Plant Cytogenetics. Katowice 1998
Prace Naukowe Uniwersytetu Śląskiego nr 1696

rRNA GENES — THEIR DISTRIBUTION AND ACTIVITY IN PLANTS

J. MALUSZYNSKA, R. HASTEROK,
H. WEISS

Department of Plant Anatomy and Cytology, Silesian University,
Jagiellońska 28, 40-032 Katowice, Poland

Introduction

Ribosomal RNA genes are one of the most extensively studied genetic units in higher eukaryotes. McCLINTOCK in 1934 demonstrated that nucleolus is always formed in association with a particular structure located on the short arm of maize chromosome 6. Because of its role in the formation of the nucleolus, this region of the chromosome has become known as the nucleolus organizer region — NOR. The demonstration that the NOR of *Drosophila* (RITOSSA, SPIEGELMAN, 1965) and *Xenopus* (BIRNSTIEL *et al.*, 1966) contained the coding region for 18S and 28S rRNA was the first direct correlation between a cytologically discernible chromosomal structure and a specific gene family. The rRNA genes offer several advantages for cytogenetic and molecular studies. Some of the most important are: (i) their presence and expression in each cell as housekeeping genes, (ii) their occurrence in many copies per genome which minimizes the plant material needed for investigation, (iii) availability of rDNA clones for analysis of genomic DNA by digestion with restriction enzyme or by *in situ* hybridization. This overview will summarise information about the structure and distribution of plant rRNA genes.

Structure and sequences of rDNA

There are two types of rDNA, the "large" rDNA which contain genes 18S, 5.8S and 25S coding 45S preribosomal RNA and 5S rDNA for 5S rRNA. Both types of rDNA belong to the genomic fraction of a middle repetitive DNA. They are separated from each other on plant chromosomes. This is different from the organisation of these genes in bacteria and in chloroplast where 5S rRNA genes are physically linked to the other rRNA genes. The ribosomal RNA genes can be present in many thousands of copies in plant genomes and located in tandem arrays at one or several chromosomal loci. The number of rRNA genes can range from 570 copies per haploid genome in *Arabidopsis* (PRUITT, MEYEROWITZ, 1986) to 31,000 in *Hyacinthus orientalis* (INGLE *et al.*, 1975). The number of 5S rRNA genes ranges in the flowering plant from 3,600 copies in *Matthiola incana* (HEMLEBEN, WERTS, 1988) to about 100,000 copies in flax (GOLDSBOURGH *et al.*, 1981). There is no correlation between the number of copies of rDNA and 5S rDNA. The number of 5S rRNA genes in plants is often much higher than that of 18S-5.8S-25S rRNA (HEMLEBEN *et al.*, 1978; APPELS *et al.*, 1980). *Arabidopsis* contains 570 copies of 18S-5.8S-25S rRNA genes (PRUITT, MEYEROWITZ, 1986) and 1,000 copies of 5S rRNA genes (CAMPELL *et al.*, 1992); in *Vigna radiata* there are 1,500 and 3,600 copies, respectively (HEMLEBEN, WERTS, 1988) but in tomato the copy number of 5S rDNA is much lower than that for 18S-5.8S-25S rDNA (LAPITAN *et al.*, 1991).

The data on rRNA genes suggest that the number of copies is not related to the genome size. As is shown in Tab. 1, the rRNA gene number estimated on

Table 1

Comparison of number of rDNA copies per one picogram of genomic DNA

Species	DNA content 1C (pg)*	rDNA copy number	copy pg	Reference
		18S-5.8-25S rDNA		
Arabidopsis thaliana	0.2	570	2 850	(1)
Hordeum vulgare	5.5	4 200	763	(2)
Triticum aestivum	17.3	4 500	264	(3)
		5S rDNA		
Arabidopsis thaliana	0.2	1 000	5 000	(4)
Linum usitatissimum	0.75	58 500	78 000	(5)
Mathiola incana	1.5	2 400	2 400	(6)
Lycopersicon esculentum	1.0	1 000	1 000	(7)

