In situ visualization of rDNA arrangement and its relationship with subnucleolar structural regions in *Allium sativum* cell nucleolus

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Summary
We used a DNA-specific staining technique to show the two states of DNA component distributed in the nucleolar region of *Allium sativum* cells. One state is the extended DNA fiber, and the other is the condensed DNA clump. In situ hybridization demonstrated that the extended DNA fiber was an rRNA gene. Anti-fibrillarin antibody immunolabeling revealed that these rRNA genes were located in the dense fibrillar component near the fibrillar center, including at the periphery of the fibrillar center. None was in the dense fibrillar component far away from the fibrillar center. The condensed DNA clump was located in the fibrillar center. Further observations showed that the rRNA genes in the nucleolus were all arranged around the fibrillar center and associated with the DNA clumps in the fibrillar center. Results of statistical analysis showed that the distribution region of rRNA genes occupied about one-third of the total dense fibrillar component region. Ag-NOR protein showed a similar distribution pattern to that of rDNA. Immunolabeling of an anti-rRNA/DNA hybrid antibody demonstrated that the transcription sites of rRNA were located at the periphery of the fibrillar center and in the dense fibrillar component near the fibrillar center, and these sites were consistent with the location and arrangement of rDNA shown in situ. These results demonstrated that transcription of rRNA takes place around the fibrillar center and at the periphery, whereas the dense fibrillar component that was far away from fibrillar center was the non-transcription region. The DNA clumps within the fibrillar center were probably the anchoring sites for rDNA arrangement.

Key words: Nucleolar ultrastructure, DNA specific staining, Ag-NOR protein, rDNA transcription, Immunocytochemistry, Plant nucleolus

Introduction
The nucleolus is the most prominent subcellular structure in eukaryotic cells. Its distinguishing characteristics are associated with the localization of rRNA gene transcription and transcription product processing. The nucleolus is a good structural model for investigations of gene expression as well as changes in the splicing processes of the transcription products. Although rRNA synthesis and pre-ribosomal processing functions were demonstrated more than 40 years ago, the structure-function relationship between the various subnucleolar regions and the rRNA synthesis remains to be elucidated (de Cácer et al., 1999; Olson et al., 2000).

One nucleolar structure-function relationship that needs to be clarified is that of rDNA arrangement in the nucleolus and its relationship with the subnucleolar structure region (Biggiogera et al., 2001). Although Miller’s early spread technique revealed the ‘christmas tree’ structure for the active transcription of rRNA genes (Miller et al., 1969), this structure in the nucleolus of living cells is in a topological constraint state. It is very difficult to analyze the relationship between the nucleolar structures and their functions by observing the ‘christmas tree’ structure in situ (Shaw et al., 1995; Scheer et al., 1997). In spite of this, Miller’s spread technique did reveal an important characteristic of the rRNA gene, that is, rDNA is of a non-nucleosomal configuration. In the nucleolus, the rRNA gene is in a highly decondensed state (Derenzini et al., 1983; Medina et al., 2000; Biggiogera et al., 2001).

The first objective of our study was, by employing the cytochemical technique of DNA-specific staining NAMA-Ur (Testillano et al., 1991), to display the extended rDNA fiber in situ in the nucleolus of *Allium sativum* cells. One state is the extended DNA fiber, and the other is the condensed DNA clump. In situ hybridization demonstrated that the transcription sites of rRNA were located at the periphery of the fibrillar center and in the dense fibrillar component near the fibrillar center, and these sites were consistent with the location and arrangement of rDNA shown in situ. These results demonstrated that transcription of rRNA takes place around the fibrillar center and at the periphery, whereas the dense fibrillar component that was far away from fibrillar center was the non-transcription region. The DNA clumps within the fibrillar center were probably the anchoring sites for rDNA arrangement.

