

Behavior of RNAs transcripts during nucleolus assembly and disassembly in *Vicia faba* root meristematic cells under normal conditions and after colchicine treatment

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Received: 28 February 2011 / Revised: 28 September 2011 / Accepted: 16 January 2012 / Published online: 2 February 2012
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Abstract To study the behavior of newly synthesized RNA molecules during unperturbed mitosis and after induction of aneuploid nuclei, a novel method based on the incorporation of 5-ethynyl uridine was applied. This approach allows revealing transcription in cytological specimens by omitting immunocytochemical procedures. The obtained results indicate that RNAs synthesized in the preceding cell cycle behave similarly as nucleolar proteins and participate in post-mitotic nucleolar assembly not only during unperturbed nuclear divisions. Colocalization of nucleolar proteins and RNAs seems also to occur in aneuploid nuclei devoid of functional nucleolar organizer regions (NORs) or NOR per se. This paper also shows the presence of nucleoplasmic gene expression in aberrant aneuploid nuclei and micronuclei, despite occurrence of their genetic material disorganization. Moreover, experiments showing the effect of transcription and replication inhibition on the induction of aneuploid nuclei were also performed. Presented data shed new light on nucleologenesis and co-existence of nucleolar proteins with RNAs in prenucleolar bodies formed in aneuploid nuclei devoid of functional NORs.

Keywords 5-Ethynyl uridine · RNA transcription · Nucleologenesis · Aneuploid nuclei · *Vicia faba*

Introduction

A great number of reviews about nucleolar architecture in animal and plant cell nuclei have been written so far, and a lot of experiments have proved close structural and functional relationships regarding three main elements discernible under an electron microscope, i.e., fibrillar centers (FCs), dense fibrillar component (DFC) and granular component (GC) (Hernandez-Verdun 2006a, b; McKeown and Shaw 2009; Olson and Dundr 2005; Raska et al. 2006; Sirri et al. 2008). Despite the vast amount of detailed information concerning nucleologenesis, still little is known about the behavior of nucleolar components throughout the course of cell division cycle.

It has been reported that during mitosis rDNA transcription machinery proteins are positioned close to the nucleolar organizer regions (NORs), whereas rRNA processing machinery proteins are located at the chromosomal peripheries (Hernandez-Verdun 2006b; Sirri et al. 2008). According to Gautier et al. (1992b), the latter position may also be implicated for ribonucleoproteins (RNPs). Using immunofluorescence techniques, Angelier et al. (2005) proved that during anaphase fibrillarin and B23 migrate toward the cell poles in the vicinity of chromosomes. Biochemical studies showed that in metaphase-arrested cells another nucleolar protein, nucleolin, remains associated with both fibrillarin and B23, and all these proteins form RNP complexes together with pre-rRNA. The integrity of such complexes is closely dependent on the presence of RNAs (Piñol-Roma 1999), indicating that proteins functioning within the nucleolar area can be held together during mitosis.

The disassembly of nucleolar structures in prophase is correlated with the repression of transcription which is complete as cells pass to metaphase. Silencing of rDNA transcription during mitosis is connected with the presence

Communicated by S. Abe.

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of active CDK1-cyclin B complexes (called also M-phase promoting factor, MPF) (ref. Olson and Dundr 2005; Sirri et al. 2008). On the contrary, exit from mitosis is correlated with the degradation of cyclin B (Xu and Chang 2007), which brings about a decrease in the amount of active MPF. Hence, it seems that nucleolar assembly coincides with the activation of rRNA transcription in early telophase, when pre-rRNAs synthesized during the preceding cell cycle together with nucleolar proteins start migrating toward NORs (Savino et al. 2001). Electron microscopic observations indicate that before reaching NORs, both components first concentrate in fibrogranular structures of the prenucleolar bodies (PNBs) containing fibrillarin, nucleolin, B23, rRNAs and some snoRNPs, such as U3, U8 and U14 (Angelier et al. 2005; Dousset et al. 2000).

Direct recruitment of nucleolar factors to NOR (independent of rRNA transcription) has also been proposed, since recruitment of processing proteins both at NORs and into PNBs can occur in transcriptionally inhibited cells. However, in such case, PNBs do not fuse with NOR and increase in size (Dousset et al. 2000; Savino et al. 2001, and ref. therein). PNBs seem to be very dynamic structures. Some proteins, such as fibrillarin (an early-processing protein), remain there transiently, while others (late-processing proteins, e.g., Nop52) become sequestered for a longer period of time (Hernandez-Verdun 2006a, b; Savino et al. 2001). Despite the fact that each protein is present in PNBs only during a specific period of time, colocalization of early- and late-processing proteins in the same PNBs was also proved (Angelier et al. 2005).

A new method, based on the incorporation of 5-ethynyl uridine allows to detect newly synthesized RNA molecules by a cycloaddition reaction without applying immunocytochemical procedures (Jao and Salic 2008). In accordance with earlier data, results presented in this work indicate that rRNA molecules seem to associate with chromosomal structures throughout some stages of an unperturbed mitosis. Furthermore, it is shown that irrespective of the presence of functional NORs, nucleolar proteins observed in PNBs (formed in aneuploid cells after colchicine treatment) may still associate with rRNA molecules. The obtained results seem to indicate that both nucleolar proteins and rRNA synthesized during the preceding cell cycle participate jointly in the early stages of nucleologenesis.