* BENNETT *et al.*, 1995; (4) CAMPELL *et al.*, 1992; (3) FLAVELL, SMITH, 1974; (6) HEMLEBEN, WERTS, 1988; (7) LAPITAN *et al.*, 1991; (1) PRUITT, MEYEROWITZ, 1986; (5) SCHNEEBERGER, CULLIS, 1992; (2) SUBRAHMANYAM *et al.*, 1994.

the genomic picogram basis can vary hundreds and thousands of times between different plant species. In some species, a large proportion of nuclear genome is comprised of rRNA genes. The *Arabidopsis* genome contains 8% of 18S-5.8S-25S rDNA and 0.7% of 5S rDNA (PRUITT, MEYEROWITZ, 1986) while in flax 5S rDNA occupies some 3% of the genome (SCHNEEBERGER, CULLIS, 1992). The copy number of rRNA genes is different not only in various species, but can also vary between loci within the genome. For example, there are essential differences in the copy number between particular loci of hexaploid wheat (Tab. 2).

Table 2

Number of rRNA genes at the nucleolus organisers in four varieties of hexaploid wheat (FLAVELL, SMITH, 1974)

Variety	Chromosome			
	1B	6B	1A	5D
Chinese Spring	1350	2750	125	350
Cappelle-Desprez	1300	500	870	400
Triticum spelta	870	1800	1667	880
Cheyenne	1500	3000	280	130

Each rDNA unit for 18S-5.8S-25S ribosomal RNA is composed of an external transcribed space region (ETS), the gene encoding for 18S rRNA, an internal transcribed spacer (ITS) on each side of the 5.8S rRNA gene and 25S rRNA gene followed by a nontranscribed spacer (NTS). This intergenic DNA separates transcribed regions in the rDNA repeat unit. The sequences for control, initiation and termination of their transcription are located in the non-transcribed region (Fig. 1). The rDNA unit is transcribed, by polymerase I,

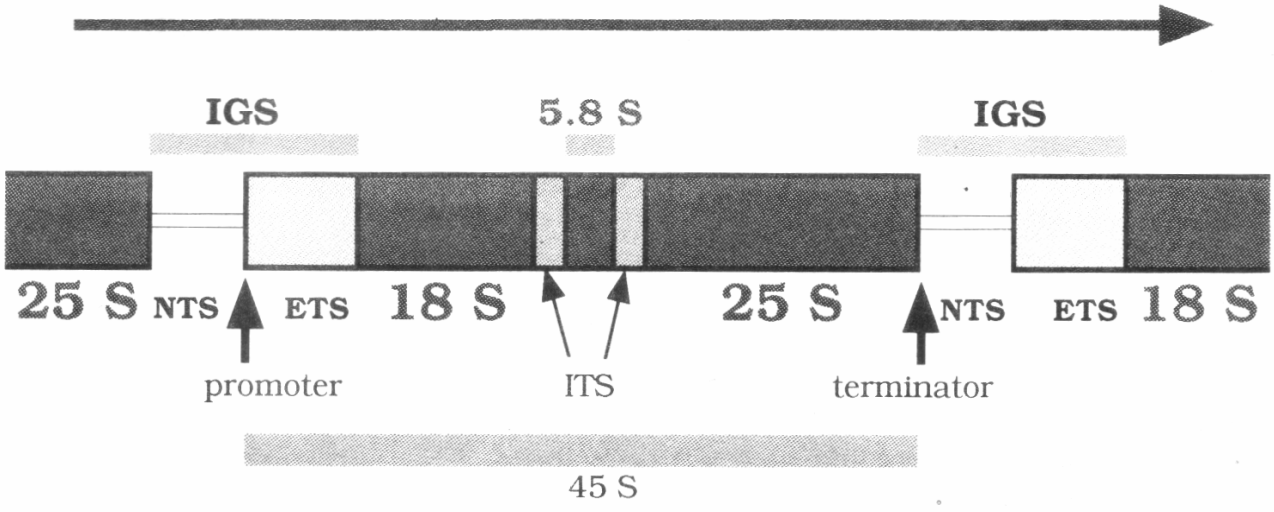


Fig. 1. General structure of the rDNA repeating units for 18S-5.8S-25S rRNA in plants
IGS — intergenic spacer, NTS — nontranscribed spacer, ETS — external transcribed spacer, ITS — internal transcribed spacer