Materials and Methods
Tissues
Root tip meristems of *Allium sativum* were used in this study. Root tip meristems were obtained from garlic bulbs grown under standard condition of 25°C.
Conventional electron microscopy

Root tips were carefully excised and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer saline (PBS) pH 7.4 for 2 hours at room temperature. After rinsing in double-distilled water for 20 minutes, they were postfixed in 1% osmium tetroxide for 60 minutes. Samples were dehydrated in an ethanol-acetone series and embedded in Epon 812. Ultra-thin sections of 60-80 nm were stained with uranyl acetate and lead citrate and examined under a Hitachi-600 transmission electron microscope.

NAMA-Ur procedure for DNA-specific staining

To study the distribution of DNA in the nucleolus, we employed the NAMA-Ur method reported by Testillano (Testillano et al., 1991). Briefly, samples were fixed in 3% glutaraldehyde and 4% formaldehyde in 0.1 M PBS for 1 hour at 4°C. After washing in 0.1 M PBS, specimens were immersed in 0.5 N NaOH in 4% formaldehyde overnight (NA) and then rinsed in double-distilled water three times for 10 minutes each, followed by 1% acetic acid three times for 10 minutes each, and finally in double-distilled water again for three times for 10 minutes each. After that, specimens were treated with a freshly prepared methanol:acetic anhydride (5:1, v:v) mixture at 25°C for 18-24 hours until the samples were bleached. Specimens were dehydrated in a methanol series and embedded in Epon 812. Semi-thin sections were stained with 2% aqueous uranyl acetate for 70 minutes at 60°C. After washing in double-distilled water and drying at 25°C, they were examined under a Hitachi EM 600-2 at 75 KV.

Electron microscopic silver staining

Root tips were immediately fixed in 4% paraformaldehyde and 2.5% glutaraldehyde in PBS pH 7.4 for 2 hours at 4°C. After a treatment with AgNO3 (50%) solution for 24-48 hours at 37°C. The sections were then treated for 10 minutes at 80°C and 2.5% glutaraldehyde in PBS pH 7.4 for 2 hours at 4°C. Root tips were immediately fixed in 4% paraformaldehyde in 0.1 M PBS, specimens were immersed in 0.5 N NaOH in 4% formaldehyde overnight (NA) and then rinsed in double-distilled water three times for 10 minutes each, followed by 1% acetic acid three times for 10 minutes each, and finally in double-distilled water again for three times for 10 minutes each. After that, specimens were treated with a freshly prepared methanol:acetic anhydride (5:1, v:v) mixture at 25°C for 18-24 hours until the samples were bleached. Specimens were dehydrated in a methanol series and embedded in Epon 812. Ultra-thin sections were stained with 2% aqueous uranyl acetate for 70 minutes at 60°C. After washing in double-distilled water and drying at 25°C, they were examined under a Hitachi EM 600-2 at 75 KV.

In situ hybridization

The rDNA probe was obtained from the pTA71 plasmid, which contains the rDNA repeat unit of wheat cloned in pUC18. A 3.6 kb fragment of pTA71 containing 18S and 25S rDNA were excised by BamHI digestion, isolated by agarose gel electrophoresis and labeled by nick translation in the presence of bio-16-dUTP (Bionic, Gibco). The probe was purified by a Spin G-25 column, precipitated by ethanol, dissolved in sterile water and stored at –20°C.

The root tips treated with the NAMA-Ur method were sectioned and directly subjected to in situ hybridization without the final step of uranyl acetate staining. In situ hybridization was performed according to the non-radioactive in situ hybridization application manual (Boehringer Mannheim). Briefly, ultra-thin sections were first treated with proteinase K (10 μg/ml) for 15 minutes at 37°C. The sections were then treated for 10 minutes at 80°C in 75% deionized formamide in 2xSSC and then placed in 100°C denatured probe solution (50% formamide, 5xSSC, 10% dextran sulfate, 10 mmol Tris-HCl, 0.5% sodium dodecyl sulphate, 250 μg/ml salmon sperm DNA and 5 mg/ml sodium pyrophosphate) and hybridized at 42°C in a moist chamber for 18 hours. Sections were floated three times for 10 minutes on drops of PBS and incubated with Streptavidin-conjugated 10 nm gold particles (Sigma). After washing and drying, sections were stained with 2% aqueous uranyl acetate for 20 minutes at 60°C. After washing in double-distilled water and drying at 25°C, they were observed under a Hitachi EM 600-2 at 75 KV.

