Chromosoma (2002) 110:559–569
DOI 10.1007/s00412-001-0178-9

ORIGINAL ARTICLE

Ricardo Acevedo · Rafael Samaniego
Susana Moreno Díaz de la Espina

Coiled bodies in nuclei from plant cells evolving from dormancy to proliferation

Received: 21 September 2000 / Revised: 21 June 2001 / Accepted: 21 August 2001 / Published online: 18 December 2001

Abstract To characterise the coiled bodies in meristematic nuclei of *Saccharum officinarum*, immunofluorescence labelling with antibodies against components of the splicing (U2B″ and Sm core protein B) and pre-rRNA processing (fibrillarin) complexes was used in cells from the dormant root primordia and from roots at different times after activation to the steady state of proliferation. The number, size and distribution of coiled bodies varied in the meristematic tissue depending on cell activity. While G0 cells in the dry primordia and proliferating cells showed a similar number of coiled bodies attached to their nucleoli, the number of nucleoplasmic coiled bodies greatly increased after the primordia were stimulated to proliferate. Their number remained steady from the time the meristematic population reached the steady state of proliferation, as estimated by flow cytometry. Fractionation studies demonstrated that coiled bodies are a part of the underlying nuclear matrix. Comparison of immunocytochemical and cytochemical data from confocal and electron microscopical studies demonstrated that the nucleolar and nucleoplasmic coiled bodies detected by confocal microscopy shared many features, suggesting that they form a family of closely related structures.

Introduction

Coiled bodies are a class of conserved nuclear bodies, first described in mammalian cells (Monneron and Bernhard 1965). Coiled bodies contain splicing ribonucleoproteins (RNPs), the phosphoprotein p80 coilin and nucleolar antigens such as fibrillarin and snoRNPs (Sleeman and Lamond 1999). Coiled bodies are highly dynamic structures. They vary in size, number, nuclear localisation (associated with the nucleolus and free in the nucleoplasm) and composition, in different cell types and under different growth conditions (Barlow 1983a; Boudonck et al. 1998). Coiled bodies have been proposed to be the sites of assembly of splicing factors (Sleeman and Lamond 1999), to play a role in histone mRNA processing (Lamond and Earnshaw 1998) and to be involved in the processing, or transport, of snoRNA precursors (Shaw et al. 1998). But the function of coiled bodies is still under debate. Recently it has been proposed that the term coiled bodies may include a family of related structures, some of them containing different proteins and performing different functions (Sleeman and Lamond 1999).

Although it was not until the 1980s that coiled bodies were characterised in plants (Moreno Díaz de la Espina et al. 1982a, b; Martín et al. 1992), in the last decade there has been a great advance in knowledge about plant coiled bodies (Beven et al. 1996; Simpson and Filipowicz 1996; Olmedilla et al. 1997; Boudonck et al. 1998, 1999; Jennane et al. 1999). At present we know that plant coiled bodies are highly dynamic structures, with a composition similar to that of animal coiled bodies, whose numbers appear to be cell and developmentally regulated (Boudonck et al. 1998) and that move and coalesce “in vivo” (Boudonck et al. 1999).

In addition to coiled bodies, two other types of plant nuclear bodies have been extensively analysed: nucleolus-associated bodies (NABs) or karyosomes (Barlow 1983a, b) and argyrophilic intranuclear bodies or dense bodies (Barlow 1981, 1983b). Nucleolus-associated bodies are typical structures of non-reticulate nuclei (Williams et al. 1983; Moreno Díaz de la Espina et al. 1992) that contain splicing factors (Chamberland and Lafontaine 1993). Their numbers apparently correlate with the DNA content and chromatin organisation of the

Edited by: M.F. Trendelenburg

R. Acevedo · R. Samaniego · S. Moreno Díaz de la Espina (✉)
Nuclear Matrix Laboratory, Department of Plant Biology,
Centro de Investigaciones Biológicas, CSIC, Velázquez 144,
28006 Madrid, Spain
e-mail: smoreno@cib.csic.es

Present address:
R. Acevedo, Instituto de Investigaciones de la Caña de Azúcar,
Avda. Van Troi 17203, Rancho Boyeros, Ciudad de La Habana,
Cuba

species and they are more abundant in slowly dividing or non-dividing cells (Barlow 1983a), while dense bodies are nucleoplasmic and were previously related to the coiled bodies (Moreno Díaz de la Espina et al. 1982a, b).

In this study we characterised the coiled bodies of sugarcane (*Saccharum officinarum*), a monocot with a 2C DNA content of 6 pg and a semireticulate chromatin organisation (Acevedo et al. 1998, 2001), by confocal microscopy using antibodies against splicing factors and the nucleolar processing protein fibrillarin, all of of which are considered to be constitutive components of coiled bodies. To analyse the correlation between coiled bodies and nuclear activity, we observed their distribution during the process of activation of the dormant root primordia after stimulation to proliferate. Electron microscopy (EM) allowed us to make a comparative ultrastructural, cytochemical and immunocytochemical analysis of coiled bodies, NABs and DBs. Our results demonstrate that plant coiled bodies are components of the underlying nuclear matrix and also that the number of nucleolar coiled bodies is independent of cell activity, in contrast to what happens with nucleoplasmic coiled bodies.

Materials and methods

Materials

Sugarcane *(S. officinarum*cv. Cristalina) stalks supplied by I.N.I.C.A. (Instituto Nacional de Investigaciones de la Caña de Azúcar) were used. Stem sugarcane cuttings containing radicle bands were cultured on wet filter paper and cotton, in a Refritherm-5 (Struers) incubator at 30°C with 85% relative humidity, under dark conditions. Samples of quiescent primordia were excised from the radicle bands before soaking. Roots of 2, 5, 7 and 15 mm length were also taken.

Antibodies

The primary antibodies employed were: 4G3 (1:5), a mouse monoclonal (Organon Teknica) recognising the spliceosomal protein U2B″ in plants (Boudonck et al. 1998); Y12 (undiluted) a mouse monoclonal recognising Sm protein B in plants (Vázquez-Nin et al. 1992), and S4 (1:200) a human autoimmune serum against fibrillarin recognising the plant protein (Moreno Díaz de la Espina 1995). Antimouse and antihuman antibodies conjugated to fluorescein isothiocyanate (1:100) (Sigma) or Cy2 (1:200) as well as to 10 nm gold particles were used as secondary antibodies for confocal and EM immunolabelling, respectively.

Confocal microscopy

Quiescent and proliferative root meristems were fixed in 4% paraformaldehyde in PEM buffer (50 mM PIPES, 5 mM $MgSO_4$, 5 mM EGTA) pH 7.0, for 1 h at room temperature and washed three times for 15 min in the same buffer. After digestion with 2% cellulase R-10 and 20% liquid pectinase (from *Aspergillus niger*, Sigma Chemical Co., St. Louis, Mo.), for 50 min at 37°C in MAN buffer [0.4 M D-mannitol, 5 mM EGTA, 1 µg/ml Triton X-100, 0.3 mM phenylmethylsulphonyl fluoride (PMSF)] pH 7.0, and washing three times for 15 min in PEM buffer, root segments were squashed on 0.1% poly-L-lysine-coated slides and solid residues were discarded. Cellular preparations were air-dried and kept at –20°C until use. After quenching aldehyde groups with a freshly prepared 1 mg/ml $NaBH_4$ solution in PBS, pH 8.0, three times for 15 min, the slides were blocked and incubated with the primary and secondary antibodies as previously described (Yu and Moreno Díaz de la Espina 1999). Experiments omitting the incubation with the primary antibody were performed as negative controls. After labelling, preparations were stained with 1 µg/ml 4′,6-diamidino-2-phenylindole (DAPI) or 10 µg/ml propidium iodide before mounting.