into a large preribosomal 45S RNA transcript (Fig. 2). The transcript consists of sequences which are present in mature rRNAs and sequences which are removed during post-transcriptional modification (Fig. 3).

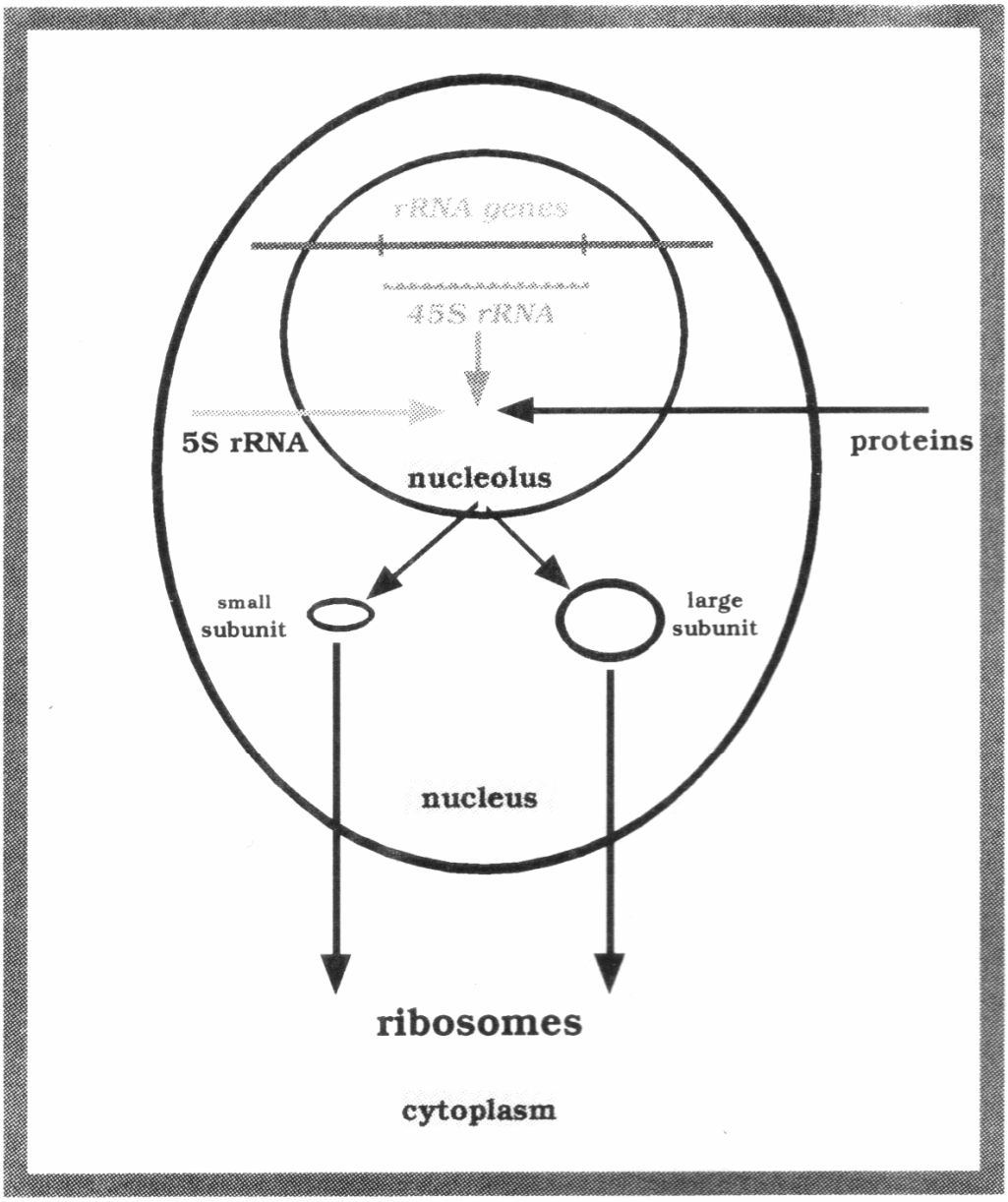


Fig. 2. The function of the nucleolus in ribosome synthesis

The length of the 18S-5.8S-25S rDNA unit varies between plant species, from 7 kbp (*Glycine max*, VARSANYI-BREINER *et al.*, 1979) to 18 kbp (*Trillium apetalon*, YAKURA *et al.*, 1983). In wheat, the rDNA unit, the most frequently used for *in situ* hybridization and known as a pTa71 probe, is about 9 kbp long (GERLACH, BEDBROOK, 1979). The length of rDNA units, composition of sequences and number of copies can vary between species, individual plants and also within a locus. This variation is mainly due to differences in the length of intergenic DNA. In some species, such as flax and soybean, the rDNA units are homogeneous. In others, such as wheat, barley, rye, faba bean, pea and rice, they show length heterogeneity.

