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rRNA distribution during microspore development in anthers of *Beta vulgaris* L.: quantitative in situ hybridization analysis

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SUMMARY

We related changes in the ultrastructural organization of the nucleoli with the results of quantitative in situ hybridizations to characterize rRNA metabolism during the development of microspore mother cells in the sugar beet anther (*Beta vulgaris* L.). In the course of meiotic prophase and early postmeiotic interphase the morphological characteristics of the nucleoli are typical of low or no transcriptional activity and a low rate of rRNA processing.

However, we found evidence of an apparent increase in the relative numbers of 18 S rRNA transcripts in some stages of microsporogenesis. This was found in both the nucleoli and cytoplasm of pachytene meiocytes, and in later stages there was a spectacular accumulation of rRNA transcripts in nucleoli of the tetrad cells. Quantitative data are analyzed in the light of morphometric findings in the cells and their compartments to elucidate the degree to which changes in cell size are related to changes in labeling density and distribution. The results are discussed in terms of rRNA synthesis, transport and degradation as processes involved in the regulation of rRNA within microsporocytes and microspores.

Key words: rRNA distribution, Microsporogenesis, *Beta vulgaris* L.

INTRODUCTION

The transition from the sporophytic to gametophytic phase of development, and processes leading to the formation of gametes, are important events in the plant life cycle. Both are known to be accompanied by striking changes in the physiology and structure of cells within the embryo sac and anther (Boer de Jeu, 1978; Medina et al., 1981; Scott et al., 1991; Majewska-Sawka et al., 1993).

Development of the male gametophyte in plants involves fluctuations in RNA content within microsporocytes, microspores and pollen grains. A conspicuous decrease in the ribosome population of male meiocyte cytoplasm in some species has been shown to occur as early as the start of meiotic prophase (Mackenzie et al., 1967; Parchman and Lin, 1972; Dickinson and Li, 1988). These observations, originally based on visible changes in 'cytoplasm density' seen with light and electron microscopy, were later confirmed in biochemical studies. The decrease in RNA content during this period affects both ribosomal and messenger RNA molecules (Porter et al., 1983a; Dickinson and Li, 1988). As meiosis proceeds, the level of rRNA shows further changes, which are partly due to peak synthesis in zygotene/pachytene (Porter et al., 1983a; Risueño and Medina, 1986). After this phase, however, a long period of transcriptional inactivity of rDNA genes ensues, and lasts until the late microspore stage (Mascarenhas, 1988, 1989). Despite the long gap in rRNA synthesis, the number of ribosomes increases markedly in the cytoplasm of tetrads in many plant species. It has been suggested that repopulation of the cell with ribosomes may involve different, species-dependent mechanisms. In several taxa this process involves the presence of cytoplasmic bodies resembling nucleoli (nucleoloids) which appear after telophase II until the young microspore stage (Dickinson and Heslop-Harrison, 1970; Rodriguez-Garcia and Fernandez, 1987).

The aim of the present study was to clarify some basic aspects of the regulation of rRNA content during meiotic prophase and postmeiotic interphase in species which do not display cytoplasmic nucleolus-like bodies. We used morphometric methods and quantitative in situ hybridization with the 18 S rDNA fragment of *Pisum sativum* to determine the relative number of rRNA transcripts within cells in successive stages of microsporogenesis in sugar beet anthers.

MATERIALS AND METHODS

Sugar beet plants (*Beta vulgaris* L.) of two diploid lines (LO8 and LO9) were supplied by the Institute of Plant Breeding and Acclimatization, Department of Genetics and Breeding in Bydgoszcz, Poland.

Conventional electron microscopy

Anthers were excised and fixed in Karnovky's fixative (4% paraformaldehyde, 5% glutaraldehyde in 0.1 cacodylate buffer, pH 7.0) overnight at 4°C, then washed several times in cold buffer and post-fixed in 1% OsO_4. They were then dehydrated in an ethanol series and embedded in Epon 812. Ultrathin sections were cut on a

Reichert-JUNG ultracut E microtome and stained with uranyl acetate/lead citrate.

In situ hybridization

Tissue preparation

The anthers in successive stages of development were simultaneously fixed with the phase-partition procedure described by McFadden et al. (1988): a solution of 4% paraformaldehyde and 0.25% glutaraldehyde in 50 mM Pipes buffer (pH 7.3) was mixed with an equal volume of *n*-heptane and shaken for 1 minute. After the phases had separated, the tissue was placed first in the heptane phase for 10 minutes at room temperature, and then in the fixative phase for 2 hours. Subsequently, the anthers were rinsed several times in Pipes buffer, dehydrated in an ethanol series and embedded in LR Gold resin (London Resin, UK). For localization of rRNA using light microscopy, anthers were sectioned at a thickness of 1 μm and the sections attached to the surface-coated slides (2% Biobond, BioCell Research Laboratories, UK) on a warming plate at 70°C. For localization of rRNA using electron microscopy, ultrathin sections were collected on Formvar-coated nickel grids.