Materials and methods

Seeds of *Vicia faba* subsp. *minor* var. *Nadwiślanski* were sown on wet filter paper in Petri dishes and germinated for 3 days at room temperature in darkness. For experiments, seedlings with 1.5–2 cm roots were selected. In order to produce cell nuclei without functional NORs, aneuploidy was

induced by 6-h incubation with 5 mM colchicine followed by 24-h post-incubation either in water, in 2.5 mM hydroxyurea (HU) or in 5 μ M actinomycin D (AMD) solutions.

Chemical agents

Hydroxyurea, actinomycin D and DAPI were supplied by SIGMA, colchicine and Triton X-100 by Fluka BioChemika, acridine orange by Windsor Laboratories Limited, glutaraldehyde by MERCK, and acetic acid by CHEMPUR. Click-iT[®] RNA Alexa Fluor[®] 488 Imaging Kit (Cat# C10329) for the visualization of RNA transcripts was supplied by INVITROGEN. Other chemicals were obtained from POCH S.A.

Ag-NOR staining

1 cm root fragments were fixed in 2.5% glutaraldehyde in PBS (pH 7.4) for 10 min, at 4°C. After four times wash in PBS (5 min each), roots were transferred to Carnoy's mixture (ethanol:acetic acid; 3:1, v/v). Following fixation (4°C; 5 min), roots were rinsed three times in 96% ethanol, rehydrated (30–70% ethanol, distilled water), and stained with 1 volume of 2% gelatin (diluted in 1% formic acid) mixed with 2 volumes of 50% aqueous silver nitrate solution. After 7 min staining at 65°C, roots were rinsed ten times in water and fixed in 5% sodium thiosulfate for 10 min. Root tips (1.5 mm long) were cut off and squashed in a drop of 45% acetic acid onto slides using dry ice method. After removing cover glasses, slides were plunged into 70% ethanol and air dried. Some specimens were mounted in Canada balsam, others were left for acridine orange staining. Photographs were made using NIKON Optiphot-2 microscope.

Acridine orange (AO) staining

To show colocalization of nucleolar proteins and ribonucleic acids, slides with silver-stained cells were counterstained for 3 min with AO solution (1 mg/ml; PBS, pH 6.0) and rinsed with PBS. Photographs were made using NIKON Optiphot-2 microscope equipped with blue excitation light (filter block B-2A; $\lambda = 460$ nm). Broad-range filter revealed simultaneously green fluorescence for DNA and red for RNA.

Chemical labeling and detection of RNA transcripts

Click-iT[®] RNA Alexa Fluor[®] 488 Imaging Kit was used for the visualization of RNA transcripts (Jao and Salic 2008). 1.5 cm root fragments were cut off and transferred to 1 mM solution of 5-ethynyl uridine (EU) for various incubation times, in dark. After labeling, 1.5 mm apical root parts were fixed in PBS-buffered 4% paraformaldehyde (4°C; pH 7.4)

for 45 min (maceration) or 20 min (isolation of cell nuclei). For maceration, meristems were rinsed twice in PBS and transferred for 45 min to the citrate-buffered mixture (pH 5.0; 40°C) containing 2.5% pectinase from *Aspergillus niger* (FLUKA), 2.5% cellulose Onozuka R-10 from *Trichoderma viride* (SERVA), and 2.5% pectolyase Y-23 (ICN). Next, root tips were rinsed twice in cold PBS, squashed onto microscope slides (Polysine™, Menzel-Gläser) in a drop of distilled water, and placed on dry ice. After 10 min, cover glasses were removed and slides were washed with PBS, distilled water and air dried. For isolation of nuclei, meristems were rinsed twice in PBS and squashed between two slides in a drop of PBS. Fraction of isolated nuclei were harvested on microscope slides and air dried. Macerated cells or isolated nuclei were then permeabilized with 0.5% Triton X-100 for 15 min. Sites of EU incorporation were detected by using Click-iT® reaction cocktail consisted of components prepared according to the vendors manual. Incubation was performed at room temperature for 60 min. After that time, slides were washed in Click-iT® reaction rinse buffer and PBS. DNA was stained with DAPI (0.5 µg/ml) for 5 min and then washed in PBS. Specimens were mounted in PBS/glycerol mixture (9:1) containing 2.5% DABCO (1,4-diazabicyclo[2.2.2]octane). Photographs were made using Eclipse E600W microscope. Alexa Fluor® 488 DM 505 filter (excitation wavelength 465–495) and DAPI DM 400 filter (excitation wavelength 340–380) were used.

Results

rRNAs localize between the chromosomes during mitosis

To visualize rRNAs during non-perturbed mitosis, EU labeling was performed for 5 h. Apart from interphase cell nuclei, the applied procedure of isolation yielded some amount of mitotic figures. Since the most intense fluorescent signal has been localized to the nucleolus, the main fraction of transcripts can seemingly be attributed to rRNAs. During nucleolar disassembly in prophase, rRNAs start to move out of the nucleolus and fluorescence signal was found between the chromosomes (Fig. 1a–a''). Although the intensity of fluorescence decreases in metaphase (and is hardly visible), in some cases faint labeling in the vicinity of chromosomes could still be observed (Fig. 1b–b''). However, no fluorescent signal for RNA could be detected in NOR region during this stage of mitosis. The fluorescence signal, localized around and between chromosomes, reappeared in anaphase and telophase (Fig. 1c–c'', d–d''). At later stages of telophase, a huge flow of rRNAs toward the nucleolus was evidenced

(Fig. 1e–e''). In early-G1 nuclei, EU labeling was confined mainly to the nucleolus, with only small amount of fluorescence signals between chromatin fibrils (Fig. 1f–f'').