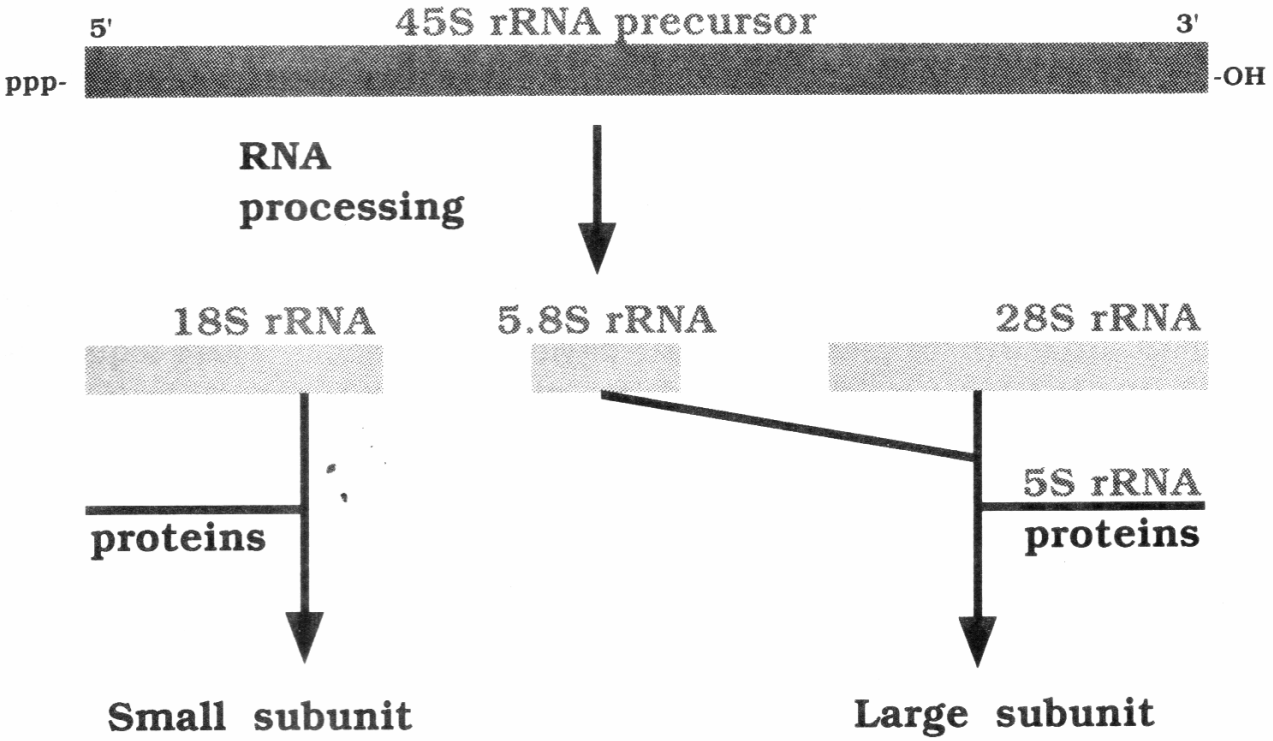


Fig. 3. Processing of preribosomal 45S RNA transcript and formation of large and small ribosomal subunits

In onion and radish they have the same length but sequence heterogeneity. Other species have both length and sequence heterogeneity (FLAVELL, 1986). The heterogeneity of rDNA repeat units at the species level, might be very useful for plant breeding and evolutionary studies (SUBRAHMANYAM *et al.*, 1994; BORISJUK *et al.*, 1997). It is possible to distinguish between different cultivars or wild populations by the restriction enzyme pattern of rDNA.

Relatively high polymorphisms in the rRNA genes within an individual genome have been reported in several conifer species. The size of the rRNA gene unit range between 21 and 40 kbp (BABOLA *et al.*, 1992). Unlike most angiosperms, the rRNA genes in conifers are divided among numerous major and minor loci. In diploid species, with 24 chromosomes, the rDNA was located on 12—16 chromosomes (BROWN *et al.*, 1993; DOUDRICK *et al.*, 1995).

The gene regions of the rDNA unit represent highly conserved sequences. They show substantial sequence homology between plant species, there is also homology between plants and species of other kingdoms. For example a pTa71 probe of rDNA from wheat hybridized with grasshopper chromosomes (LOPEZ-LEON *et al.*, 1994). In contrast, the intergenic region shows sequence divergence between plant species, even closely related ones. The intergenic region contains tandemly repeated sequences with different length in different species, such subrepeat sequences are about 135 bp in wheat (APPELS, DVORAK, 1982) and 325 bp in faba bean (YAKURA, TANIFUJI, 1983).