Preparations were examined with an MCR 1024 BioRad confocal system (BioRad, Richmond, Calif.) mounted on a Zeiss Axiovert 135 microscope (Zeiss, Oberkochen, Germany) equipped with a ×63, 1.4 NA planapochromat immersion objective. A 25 mW multiline argon laser producing two major lines at 488 and 514 nm was used. For each nucleus, a series of optical sections was obtained at a pitch of 0.3–0.5 µm. An average of two images (Kalman filter) recorded at a slow speed for 1024×1024 pixels was enough to obtain images with a good signal-to-noise ratio and avoid bleaching. The scanning zoom was ×2. Images were processed with the Lasersharp software package (BioRad, UK).

Quantitative evaluations

The numbers of coiled bodies per nucleus were counted in the projections of the confocal sections corresponding to each whole nucleus, after immunostaining with 4G3 or Y12 antibodies. Thirty nuclei from cells taken from three different preparations of the dormant primordia, reactivating meristems of 2 mm roots and steady state meristems of 15 mm roots were used in each case. The data were statistically analysed by calculating the differences between percentages in the different populations (Sokal and Rohlf 1969) and by the Kruskal-Wallis non-parametric method with a Dunn test (Dickinson 1985).

Electron microscopy

For conventional EM, roots were fixed in 2% glutaraldehyde in 0.025 M cacodylate buffer, pH 7.2 for 1 h, post-fixed in 1% osmium tetroxide in the same buffer and dehydrated in an ethanol series to absolute ethanol. After 1 h infiltrations in ethanol:resin mixtures (2:1, 1:2) and in pure resin for 3 days at 4°C, samples were embedded in LRW resin in closed gelatine capsules and cured at 60°C for 20 h.

Cytochemical staining

EDTA regressive staining (Bernhard 1969) was performed in sections from samples fixed only in 3% glutaraldehyde and processed as previously described (Moreno Díaz de la Espina et al. 1992). For bismuth oxynitrate staining, roots were fixed in 6% glutaraldehyde in 0.025 M cacodylate buffer and processed as described. For staining, sections were floated on a 2% bismuth oxynitrate solution in 0.1 M triethanolamine buffer, washed in distilled water and air dried (Moreno Díaz de la Espina et al. 1992, 1993). Silver staining was performed on sections as previously described (Moreno Díaz de la Espina et al. 1992). Briefly, roots were fixed in 3% glutaraldehyde as for conventional preparations, post-fixed in Carnoy's fixative (3:1 ethanol:acetic acid) in 0.5 M NH_4Cl in cacodylate buffer for 1 h, dehydrated in an ethanol series and embedded in LRW resin as described above. Sections were mounted on 300 mesh gold grids and floated on a silver solution containing 1 vol of 2% gelatine in 1% formic acid, and 2 vol of 50% silver nitrate solution, for 10 min at 37°C. After rinsing in distilled water, grids were floated for 10 min on a 5% sodium thiosulphate solution and washed again in distilled water. Sections were observed either unstained or stained in a 5% aqueous uranyl acetate solution.

Immunogold labelling

Immunogold labelling was performed on ultrathin sections mounted on nickel grids using the same primary antibodies and dilutions as described above for immunofluorescence. After quenching in a fresh 1 mg/ml solution of $NaBH_4$, grids were incubated on the blocking solution (PBS, 0.05% Tween 20, 2% BSA) for 30 min at room temperature in a humid chamber, and then with the primary antibodies diluted in the blocking solution overnight at 4°C. After washing in PBS containing 0.05% Tween, incubation with 10 nm gold-conjugated anti-mouse antibodies in blocking solution was performed in the dark at room temperature for 30 min. Controls were performed by omitting incubation with the primary antibody.

Nuclear fractionation

Nuclear matrices were prepared from isolated nuclei of *Allium cepa* meristematic root cells as previously described (Yu and Moreno Díaz de la Espina 1999). Briefly, isolated nuclei were incubated in cytoskeleton buffer [CSK, 10 mM PIPES, pH 6.8, 100 mM KCl, 300 mM sucrose, 3 mM $MgCl_2$, 1 mM EGTA, 1.2 mM PMSF, 20 mM dithiothreitol (DTT)] containing 0.5% Triton X-100. After 5 min soluble nuclear proteins were removed by centrifugation at 690 *g* for 10 min. The pellet containing the insoluble nuclear proteins was then digested with 200 µg/ml RNase-free DNase I in digestion buffer (DB, 10 mM PIPES, pH 6.8, 50 mM KCl, 50 mM NaCl, 300 mM sucrose, 3 mM $MgCl_2$, 1 mM EGTA, 1.2 mM PMSF, 0.5% Triton X-100, 20 mM DTT) for 30 min at room temperature. Then 1 M ammonium sulphate was added to a final concentration of 0.25 M. At this step DNA and its associated proteins were removed, leaving the nuclear matrix containing nuclear RNP complexes. The nuclear matrix was further extracted by adding 4 M NaCl to a final concentration of 2 M. This extraction releases the outer nuclear matrix proteins and reveals the nuclear matrix. Except where mentioned, all steps were done at 4°C. After extraction, nuclear matrix pellets were fixed and processed for either confocal microscopy or EM as previously described (Samaniego et al. 2001).

Results

Sugarcane coiled bodies contain the spliceosomal components U2B″ and the Sm core protein B as well as fibrillarin

As we were unable to detect p-80 coilin in plants in spite of several trials with different antibodies, we used the anti-U2B″ antibody 4G3, which has proved to be a good marker for coiled bodies in plants (Boudonck et al. 1998, 1999), to analyse these structures by confocal microscopy.

Labelling for U2B″ was localised to prominent spherical structures corresponding to coiled bodies, as identified by transmission microscopical images of the same sections, which presented variable numbers, sizes and localisations in the nucleus (Fig. 1a–d). They appeared either free in the nucleoplasm, attached to the nucleolar periphery or deep inside the nucleolar body. Diffuse nucleoplasmic staining was also evident that was below the resolution of the confocal microscope. This nucleoplasmic staining is very similar to that revealed by anti-PANA antibody, which recognises a marker protein of interchromatin granules in onion cells (Cui and Moreno Díaz de la Espina, in preparation). Labelling was excluded from the nucleolus and cytoplasm (Fig. 1a–c, a'–c').

Labelling with the Y12 antibody, which detects a different spliceosomal protein (Sm core protein B) in plants (Vázquez-Nin et al. 1992), produced a similar staining pattern to that of 4G3, that is, very intense in coiled bodies and diffuse in the nucleoplasm (data not shown).

Incubation with serum S4, which recognises the protein fibrillarin involved in the processing of pre-ribosomal precursors in plant cells (Moreno Díaz de la Espina 1995), stained both the nucleolus and coiled bodies, revealing that the latter also contained fibrillarin. Although it was very difficult to discriminate between coiled bodies embedded in the nucleolus after fibrillarin staining, the staining was very strong in the coiled bodies budding from the nucleolus. On the other hand, only a fraction of the nucleoplasmic coiled bodies showed any reaction. The nucleoplasm never appeared stained, even in those cases that showed faint cytoplasmic staining (Fig. 1e, e'). In the last experiments the strong nucleolar staining made the discrimination of the coiled bodies embedded in or closely attached to the nucleolus very difficult. For this reason we used only the two other antibodies for the quantitative analysis.