Colchicine effectively induces polyploid and aneuploid cell nuclei in root meristems of *V. faba*

The 6-h incubation of *V. faba* primary root meristems in 5 mM colchicine followed by 24-h post-treatment in water gives rise to a small number of aberrant cells having either aneuploid nuclei or/and micronuclei, resulting probably from the transition of tetraploid cells (created previously due to microtubular disruption) throughout the next mitotic division. To assess the role of an unperturbed DNA or RNA synthesis in the presence of such abnormalities, another two parallel series of experiments have been performed using hydroxyurea (HU), an inhibitor of ribonucleotide reductase, or actinomycin D (AMD), a blocker of RNA polymerase activity. Based on a variety of earlier data (Morcillo and Torre 1980; Stockert et al. 1970), the silver-stained cells showing abnormal nuclear morphology (irrespective of the experimental series) were classified into three classes, including: A-type binucleate cells, with one nucleus having a fully organized nucleolus and the other nucleus showing a mass of non-fused argyrophilic proteins corresponding to PNBs (Fig. 2a); B-type diploid or polyploid cells, with a single nucleus containing one or more nucleoli and some amount of argyrophilic material scattered in the nucleoplasm (Fig. 2b–d); and C-type binucleate cells, with normally organized nucleoli (Fig. 2e). Moreover, some of the A-type cells were found to contain one or more small aneuploid nuclei termed as micronuclei, having PNBs. During statistical analysis these cells were also rated as A-type.

Quantitative analysis (Fig. 3) shows that in seedlings incubated with colchicine and post-treated with water, A-type cells constitute 3.5%, B-type cells 3.0%, and C-type 0.7% of all root meristem cells. Post-incubation in HU brings about reduction of A-type cells up to 0.8%, almost complete decrease of B-type cells (0.01%), and a slight increase in relative number of C-type cells (0.99%), as compared to roots post-incubated in water. AMD treatment also leads to a slight drop in number of A-type cells (2.6%) and to a significant increase in cells categorized to type B (13.4%). C-type cells are nearly as frequent as in root meristems post-incubated in water (0.72%).

In aneuploid nuclei lacking functional NORs, argyrophilic proteins colocalize with RNAs during PNBs formation

As indicated before, rRNAs synthesized prior to mitosis show similar behavior to nucleolar proteins. To find out whether RNA colocalize with silver-stained proteins in aneuploid

Fig. 1 Localization of EU-labeled rRNA during: **a** prophase, **b** metaphase, **c** anaphase, **d** telophase, **e** late telophase and **f** early-G1, **g** negative control (without EU). **a'–g'** represent *inverted grayscale*, **a''–g''** DAPI staining. *Arrowheads* in that panel show nucleoli, *arrows* mark NOR during late telophase. *Bar* 10 μ m