Study of the rDNA spacer length polymorphism in different population of three grass species indicates that this polymorphism may be of great importance for adaptation of plant populations, and is not just a random variability in multicopy genetic loci (POLONCO, PEREZ de la VEGA, 1995). The rDNA may be useful at different levels of the taxonomic hierarchy studies, depending on what part of the basic unit is investigated.

The internal transcribed spacer region (ITS) of rDNA seems to be one of the most attractive for plant molecular phylogenetic studies. These sequences are transcribed but not incorporated into mature rRNA. Several features of ITS make them very convenient for investigation. They are small and easy to obtain, are present in high copy number with highly conserved flanks and can be amplified by PCR. The ITS sequences have been used for phylogenetic studies in several angiosperm taxa and have shown that they are a particularly valuable resource for plant systematic (BALDWIN *et al.*, 1995).

The number of copies and the structure of rRNA genes, as in phylogenetic studies, was investigated in relation to somaclonal variation among plants regenerated from *in vitro* culture. Sequence changes of rDNA were more frequently reported for non transcribed regions than for the coding region. Several studies have shown a decrease in the number of rRNA genes in plants resulting from tissue cultures of potato and triticale (LANDSMANN, UHRIG, 1985; BRETTELI *et al.*, 1986). In wheat, variation in the organization of non transcribed regions of rDNA has been found in doubled haploid lines and in plants regenerated from callus (RODE *et al.*, 1987; BENSLIMANE *et al.*, 1988; BREIMAN *et al.*, 1987).