Ribosomal RNA and DNA probes

The 18 S fragment (about 1 kb) of *Pisum sativum* L., originally cloned by Dr William Thompson of North Carolina State University, was kindly provided by Dr Goeffrey McFadden from Melbourne University.

The pBLUESCRIPT KS(+/–) vector was linearized using *Bam*HI and *Eco*RI restriction enzymes. DIG RNA labeling kit (Boehringer Mannheim, Germany) was used to label RNA with digoxigenin (DIG-11-UTP) by in vitro transcription for 2 hours at 37°C using T7 and T3 RNA polymerases to create antisense and sense (control) strands, respectively.

Double-stranded DNA was excised from the plasmid by digestion with *Bam*HI and *Eco*RI restriction enzymes, isolated by agarose gel electrophoresis, and recovered from the gel with the phenol squeeze method. rDNA was labeled with digoxigenin (DIG-11-dUTP) by random priming with a labeling kit from Boehringer Mannheim. Labeled DNA was resuspended in hybridization buffer (50% formamide, 0.75 M NaCl, 0.3 M sodium citrate, 5 mM phosphate buffer, pH 7.2, 5 mM EDTA, 1 mM sodium pyrophosphate, 0.1% Ficoll, 0.1% polyvinyl pyrrolidone, 0.1% bovine serum albumin, 100 μg ml^{-1} herring sperm DNA) at 5 μg/ml. Before use, the probe was denaturated at 95°C for 10 minutes.

Hybridization protocols

Semithin sections were usually used in RNA-RNA hybridization experiments, and ultrathin sections for DNA-RNA hybridization and quantitative analysis. Semithin sections were pretreated with 20 μg ml^{-1} proteinase K (Boehringer Mannheim) in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) for 30 minutes at 37°C, and then washed several times in TE buffer. Small drops of RNA probe were placed over each section, which was covered with a coverglass. Whole slides were then placed in a small chamber moistened with the hybridization buffer. RNA-RNA hybridization took place at 55°C for 16 hours. Posthybridization washing was done by dipping whole slides into glass vessels containing the following solutions: 4× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.2; 3× 10 minutes) at room temperature (RT), 2× SSC (3× 10 minutes, RT), 1× SSC (2× 30 minutes, 55°C), SC buffer (50 mM Pipes pH 7.2, 0.5 M NaCl, 0.5% Tween-20; 15 minutes, RT). Nonspecific antigens were blocked with SC buffer containing 1% BSA for 1 hour. RNA-RNA hybrids were detected with a sheep anti-digoxigenin monoclonal antibody (1/20 in SC buffer for 1 hour, RT) coupled to 5 nm colloidal gold (BioCell Research Laboratories, UK). The hybridization signal was enhanced by applying silver enhancing kit (BioCell Research Laboratories, UK) to the sections for 15 minutes at 20°C, in accordance with the manufacturer's instructions.

Grids with ultrathin sections were pretreated with 10 μg ml^{-1} proteinase K in TE for 20 minutes at 37°C, and then placed on 3 μl drops of denaturated DNA probe. Hybridization took place in a sealed, moistened chamber for 18 hours at 37°C. Posthybridization washing was done by floating the grids on the drops of solutions noted above for semithin sections, except that the blocking time in 1% BSA was reduced to 15 minutes. DNA-RNA hybrids were located with a two-step monoclonal antibody (mAb) technique: incubation with mouse-anti-digoxigenin from Boehringer Mannheim (1/15 in SC buffer for 1 hour, RT) was followed by a second treatment with rabbit anti-mouse mAb coupled to 10 nm colloidal gold particles (BioCell Research Laboratories, UK) (1/15 in SC buffer for 1 hour, RT). The grids were examined and photomicrographs taken under Zeiss 10C and Tesla BS 500 electron microscopes at 60 kV.

Statistical analysis

The numbers of gold particles attached to the microsporocyte/microspore compartments (cytoplasm and nucleoli) in successive stages of development were compared. For this purpose ultrathin sections of numerous anthers representing the four developmental stages: (I) leptotene/zygotene, (II) pachytene, (III) mature tetrads and (IV) young microspores were simultaneously subjected to hybridization with the same batch of DNA probe as described above. Generally, 18-56 cells from at least 3 different anthers were photographed and analyzed per stage. Morphometric analyses of cell sections was done with the IPS 512 image analysis system (Imal, Poland) to obtain precise information on the area of the cells themselves, and of the cell's compartments.