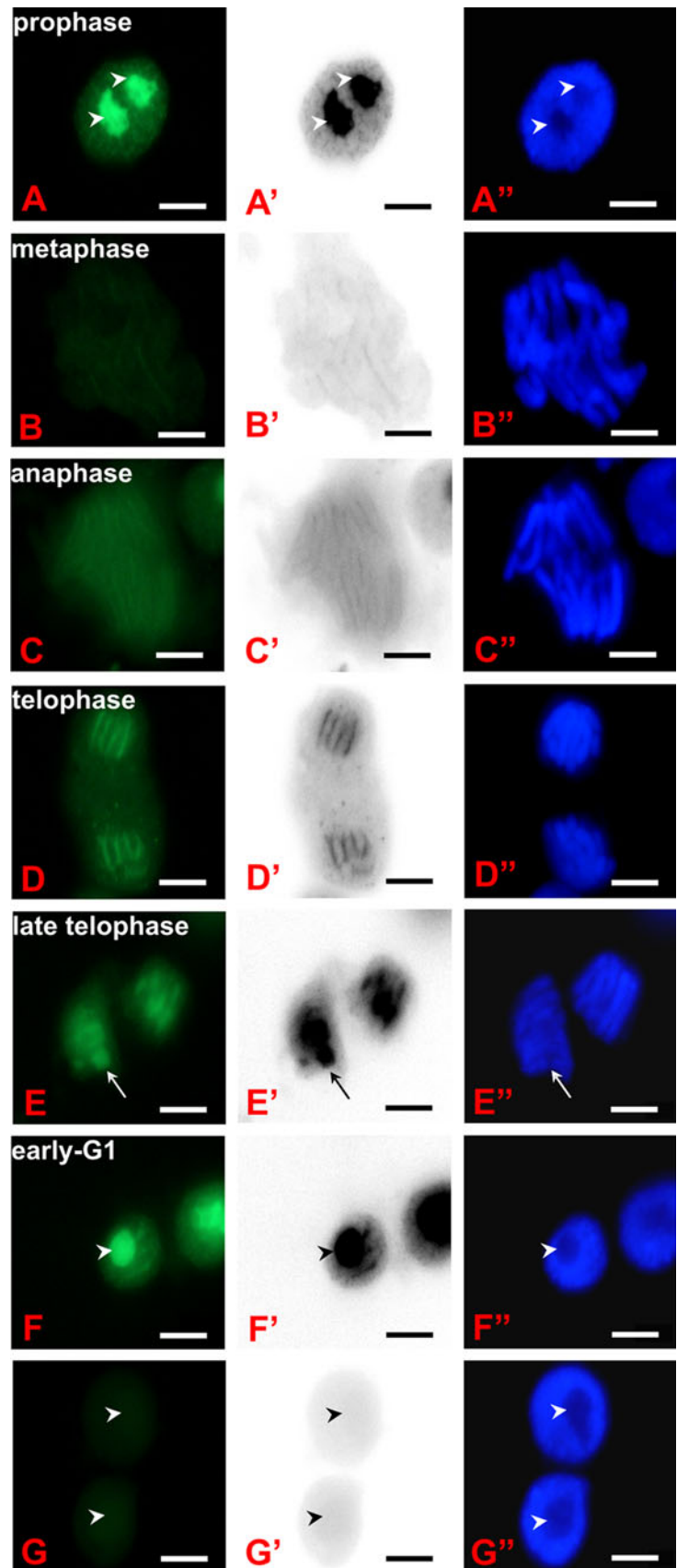


Fig. 2 Different types of cells showing abnormal nuclear morphology after 6 h colchicine treatment: **a** cells with two aneuploid nuclei (A-type cells) obtained during post-incubation in water (*arrow* shows aneuploid nuclei devoid of functional NOR), **b** B-type cells after post-incubation in water, **c**, **d** B-type cells after post-incubation in AMD, **e** binucleate cells (C-type cells) after post-incubation in water. *Dashed lines* mark single cell, *asterisks* nucleoli, *arrowheads* PNBs. *Bar* 10 μ m

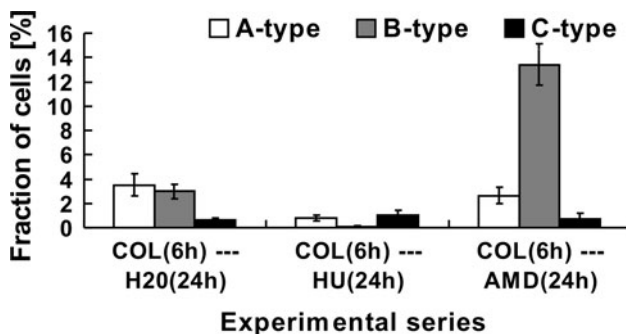
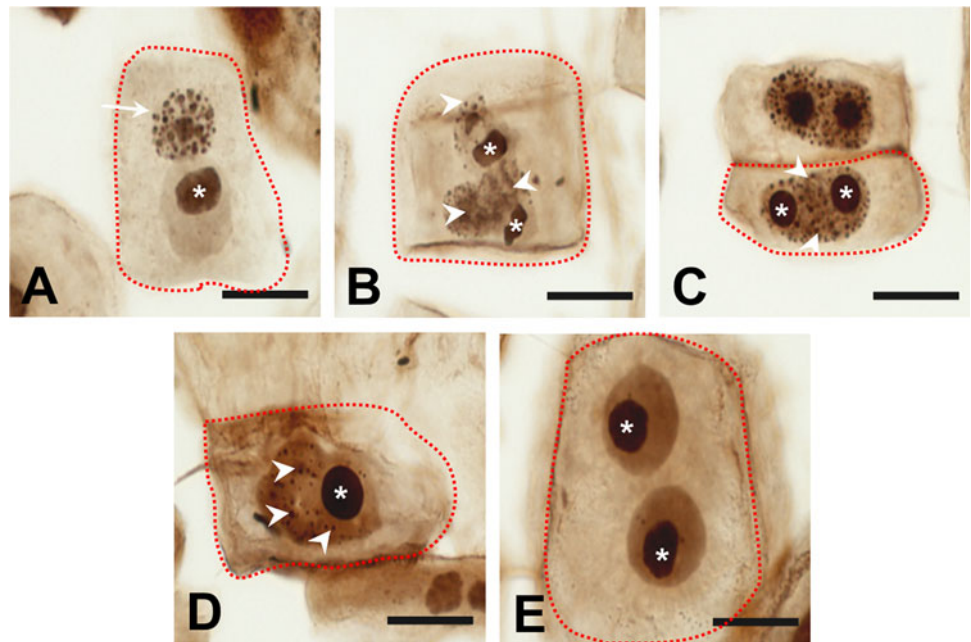


Fig. 3 Frequency of three types of cells obtained after incubation in colchicine followed by post-incubation in H₂O, HU or AMD; revealed by silver staining

nuclei and micronuclei, Ag-NOR staining followed by acridine orange were introduced. Microscopic examination clearly reveals colocalization of silver-stained nucleolar proteins and RNA molecules in aneuploid nuclei lacking functional NORs (Fig. 4, arrowheads). The term “functional NOR” is used to indicate the occurrence of active nuclear elements capable to congregated dispersed PNBs into nucleoli. Interestingly, aneuploid nuclei (Fig. 4c, c', arrows) and small micronuclei (Fig. 4b, b', arrows) containing no argyrophilic protein and RNAs have also been found.

In aneuploid cell nuclei lacking functional NOR, the pre-mitotically EU-labeled rRNA molecules seem to colocalize with silver-stained proteins

To find out whether RNAs localized in PNBs of aneuploid nuclei are synthesized prior to mitosis, root meristem cells were incubated for 6 h in the mixture of EU and colchicine

and then transferred to water for 2, 4 or 12 h. During the second and the fourth hour of post-incubation, most cell nuclei displayed one to four nucleoli (Fig. 5a, a'), while some of nuclei were found in the progress of nucleolar assembly (Fig. 5b, b', c, c'). Throughout that period of time, no labeled aneuploid nuclei lacking functional NOR could be found. However, a small population of aneuploid cell nuclei with EU-labeled PNBs (with a relatively low fluorescence signal compared to negative control cells; Fig. 6d, d') has been found after the prolonged 12-h post-incubation in water (Fig. 6a, a', b, b'), showing the same localization as DAPI-negative spots. Moreover, in some cells PNBs could be seen in vicinity of the chromosomes (Fig. 6c, c').

