NUCLEOLAR FIBRILLAR CENTRES IN PLANT MERISTEMATIC CELLS:
ULTRASTRUCTURE, CYTOCHEMISTRY AND AUTORADIOGRAPHY*

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SUMMARY

In plant cells nucleolar fibrillar centres (FCs) undergo ultrastructural changes, depending on the nucleolar activity. We have found two types of FC structure in nucleoli with either high or low activity, to which we have given the conventional names of homogeneous and heterogeneous, respectively. The first type is characterized by the presence of fibres that we describe structurally and cytochemically as decondensed chromatin; the second type, in addition to these fibres, contains a variable number of dense cores made up of condensed chromatin. Moreover, RNA does not appear to be present in FCs, while proteins are a major component.

Autoradiography after tritiated uridine incorporation shows that FCs are not the site of transcription, but that this takes place in the fibrillar component; the same result is obtained using the lead acetate fixation technique for detecting orthophosphate ions. This fact leads us to think that FCs are not the whole interphase counterpart of the mitotic nucleolar organizing region (NOR), as stated by other authors, but only the portion of the NOR that is temporarily inactive in transcription; the transcriptionally active part of the NOR is in the fibrillar component, bound to its earliest product of transcription. Thus, FCs and the fibrillar component constitute a functional unit.

INTRODUCTION

The nucleolus is one of the best models in which studies on the structure–function relationship at the cellular level can be carried out in eukaryotic organisms.

In plant cells as well as animal cells both the fibrillar component and the granular component are typical structural elements of the interphase nucleolus whose molecular counterpart has been very well established (see, for example, recent reviews by Fakan & Puvion, 1980; Jordan, Timmis & Trewavas, 1980). Another occasional component of the interphase nucleolus is the nucleolar vacuole, which has also been correlated with enhanced nucleolar activity (Moreno Díaz de la Espina, Medina & Risueño, 1980).

However, there is a fourth component in the typical interphase nucleolus; namely, light or electron-lucid areas immersed in the fibrillar component. Nomenclature (always a problem in defining nucleolar constituents; see Swift, 1966) has been confusing in these areas (Goessens & Lepoint, 1979). Recently, the term ‘fibrillar

* This paper is dedicated to the memory of the late Dr G. Moyne.
centres' (Recher, Whitescarver & Briggs, 1969) has become the name most commonly used for them.

There is abundant literature about fibrillar centres (FCs) and their relationship to the intranucleolar chromatin that contains ribosomal cistrons (rDNA) (see reviews by Goessens & Lepoint, 1979; Franke et al. 1979; Fakan & Puvion, 1980; Mirre & Stahl, 1981, among others). It seems to be well-established that this chromatin is the same as that of the nucleolar-organizing region (NOR) around which the nucleolus re-organizes at the end of mitosis. In this paper both of these will be treated as the same.

Nevertheless, in spite of this abundant literature, some important items on fibrillar centres remain open to question, such as: (1) What is the precise relationship between fibrillar centres and NORs? In other words, is the whole NOR contained in the set of fibrillar centres of a nucleolus? (2) Why are the fibrillar centres only a 'frequent' and not a 'general' nucleolar component? (3) Is the structure of the fibrillar centre always the same?

Moreover, the information available on plant cell fibrillar centres has not reached the same level as that on animal cells, even if we consider some relevant papers dealing with them (Lafontaine & Lord, 1973; Jordan et al. 1980). In addition, plant cells have a particular important feature; namely, a greater amount of ribosomal cistrons than animal cells (Ingle & Sinclair, 1972).

**MATERIALS AND METHODS**

The material used was root meristems of *Allium cepa* L. The onion bulbs were grown under standard conditions at 15 °C according to the procedure described by Risueño et al. (1978). In the case of dormant cells, root meristems were dissected from the bulbs and fixed without soaking.

