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SHORT COMMUNICATION

DISTRIBUTION AND TRANSCRIPTION ACTIVITY OF NUCLEOLAR DNA IN HIGHER PLANT CELLS

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By using the NAMA-Ur DNA selective staining method, we have observed *in situ* the location of nucleolar DNA in onion cells and found it at the boundary between fibrillar centres (FC) and dense fibrillar component (DFC) in transcriptionally active nucleolus. We have also used anti-NOR serum, which is identified as the RNA Polymerase I transcription factor (UBF) antibody, to study its reactivity with higher plant cells and demonstrated this factor associated to the DFC but not present at the interior of FC. Finally, by employing anti-DNA/RNA hybrid antibodies, we labeled the transcriptionally active rRNA genes in active nucleolus and testified that at the boundary between FC and DFC. The results provide the evidence that the boundary between FC and DFC is the genuine transcription site of rRNA genes in nucleolus.

KEYWORDS: nucleolus; location of nucleolar DNA *in situ*; transcription sites of rRNA genes; RNA Polymerase I transcription factor (UBF); *Alllium cepa*.

INTRODUCTION

In nucleolus, ribosomal DNA (rDNA) comprises the genes from which the precursor of ribosomal RNA is synthesized. Basic structure of nucleolus in eukaryote cells can be divided into fibrillar centres (FC), dense fibrillar component (DFC) and granular component (GC). A better understanding of the structural organization of the nucleolus is directly dependent upon the precise localization of rRNA genes and their transcription sites in distinct subnucleolar components.

Early electron microscope autoradiographical results followed by short pulse and pulse-chase experiments with radioactive uridine established that the DFC is the site of active transcription and the GC contains the pre-rRNA maturation product. However, the immunoelectron microscopic localization results clearly showed that RNA polymerase I was present in the interior of the FC, lead to an argument for assuming that the FC represents the exclusive site of rDNA transcription. From then on, many approaches have been employed to pinpoint the location of nucleolar DNA and the rRNA gene transcription sites in various plant and animal cell systems. These included *in situ* hybridization with appropriate rDNA probes, selective DNA staining, incorporation of Br-UTP as a trace marker and immunodetection of nucleolar proteins involved in the activation of rRNA gene transcription (Shaw *et al.*, 1995).

Unfortunately, although there have been many attempts to describe the structure of the nucleolus, in particular the location of the nucleolar DNA and the rRNA gene transcription sites, the situation still remains obscure (Mais and Scheer, 2001). To this end, we report here the results of our

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studies on the transcription sites of rRNA genes in onion cells.

MATERIALS AND METHODS

Tissue

Root tip meristem of *Allium cepa* was used as the material in this study. Root tip meristem was obtained from onion bulb grown under standard conditions at 15°C.

NAMA-Ur Procedure

As far as the distribution of DNA in nucleolus was concerned, we employed the NAMA-Ur method reported by Testillano (Testillano *et al.*, 1991). Specimens were observed in a Hitachi-600 transmission electron microscope.

Isolation of nuclei

The isolation of nuclei was carried out according to the report from Ghosh and Dey (Ghosh *et al.*, 1986).

SDS-PAGE analyses and immunoblotting

The anti-NOR serum which corresponds to the rRNA polymerase I transcription factor UBF (Rodrigo *et al.*, 1992), was provided by Prof. M. Waldivia.

Isolated nuclei were treated with SDS-PAGE sample buffer for 4 min at 100°C, Electrophoresis was performed on 12% separation gel and 5% concentration gel. The gels were stained with Coomassie Brilliant Blue (R-250) and destained in alcohol-acetate.

Proteins were transferred electrophoretically from polyacrylamide gel to nitrocellulose sheet and incubated in PBS containing 0.05% Tween 20 and 5% skimmed milk powder at room temperature for 1 h, washed three times in PBST (PBS containing 0.05% Tween-20), 10 min each time. Nitrocellulose sheets were immerged in PBS containing 1% BSA and anti-NOR serum with a dilution of 1:500 at 37°C for 2 h. Washed in PBS, incubated with horseradish peroxidase conjugated sheep anti-human IgG antibody at a dilution of 1:500 in PBST/1%BSA at 37% for 1 h, washed as above. The antigen-antibody complex was visualized by incubation of the nitrocellulose sheet with 0.5 mg/ml DAB (diaminobenzioline) in PBS containing 0.006% H_2O_2 for 1–2 min.

Control trials were performed by omitting the incubation with the anti-NOR serum.

Immuno-gold labelling of anti-NOR serum and anti-DNA/RNA antibody

The anti-DNA/RNA hybrid antibody was kindly provided by Prof. B. D. Stollar (Rudkin *et al.*, 1977). Lowicryl K4M was obtained from Chemische Werke Lowi GMBH & Co in Germany. Protein A conjugated to colloidal gold particles was obtained from Sigma.

Samples were fixed in 3% glutaraldehyde and 4% formaldehyde in PBS for 2 h. After washing in bidistilled water, they were dehydrated in a ethanol series and embedded in Lowicryl K4M at −30°C under UV irradiation.