Immunocytochemistry

The anti-DNA/RNA hybrid antibody was kindly provided by B. D. Stollar (Rudkin et al., 1997). The anti-fibrillarin antibody was purchased from Santa Cruz. Lowicryl K4M was obtained from Chemische Werke Lowi GMBH & Co., Germany. Protein A conjugated to 10 or 15 nm colloidal gold particles was purchased from Sigma.

Processing for Lowicryl K4M embedding

Samples were fixed in 3% glutaraldehyde and 4% formaldehyde in PBS for 2 hours. After washing in double-distilled water three times, for 30 minutes each, they were dehydrated in an ethanol series and permeated by 100% ethanol: K4M (1:1) mixture for 12 hours at 0°C, 100% ethanol: K4M (1:2) mixture for 1 hour at –10°C, and 100% K4M for 60 hours at –30°C. After that specimens were embedded in Lowicryl K4M at –30°C under UV for over 24 hours, and then irradiated again for 2-3 days at room temperature.

Immunogold labeling of an anti-fibrillarin antibody and an anti-DNA/RNA hybrid antibody

Immunogold labeling of an anti-fibrillarin antibody and DNA/RNA hybrid antibody were carried out as described previously (Testillano et al., 1994). Briefly, ultra-thin Lowicryl sections were mounted on
Formvar nickel grids and washed in PBS three times for 1 minute each, and in 5% BSA (PBS, 0.05% Triton X-100) for 10 minutes. Then the grids were incubated with the anti-fibrillarin antibody or anti-DNA/RNA hybrid antibody diluted 1:300 in PBS for 1 hour at room temperature. After several washings in PBS, they were floated in protein A conjugated to 15 nm colloidal gold particles diluted 1:25 in PBS for 45 minutes at room temperature. They were washed in PBS and in double-distilled water. After drying, some sections were stained with 5% uranyl acetate, and others were subjected to the NAMA-Ur method and observed under a Hitachi EM H-600-2 at 75 KV. Controls were done by replacing the primary antibody with diluents.

Nucleolar component measurement

The areas of components in sections were measured using an IBAS image analysis system from Germany. 30 micrographs were chosen from different grids. Among the 30 micrographs, 15 were taken from NAMA-Ur-stained sections. For a more accurate measurement of the areas, micrographs of the subnucleolar regions were enlarged to as high a magnification as 120,000×.

Results

With Epon812 embedded sections, after conventional staining, three nucleolar regions may be recognized under an electron microscope: the lowest electronic density region, known as the fibrillar center (FC); the dense fibrillar component (DFC), the high electronic density region arranged around the FC; and the granular component (GC), which is distributed between the DFC and the periphery of nucleolus (Fig. 1A). In addition, vacuoles (V) were also observed within the nucleolus, and they were characterized by a region with low electronic density similar to the FC but not enveloped by the DFC at its periphery (Fig. 1A).

To demonstrate and locate nucleolar DNA components, we first employed the NAMA-Ur DNA-specific staining technique to treat the cells and then observed them under the electron microscope after semi-thin sectioning. We found that the semi-thin sections produced the better contrast of the DNA components than the ultra-thin sections, and this allowed a clearer visualization. The cytoplasm was basically bleached; the DNA in the nucleoplasm was intensely and specifically stained and mainly present as high-density clumps. In the nucleolus, although the background of the medium electronic density was retained, the specifically stained high electronic density component of DNA can still be seen. We found that the DNA components in the nucleolus existed in two forms, one was the decondensed DNA fiber, which was distinctly different from the DNA component in nucleoplasm (Fig. 1B,D), and the other was the condensed DNA clumps, which appeared to be composed of tightly coiled DNA filaments and surround by DNA fibers (Fig. 1C, arrows). In addition, the DNA clumps were only located in the regions with low electronic density in the nucleolus. These low-density regions seemed to correspond to FCs. Since fibrillarin protein is the marker protein of DFC, the anti-fibrillarin antibody immunolabeling experiment was carried out to prove that these low-density regions were FCs. Moreover, we also found that the region of fibrillarin labeling was bigger than that occupied by DNA fibers, suggesting that not all DFC contained DNA (Fig. 2).