Samples were processed for electron microscopy either by conventional techniques (glutaraldehyde-osmium fixation, uranyl-lead staining) or by cytochemical procedures. The latter included: (a) preferential staining methods: EDTA (Bernhard, 1969) for ribonucleoprotein; osmium ammine (Cogliatti & Gautier, 1973) for deoxyribonucleoprotein (DNP); and lead acetate (Tandler & Solari, 1969) for inorganic phosphate; (b) enzymic digestion (RNase, DNase and trypsin), including a parallel control series without enzyme. All these techniques were carried out as previously described (Risueño et al. 1978).

Tritiated uridine incorporation and further autoradiography were performed as described by Moreno Diaz de la Espina et al. (1980). Tritiated thymidine was supplied to the roots at a concentration of 10 μCi ml⁻¹ for 18 h. After incorporation, the samples were washed in distilled water and prefixed with glutaraldehyde in 0.025 M-cacodylate buffer for 1 h. They were then washed extensively in 0.025 M-cacodylate and extracted with 1 mg ml⁻¹ DNase-free RNase in Gomori's buffer at pH 7.2 at 37 °C for 1 h. After two washings at 37 °C in the same buffer and

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**Figs. 1 to 4: Glutaraldehyde-osmium fixation.**

**Fig. 1.** An active nucleus with a high proportion of granular component (g) in relation to the fibrillar component (f), showing numerous fibrillar centres (arrows). Chromatin (chr). x 24 282.

**Fig. 2.** High magnification of a portion of an active nucleolus, showing two fibrillar centres of the homogenous type immersed in the fibrillar component (f). Within them, the 8-11 nm densely stained fibres (arrows), which show continuity at some points with the fibres of the fibrillar component (arrowheads), stand out clearly. x 92796.
before postfixation with osmium tetroxide the samples were washed in sodium cacodylate buffer until the non-incorporated label had been eliminated, as determined by monitoring the wash buffer in a scintillation counter. They were then processed as described (Moreno Díaz de la Espina et al. 1980) for tritiated uridine incorporation experiments. Quantitative data, either from conventionally processed sections or from autoradiograms, were collected using a Kontron MOP-AM/03 semi-automatic image analyser. Autoradiographic data were processed in an IBM 5100 mini-computer with a program that included their expression in histograms.

RESULTS

Ultrastructure

Nucleolar fibrillar centres are seen in sections as nearly rounded areas immersed in the fibrillar component and less electron-opaque than it (Figs. 1-4). The material of the FCs is continuous with the fibrillar component that surrounds it; therefore, it is usually very difficult to distinguish the boundary between the FC and the fibrillar component (Figs. 1-4).

Table 1. Fibrillar component/whole nucleolus*

| Nucleoli with homogeneous fibrillar centres | 0.42 ± 0.04 |
| Nucleoli with heterogeneous fibrillar centres | 0.71 ± 0.06 |

* Expressed as the ratio of areas on random sections

Two clearly distinct types of FC structure can be described in plant meristematic cells:

1. Apparently homogeneous, fibrous and medium electron-dense structures when observed under low magnification (Fig. 1). Using higher magnifications, we can distinguish dispersed fragments of densely stained fibres 8-11 nm surrounded by a lighter material. These denser fibres are continuous with the fibrillar component (Fig. 2). This kind of FC structure is observed only in nucleoli of cells grown under standard conditions when the ratio of granular component/fibrillar component is high (see Table 1), but never in dormant cells. In order to simplify the terminology, we will use the conventional name 'homogeneous' for this type of FC structure.

Fig. 3. Nucleolus corresponding to the \( G_i \) interphasic period, which has a reduced biosynthetic activity; consequently, it shows a low proportion of granular component (g) in relation to fibrillar component (f). The fibrillar centres (arrows) are large in size and correspond to the heterogeneous type, showing a variable number of dense cores in their interiors. Some of the fibrillar centres are interconnected by a large clear area (star), which contains 10-14 nm loosely arranged fibrils (arrowheads). \( \times 34,900 \).