Variation of coiled body numbers during reactivation of the dormant root primordia

Due to the lack of a specific marker for plant coiled bodies, these structures were examined during the process of reactivation of the dormant root primordia up to the steady state of the proliferating meristem by using the two antibodies against the spliceosomal components (4G3 and Y12) and confocal microscopy. In this case, confusion of coiled bodies with interchromatin granule speckles is not possible for two reasons: (1) interchromatin granules never form speckles in plant cells similar to those in animal cells (see Medina et al. 1989; Moreno Díaz de la Espina et al. 1993). (2) The anti-PANA antibody, a specific marker of interchromatin granules, which recognises a double band of M_r 100,000 and 110,000 in onion cells, never reacted with coiled bodies. Instead it revealed delicate staining in the nucleoplasm, excluding the nucleolar domains and sometimes small dark areas by immunofluorescence, and clearly marked interchromatin granules by immunogold labelling (Cui and Moreno Díaz de la Espina, in preparation).

In the dormant primordia before soaking over 90% of the cells are accumulated in G0 with a 2C DNA content, as demonstrated by flow cytometry (Acevedo et al. 2001). Their nuclei displayed between 1 and 10 large coiled bodies deeply attached to the nucleolus with a mean value of 3.51±0.29. Nucleoplasmic coiled bodies were very rare in these cells (Figs. 1a, a′, 2). After the primordia had been stimulated to proliferate and the mitotic index had reached a value of 4 in the meristematic population, i.e. in the 2 mm root (Acevedo et al. submitted), the number of coiled bodies per cell increased to a maximum of 17 (mean value 7.20±0.4). The increase in the number of coiled bodies was accompanied by a de-

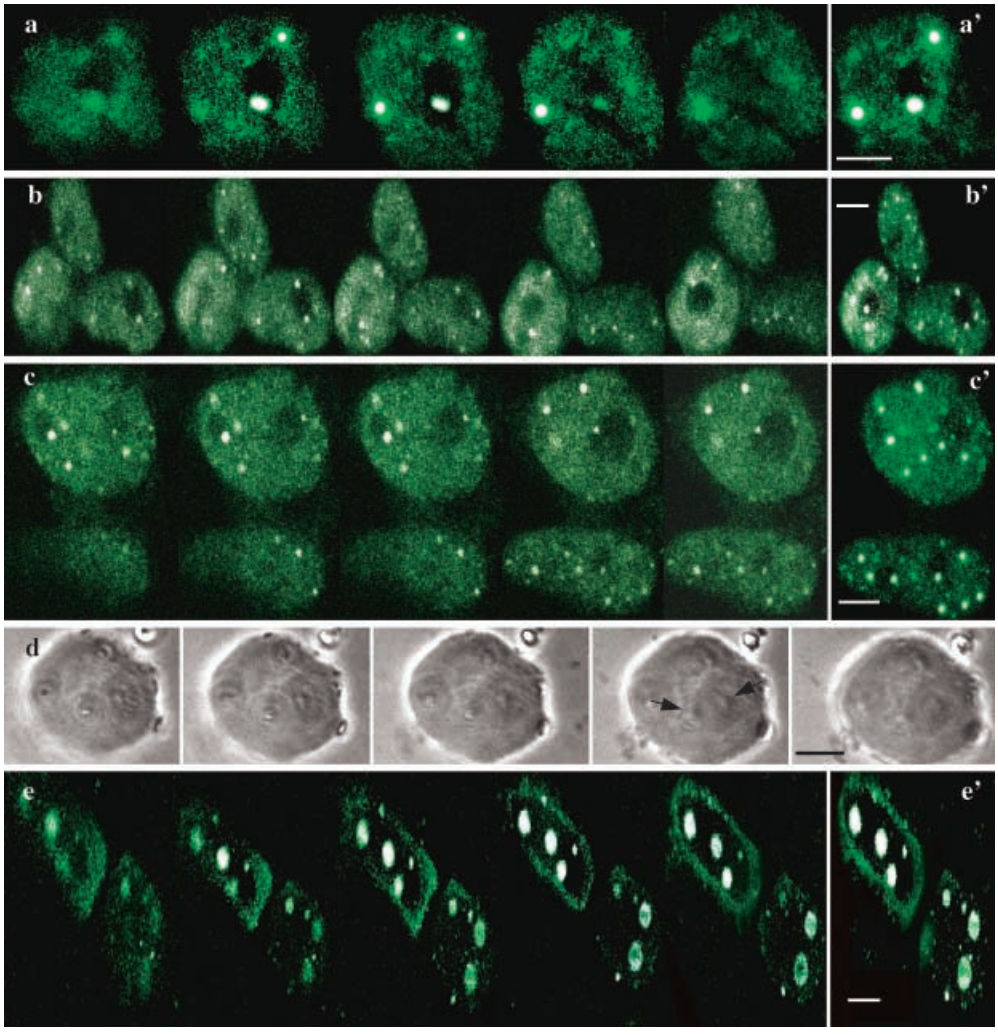


Fig. 1 Labelling of sugarcane meristem root cells in different states of activity with 4G3 (**a, b, c**), and S4 (**e**) antibodies. The bright spots, sometimes associated with the nucleolus, are coiled bodies. Diffuse staining of the nucleoplasm is also observed. In each case several confocal images through a cell and the corresponding projection (on the *right*) are shown. **d** A series of transmission images corresponding to mid-sections of the top nuclei shown in **c**. Coiled bodies are shown within them (*arrows*) that differ in contrast from those of the nucleolus and nucleoplasm. Dormant (G0) cells of the primordia display a few large coiled bodies clearly associated with or even embedded in the nucleolus (**a, a'**). Reactivating meristem cells in the 2 mm root have more but smaller coiled bodies, many of them lying in the nucleoplasm (**b, b'**). The steady-state proliferating cells of the 15 mm root present a distribution of coiled bodies similar to that in the 2 mm root (**c, c'**). Antifibrillarin staining of proliferating cells in the 15 mm root (**e, e'**). The nucleoli are strongly stained, making the detection of nucleolar coiled bodies difficult and these are evident only when detached from the nucleolus. Nucleoplasmic coiled bodies stand out clearly from the negative nucleoplasm, but on average are less abundant than after 4G3 or Y12 staining. *Bar* represents 5 µm

crease in their size. Thus in the 2 mm proliferating meristem cells, coiled bodies were smaller and appeared almost equally distributed between the nucleolus and nucleoplasm (Figs. 1b, b′, 2). The numbers and nuclear distribution of coiled bodies were maintained when the steady state of proliferation was finally achieved (mean value 6.44±0.4) (Figs. 1c, c′, 2).

Statistical analysis of the recorded numbers of coiled bodies in the dormant, reactivating and steady state proliferating cells of the root meristem revealed that the number of nucleoplasmic coiled bodies clearly correlates with cell activity, as there were significant differences between the dormant and active cells but not between the two proliferating populations (Fig. 2). On the contrary, the number of nucleolus-associated coiled bodies was dependent upon neither nucleolar nor cell activity, as there were no significant differences ($P<0.05$) in their values among the dormant, reactivating and steady state dividing cells (Fig. 2), although the distribution of nucleolus-associated coiled bodies per nucleolus varied in the three cell populations due to nucleolar fusion (Fig. 3).