To find out if the DNA component in the nucleolus is an rRNA gene, we used the biotin-labeled rDNA fragments containing 25S and 18S as probes, as well as the biotin antibody conjugated with 10 nm colloid gold as a labeling
signal to perform in situ hybridization. After hybridization, the sections underwent the final step of specific staining. The labeling signals of gold particles appeared mainly on the extended DNA fibers, and none appeared on DNA within FC (Fig. 3). This result clearly shows that the DNA fibers distributed around the FC represent the sites of rRNA genes, whereas the DNA clumps located within the FC were not rRNA genes.

After determining that the fibrillar DNA component in the nucleolus was an rRNA gene, we further investigated its location and arrangement and found that the highly extended rDNA fibers were mainly arranged and distributed around the FC, with their initial positions at the boundary of FC. The DNA in the FC was kept in a condensed state (Fig. 4). In general, rDNA was arranged in a circular configuration with each FC as its center (Fig. 4, arrows).

Our observations also showed that rDNA was always arranged in an identical configuration, with FC as the center, regardless of its size and quantity (Fig. 5A). In some sections of the nucleolus, parts of the DFC region at the periphery of the FC were not surrounded by rDNA, whereas others were completely devoid of rDNA (Fig. 5B), indicating that the arrangement of rDNA in the DFC was not uniform. Besides, in some sections of nucleolus, the rDNA in the nucleolus was connected to the extranucleolar DNA at many sites, as shown in Fig. 5D.

When viewed under a high magnification (Fig. 6A,B), we observed some DNA fibers that stretched out of the aggregated DNA clumps within FC while still connected to DNA clumps with the extended rDNA in DFC regions. The rDNA fibers surrounding the FC were variable in diameter. This variation may be ascribed to the presence of many ‘arrow-like structures’ along the rDNA fibers (Fig. 7A, arrows). An enlarged image of FC and its surrounding rDNA shown in Fig. 7A enabled us to take a closer look at these structures (Fig. 7B), and it can be seen that the arrow-like structures were arranged along the rDNA fibers with a certain distance interval. Owing to the variable angles of sectioning, the appearance of these structures varied. The maximum length of the arrow-like structures measured about 200 nm (Fig. 7C,D). Fine fibers ~6 nm in diameter extending laterally from these structures can occasionally be detected (Fig. 7D, arrows). The thinner rDNA fibers were located in between the arrow-like structures. Meanwhile, transversal views of the arrow-like structures were also found on these sections (Fig. 7E). On the basis of these images and measurements, we postulate that these structures represent in situ rDNA transcription units, equivalent to the tightly packed in situ Christmas tree structures (Scheer et al., 1997; Gonzalez-Melendi et al., 2001). This phenomenon may reflect the morphology of elongating rRNA in transcribing regions, even though the transcripts were tightly packed with rDNA.

Early studies found that some argyrophilic proteins specifically appeared in the metaphase NOR and interphase nucleolus (Goodpasture et al., 1975). Under acidic condition, these proteins can be stained specifically by AgNO₃ and readily...
visualized. Later it was found that the Ag-NOR protein was the marker of active rRNA gene and that rDNA transcription did not take place in the absence of Ag-NOR protein (Fakan et al., 1986; Pession et al., 1991; Derenzini et al., 2000). In our experiment, we observed the in situ arrangement of rDNA. If this arrangement truly exists, then the distribution of Ag-NOR proteins that are closely related to rDNA transcription will conform with such an arrangement. To prove this assumption, we analyzed the distribution of silver-stained proteins. After the intact cells were silver-stained, the silver-stained proteins mainly appeared in the nucleolus (Fig. 8B). Under a high magnification, Ag-NOR proteins in the nucleolus could be seen in many circular configurations. Each circle was a relatively independent unit, but these circles were interconnected, and no silver-stained protein was present in the center of the circles (Fig. 8A). Furthermore, we compared one rDNA configuration image with that of Ag-NOR under the same magnification (Fig. 9A,B). It can be seen that the distributions of rDNA and Ag-NOR proteins were very similar, and the result of statistical analysis demonstrated that the areas of the regions occupied by the nucleolar Ag-NOR proteins and rDNA fibers were fundamentally identical (Fig. 11). This analysis suggested that our inference was correct. However, we also noticed that there was a distinct difference between these two arrangement configurations. The central portion of the rDNA configuration had DNA components in an aggregated state, whereas the central portion of the circular configuration had no silver-stained protein.