Fig. 4. Portion of a slightly active nucleolus at high magnification with two heterogeneous fibrillar centres immersed in the fibrillar component (f). The dense cores within them are made up of 16-22 nm densely packed fibres (white arrow), which show continuity with other densely stained 8-11 nm fibres (arrows) identical to those present in the homogeneous fibrillar centres. \( \times 79,321 \).
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(2) Heterogeneous, even under low magnification (Fig. 3), showing a variable number of dense cores intermingled with a light fibrous material. At higher magnification (Fig. 4) the cores are seen to be made up of 16-22 nm densely packed fibres, while the rest of the FC is identical to the so-called homogeneous FCs. This structure is typical in nucleoli of dormant cells and in those of cells grown under standard conditions with a low ratio of granular/fibrillar component (Table 1). Frequently, several individual heterogeneous FCs are found in larger and clearer areas that, in their turn, have 10-14 nm fibres very loosely arranged in them (arrowheads in Fig. 3).

Cytochemistry

Under EDTA regressive staining, which bleaches chromatin and contrasts ribonucleoprotein (RNP) structures preferentially, FCs appear bleached, though some stained material can be found in them, notably some granules similar to those of the granular component (Fig. 5). In the case of heterogeneous FCs, the internal cores are bleached in the same way as the chromatin masses (Fig. 6).

After digestion of sections with RNase we can see, at low magnification, that chromatin masses are densely contrasted while the nucleolus appears electron-lucid as a result of the digestion of a large part of its components (Fig. 7). Nevertheless, the dark cores corresponding to the heterogeneous FCs show a contrast very similar to that of the condensed chromatin masses (Fig. 7). At higher magnification, in addition to those described as type (2), the homogeneous FCs appear well-preserved from digestion (arrows in Fig. 8), showing some fibres similar to those of the rest of the digested nucleolus.

Osmium ammine staining, preferential for DNP, strongly contrasts the cores of heterogeneous FCs, as well as the condensed chromatin masses (Fig. 9). It is not possible to demonstrate the outline of homogeneous FCs by this technique; the nucleoli that presumably contain this type of FC show short fragments of preferentially contrasted 8-11 nm fibres occupying large areas of the nucleolar space. They are not distributed uniformly (Figs. 10, 11).

In heterogeneous FCs, DNase-digestion experiments resulted in the bleaching of internal cores as well as some 8-11 nm fibres in the same way as with condensed chromatin masses (Fig. 3).

Figs. 5, 6. EDTA regressive staining preferential for RNP structures. In these preparations the nucleolus (nu) appears contrasted while the condensed chromatin masses (chr) are devoid of stain. Fig. 5 corresponds to an active nucleolus. Fibrillar centres (arrow) appear bleached, though some stained material can be found in them. Fig. 6 shows a portion of a very slightly active nucleolus from a dormant root cell, and a heterogeneous fibrillar centre (arrow), whose internal cores are bleached in the same way as the condensed chromatin masses. Fig. 5, x 23826. Fig. 6, x 50648.

Figs. 7, 8. After RNase digestion the chromatin masses (chr) are densely contrasted, while the nucleolus (nu) shows a much lower contrast as a result of the digestion. At higher magnification (Fig. 8) the dense cores within the heterogeneous fibrillar centres (arrowhead) have the same contrast as the condensed chromatin masses of the nucleus. The homogeneous fibrillar centres appear well preserved from the digestion (arrow in Fig. 8). Fig. 7, x 12141. Fig. 8, x 46136.
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chromatin masses. In homogeneous FCs, only the 8–11 nm fibres appeared to be digested. In both types of FC some material different from the aforementioned cores and fibres persisted after digestion (Fig. 12).

After lead acetate fixation, which detects phosphate ions by precipitation as lead orthophosphate, the precipitate accumulates in the fibrillar component of the nucleolus, except for some zones that appear devoid of it (Fig. 13). After washing these preparations in cold trichloroacetic acid and staining them with the conventional stain of uranyl acetate and lead citrate, it becomes clear that the zones devoid of precipitate correspond to the FCs (Fig. 14).

Finally, trypsin almost completely digests FCs, leaving only a few 20 nm fibres (Fig. 15).

**Autoradiography**

1. After 18 h of incorporation of tritiated thymidine (Figs. 16, 17), the extranucleolar chromatin shows a high density of silver grains; within the nucleolus, the three structural components have different patterns of incorporation. FCs and the fibrillar component appear conspicuously labelled, while the granular component lacks any significant labelling.