Ultrastructural characterisation of the two types of coiled bodies

Due to the lack of reactivity of 4G3 antibody on LRW sections, we used immunogold labelling with the Y12 antibody on sections of cells of the primordia and the two proliferating meristems to identify in the EM the nu-

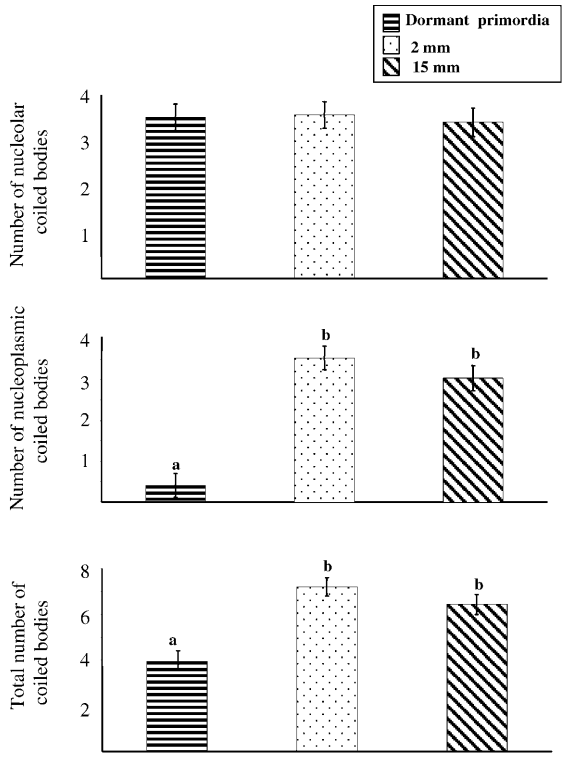


Fig. 2 Histograms showing the frequencies of nucleolar, nucleoplasmic and total coiled bodies present in meristematic nuclei of roots of different lengths, i.e. presenting different levels of proliferation. Different *letters* on top of the *bars* represent their statistical differences at $P<0.05$

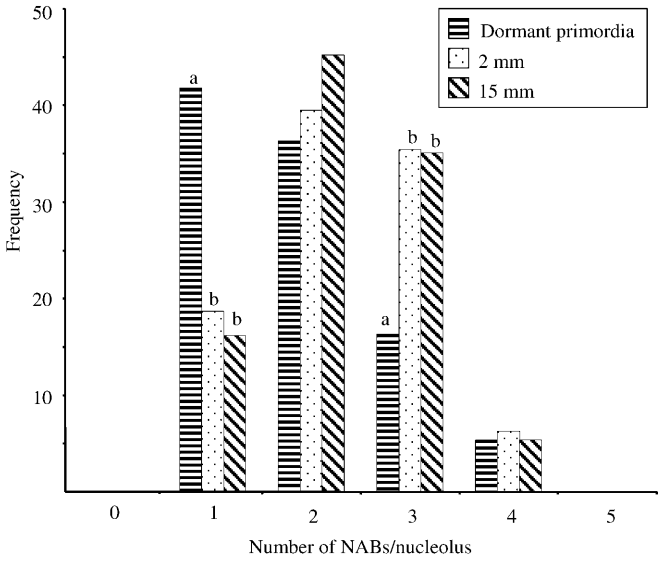


Fig. 3 Comparison of the frequency of nucleolar coiled bodies (NABs) per nucleolus in roots of three different lengths. Most nucleoli have between 1 and 3 associated coiled bodies and up to 4 were observed in a small proportion of cells. Taking into account the fact that the total number of nucleolus-associated coiled bodies remains a constant independently of cellular activity (see Fig. 2), and also that in this species all the nucleolar organising loci are active in organising a nucleolus (Acevedo et al. submitted), the significant differences observed probably correspond to nucleolar fusions produced during cell activation as quantified by silver staining (Acevedo et al. submitted). Different *letters* on the tops of *bars* represent their statistical differences at $P<0.05$

clear bodies corresponding to the coiled bodies previously analysed by confocal microscopy.

Two distinct types of nuclear bodies were revealed by Y12 immunogold staining (Figs. 4b, 5b, 6b). The first consisted of more or less spherical nuclear bodies with a diameter of 0.76±0.13 µm in dormant cells and 0.35±0.1 µm in proliferative cells, which were attached to the nucleolus, whose 0.08±0.02 µm fibres displayed a coiled-coil organisation (Figs. 4a–c, 5a, b, 6e). They looked very similar to the previously described NABs and shared some cytochemical characteristics with them.

In dormant G0 cells from the primordia, the nucleolar coiled bodies/NABs appeared deeply immersed in the rudimentary inactive nucleolus. They were very large in relation to it, had a lower contrast than the inactive dense fibrillar component and were in close contact with the intranucleolar chromatin, which appeared bleached after EDTA staining, but they were clearly different from it (Fig. 4a–c).

After reactivation, the nucleolus of the proliferating cells showed a looser organisation with three distinct nucleolar components: a peripheral granular component, a dense fibrillar component and numerous small fibrillar centres (Figs. 5a, 6a). At these stages, the nucleolar coiled bodies/NABs were smaller in relation to the size of the nucleolus, and they appeared at the nucleolar periphery rather than immersed in the nucleolar body, often loosely bound to the nucleolus (Fig. 5b, 6c, e). Nevertheless, they presented the same cytochemical characteristics as those in the dormant nuclei (Figs. 4, 5, 6).

Correlating with cell reactivation, nucleoplasmic coiled bodies containing spliceosomal proteins were observed free in the nucleoplasm, in agreement with the data recorded by confocal microscopy (Fig. 6a, b). These were roundish bodies, 0.31±0.13 µm in diameter, and presented many similarities to the plant nuclear bodies previously described as nucleoplasmic coiled bodies (Moreno Díaz de la Espina et al. 1982a) and dense bodies (Barlow 1983a).

The immunocytochemical and cytochemical analysis proved that both types of coiled bodies shared the same characteristics (Table 1). Thus both contained spliceosomal proteins (Figs. 4b, 5b, 6b) and showed strong contrast after EDTA staining, like the nucleolus and the rest of the nuclear RNPs, but in contrast to the bleached chromatin (Fig. 4c, 6a). Besides, both types of nuclear bodies were argyrophilic, with a density of silver deposits similar to that of the nucleolar granular component, as reported for NABs and DBs (Fig. 6c) (Moreno Díaz de la Espina et al. 1992) and they contained highly phosphorylated proteins (Fig. 6d, e). All these data suggested that both NABs and DBs could be the counterpart of the nucleolar and nucleoplasmic coiled bodies detected by confocal microscopy.