The 5S rDNA consists of highly conserved 120 bp coding regions and a variable nontranscribed spacer region of 80-900 bp. The transcription of 5S rDNA is directed by polymerase III. Similarly to 18S-5.8S-25S rDNA, the intergenic spacer exhibits considerable interspecific divergence. The nucleotide sequence of 5S DNA units has been determined in many plants and these data suggest that 5S DNA can provide a good model system for studying the nature of evolutionary changes at a locus composed of tandem arrays of units. The units at a given locus are very similar in the DNA sequences but differences have been observed between loci. Evolutionary studies in the *Triticeae* showed that 5S rRNA genes consist of two size classes, classified as "short" (units ranging from 327 to 469 bp) and "long" (units greater than 469 bp) (BAUM, APPELS, 1992; BAUM, JOHNSON, 1994). These two different repeat units are not intermixed within a tandem array but they form separate clusters, each on separate chromosomes. In most species of *Triticeae*, 5S rRNA genes have been located on homoeologous chromosome group 1 and 5. The short repeat sequences were localized on chromosome 1 and the long on chromosome 5 (APPELS *et al.*, 1992).

The studies of 5S RNA gene structure and copy number in gymnosperms have also shown the presence of two size classes of 5S rDNA. Gene units were about 525 and 850 bp long in *Pinus radiata*. The difference in the two size classes was due to 330 bp insertion in the spacer region of the long unit. Among thirty examined *Pinus* species, the New World species had both short and long units whereas the Old World species had only long units (MORAN *et al.*, 1992).

NOR — chromosomes

Chromosomes with a nucleolar organizer region, NOR chromosomes, possess secondary constriction which makes it easy to distinguish them with conventional cytological methods even among such small chromosomes as chromosomes of *Arabidopsis* (MALUSZYNSKA, HESLOP—HARRISON, 1991). There are specific methods which allow detection of the rDNA loci on chromosomes and interphase nuclei. The following methods of selective staining are most frequently applied:

— FISH — fluorescent *in situ* hybridization — for detection and localization of rDNA sequences on chromosomes and interphase nuclei (MALUSZYNSKA, HESLOP—HARRISON, 1992; LEITCH *et al.*, 1994).
— Ag — staining of NOR — to indicate the activity of rDNA (BLOOM, GOODPASTURE, 1976; HIZUME *et al.*, 1980). Chemical groups responsible for silver staining have not yet been identified with certainty. Most of the nucleolar proteins, which are substrates for silver staining, are phosphoproteins associated with metaphase chromosomes at the NOR which were active at the previous interphase. One of these proteins is e.g. RNA polymerase I (HERNANDEZ-VERDUN *et al.*, 1993). It was shown that the sites of Ag-NOR staining are not within the chromatin itself but lie at the sides of the chromatids (SCHWARZACHER *et al.*, 1978).
— CMA — (fluorescent staining with chromomycin A3) for detection of G+C rich chromosomal segments. It usually stains the NOR region but does not distinguish between NOR and other G−C rich chromosomal segments (SCHWEIZER, AMBROS, 1994).
— N — banding — for NOR staining. In this technique the extraction of nucleic acid and histons from chromosomes is followed by Giemsa staining. It is less sensitive than silver staining (FUNAKI *et al.*, 1975).

Fluorescent *in situ* hybridization provides high resolution for chromosome mapping. Since this technique was adopted knowledge of the location of rDNA on metaphase chromosomes and interphase nuclei has increased significantly.

This is the only method for the localization of 5S rRNA genes. Using this method it is also possible to detect new 18S-5.8S-25S rDNA loci. The application of FISH enabled detection of the rDNA sequences on specific chromosomes such as the B chromosome of *Crepis capillaris* and *Triticum* (MALUSZYNSKA, SCHWEIZER, 1989; FRIEBE *et al.*, 1995). The development and improvement of hybridization and detection methods provide a new possibility to visualise subsequent loci of rDNA. Using immunocytochemical detection of hybridization sites in *B. napus* it was possible to identify six loci of the rDNA (MALUSZYNSKA, HESLOP-HARRISON, 1993), while direct Cy3 labelled DNA probe enabled localization of seven loci (MALUSZYNSKA, HASTEROK, 1997). Similarly, the high sensitivity of the technique enabled the identification of new rDNA loci on tomato chromosomes (XU, EARLE, 1994).