Morphometric data were correlated with direct counts of gold particles attached to the cytoplasmic and nucleolar areas. The signal obtained as a result of RNA-DNA hybridization was good enough to perform computer counting of gold particles. Cytoplasmic labeling was determined for each stage by counting the number of gold particles in 140-301 randomly chosen squares corresponding to 1 μm^2. Nucleolar labeling was determined per whole section area. The data for each stage of microsporocyte/microspore development were compared using Student's *t*-test. The least significant differences (LSD) in labeling intensity between cytoplasmic compartments in successive stages of development, and between nucleolar compartments, were estimated for α=0.05.

RESULTS

Nucleolar ultrastructure

During the early period of meiotic prophase, i.e. at leptotene, zygotene and pachytene, nucleoli show a segregated structure, with the dense fibrillar component occupying the central part, and the granular component lying at the nucleolar periphery (Fig. 1A). The fibrillar component contains a few large fibrillar centers, which frequently display darkly stained inclusions (Fig. 1B). The nucleolar organizing region (NOR) is observed at the nucleolar periphery as a lighter stained structure usually in contact with the fibrillar component (Fig. 1B). Nucleoli with a large, centrally located vacuole are also observed at this phase of meiosis, and some perichromatin granules are present within the nucleolar mass (Fig. 1C). At this time, the nucleolus is located either centrally or in a slightly acentric position, close to the nuclear envelope.

As meiosis proceeds in diplotene and diakinesis, nucleoli hardly ever contain the granular component, and are characterized as a compact fibrillar structure with very few fibrillar centers. By the end of meiotic prophase, the nucleoli disappear as such, and the nucleolar material becomes reorganized after

telophase II. The beginnings of new nucleoli can be distinguished as prenucleolar bodies which subsequently join to form true nucleoli.

During the tetrad stage, as well as in young microspores after they are released from the callose wall, the nucleoli are composed only of compact fibrillar material (Fig. 1D). Fibrillar centers are either absent or only occasionally observed, but they are generally no more numerous than one per section.

Location of 18 S rRNA transcripts in microsporocytes/microspores

Both RNA-RNA and DNA-RNA hybridizations in sections of sugar beet anthers resulted in a very specific localization of 18 S rRNA fragments. Light microscopic observations showed that the most intense hybridization signal was visualized in the nucleoli, and less conspicuous labeling could be seen in the cytoplasm. Some labeling was also found in the nucleoplasm, whereas vacuoles and cell walls were clearly devoid of silver deposits (Fig. 2A,B). Comparisons of semithin sections from anthers in successive developmental stages showed that the tapetum was the tissue where the most intense labeling was observed in each stage, whereas the cytoplasm of microsporocytes/microspores showed a less intense signal (Fig. 2B).

Electron micrographs showed that cytoplasmic organelles (vacuoles, mitochondria) and the callose wall contained very few gold particles (Fig. 3A). Labeling in the nucleoli was observed in both the fibrillar and granullar components. In most sections, fibrillar centers were devoid of label, although a few gold particles could occasionally be found. The amount of label in fibrillar centers was not estimated. No signal was visualized in nucleolar vacuoles or in NOR chromatin (Fig. 3B).

Quantitative distribution of rRNA transcripts in cell compartments

Morphometric data on cell section and nucleolar areas are shown along with statistical estimates of the results in the four stages of microsporogenesis in Fig. 4A and B. These measurements were correlated with quantitative analysis of the numbers of gold particles attached to the cytoplasm and nucleolar areas to determine the precise pattern of labeling density and distribution in each stage. Relative content of ribosomal transcripts was estimated from the number of gold particles.

In meiocytes representing two successive stages of meiotic prophase (leptotene/zygotene and pachytene) we found no dif-