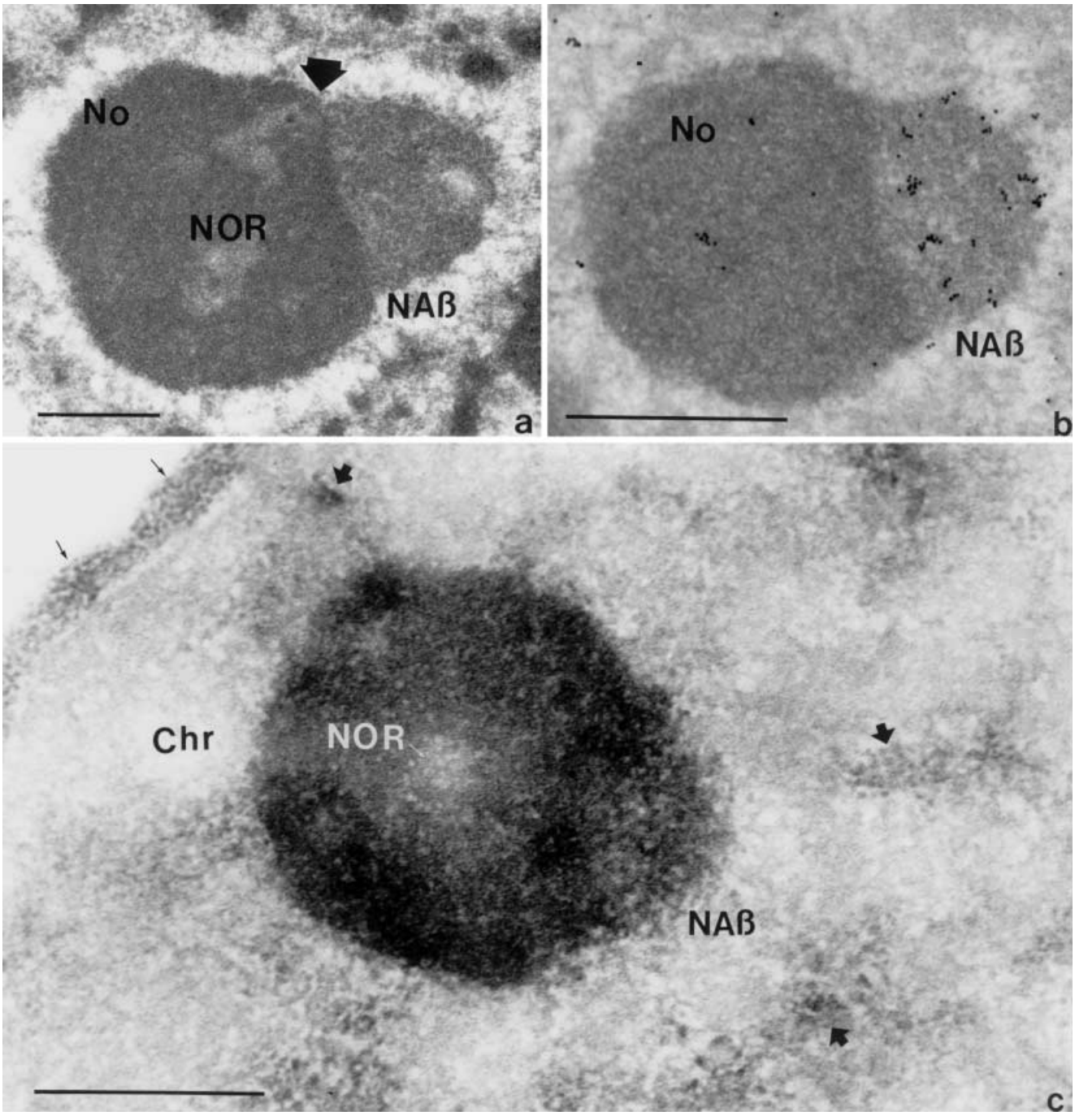


Fig. 4a–c Characterisation by electron microscopy of the nucleolar coiled bodies (NABs) in dormant cells. **a** Conventional staining. A large nucleolar coiled body, similar to that shown in Fig. 1a, made up by dense coiled threads protrudes from the dormant fibrillar nucleolus (*No*). Inside the nucleolar body, a section through the pale nucleolar organiser (*NOR*) in close contact with the NAB (*arrow*) is shown. **b** Y12 immunolabelling of the NAB. **c** EDTA staining for ribonucleoproteins (RNPs) produces strong staining of the NAB, the inactive nucleolus and the packed ribosomes in the cytoplasm (*small arrows*). The big masses of condensed chromatin are bleached by the procedure. Note the rudimentary organisation of RNP structures in the interchromatin and perichromatin nuclear regions (*arrows*). *Bars* represent 0.5 µm

Coiled bodies are components of the plant nuclear matrix

To investigate the association of coiled bodies with the nuclear matrix, we used the same tissue from another monocot, *A. cepa*, a species in which the nuclear matrix is well characterised (Moreno Díaz de la Espina 1995; Yu and Moreno Díaz de la Espina 1999). Confocal microscopy of nuclear matrices after Y12 staining demonstrated that both nucleolar and nucleoplasmic coiled bodies were associated with the nuclear matrix (Fig. 7). Electron microscopy of nuclear matrix sections confirmed that coiled bodies with a similar structure to those in intact cells were associated with the filaments of the internal matrix (Fig. 6f).

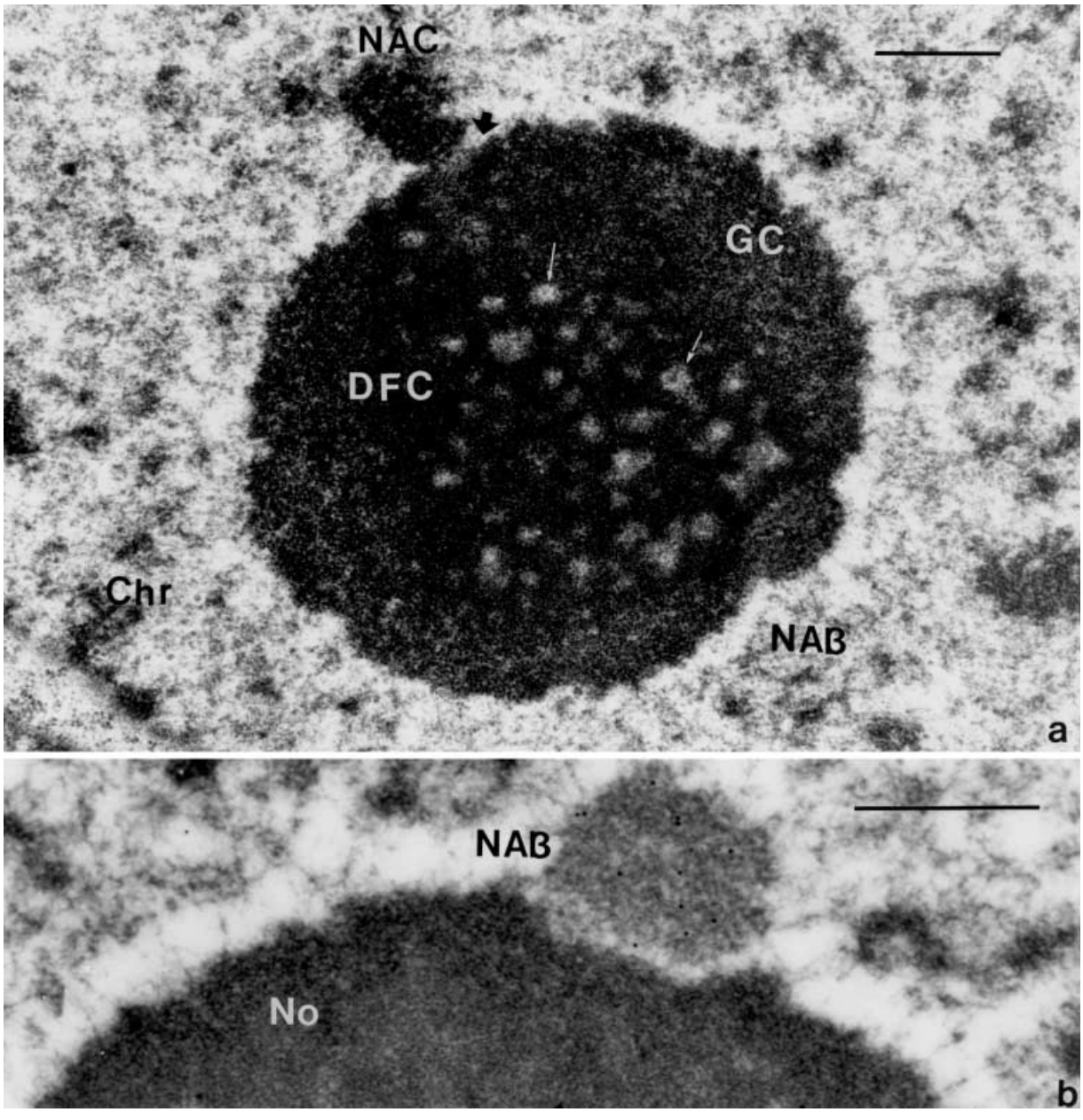
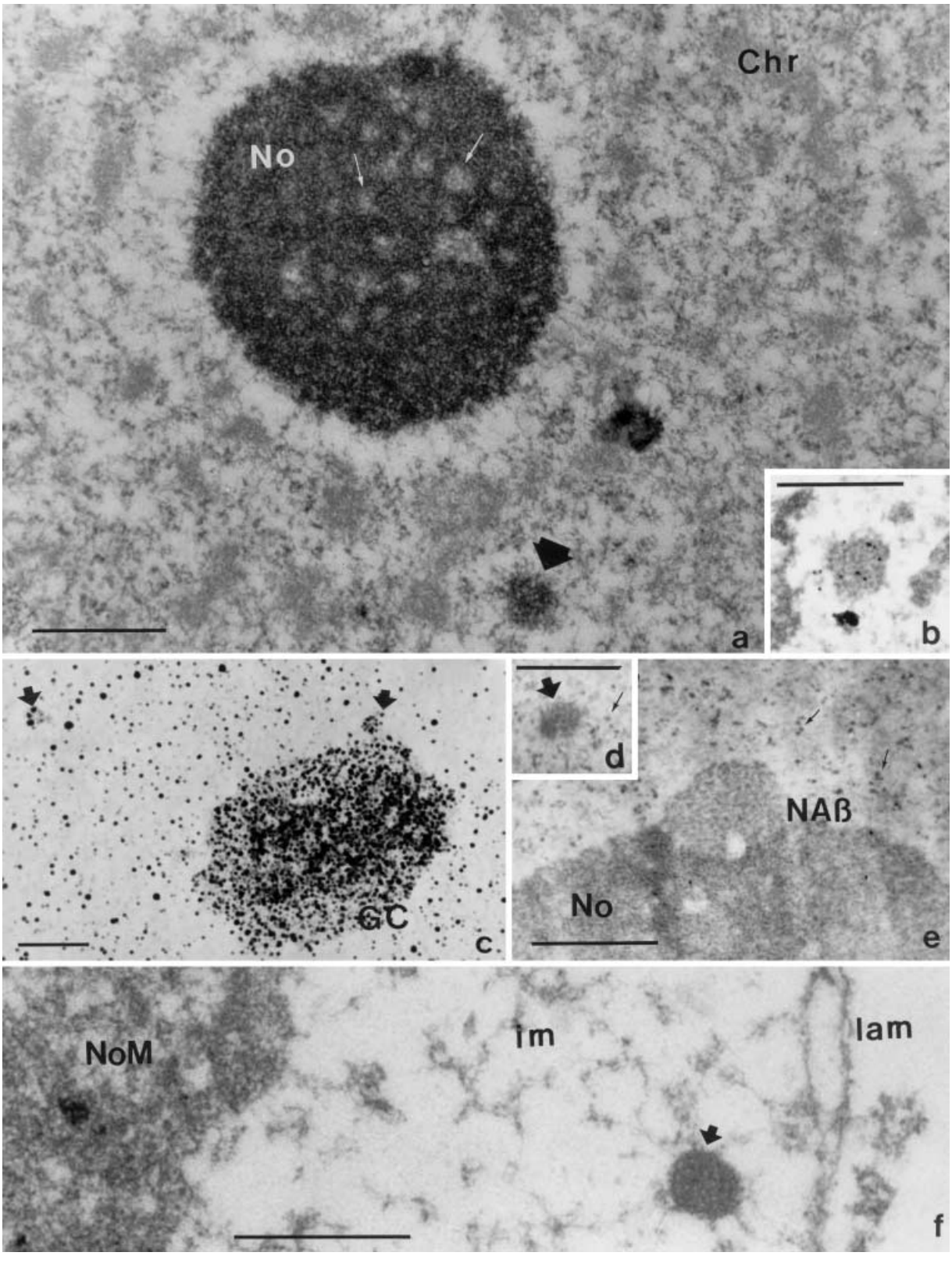


