that the controversial nature of these results is due to different interpretations of the nucleolar structures, as well as the possibility of the existence of differences in the functional organization of nucleoli in the different cells and species (Schwarzacher and Wachtler, 1991).

We have shown that the anti-NOR serum has a high specificity for the UBF transcription factor, which remains joined to the rDNA in different cell types. Moreover, we have demonstrated a strong conservation of this protein throughout evolution, disclosing different relative molecular masses, depending on the level of cell complexity, but without modification of the neutral character of their isoelectric points. Furthermore, the immunolocalization of UBF to the fibrillar component in all the species studied can make the anti-NOR serum a useful tool for the investigation of both the nucleolar organization and the localization of ribosomal genes.

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