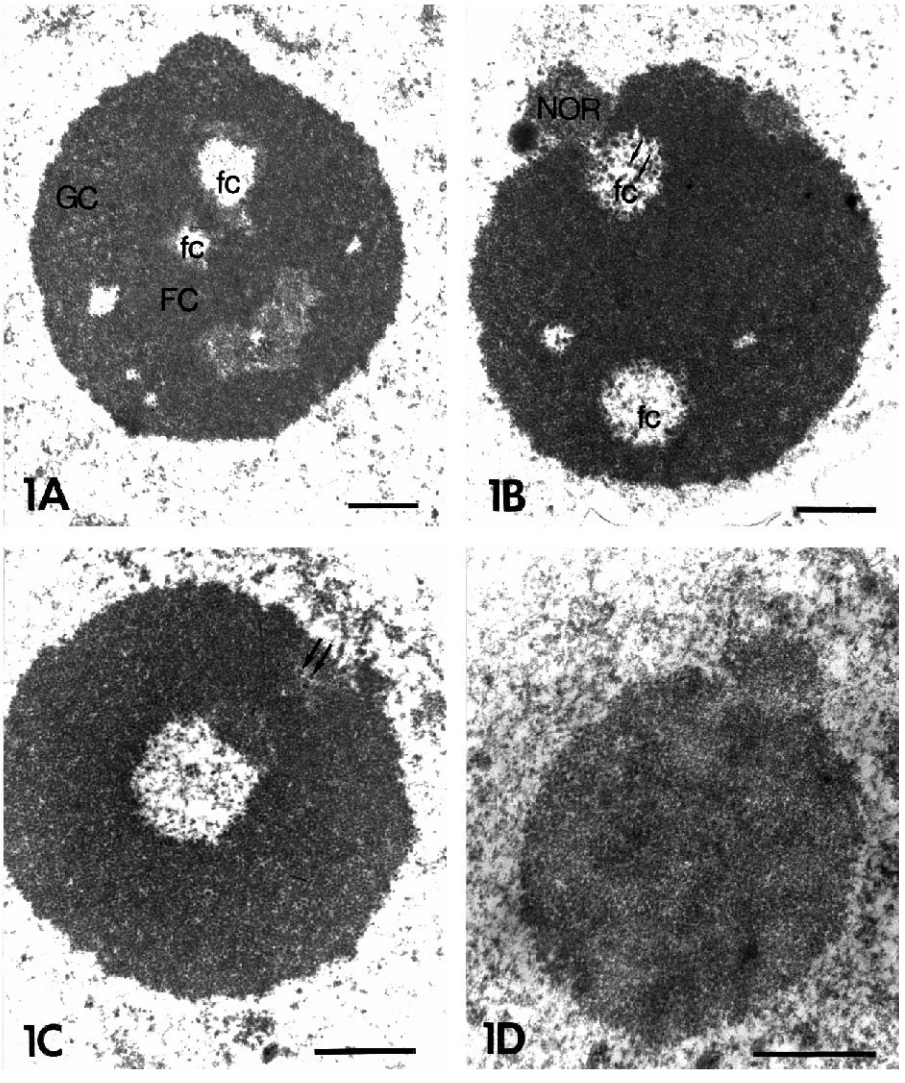

Fig. 1. Ultrastructural organization of the nucleoli during meiotic prophase and postmeiotic interphase: (A) 'segregated' nucleolus with a fibrillar component (FC) occupying the central portion, and granular component (GC) at the periphery. Large fibrillar centers (fc) are visible; (B) nucleolus with the NOR at the periphery. Fibrillar centers (fc) display darkly stained inclusions (arrows); (C) vacuolated nucleolus with visible perichromatin granules (arrows); (D) compact, fibrillar nucleolus typical of the late prophase, tetrad stage and young microspore stage. Bars, 0.5 μm.

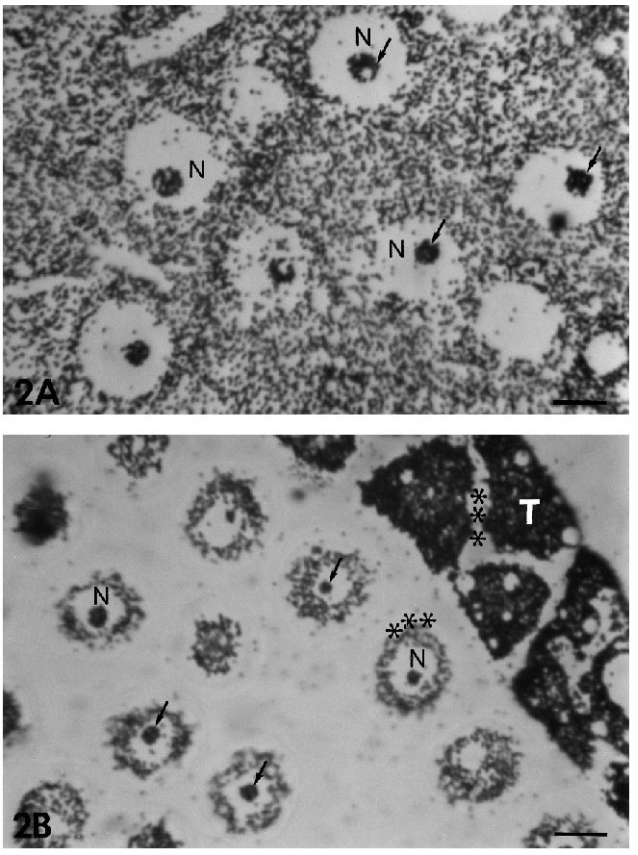

Fig. 2. Labeling of meiocytes (A) and microspores (B) obtained with in situ hybridization with an 18 S rRNA probe. Reaction enhanced by silver, no additional staining. Nucleoli (arrows) show the most intense signal whereas the nuclei (N) are only slightly labeled. Difference in labeling intensity of the tapetum (T) and microspores is visible. Cell walls (asterisks) are devoid of silver deposits.Bars, 5 μm.

ferences in average area of cell section or in nucleolar area. During this time, however, the relative number of transcripts increased significantly (α=0.05) in both the cytoplasmic and nucleolar compartments (Fig. 4C,D; Tables 1, 2). The quantitative distribution of 18 S rRNA transcripts in two cell compartments also changed markedly (Table 3). In leptotene/zygotene, the nucleolus occupies an average of 3.4% of the cell area, and contains 7.9% of all transcripts. In pachytene, it occupies a slightly larger area (4%), but contains up to 15.1% of ribosomal transcripts.