Fig. 5a, b High magnification images of reactivating meristem cells from the 2 mm root, similar to those shown in Fig. 1b. **a** Conventional electron microscopical preparation showing a typical highly active nucleolus with small and numerous fibrillar centres (*arrows*) immersed in the dense fibrillar component (*DFC*), both surrounded by abundant granular component (*GC*). Note the different ultrastructural organisation of the nucleolus-associated chromatin (*NAC*), which contains ribosomal genes (Acevedo et al. submitted) and makes contact with the fibrillar centres, and the nucleolar coiled body (*NAB*), which is budding from the nucleolus. The organisation of the semireticulate nucleus is typical of high activity, with decondensed chromatin masses (*chr*) and numerous fibrils and granules in the interchromatin regions. **b** Y12 immunogold labelling of a nucleolar coiled body from a similar stage of activation. (*No* nucleolus). *Bars* represent 0.5 µm

Table 1 Survey of the main cytochemical and immunocytochemical characteristics of both the nucleolar and nucleoplasmic coiled bodies. (Fib, fibrillarin; G0, presence in the dormant G0 cells; P, presence in proliferating cells)

	EDTA	Ag	Bis^{+3}	Fib	Y12	U_2B''	G0	P
Nucleolar coiled body (NAB)	+	+	+	+	+	+	+	+
Nucleoplasmic coiled body	+	+	++	+/–	+	+	+/–	+


Chr
No
a
b
GC
c
d
NAß
No
e
NoM
Im
lam
f

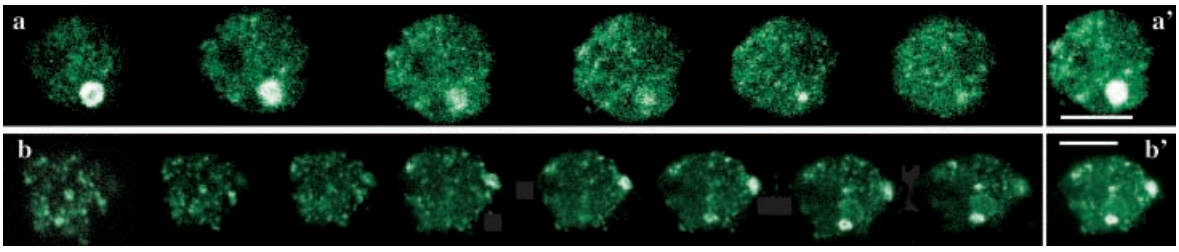


Fig. 7a, b Labelling of the nuclear matrix from onion proliferating cells with Y12. Several confocal images through the matrix and the corresponding projections (on the *right*) are shown. A large nucleolar coiled body connected to one of the nucleoli (**a**) and a few small nucleoplasmic coiled bodies (**b**) are shown. *Bar* represents 5 µm

Discussion

Nucleolar and nucleoplasmic coiled bodies are similar structures

The structures reported here as coiled bodies in the confocal microscope after immunostaining against components of the splicing and rRNA processing complexes share many features with the previously described plant coiled bodies and NABs (Barlow 1981; Moreno Díaz de la Espina et al. 1982a, b; Barlow 1983a, b; Williams et al. 1983; Moreno Díaz de la Espina et al. 1992; Chamberland and Lafontaine 1993; Jennane et al. 1999). Both nucleoplasmic and nucleolar coiled bodies display the same ultrastructural organisation consisting of the typical coiled threads (Monneron and Bernhard 1965; Moreno Díaz de la Espina et al. 1982a), which is different from that of G1 prenucleolar bodies containing fibrillarin (Medina et al. 1995) and also from the nuclear bodies corresponding to replication factories in plants (Samaniego and Moreno Díaz de la Espina 2000; Samaniego et al. 2001). Nucleolar coiled bodies are also ultrastructurally different from the nucleolus-associated chromatin masses that contain the ribosomal cistrons (Acevedo et al. submitted). Their composition is also similar. Both nucleolar (NABs) and nucleoplasmic coiled bodies are devoid of DNA (Moreno Díaz de la Espina et al. 1982a; Martín et al. 1992; Jennane et al. 1999). We demonstrate here that both contain RNPs, argyrophilic and highly phosphorylated proteins, splicing factors and fibrillarin, though displaying an intraclass heterogeneity (Alliegro and Alliegro 1998). All the aforementioned data and also the evidence that nucleolar and nucleoplasmic coiled bodies move and coalesce (Boudonck et al. 1999) suggest that they correspond to closely related structures.