RNA synthesis occurs in aneuploid nuclei devoid of functional NOR

Using autoradiography, Labidi et al. (1987) have shown that transcription can occur in micronuclei. By applying a novel method based on EU incorporation, a similar result was also observed in 6 h colchicine-treated root meristems post-incubated in water (24 h). Transcription was noticed both in aneuploid nuclei (Fig. 7a, rectangle) without functional NOR, where fluorescence could be seen in specific zones around DAPI-negative spots (Fig. 7b, c, arrowheads), as well as in some large micronuclei (Fig. 8, arrows). Transcriptionally active aneuploid nuclei and micronuclei have also been observed during 24-h post-incubation in HU (data not shown). However, no EU incorporation could be seen in aneuploid root cell nuclei when seedlings released from colchicine block were treated with AMD for 24 h (data not shown).

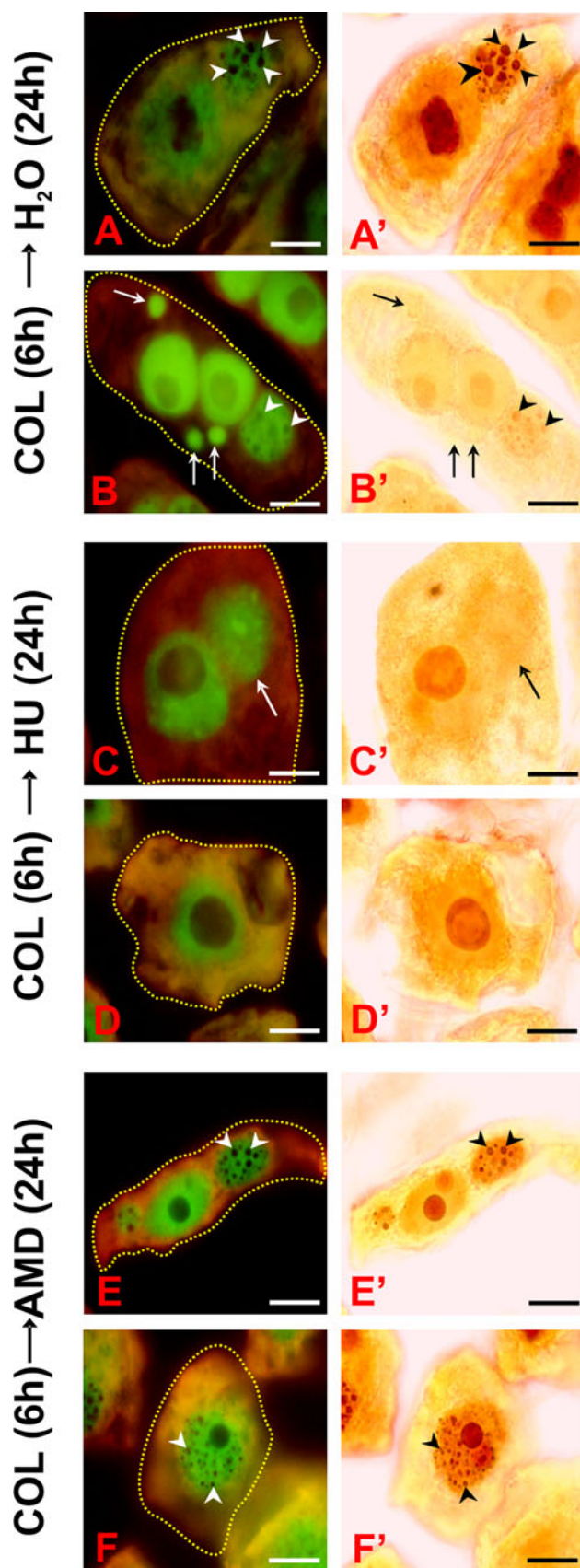


Fig. 4 Colocalization of argyrophilic proteins and RNAs. Cells obtained after 6 h colchicine treatment followed by 24-h incubation in: **a, a'**, **b, b'** water, **c, c'**, **d, d'** hydroxyurea or **e, e'**, **f, f'** actinomycin D. *Dashed lines* mark single cell. *Arrowheads* show PNBs and *arrows* point micronuclei (**b, b'**) and aneuploid nuclei (**c, c'**) without PNBs. *Bar* 10 μ m

Discussion

Post-incubation of colchicine-treated root meristem cells in water, HU or AMD gives rise to different types of nuclear ploidies

Nucleolar assembly is a very complicated process proceeding in two steps. At the beginning, NOR recruits early-processing proteins, e.g., fibrillarin, and then late-processing proteins, like B23 or Nop52 (Angelier et al. 2005; Savino et al. 2001). Although it has been proposed that RNA polymerase I (Pol I) activity is necessary for the assembly of nucleolar material around NOR (Benavente et al. 1987), more recent data suggest that recruitment of early-processing proteins in some cases may be independent of transcription activation (Sirri et al. 2000, 2002). However, it has been shown that in the presence of AMD only initial and direct recruitment of nucleolin and fibrillarin is possible, while full nucleolar assembly still requires Pol I-mediated transcription since PNBs fail to fuse with NOR in such conditions (Dousset et al. 2000). Similar effect of AMD was also described by Stockert et al. (1970). The performed observations are consistent with the above results and support the idea that under transcription inhibition an initial assembly of nucleoli is possible, since during post-incubation in AMD, apart from A-type cells, a considerable number of diploid cells having homogeneously silver-stained proteins in the nucleolar area and dispersed argyrophilic material in nucleoplasm (they are rated as sort of B-type cells) were also observed. It still remains to be solved whether Pol I activity per se is essential for nucleolar assembly or if there is a need for specific gene products, functional, only when new ribosomes are formed.