When meiotic division is complete, cell size (Fig. 4A) and nucleolar size (Fig. 4B) are both reduced in single cells of the tetrad. The relative number of transcripts in the tetrad cytoplasm remained similar to that observed during pachytene (Fig. 4C; Table 1). However, the number of gold particles attached to the nucleoli showed that labeling density increased significantly in comparison with the pachytene stage (Fig. 4D; Table 2). At this time the nucleolus contains 17.2% of the total number of transcripts (Table 3).

After the microspores are released from the callose wall, whole cells and nucleoli grow conspicuously (Fig. 4A,B). The relative content of transcripts in both compartments is similar to that found in the tetrad stage (Fig. 4C,D). The small discrepancies with the direct counts of gold particles were not statistically significant (Tables 1, 2). At this stage the relative

Table 1. Relative numbers of 18 S rRNA transcripts in the cytoplasm (log number/μm²)

Stages of development	$\bar{x}_1$	$\bar{x}_2$	$\bar{x}_1$-$\bar{x}_2$	LSD for α=0.05
I - II	1.30	1.53	(–) 0.24*	0.06
I - III	1.30	1.35	(–) 0.05	0.12
I - IV	1.30	1.27	0.03	0.27
II - III	1.53	1.35	0.19	0.33
II - IV	1.53	1.27	0.26*	0.17
III - IV	1.35	1.27	0.08	0.22

number of rRNA transcripts in the nucleoli decreases to 12.5% of the total content (Table 3).

Control experiments were done in sections of anthers in the microspore stage, by hybridization in buffer without the DNA probe. Gold particles appeared scattered lightly throughout the sections, reflecting nonspecific binding of the antibodies. The average number of gold particles attached to 1 μm² of nucleolar area was 0.19, and the number of particles per 1 μm² of cytoplasm was 0.25. These low values, representing 'hybridization background', were not analyzed statistically.

DISCUSSION

Nucleolar ultrastructure

Nucleolar organization during meiotic prophase and at the beginning of postmeiotic interphase indicates little or no rDNA transcription and transcript maturation. This finding is based on several morphological features typical of the nucleoli during this period of microsporogenesis: segregation of fibrillar and granular components, little or no granular component in some stages, appearance of vacuolated nucleoli with perichromatin granules, and condensation of NOR chromatin (Bernhard, 1971; Hadjiolov, 1985).

Segregation of the nucleolar components during meiotic prophase has been described in microsporogenesis and megasporogenesis in different plant species (Luck and Jordan, 1977; Risueño et al., 1980; Medina and Risueño, 1981; Medina et al., 1981, 1983; Polowick and Sawney, 1992). Such morphology may be artificialy induced by chemical treatment with inhibitors that specifically block certain steps of rRNA synthesis or processing (Fernandez-Gomez et al., 1983; Sanchez-Pina et al., 1984; Puvion-Dutilleul et al., 1992).

Vaculolated nucleoli also seem to appear as a consequence

Table 2. Relative numbers of 18 S rRNA transcripts in the nucleoli (log number/μm²)

Stages of development	$\bar{x}_1$	$\bar{x}_2$	$\bar{x}_1$-$\bar{x}_2$	LSD for α=0.05
I - II	1.68	2.04	(–) 0.36*	0.06
I - III	1.68	2.17	(–) 0.50*	0.07
I - IV	1.68	2.06	(–) 0.39*	0.17
II - III	2.04	2.17	(–) 0.14*	0.05
II - IV	2.04	2.06	(–) 0.03	0.10
III - IV	2.17	2.06	0.11	0.13