◀ **Fig. 6a–f** Cytochemical characterisation of coiled bodies from actively proliferating cells similar to those shown in Fig. 5a. **a** EDTA preferential staining for ribonucleoproteins. The nucleoplasmic coiled body (*arrow*) is deeply contrasted as are the nucleolus (*No*) and the abundant ribonucleoprotein (*RNP*) fibrils and granules in the interchromatin regions. The nucleolar fibrillar centres (*small arrows*) and the condensed chromatin masses (*chr*) appear bleached by the staining. **b** Y12 immunogoldstaining of a nucleoplasmic coiled body. **c** Silver impregnation. Both the nucleolar and nucleoplasmic coiled bodies (*arrows*) appear covered by silver deposits, with a similar density to that of the nucleolar granular component (*GC*). **d, e** Bismuth oxynitrate staining. The interchromatin granules (*small arrows*) are preferentially contrasted while the chromatin is not. The nucleolus and coiled bodies show intermediate staining. Note that the nucleoplasmic coiled body (**d**) is more strongly stained than the nucleolar coiled body. **f** Portion of a nuclear matrix from an actively proliferating onion cell extracted as described in Materials and methods, displaying a coiled body (*arrow*) connected to the internal matrix (*im*) elements. (*lam* lamina, *NoM* nucleolar matrix) *Bars* represent 0.5 µm

Variation of coiled body numbers during activation differs for the two types of coiled bodies

Coiled body numbers have been reported to depend on a series of factors such as cell type and activity, cell cycle position, etc., in plant cells (Boudonck et al. 1998, 1999). Our analysis using the same cell system from a dormant condition through reactivation to the steady state of proliferation allowed us to fix many of these variables and follow the evolution of coiled bodies during the process of cell reactivation from G0 to a steady state interphase cell cycle (G1, S and G2) (Acevedo et al. submitted). Although there is an overall increase in coiled body number correlating with cell activation, as reported in other plant systems (Boudonck et al. 1998, 1999), our data demonstrate for the first time different behaviour for the two types of coiled bodies. Nucleoplasmic coiled bodies, which are very rare in dormant cells from primordia, account for the general increase in coiled bodies during activation, although their numbers do not vary significantly between the stabilised and non-stabilised proliferating populations. In contrast, the number of nucleolar coiled bodies in plant cells appears to depend rather on nuclear organisation and/or genetic characteristics than on cell activity (Barlow 1981, 1983a; Lafontaine et al. 1991; Lafontaine and Chamberland 1995; Jennane et al. 1999).

The number of coiled bodies is inversely correlated with their size. Nucleolar coiled bodies of G0 dormant cells appear as large recruitment sites for spliceosomal components when the nucleolus and the splicing machinery are not active, as inferred from the rudimentary organisation of the interchromatin nuclear regions and nucleoli in these cells after EDTA staining. This is in agreement with the results reported in meiosis (Olmedilla et al. 1997) and with the redistribution of coiled bodies af-

ter inhibition of transcription or Ser/Thr protein phosphatases in cultured plant cells (Boudonck et al. 1999). Thus, our results stress the newly ascribed role of the nucleolus sequestering some proliferation relevant proteins to prevent their activity (Visintin and Amon 2000). But the maintenance of their number in proliferating cells, independently of cell cycle equilibrium, indicates the existence of a fixed number of sites for recruitment of spliceosomal components in the nucleolus, independently of cell activity levels.

Based on their recorded numbers and proximity to the nucleolar organisers (NORs), it has been suggested that nucleolar coiled bodies (NABs) correspond to the secondary constrictions of nucleolar chromosomes (Lafontaine and Chamberland 1995). In this sugarcane cultivar, an octoploid with a low DNA content (6.0 pg), and a semireticulate nuclear organisation (Acevedo et al. 1998), the recorded numbers of NABs are compatible with those of chromosomes bearing an NOR, as detected by fluorescence in situ hybridisation (FISH) (D'Hont et al. 1996; Acevedo et al. submitted). Nevertheless the nucleolar coiled bodies are different from the NOR signals and do not contain DNA as demonstrated by FISH and their DAPI-negative staining (Acevedo et al. submitted).

Coiled bodies correspond to specific nuclear and nuclear matrix domains

Nucleic acid metabolism is spatially ordered in the nucleus by association with the nuclear matrix (Ma et al. 1999) in such a way that the cell nucleus is organised into several territorial domains with specific functions (Jackson and Cook 1995; Moreno Díaz de la Espina 1995). Considering that coiled bodies are components of the underlying nuclear matrix, they could correspond to specific domains of the nuclear/chromosomal matrix as occurs with the replication factories (Jackson and Cook 1995; Samaniego and Moreno Díaz de la Espina 2000; Samaniego et al. 2001). Additional results supporting this idea are the association of coiled bodies with specific chromosomal foci like those for the UsnRNAs, histone mRNA clusters (Sleeman and Lamond 1999) and NORs in some plant species (Lafontaine et al. 1991), and also the observation that coiled bodies reform in the same locations during heat shock recovery in living plant cells (Boudonck et al. 1999).

Coiled body dynamics during the reactivation of primordia

The above observations are consistent with a model in which coiled bodies present a fixed basic number per nucleus, depending on the genetic and structural characteristics of cells, but independently of cell activity. Nuclei from the dormant primordia cells should have this basic number of large nucleolar coiled bodies. During reactivation the increase in the number of nucleoplasmic coiled bodies is accompanied by a decrease in size but not in the number of the nucleolar coiled bodies. It should be very interesting to find out whether coiled bodies can not only fuse but also divide at the nucleolar periphery in vivo (Boudonck et al. 1999).

In spite of the close similarities between the two types of coiled bodies in plants, nucleolar coiled bodies should have some special characteristics in relation to the nucleoplasmic ones as: (1) they are strongly dependent on DNA content and nuclear organisation in plants (Barlow 1983a; Moreno Díaz de la Espina et al. 1992; Lafontaine and Chamberland 1995); (2) fusion of coiled bodies has been detected at the nucleolar periphery in vivo (Boudonck et al. 1999); and (3) the inhibition of transcription or phosphatase activity produces relocation of the nucleoplasmic coiled bodies to the nucleolus (Boudonck et al. 1999). Although a functional interaction between the nucleolus and coiled bodies has long been suggested, the mechanisms involved in this process remain unclear (Olson et al. 2000; Visintin and Amon 2000).

Acknowledgements We wish to thank to Dr. C. de la Torre for critical reading of the manuscript, Dr. T. Martin (Chicago, Illinois) for the Y12 serum, Dr. R. Ochs (La Jolla, California) for the S4 serum, the Instituto de Investigaciones de la Caña de Azúcar (INICA) (La Habana, Cuba) for providing the sugarcane cultivar used in this work, Mrs. M. Carnota and Mrs. N. Fontúrbel for expert technical assistance and Mrs. Beryl Ligus Walker for her revision of the English. This work has been supported by the Spanish DGES (projects PB96-0909 and PB98-0647) and by the CSIC/CITMA agreement (project 99CU0010). Ricardo Acevedo is a Mutis fellow financed by the Spanish Agency of International Cooperation (AECI). Experiments done for this publication comply with the current laws of Spain.