When the rDNA was under transcription, the transient DNA/RNA hybrid double-stranded structure is sometimes formed. With the aid of an anti-DNA/RNA hybrid antibody, we were able to directly and selectively label the transcription sites of the rRNA gene. Comparative experiments proved that the antibody labeling system had a very good specificity (data not shown).

The result of the labeling study of the rRNA gene transcription sites showed that in the nucleus some signals indicating DNA transcription activities appeared on the decondensed chromatin or the boundary area of the condensed chromatin. However, in the nucleolus, the signals were located at the periphery of the FC and the DFC region near the FC. We noticed that the signals were never seen in the nucleolus-associated chromatin, granular component and the interior of the FC. Hence, it can be inferred that the transcription sites of rRNA in the nucleolus are

**Fig. 6.** Micrographs of rDNA arrangement at high magnifications. (A,B) DNA clumps within FCs are connected with rDNA surrounding the FC by DNA fibers (white arrows). A and B are partial enlargements of C. Bar, 0.3 μm.

**Fig. 7.** rDNA ultrastructures after specific staining, showing the variation in thickness of rDNA fibers (A). Note the ‘arrow-like structures’ along the thicker parts of the rDNA fiber (B,C,E). The maximum length of the arrow-like structures is 200 nm, whereas the fibers extending from these structures measure about 6 nm in diameter (E, arrows). D represents the transverse section of the arrow-like structure. Bar, 0.5 μm (A), 0.3 μm (B) and 30 nm (C-E). Asterisks indicate FCs.
located at the periphery of the FC and DFC regions near the
FC (Fig. 10).

To estimate the areas of the DFC occupied by rDNA, we
randomly selected 30 nucleolus sections, of which 15
nucleolus sections were treated with DNA-specific staining,
and analyzed statistically the total area of the nucleolus,
granular regions, FC, DFC and rDNA filaments as well as the
area occupied by the Ag-NOR proteins (Fig. 11). The
statistical results showed that the area occupied by the highly
decondensed rDNA fibers accounted for about one-third of the
total area of the DFC, implying that one-third of the DFC
region near the FC (including the periphery of the FC) was the
transcription domain for the rRNA gene.

**Discussion**

The granular region is mainly composed of pre-ribosomes
without rDNA components (Thiry and Goessens, 1996). The
possible distribution regions of DNA components in the
nucleolus may be the DFC and FC. For a long time it has not
been clear whether the rDNA is situated at the DFC or at the
FC (Wachtlar et al., 1989; Thiry and Thiry-Blaise, 1991;
Puvion-Dutilleul et al., 1991; Hozak et al., 1995; Lazdins et
al., 1997). In plant cells, the FC-containing DNA components
are visualized under conventional staining conditions (Fig.
1A). However, it was proved by electron microscopy and in
situ hybridization that DFC (especially the DFC near FC)
contains rDNA (Jordan et al., 1990; Derenzini et al., 1993).

In this regard, the interesting questions are how is the rDNA
arranged and distributed in DFC and what is the relationship
between rDNA in DFC and DNA components in FC. In order
to observe the distribution of rDNA in the nucleolus in situ, we
have employed NAMA-Ur DNA-specific staining in an attempt
to verify two points in this regard. One is that since rDNA has

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**Fig. 8.** The distribution of Ag-NOR protein in the nucleolus. (A) Ag-NOR proteins are mainly distributed in the
interior of the nucleolus and present as circular configurations with hollow centers. A is the enlarged image of the
nucleolar region of B. (B) The complete picture of the cell nucleus after Ag-NOR staining. Ag-NOR proteins are mainly
located in the nucleolus. N, nucleus; Nu, nucleolus; Cyt, cytoplasm. Bar, 0.3 μm.