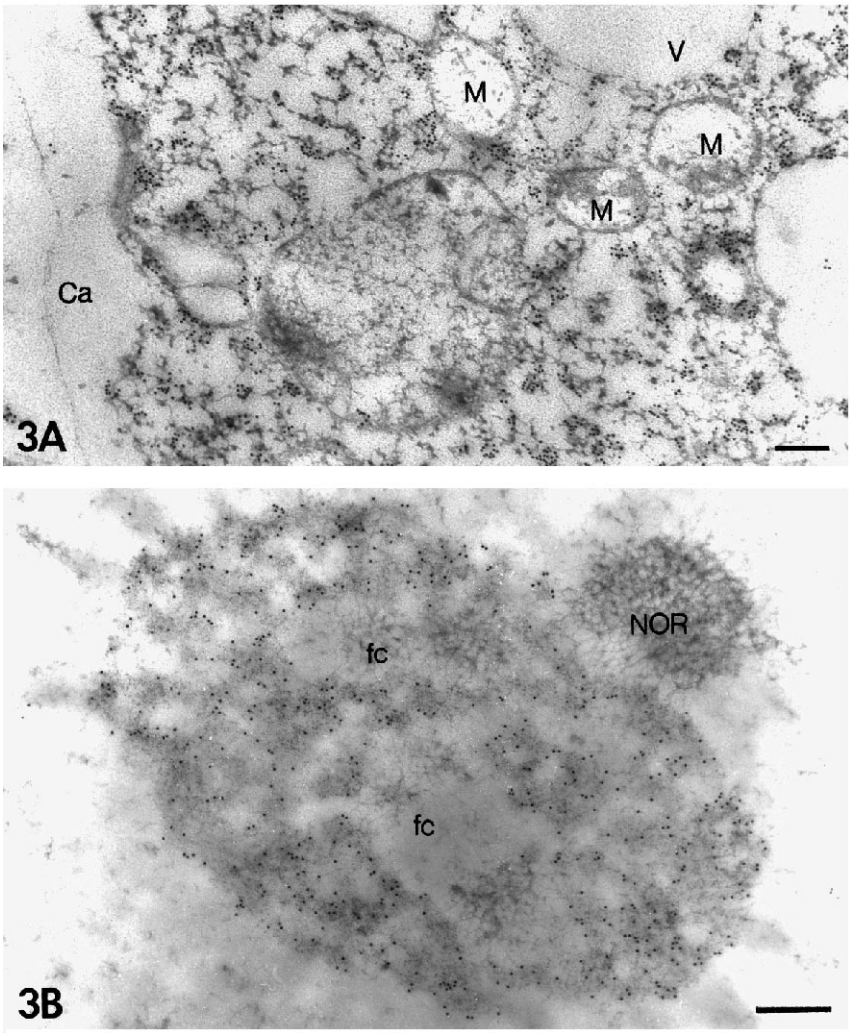

Fig. 3. (A) Electron micrograph of specific labeling of meiocyte cytoplasm as a result of in situ hybridization with an 18 S rDNA probe. Callose wall (Ca) and cytoplasmic organelles: mitochondria (M) and vacuoles (V) are free from gold particles. No uranyl acetate/lead citrate staining. Bar, 0.2 μm. (B) Labeling of the nucleolus at pachytene stage. Fibrillar centers (fc) and nucleolar organizing region (NOR) are free from gold particles. No uranyl acetate/lead citrate staining. Bar, 0.2 μm.

of disturbances in posttranscriptional changes of rRNA, as suggested by the presence of nucleolar perichromatin granules, which probably correspond to incorrectly processed rRNA particles (Risueño and Medina, 1986). The condensed NOR chromatin located at the periphery of the nucleolus throughout the period of microsporogenesis studied here is typical of nucleoli in which transcription is impaired (Medina et al., 1983; Risueño and Medina, 1986: Highett et al., 1993).

Location of rRNA transcripts within microsporocytes/microspores

Several authors have shown that in situ hybridization for light microscopic observation is a useful method for estimating the intensity of labeling in different tissues (Raghavan and Olmedilla, 1989; Sekhar and Williams, 1992), and for determining the relative number of rDNA genes or their transcripts in cells (Porter et al., 1983b; Kwiatkowska et al., 1987; Raghavan, 1989; Dow and Mascarenhas, 1991a,b).

The location of genes or transcripts at the ultrastructural level provides more detailed information about their subcellular distribution. However, statistical analysis has rarely been applied to label intensity evaluation. In only a few cases, the gold particles attached to particular domains of the nucleoli have been quantified to determine the sites where rDNA transcription is initiated (Olmedilla et al., 1993; Hozák et al., 1993; Thiry, 1993). As far as we have determined, few studies have combined in situ hybridization with morphometric analyses and ultrastructural observation to follow the changes in the relative numbers of ribosomal transcripts and their subcellular distribution during cell growth and differentiation. This lack of information may be due to the fact that the method itself, although precise, is also laborious and technically demanding. In the present study we took advantage of the fact that sugar beet anthers representing successive developmental stages are available simultaneously. Moreover, the simultaneous applica-

Table 3. Proportional content of 18 S rRNA transcripts in the nucleoli

Stage of development	Percent contribution of nucleolar area to cellular area	Percent content of the total number of transcripts within the nucleolus
I	3.4	7.9
II	4.0	15.1
III	2.6	17.2
IV	2.3	12.5