Ultra-thin Lowicryl sections were mounted on formvar nickel grids and washed in 5% BSA (PBS, 0.05% Triton X-100) for 10 min. Then the grids were incubated in the anti-NOR serum and anti-DNA/RNA hybrid antibody diluted 1:300 in PBS for 1 h at room temperature. After washing in PBS, they were floated in protein A conjugated to 10-nm colloidal gold particles (Sigma), diluted 1:25 in PBS for 45 min at room temperature. They were washed in PBS and then bidistilled water. Finally, the sections were counterstained with 5% uranyl acetate and observed in a Hitachi EM H-600-2 at 75 kV.

Control measurements were taken by replacing the anti-NOR serum and anti-DNA/RNA hybrid antibody with antibody diluent.

RESULTS

In a transcriptionally active nucleolus, there are a few (usually >3 per section) FCs, and the rDNA transcription levels are high. After the semi-thin sections of active nucleolus were stained using the NAMA-Ur method, unexpectedly, we found that nucleolar DNA was restricted to the boundary regions between FC and DFC, whereas FCs did not contain any detectable DNA (Fig. 1).

Since the distribution of transcription factor (UBF) might reflect the sites where the rRNA transcription units are located, we located the UBF in the nucleolus at the ultrastructural level by anti-NOR serum. Previously, we had detected the UBF antibody specific reactivity by immunoblotting.

Having isolated the nuclei of onion cells, SDS-PAGE gel electrophoresis was carried out on nuclei protein extracts, followed by electrophoretic

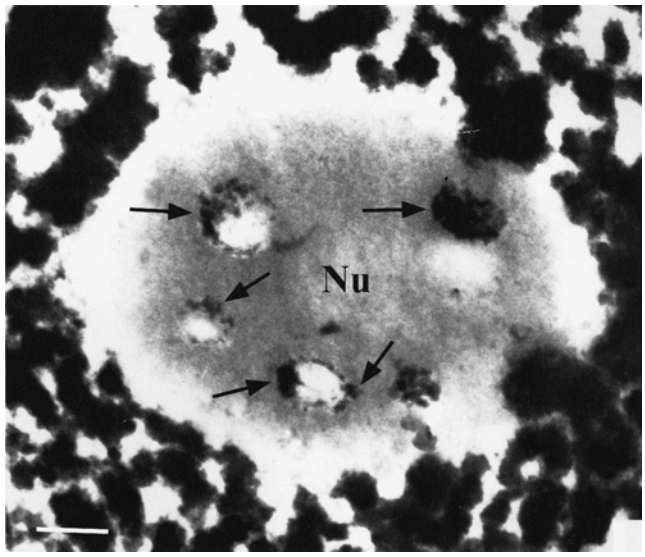

Fig. 1. The semi-thin section of an active nucleolus of onion cells after DNA-specific staining, showing the location of nucleolar DNA. Nucleolar DNA was present at the boundary between FC and DFC. Arrows indicate DNA. Nu: nucleolus. Bar: 0.5 μm.

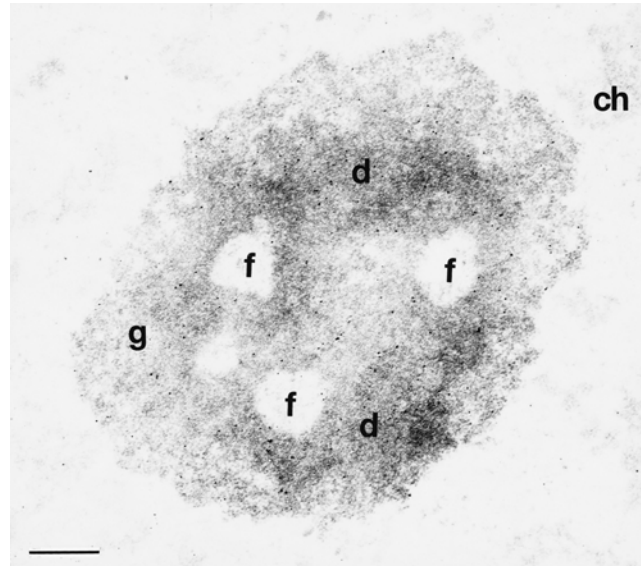

Fig. 3. Electron microscopic immunolocalization of UBF in an active nucleolus of onion cells. Gold particles were found mostly distributed over the DFC and the boundary between DFC and FC. Few gold particles were present at the GC, but the FC was mainly devoid of labelling. ch: chromatin; d: dense fibrillar component; f: fibrillar centre; g: granular component. Bar: 0.3 μm.

transfer and immunoblotting. Only one polypeptide was detected—which migrated at ~97 kD—when immunoblotted with anti-NOR serum (Fig. 2).

After localization at the ultrastructural level by immuno-gold labelling, labelling with protein A-gold was restricted to the nucleoli. Gold particles were found mostly distributed over the whole DFC (Fig. 3). The quantitative comparison of the label present in the boundary region was highest in the nucleolus (Table 1). Few gold particles were present at the GC, and the interior of the FC was largely devoid of labelling (Fig. 3).