In agreement with earlier observations made both on plant and animal cells (Caperta et al. 2006; Hernandez-Verdun et al. 1991), the obtained results indicate that apart from cells having regular diploid and polyploid nuclei, colchicine-induced disorganization of microtubules brought about a number of cells with aneuploid nuclei devoid of functional NORs or NORs per se. Due to significant reduction of A-type cells formed after HU treatment, it seems reasonable to suppose that they appear mostly as a result of the second round of mitosis, i.e., following nuclear division of tetraploid cells. The dispersed argyrophilic material observed in aneuploid cell nuclei support previous data pointing both to the necessity of transcription activation and to the presence of NORs for

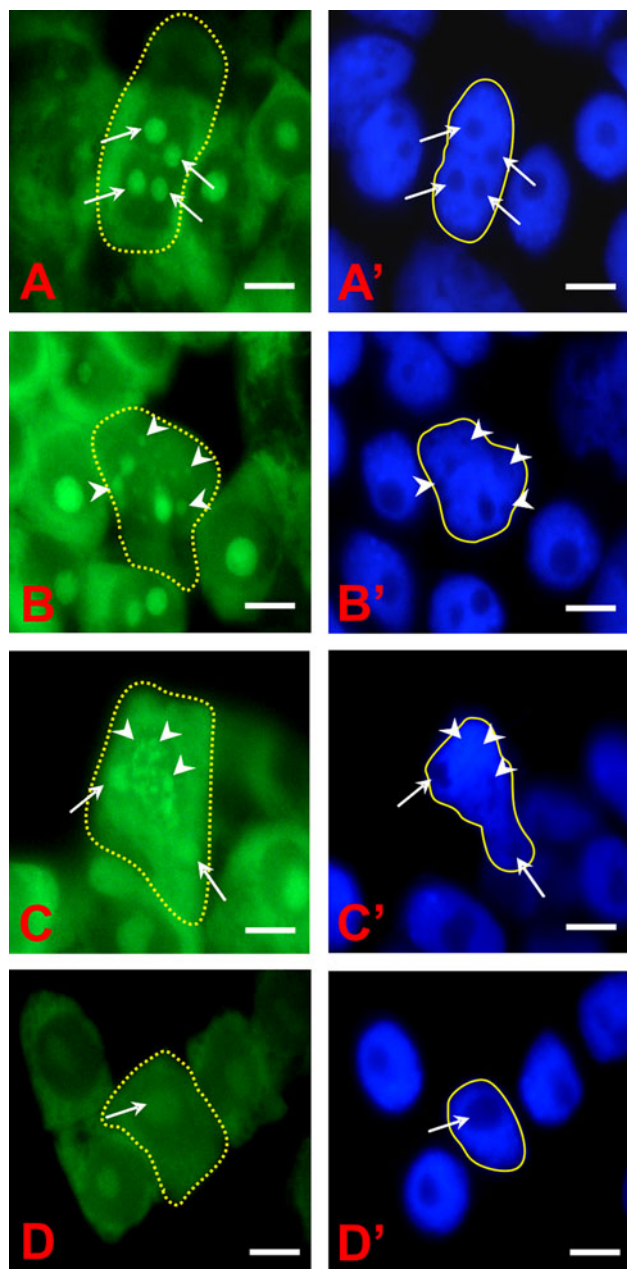


Fig. 5 Localization of EU-labeled rRNA: in nucleoli of tetraploid nucleus obtained after 2-h post-incubation in water (**a**) and in PNBs during nucleoli assembly followed by post-incubation in water for 2 (**b**) or 4 h (**c**). EU was introduced during 6 h colchicine pre-treatment. **d** negative control (without EU) for Figs. 5, 7 and 8. **a'–d'** represent DAPI staining. *Dashed lines* mark single cell, *continuous lines* mark areas of DAPI-stained DNA. *Arrowheads* show PNBs and *arrows* point to nucleoli. *Bar* 10 μ m

nucleolar assembly (Dousset et al. 2000; Morcillo and de la Torre 1980; Stockert et al. 1970).