Table 3

The number of rDNA loci at different plant species estimated by *in situ* hybridization

Species	Chromosome number, 2n	18S-5.8S-25S rDNA	5S rDNA	References
Aegilops umbellulata	14	2	1	(1)
Arabidopsis thaliana	10	2	3	(12; 16)
Avena strigosa	14	2	1	(10)
Beta vulgaris	18	2	1	(18)
Brassica campestris	20	5	3	(13)
Brassica nigra	16	2	1	(13)
Brassica oleracea	18	2	2	(13)
Crepis capillaris	6	1	1	(14; 7)
Glycine max	40	1	1	(19)
Gossypium hirsutum	52	11	2	(5)
Hordeum vulgare	14	4	4	(9)
Larix decidua	24	3	1	(11)
Lycopersicon esculentum	24	5	1	(20)
Pennisetum glaucum	14	2	1	(15)
Phaseolus vulgaris	22	4	4	(19)
Picea abies	24	6	1	(11)
Pinus elliottii	24	8	3	(3)
Pinus silvestris	24	7	2	(11)
Secale cereale	14	1	3	(2)
Triticum aestivum	42	8	3	(10)
Vicia faba	12	1	4	(6; 7)
Vigna unguiculata	22	5	2	(4)
Zea mays	20	1	1	(17)

(1) CASTILHO, HESLOP-HARRISON, 1995; (2) CUADRADO *et al.*, 1995; (3) DOUDRICK, 1996; (4) GALASSO *et al.*, 1995; (5) HANSON *et al.*, 1996; (6) HIZUME, 1992; (7) HIZUME, 1993; (8) JIANG, GILL, 1994; (9) LEITCH, HESLOP-HARRISON, 1993; (10) LINARES *et al.*, 1996; (11) LUBARETZ *et al.*, 1996; (12) MALUSZYNSKA, HESLOP-HARRISON, 1991; (13) MALUSZYNSKA, HESLOP-HARRISON, 1993, unpublished; (14) MALUSZYNSKA, SCHWEIZER, 1989; (15) MARTEL *et al.*, 1996; (16) MURATA *et al.*, 1997; (17) McMULLEN *et al.*, 1991; (18) SCHMIDT *et al.*, 1994; (19) SHI *et al.*, 1996; (20) XU, EARLE, 1996.

What is the distribution of rRNA genes within the NOR region? Investigation of double translocation lines of maize, where either the heterochromatin or secondary constriction regions of NOR were duplicated, indicated that the majority of the rRNA genes were located in the NOR heterochromatin. On the basis of this study it was estimated that 70—90% of rRNA genes were in heterochromatin. Heterochromatic DNA is associated with transcriptional inactivity. Apparently, only a small fraction of rRNA genes located in the secondary constriction are transcribed (McMULLEN *et al.*, 1991).

5S rDNA does not occupy a structurally differentiated site on chromosomes and has been shown to have no distinctive staining properties. The distribution of 5S rDNA can be determined by *in situ* hybridization. The 5S rRNA genes are most often located at the telomeric or subtelomeric chromosome region (APPELS *et al.*, 1980) but other positions, for example in tomato — near the centromer — were also reported (LAPITAN *et al.*, 1991). *Arabidopsis* contains one interstitial locus and two loci of 5S rDNA near the centromer (MURATA *et al.*, 1997).

There is no correlation between the number of rDNA and 5S rDNA loci nor between loci number and chromosome number (Tab. 3). Two species, with low and high numbers of chromosomes (*Crepis capillaris*, $2n = 6$ and *Glycine max*, $2n = 40$) possess the same number of loci, one of rDNA and one of 5S rDNA, while *Brassica campestris* ($2n = 20$) has 5 pairs of chromosomes with rDNA sequences and 3 pairs with 5S rDNA.