References

Acevedo R, Fernandez-Gómez ME, De la Torre C, Moreno Díaz de la Espina S (1998) Analysis of the nucleus in the development of roots in *Saccharum* spp. (sugarcane). In: Calderón-Benavides HA, Yacamán MJ (eds) Electron microscopy, vol IV, pp 779–780

Acevedo R, Moreno Díaz de la Espina S, Fernández-Gómez ME, Cuadrado A, Jouve N, De la Torre C (2001) Dormancy and proliferation in *Saccharum officinarum* × *S. spontaneum* hybrids which differ in the number of the introgressed *S. spontaneum* chromosomes. J Exp Bot 52:1203–1208

Alliegro MC, Alliegro MA (1998) Protein heterogeneity in the coiled body compartment. Exp Cell Res 239:60–68

Barlow PW (1981) Argyrophilic intranuclear bodies of plant cells. Experientia 37:1017–1018

Barlow PW (1983a) Changes in the frequency of two types of nuclear body during the interphase of meristematic plant cells. Protoplasma 18:104–113

Barlow PW (1983b) Nucleolus-associated bodies (karyosomes) in dividing and differentiating plant cells. Protoplasma 115:1–10

Bernhard W (1969) A new staining procedure for electron microscopical cytology. J Ultrastruct Res 27:250–265

Beven AF, Lee R, Razaz M, Leader DJ, Brown JWS, Shaw PJ (1996) The organization of ribosomal RNA processing correlates with the distribution of nucleolar snRNAs. J Cell Sci 109:1241–1251

Boudonck K, Dolan L, Shaw PJ (1998) Coiled body numbers in the *Arabidopsis* root epidermis are regulated by cell type, developmental stage and cycle parameters. J Cell Sci 111:3687–3694

Boudonck K, Dolan L, Shaw PJ (1999) The movement of coiled bodies visualized in living plant cells by the green fluorescent protein. Mol Biol Cell 10:2297–2307

Chamberland H, Lafontaine JG (1993) Localization of snRNP antigens in nucleolus-associated bodies: study of plant interphase nuclei by confocal and electron microscopy. Chromosoma 102:220–226

D'Hont A, Grivet L, Feldmann P, Rao S, Berding N, Glaszmann C (1996) Characterisation of the double genome structure of modern sugarcane cultivars (*Saccharum* spp.) by molecular cytogenetics. Mol Gen Genet 250:405–413

Dickinson J (1985) Nonparametric methods for quantitative analysis, 2nd edn. American Sciences Press, Columbus, Ohio

Jackson DA, Cook PR (1995) The structural basis of nuclear function. Int Rev Cytol 162:125–149

Jennane A, Thiry M, Goessens G (1999) Identification of coiled body-like structures in meristematic cells of *Pisum sativum* cotyledonary buds. Chromosoma 108:132–142

Lafontaine JG, Chamberland H (1995) Relationship of nucleolus-associated bodies with the nucleolar organizer tracks in plant interphase nuclei (*Pisum sativum*). Chromosoma 103:545–553

Lafontaine JG, Luck BT, Gugg S (1991) Nucleolus-associated bodies in meristematic cells of two plant species (*Cicer arietinum* and *Leucaena glauca*) with different ploidy levels. Can J Bot 69:1329–1336

Lamond AI, Earnshaw WC (1998) Structure and function in the nucleus. Science 280:547–553

Ma H, Siegel AJ, Berezney R (1999) Association of chromosome territories with the nuclear matrix: disruption of human chromosome territories correlates with the release of a subset of nuclear matrix proteins. J Cell Biol 146:531–541

Martín M, Moreno Díaz de la Espina S, Jiménez-García LF, Fernández-Gómez ME, Medina FJ (1992) Further investigations on the functional role of two nuclear bodies in onion cells. Protoplasma 167:175–182

Medina FJ, Cerdido A, Fernández-Gómez ME (1995) Components of the nucleolar processing complex (pre-rRNA, fibrillarin and nucleolin) colocalize during mitosis and are incorporated to daughter nuclei. Exp Cell Res 211:111–125

Medina MA, Moreno Díaz de la Espina S, Martín M, Fernández-Gómez ME (1989) Interchromatin granules in plant nuclei. Biol Cell 67:331–339

Monneron A, Bernhard W (1965) Fine structural organisation of the interphase nucleus in some mammalian cells. J Ultrastruct Res 27:266–288

Moreno Díaz de la Espina S (1995) Nuclear matrix isolated from plant cells. Int Rev Cytol 162b:75–139

Moreno Díaz de la Espina S, Sánchez-Pina MA, Risueño MC (1982a) Localization of acid phosphatasic activity, phosphate ions and inorganic cations in plant nuclear coiled bodies. Cell Biol Int Rep 6:601–607

Moreno Díaz de la Espina S, Risueño MC, Medina FJ (1982b) Cytochemical and autoradiographic characterization of coiled bodies in the plant cell nucleus. Biol Cell 44:229–238

Moreno Díaz de la Espina S, Mínguez A, Vázquez-Nin GH, Echeverría OM (1992) Fine structural organization of a non-reticulate cell nucleus. An ultracytochemical and immunocytochemical study. Chromosoma 101:311–321

Moreno Díaz de la Espina S, Medina MA, Mínguez A, Fernández Gómez ME (1993) Detection of highly phosphorylated nucleoproteins in plants by bismuth staining at the EM: X-Ray. Biol Cell 77:297–306

Olmedilla A, Alché JD, Rodríguez-García MI (1997) Nucleolar evolution and coiled bodies during meiotic prophase in *Olea europaea*: differential localization of nucleic acids. Eur J Cell Biol 74:181–189

Olson MOJ, Dundr M, Szebeni A (2000) The nucleolus: an old factory with unexpected capabilities. Trends Cell Biol 10:189–196

Samaniego R, Moreno Díaz de la Espina S (2000) Organisation and composition of the plant nuclear matrix. Characterisation and subcellular distribution of a MAR-binding protein: AcMFP1. Cell Biol Mol Lett 5:264–266

Samaniego R, Yu W, Meier I, Moreno Díaz de la Espina S (2001) Characterisation and high-resolution of a MAR-binding protein (MFP1) in onion proliferating cells. Planta 212:535–546

Shaw PJ, Beven AF, Leader DJ, Brown JWS (1998) Localization and processing from a polycistronic precursor of novel snoRNAs in maize. J Cell Sci 111:2121–2128

Simpson GG, Filipowicz W (1996) Splicing of precursors to mRNA in higher plants: mechanism, regulation and sub-nuclear organisation of the spliceosomal machinery. Plant Mol Biol 32:1–41

Sleeman JE, Lamond AI (1999) Nuclear organization of pre-mRNA splicing factors. Curr Opin Cell Biol 11:372–377

Sokal RR, Rohlf FJ (1969) Biometry. WH Freeman, San Francisco

Vázquez-Nin GH, Echeverría OM, Mínguez A, Moreno Díaz de la Espina S, Fakan S, Martin TE (1992) Ribonucleoprotein components of root meristematic cell nuclei of the tomato characterized by application of mild loosening and immunocytochemistry. Exp Cell Res 200:431–438

Visintin R, Amon A (2000) The nucleolus: the magician's hat for cell cycle tricks. Curr Opin Cell Biol 12:372–377

Williams LM, Jordan EG, Barlow PW (1983) The ultrastructure of nuclear bodies in interphase plant cell nuclei. Protoplasma 118:95–103

Yu W, Moreno Díaz de la Espina S (1999) The plant nucleoskeleton: ultrastructural organization and identification of NuMA homologues in nuclear matrix and mitotic spindle of plant cells. Exp Cell Res 246:516–526