2. After short pulses with tritiated uridine (Figs. 18, 19) the nucleolus shows a high level of labelling. The fibrillar component has the highest level of labelling. Frequently, silver grains on the fibrillar component are located peripherally to the FCs. A quantitative study of the autoradiograms shows an absence of significant labelling in FCs (Fig. 19).

Outside the nucleolus, labelling is located preferentially in inter- and perichromatin nuclear regions.
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DISCUSSION

The presence of DNA in FCs is evident after our cytochemical and autoradiographic studies, since FCs show some material that stains with osmium ammine and is digested with DNase; also, they are partially bleached by EDTA and incorporate tritiated thymidine. However, there is something other than DNA in FCs, since treatment with DNase does not digest them completely.

RNA could also be present in FCs because of their partial staining with EDTA, but neither experiments with tritiated uridine incorporation nor RNase digestion yield positive results to support this idea. It seems important to note here the absence of a definite boundary between FCs and the fibrillar component. This fact could be responsible for the results of EDTA staining, though we cannot rule out the possibility of some RNA (not recently synthesized) being located in the FCs. However, proteins are a major component of FCs, because of the almost complete digestion of FCs by trypsin.

In FCs there is no transcriptional activity, as demonstrated directly by experiments with tritiated uridine incorporation, and indirectly by lead acetate fixation. The absence of orthophosphate ions correlates well with the lack of both divalent cations (Tandler, Risueño & Fernández-Gómez, 1973) and acid phosphatase activity (Sánchez-Pina, Risueño & Rodríguez-García, 1980) in FCs, as all of these are related to transcriptional activity.

On the contrary, the fibrillar component contains RNA (EDTA staining, RNase digestion) that has been recently synthesized (tritiated uridine incorporation), as well as DNA (tritiated thymidine incorporation) that is transcriptionally active (uridine incorporation, lead acetate fixation).

All the above data allow us to confirm unequivocally for plant cells the previous reports made on this subject for animal cells (Goessens & Lepoint, 1979; Fakan & Puvin, 1980; Mirre & Stahl, 1981). Plant cell FCs contain intranucleolar chromatin (Lafontaine & Lord, 1973; Chouinard, 1974; Jordan et al. 1980) that is in a transcriptionally inactive state. The transcriptionally active nucleolar chromatin is included
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Fig. 17. The histograms represent the distribution of the number of silver grains per μm² in the different nuclear compartments after 18 h incorporation of tritiated thymidine. Note that all the nuclear structures containing DNA (fibrillar centres (A), nucleolar fibrillar component (B) and the rest of the nucleus (D)) are significantly labelled, in contrast to the granular component (C), which is known to have no DNA.
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Fig. 18. After a 30 min pulse of incorporation of tritiated uridine the labelling appeared mostly within the nucleolar fibrillar component (f) although some grains appeared within the granular component (g). Fibrillar centres (arrows) are devoid of silver grains, even though some grains can be located at their periphery. The perichromatinic region of the nucleus also shows considerable labelling. × 13,965.

in the fibrillar component, closely bound to its transcription products, as has been demonstrated using spreading techniques (Miller & Beatty, 1969; Franke et al. 1979). Thus, we cannot equate the concepts 'fibrillar centres' and 'nucleolar organizing region' (Jordan & Loening, 1977; Goessens, 1978; Pebusque et al. 1981). In other words, the set of FCs of any interphase nucleolus does not contain the whole NOR, but only the portion of it that is at that moment inactive in transcription. In addition, the high content of protein in FCs suggests that there are some proteins present other than those associated with NOR chromatin structure and transcription.

Consequently, FCs would not be a general component of all nucleoli, since they would not be present when all ribosomal cistrons were transcribing at the same time.