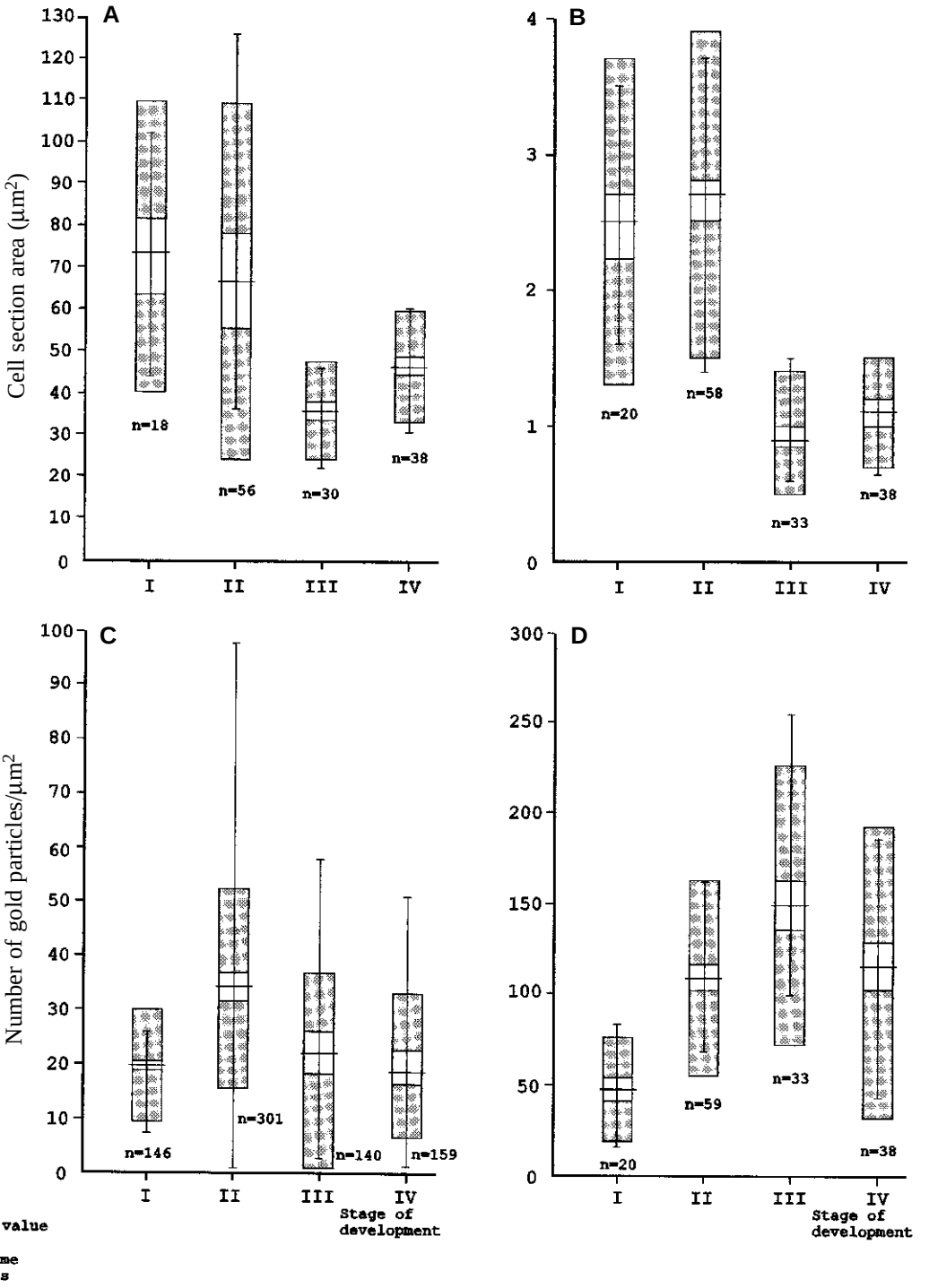

Fig. 4. (A) Statistical analysis of cell section area in four developmental stages of the *Beta vulgaris* microsporocyte. (B) Statistical analysis of nucleolar section area in four developmental stages of the *Beta vulgaris* microsporocyte. (C) Statistical analysis of the number of gold particles in the cytoplasmic area in four developmental stages of the *Beta vulgaris* microsporocyte. (D) Statistical analysis of the number of gold particles in the nucleolar area in four developmental stages of the *Beta vulgaris* microsporocyte.

tion of the hybridization procedure to many anther sections in the course of the same experiment, as well as the use of the same batch of probe, guaranteed the uniform processing of all materials, an important aspect of quantitative studies.