**Fig. 9.** The similarity between rDNA and Ag-NOR arrangement configurations. A is one of the arrangements
of rDNA. B is the circular distribution configuration of Ag-NOR protein. The magnification is the same for A and B. It
can be seen that the arrangements of rDNA and Ag-NOR proteins are similar. Bar, 0.1 μm.

**Fig. 10.** The nucleolus labeled with the RNA/DNA hybrid antibody. The signals of colloid gold particles for transcription sites are located
at the periphery of FC and DFC regions near the FC (asterisks). Chr, chromatin; G, granular component. Bar, 0.5 μm.
The characteristics of non-nucleosomal configuration, the decondensed rDNA fibers should be seen in the nucleolar region after DNA-specific staining; and the other is that the subnucleolar regions of the nucleolus should still be identified after DNA-specific staining. This would provide a basis for analyzing directly the structural relationship between the distribution of rDNA and the subnucleolar regions. By making use of a combination of several ultrastructural techniques (such as cytochemistry, immunocytochemistry and in situ hybridization), we have been able to show that rDNA was mainly situated in DFCs near FCs, and occupy one-third of the DFC, and the synthesis of rRNA took place there. The DNA within FC was in a condensed state and no transcriptional activity occurred there. The distribution of Ag-NOR proteins relative to transcription of rDNA was similar to that of rDNA.

Sirri et al. identified nucleolin and protein B23 as the two major Ag-NOR proteins in the nucleolus (Sirri et al., 2000). It was thought that Ag-NOR proteins were distributed in both FCs and DFCs (Fakan et al., 1986). However, extensive studies on the localization of nucleolin and B23 using electron microscopy have reached a general agreement that nucleolin and B23 are located in the DFC around the FC, whereas FC is devoid of these proteins (Ginisty et al., 1999; Biggiogera et al., 1990). In this paper, after the nucleolus was treated with the procedure described in Materials and Methods, it was found that the arrangement and distribution of Ag-NOR proteins were very similar to those of rDNA, that is, Ag-NOR proteins were present as circular configurations with hollow centers. The results of statistical analysis showed that the distribution areas of Ag-NOR proteins and rDNA were basically identical. Although we did not perform a labeling study of the Ag-NOR protein distribution sites, on the basis of the distribution pattern of Ag-NOR proteins and previous results, we conclude that locations of Ag-NOR proteins were consistent with that of rDNA and are arranged and distributed around the FC.

Many reports indicated that the active transcription regions were located at the periphery of the FC and DFC near the FC (Testillano et al., 1994; Meecak et al., 1996; Lazzins et al., 1997; de Carcer et al., 1999). Data presented in this paper indicate that in *Allium sativum* cells, the distribution of rDNA was restricted to the limited areas near to the FC, including the periphery of the FC (Fig. 1C, Fig. 4). Our results also demonstrated that fibrillarin-labeled regions that were distal to FC were devoid of rDNA (Fig. 4). Moreover, the immunolabeling with anti-RNA/DNA antibody showed that no transcription took place in DFC regions away from the FC (Fig. 10). Hence, the result of this paper supports the notion that only the DFC near the FC contains active sites of rDNA transcription, and no transcription activity occurs at DFCs far away from the FC. Thus, the next question is what is the proportion of transcriptionally active DFC in the total DFC? At present, there are no reports of the precise structural subdivisions of the DFC. Our statistical analysis in this study is the first attempt, and we put forward the notion that only about one-third of the DFC near the FC is distributed with rDNA and that no transcriptional activities exist in the remaining two-thirds of the DFC region. Data from early studies demonstrated that the DFC far away from the FC is involved in the splicing and processing of the transcription products of rRNA genes (Shaw et al., 1995).