Performed experiments point to another important problem concerning the process of nucleolar assembly. Because of nearly complete disappearance of B-type cells in colchicine-treated root meristems post-incubated with

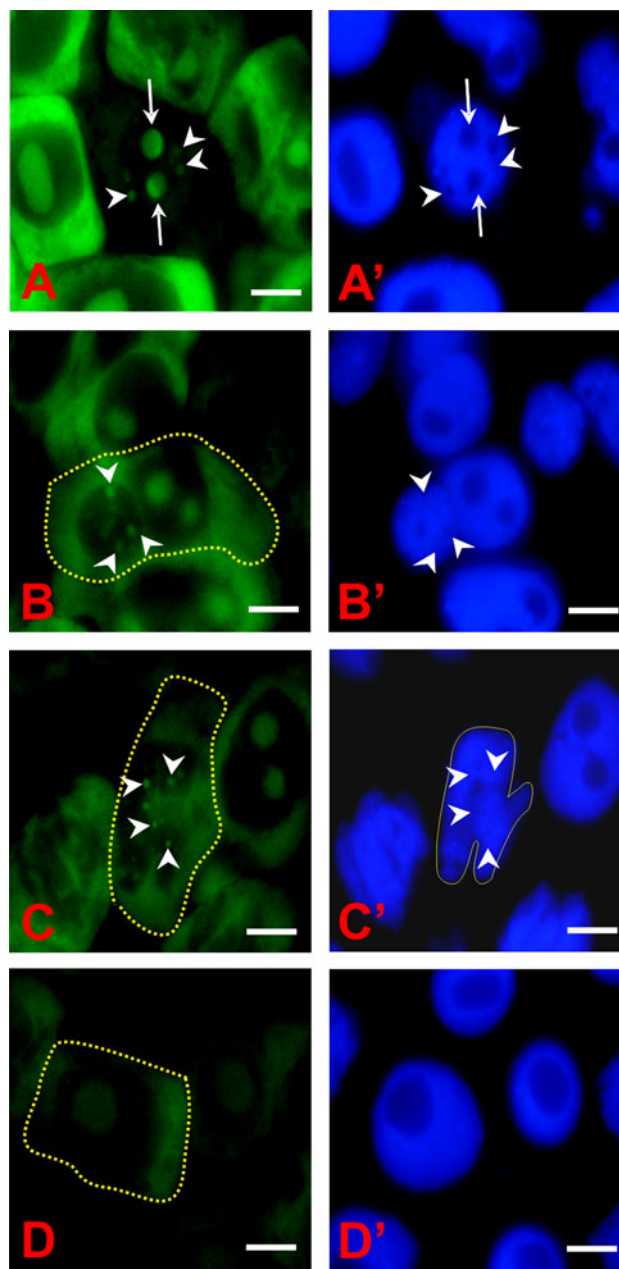


Fig. 6 PNBs with labeled RNAs in: aneuploid nuclei without functional NOR (**a**, **b**) and in vicinity of chromosomes (**c**) obtained after 6 h colchicine treatment followed by 12-h post-incubation in water. EU was introduced during colchicine treatment. **d** negative control (without EU). **a'–d'** represent DAPI staining. *Dashed lines* mark single cell, *continuous lines* mark areas of DAPI-stained DNA. *Arrowheads* shows PNBs and *arrows* point to nucleoli. *Bar* 10 μ m

HU, it seems possible that polyploid cells need additional time for the organization of the nucleoli.

Behavior of RNAs during intact cell cycle

According to the best knowledge, the present data give—for the first time—a cytochemical insight into the behavior

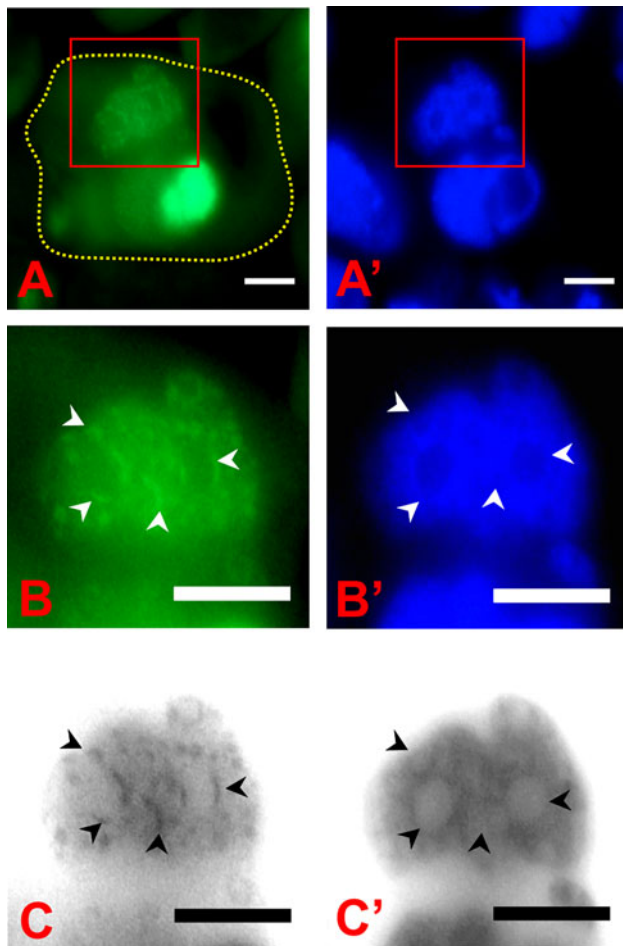


Fig. 7 Localization of EU-labeled RNA synthesis in aneuploid nucleus devoid of functional NOR: **a** whole cell marked by *dashed line*; *rectangle* shows aneuploid nuclei, **b** enlarged aneuploid nuclei, **c** *inverted grayscale* of enlarged nuclei. **a'**–**c'** represent DAPI staining. Cells were incubated in EU during last 5 h of 24-h post-incubation in water after 6 h colchicine treatment. *Bar* 10 μ m

of previously labeled RNAs during mitosis showing much similarity with the fate of nucleolar proteins (Angelier et al. 2005; Gautier et al. 1992a). The research of Gautier et al. (1992a) revealed a very high fluorescence signal for nucleolar proteins in every stage of mitosis, even in metaphase. Also Savino et al. (2001) presented data showing Nop52 localized at the chromosome periphery during metaphase. Current results indicate that metaphase chromosomes are either totally free of labeled RNA or reveal only weak fluorescence, when compared to other stages of mitosis. Weaker labeling observed during metaphase can be an artifact or can present different behavior of RNAs and nucleolar proteins, as the complete pre-mitotic phosphorylation of B23.1 represses its RNA binding activity. Contrarily, the phosphorylation of RNA-associated B23.1 is inhibited (Okuwaki et al. 2002). Possibly, different behavior of these molecules in metaphase may be explained by fluctuations in the concentration of active CDK1-cyclin B