When transcribed, DNA could form a temporary DNA/RNA hybrid double-chain structure. With the help of anti-DNA/RNA hybrid antibody, transcription sites of rRNA genes could be selectively and directly labelled (Fig. 4). The results indicated that the labelling signals of DNA transcription activity appear at the boundary between the FC and DFC.

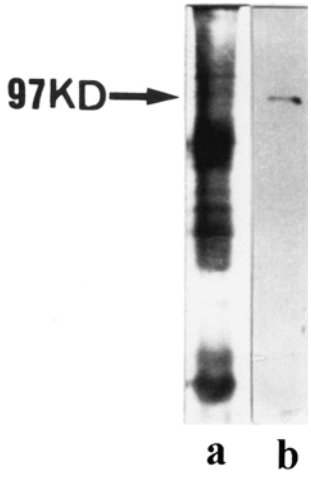

Fig. 2. Gel pattern of nuclear protein (a) and immunoblotting test for recognizing anti-NOR serum, (b) a single 97 kDa polypeptide was revealed.

DISCUSSION

To date, no definite conclusion has been reached about the location of the transcription site of rRNA genes in the nucleolus (Mais and Scheer, 2001). The present methods for studying transcription sites of rRNA genes are generally based on incorporation of labelled UTP as a trace marker, although some have suggested that this method is not very reliable. One important reason is that this method, based on an uncertain time of incorporation—especially the quick extension of transcription template—may mark the signal which has already left the original transcription site (Jackson *et al.*, 1993). To overcome the disadvantages of the present method, we have used anti-DNA/RNA hybrid antibody directly and

Table 1.
Quantitative study on the gold densities of immunogold labelling by anti-NOR serum

	Gold particles/m² se				
	Nucleoplasm	*DFC*	*GC*	*Boundary between FC and DFC*	*FC*
Immunolabelling	0.36 ± 0.04	16.09 ± 2.13	4.07 ± 0.37	23.09 ± 2.65	0.67 ± 0.20
Control	0.25 ± 0.01	1.23 ± 0.05	1.67 ± 0.12	4.21 ± 0.15	0.70 ± 0.14

Densities of gold particles were counted with IBAS image process system. The boundary between FC and DFC in nucleolus was defined according to the report from Guillermo *et al.* (1999). DFC: dense fibrillar component; FC: fibrillar centre; GC: granular component.

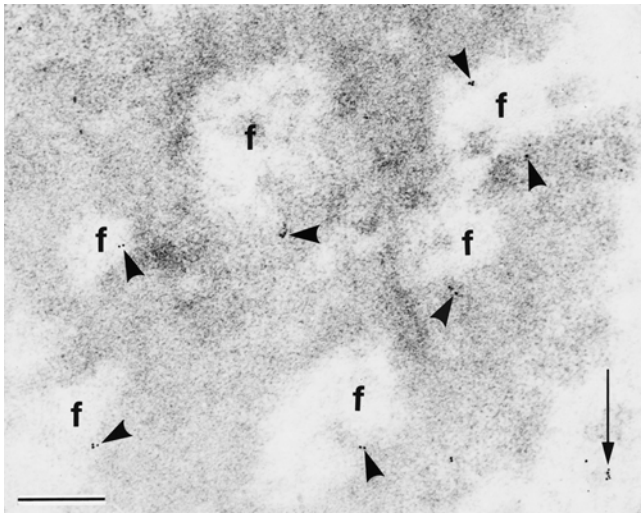

Fig. 4. Nucleolus labelled by anti-DNA/RNA hybrid antibody. Labelling signals (short arrows) of rDNA transcription activity located at the boundary between FC and DFC. Long arrows indicated the transcription sites occurred in nucleoplasm. f: fibrillar centre. Bar: 0.5 μm.

selectively to label the rRNA genes which are being transcribed. Our results confirm that the transcription sites of rRNA genes in the onion cell nucleolus are at the boundary between the DFC and FC. This is in accord with our result of nucleolar DNA location, which were observed *in situ* by the NAMA-Ur method. Although the UBF was localized at the whole DFC by immuno-gold labelling, the result from the quantitative study indicated it was mainly distributed in the boundary region between the FC and DFC. Thus, we conclude that the boundary region between the FC and DFC is the genuine transcription site of rRNA genes.

UBF has been shown to consist of two polypeptides with apparent molecular masses of 94–97 kDa in mammalian cells (Jantzen *et al.*, 1990) and 82–85 kDa in frogs (Pikaard *et al.*, 1989). However, in *Allium cepa* root cells, Rodrigo *et al.* (1996) showed that UBF corresponds to a 58 kDa polypeptide. Our results, also in onion cells, indicate that UBF is a 97 kDa polypeptide. We do not know if this represents a single UBF polypeptide in plants because of the absence of alternative splicing of the UBF gene in plants, unlike in mammals (Bolivar *et al.*, 1996). Our finding clearly show that cloning of UBF in plants is needed to answer this question.

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