It should be noted that the thickness of rDNA fibers revealed in our experiments varied, and we reason that the cause of this variation was the occurrence of the arrow-like structures, which we postulate to be the rDNA in the form of tightly packed transcription units. We make this hypothesis on the following grounds. (1) The arrow-like structures occurred only on rDNA fibers. (2) They closely resembled the densely packed christmas tree structures in morphology. (3) They measured ~200 nm in length, close to the dimensions of the in situ Christmas tree reported previously (Scheer et al., 1997; Gonzalez-Melendi et al., 2001). (4) They were specifically distributed in DFC regions near to the FC. However, the important question arising from this phenomenon is what caused the occurrence of this structure. We postulated that the tightly compacted rDNA transcription units contained RNA and protein that are tightly associated with rDNA, and this prevented reagents from penetrating into the structures in DNA-specific staining processes, resulting in an overall staining and visualization of the transcription units by the final step of uranyl acetate staining. This effect may be more prominent in our semi-thin sections. Nevertheless, the in situ christmas tree structures revealed in our preparations provided direct evidence that rDNA transcription units are located only at DFC regions near to the FC and that native rDNA transcription units are linear compacted christmas trees (Gonzalez-Melendi et al., 2001). In our preparations, although the appearance of the in situ christmas tree resulting from the elongation of rRNA could vaguely be recognized, the structural details of the rRNA and rDNA fibers inside in situ ‘christmas trees’ were not clearly distinguished, presumably owing to the tight association between rRNA and rDNA. The length of these rDNA transcription units ranged between 100 nm and 200 nm, shorter than those previously reported for plant rDNA transcription units (Gonzalez-Melendi et al., 2001). One possible explanation is that the method we have used to observe the in situ ‘christmas tree’ is not the immunogold labeling technique (Gonzalez-Melendi et al., 2001), which may result in an error owing to the size of gold particles. However, our observations may imply a higher compaction ratio of rDNA transcription units than expected (Koberna et al., 2002).

In plant cells, FCs may be divided into the heterogeneous FC and the homogeneous FC; the former contains chromatin...
in a condensed state whereas the latter contains chromat in a dispersed state (Risueno et al., 1982). Recently, Biggiogera et al. used EFTEM (energy filtering transmission electron microscopy) to detect that the DNA component within the nucleolar FC of a murine P815 animal cell was distributed as ‘DNA cloud’. Biggiogera et al. believed that the rDNA in the FC extended to the DFC to initiate transcription activities (Biggiogera et al., 2001). The conclusion from their experiments is fundamentally consistent with the results of this paper. However, we have found that all DNA components within FC are in a tightly condensed state, or composed of tightly coiled DNA fibers, and no any decondensed DNA components were observed in the FC of the nucleolus. The reason for this phenomenon is still unclear to us. One possible explanation is that it may be a result of the different experimental materials used.

As to the functions of FC, at present there are at least two hypotheses, one is that FC is the anchoring site for the inactive rDNA storage, and the other is that FC is the assembly site of the rRNA gene transcription machinery (Medina et al., 2000). So far, there has been clear evidence showing that RNA polymerase I is located in the FC (Scheer et al., 1984). The reliability of this viewpoint cannot be verified in this paper, but results from our experiments and many others show that FC contains DNA that has no transcriptional activities (Medina et al., 2000; Tao et al., 2001). Our results have also demonstrated that some extended DNA fibers stretch out of DNA clumps in FC to connect with the extended rDNA. This has led us to believe that the DNA within FC is the anchoring site for the rDNA special arrangement.

On the basis of the data from this paper and other researches, we have constructed a model for the nucleolus of plant cells (Fig. 12). This model mainly illustrates that FC contains DNA components in a highly condensed state, which may serve as the anchoring site for rDNA arrangement. The transcribing rRNA genes are present in a non-nucleosomal configuration in a highly decondensed state and are arranged in one-third of the DFC region, near the FC, which includes the periphery of FC and Ag-NOR proteins, as well as the protein molecules needed for transcription, such as RNA polymerase I transcription factor UBF, which are also distributed in this region (de Carcer et al., 1999).

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