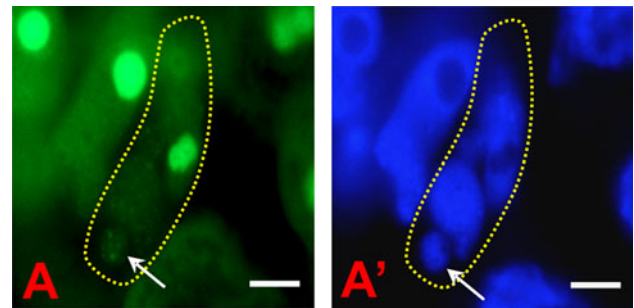


Fig. 8 Localization of EU-labeled RNA synthesis in micronuclei (*arrows*): **a** EU labeling, **a'** DAPI staining. *Dashed line* shows single cell. Cells were incubated in EU during last 5 h of 24-h post-incubation in water after 6 h colchicine treatment. *Bar* 10 μ m

complexes which, after reaching maximum level in prophase, phosphorylate B23.1. It is likely that one pool of RNAs, visible around metaphase chromosomes, remains associated with B23, whereas the other, not associated with nucleolar proteins, is dispersed in the cytoplasm. Moreover, nucleolin, another nucleolar phosphoprotein phosphorylated during mitosis by CDK1 kinase (Srivastava and Polard 1999) may also play a role. Even so, the results obtained in the current work clearly indicate that nucleolar proteins and RNAs synthesized during the preceding cell cycle reveal similar behavior at least in some phases of mitosis. It is noteworthy to mention that fibrillarin, nucleolin and B23 are associated with precursor rRNA in immunopurified complexes extracted from metaphase-arrested cells (Piñol-Roma 1999).

Behavior of RNAs during aneuploid nuclei formation

It was proved that pre-rRNA transcripts produced in the previous cell cycle are stable and appear in PNBs (Doussset et al. 2000; Dunder et al. 2000), which may also contain U3 and U8 small nucleolar RNAs (Verheggen et al. 2000). Moreover, by using immunofluorescence, micronuclei similarly to nuclei have been shown to include a number of nucleolar proteins (Hernandez-Verdun et al. 1991). Simultaneous staining with acridine orange and silver nitrate reveals that RNA transcripts also colocalize with nucleolar proteins in aneuploid nuclei devoid of functional NOR, as it occurs in PNBs formed under normal conditions. To ascertain that RNAs present in aneuploid nuclei have been synthesized before mitosis, the newly formed transcripts were labeled by EU in the presence of colchicine (6-h incubation). The obtained data indicate that RNAs synthesized during the preceding cell cycle on the one hand participate in post-mitotic nucleolar assembly since previously localized in PNBs could be seen while traveling toward the nucleoli; on the other hand they are dispersed in aneuploid nuclei lacking functional NOR,

since RNAs labeled during colchicine treatment were found organized in PNBs, in these nuclei 12 h after the release from metaphase block.

The appearance of aneuploid nuclei and micronuclei without any silver-stained nucleolar proteins and RNAs questions whether these molecules are really attached to chromosomal periphery during mitosis. However, earlier reports showed that nucleolar antigens can be found around most chromosomal entities, but they do not cover their entire surface (Gautier et al. 1992b). Hence, this may explain the absence of nucleolar proteins and RNAs in some aneuploid nuclei and micronuclei formed after aberrant mitosis.

RNA transcription can proceed in micronuclei and aneuploid nuclei devoid of functional NORs

Performed experiments confirmed that despite genetic material disorganization in aneuploid nuclei and micronuclei, apart from nucleolar rDNA genes, other parts of nuclear areas may still reveal transcription. Detailed analysis of such process was described previously by Labidi et al. (1987). Current experiments using EU labeling indicate that either not all micronuclei show transcriptional activity or not in all micronuclei this activity can be proved. It is worth to mention that in aneuploid nuclei without functional NOR, transcription was limited to specific zones around DAPI-negative areas, which probably correspond to the clusters of nucleolar proteins trapped inside these nuclei.

When summing up, the obtained results indicate that in root meristem cells of *V. faba* RNAs molecules seem to behave during mitosis in the same way as nucleolar proteins. Similar to intact nuclei they appear also in PNBs dispersed in aneuploid nuclei devoid functional NOR or NOR per se. Presented results allow to conclude that RNAs are transported to aneuploid nuclei due to their localization in the perichromosomal region during mitosis. This paper supports the idea that not only activation of transcription but also RNA transcripts synthesized during previous cell cycle may play an important role during plant nucleologenesis.

Acknowledgments I thank Professor J. Maszewski for helpful comments and critical reading of the manuscript.

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