RNA content during microsporogenesis

The most widespread feature of plant microsporogenesis seems to be the reduction of RNA synthesis and RNA content as soon as cells enter meiotic prophase (Das, 1965; Mackenzie et al., 1967; Parchman and Lin, 1972; Knox et al., 1973; Dickinson and Li, 1988). As meiosis proceeds, there is a transitory increase in rRNA and mRNA synthesis during zygotene and pachytene in several species (Porter et al., 1983a; Risueño and Medina, 1986; Pennel and Bell, 1987). However, other studies indicate that changes in RNA content during MMC development may differ greatly not only within the species, but also depending on the methods used, making it difficult to find common characteristics of RNA metabolism during MMC development (Porter et al., 1983a,b; Sekhar and Williams, 1992). Moreover, most of these studies did not obtain morphometric data and thus did not ascertain the changes in RNA content as a result of either cell growth or real processes of synthesis, transport and degradation.

In sugar beet anthers, during the period of MMC development between leptotene/zygotene and pachytene, relative transcript density increases conspicuously in both the nucleoli and the cytoplasm. Because this change occurred when the morphometric changes in both whole cells and nucleoli were not statistically significant, there must have been an actual increase in rRNA synthesis. This datum probably reflects the transitory activation of transcription of rDNA genes in zygotene/pachytene, which typifies meiotic prophase in many plant species (Risueño and Medina, 1986). Nucleolar size was not correlated with transcriptional activity in sugar beet meiocytes. Although a positive correlation between these two features has been found in several plant and animal cells, it is not a general phenomenon (Kwiatkowska et al., 1987). In sugar beet, the lack of any correlation may have been due to the rapid transport of rRNA to the cytoplasm, as reflected by the increase in the number of transcripts in this compartment. The changes in rRNA transcript content occurred during the period when ultrastructural organization of the nucleoli indicates rather low transcriptional activity of rDNA genes. However, autoradiographic studies in other species proved that rRNA synthesis during zygotene/pachytene is evidenced by the incorporation of [^{3}H]uridine only in the NOR area, but not in the nucleolar mass. This process frequently correlates with the formation of nuclear bodies (Risueño and Medina, 1986).

Ribosomal transcripts in nucleoli of sugar beet microsporocytes were most numerous in the tetrad stage, i.e. when nucleoli are composed of compact fibrillar mass. These structures are considered to reflect the near absence of rRNA synthesis and processing. Therefore the increase in nucleolar rRNA at this stage may not reflect actual synthesis, but rather the accumulation of transcripts during nucleologenesis (De la Torre and Gimenez-Martin, 1982). The amount of cytoplasmic rRNA is the same in pachytene meiocytes and in cells of mature tetrads, but it should be emphasized that this refers to comparisons of single tetrad cells with single meiocytes. If all four cells of a tetrad are considered, their total size is about double that of a single meiocyte, and rRNA content is about threefold greater than at pachytene. The increase in rRNA content just after the second meiotic division accompanies microsporogenesis in several plant species. Because rRNA increases during the period of low or absent transcription of rDNA, the cytoplasm may be repopulated with ribosomes that develop from ribonucleoproteic structures termed nucleoloids (Williams et al., 1973; Dickinson and Willson, 1985; Rodriguez-Garcia and Fernandez, 1987; Górska-Brylass et al., 1988). This hypothesis was confirmed recently by in situ hybridization in lily and olive tetrads with ribosomal probes (Sato et al., 1991; Alché et al., 1994).

Sugar beet tetrads, when observed by electron microscope, hardly ever show such ribonucleoprotein structures. Two mechanisms of regulation of the rRNA level in sugar beet MMC may therefore be proposed. Nucleoloids in this species may form and then disapear very rapidly, making it impossible to detect them with the electron microscope. Another possibility is that the amount of rRNA in sugar beet tetrads may be regulated by processes that do not involve nucleoloids.

After the microspores are released from the callose wall, no significant changes were found in relative transcript density per unit area of the nucleoli or cytoplasm, although both whole cells and nucleoli had grown conspicuously. During the early period of postmeiotic interphase, little or no transcriptional activity of rDNA genes takes place (Mascarenhas, 1988, 1989): the structure of nucleoli in sugar beet microspores is that typical of inhibition of RNA synthesis at this time. The lack of change in transcript density despite marked cell growth is difficult to interpret. The almost stable number of cytoplasmic ribosomes seems to be a result - at least in part - of transport of rRNA from the nucleolus. This process may be explained by the decrease in the percentage content of total ribosomal transcripts within nucleoli of the microspore, in comparison with the nucleoli of tetrad cells.

The results of our studies show that in situ hybridization for TEM can be used not only to locate RNA sequences in specific subcellular compartments, but also to precisely quantify the relative numbers of transcripts. The changes in RNA content in the course of development can be followed by comparing the intensity of labeling in successive phases of cell growth and differentiation.

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