In active nucleoli, determined by their rate of incorporation of tritiated uridine and by a high ratio of granular/fibrillar component (Smetana & Busch, 1974), the so-called homogeneous FCs show denser 8–11 nm fibres similar to those described by Jordan et al. (1980). Our cytochemical tests have revealed that these fibres are digested by DNase and seem to be spread throughout large areas of the nucleolus under osmium ammine staining, which does not contrast nucleolar products other than the DNP. The range of the dimensions of these fibres is the same as that described by Derenzini (1979) for
No. of silver grains/μm² in different cell compartments
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decondensed chromatin fibres. Therefore, we postulate that the inactive chromatin of FCs would be in a decondensed state, similar to that of typical transcriptionally active chromatin, so that by osmium ammine staining the inactive chromatin of FCs would be cytochemically indistinguishable from the active chromatin of the fibrillar component.

This particular conformation of the chromatin in FCs could be due to the high dynamics of such active nucleoli, in which there are ribosomal cistrons continuously initiating transcription and others terminating it. From a structural point of view this would correspond to continuous interconversion between fibrillar centres and fibrillar component. Thus, it would be advantageous for the cell not to start the complicated process of chromatin condensation and decondensation in the particular case of nucleolar chromatin, but to keep the temporarily inactive regions in a decondensed state, ready to be used for transcription. This idea is supported by the observations of other authors such as Franke et al. (1979); after inhibiting nucleolar transcription, the nucleosomal and supranucleosomal structures are not restored immediately, but only after a certain time.

Derenzini, Pession-Brizzi & Novello (1981) have also demonstrated that the shift of the chromatin from the dispersed state to the condensed form is not strictly related to the inhibition of RNA synthesis. On the other hand, in oocytes, contiguous regions of inactive chromatin have different structural conformations, as in the case of the chromocentre and the fibrillar centre (Mirre & Stahl, 1976, 1978).

FCs in plant cell nucleoli undergo structural changes, depending on the nucleolar activity. In cells with low activity, such as dormant cells or cells grown under standard conditions with a low granular/fibrillar components ratio in the nucleolus, as well as the components of FCs described above there are small cores, demonstrated unequivocally as being made up of condensed chromatin. This structural change (homogeneous to heterogeneous and vice versa) in FCs has not been described before in animal cells. The heterogeneous structure of FCs is present also in late prophase and telophase of mitosis (Moreno Díaz de la Espina, Risueño, Fernández-Gómez & Tandler, 1976) and this structure is the same as that of the NOR in meiotic metaphase I (Rodríguez-García, 1977; Risueño et al. 1980). The presence of condensed chromatin cores in FCs could be due to the greater amount of ribosomal DNA in plants than in animals (Ingle & Sinclair, 1972). This would mean that an inactive or slightly active plant NOR would have only part of the ribosomal cistrons ready for transcription (decondensed), while the rest would be in the usual condensed state of inactive chromatin.

Frequently, large clear areas are seen in slightly active nucleoli containing two or more heterogeneous FCs. At present we cannot explain the nature of these spaces although, unequivocally, they are not homologous to the nucleolar vacuoles, previously characterized by us in highly active nucleoli (Moreno Díaz de la Espina et al. 1980).

Fig. 19. The histograms represent the distribution of the number of silver grains/μm² in the different cellular compartments after a 30-min pulse with tritiated uridine. As shown in A, the fibrillar centres have no significant labelling, while the fibrillar component is the most labelled compartment. B. Nucleolar fibrillar component (excluding fibrillar centres); C, nucleolar granular component; D, rest of nucleus; E, cytoplasm.
They could be ascribed tentatively to the morphological counterpart of the nucleolar skeleton (Franke et al. 1981).

In conclusion, the nucleolus has a basic structural component, the fibrillar component, made up of active ribosomal cistrons bound to the earliest product of their transcription. However, not all ribosomal cistrons are usually transcribing at the same time; when they are not transcribing, they could form fibrillar centres with decondensed chromatin; when the time of inactivation is prolonged, a portion of nucleolar chromatin may condense fully. Both active and inactive chromatin are obviously continuous. Therefore, FCs and the fibrillar component would constitute a functional unit; this idea is in accord with all experimental data reported to date, and has already been suggested by one of us (Moreno Díaz de la Espina, 1976). That functional unit is surrounded by the final product of its activity: the granular component.

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