Nucleolus

The nucleolus is the intracellular site where ribosomal genes (rDNA) are transcribed and where biogenesis of ribosomes occurs at interphase. Besides tandemly repeated rRNA genes, the presence of more than 100 ribosomal proteins, nucleolar proteins and ribonucleoproteins (RNPs) is required for the nucleolar function including RNA polimerase I, DNA topoisomerases, transcription factors, processing enzymes, 5S rRNA and small nucleolar RNAs (snoRNAs).

A functionally active nucleolus is composed of three major structural domains: (i) fibrillar centre — FC — which is the interphasic counterpart of the nucleolar organizer regions (NOR) of metaphase chromosomes, (ii) dense fibrillar component — DFC — usually surrounding FC and apparently containing transcripts of rRNA genes, (iii) a granular component — GC — which is associated with specific proteins and snoRNAs (RASKA, DUNDR, 1993).

The behaviour of the nucleolus in proliferating cells is linked to phases of the cell cycle and is called the nucleolar cycle. When cells enter mitosis and rDNA is not transcribed, nucleoli structurally disintegrate and a major portion of nucleolar proteins and RNAs are translocated to the cytoplasm. A relatively small proportion of nucleolar proteins remains associated with the nucleolus organizing region of mitotic chromosomes. After chromosome segregation, at telophase, the rDNA transcription is reactivated and nucleoli start to reassemble (SCHEER *et al.*, 1993; SHAW, JORDAN, 1995). This process is accompanied by the formation of extranucleolar bodies called the prenucleolar bodies (PNBs). The precise role of PNBs in nucleologenesis is not clear (ZATSEPINA *et al.*, 1997). The pre-rRNA has been found in PNBs of onion telophase cells (MEDINA *et al.*, 1995).

The umber of nucleoli per nucleus can be as high as the number of NOR chromosomes but this is seldom the case, usually the number of nucleoli is much less. There are two ways to explain this observation: either not all NORs are active or more than one NOR takes part in the formation of a nucleolus. In fact, both possibilities may be realized. Numbers of nucleoli have been extensively investigated in wheat. It was observed that four major nucleoli can fuse in different combinations. There were nine combinations, but the frequency of various fusions was different in different genotypes of wheat. This observation raises the question of whether fusion is random or not. The conclusion from wheat study was that genotype may play a major role in determining the pattern of nucleolar fusion. The fusion depends on the length of the cell cycle. The fusion frequency increases and the number of nucleoli per cell is lower in certain genotypes with a longer cell cycle. It should be noted that even when only one major pair of NORs is active the number of nucleoli per nucleus is usually more than one, for example in a substitution line where 1B is replaced by 1U from *Aegilops* and 6B is strongly suppressed (JORDAN *et al.*, 1982). The situation is different in chromocentric nuclei, where, with much less chromatin there is usually a single nucleolus. Cells with one nucleolus dominate in *Brassica* species. However, in *Arabidopsis* meristematic cells four nucleoli are frequently observed in addition to nuclei with three, two and one nucleolus (FRAS, MALUSZYNSKA, unpublished).

The nucleolar volume is variable in different cell types of an individual plant. This can be related to the phase of cell cycle, transcriptional activity and fusion of nucleoli. Usually, nucleolar volume increases during early stages of the cell cycle. The relationship between total nucleolus volume and the number of rRNA genes at the nucleolus organiser has been studied. Results from different wheat genotypes indicate that the volume of the nucleolus, formed at a given NOR in a given cell type, is more closely related to the proportion of the total active rRNA genes than to the absolute number of rRNA genes at

NOR (FLAVELL, O'DELL, 1979; MARTINI, FLAVELL, 1985). There is some evidence that the volume of a nucleolus can be controlled by genes other than rRNA genes (FLAVELL, O'DELL, 1979).

Small spherical bodies associated with the nucleolus known as nucleolus associated bodies or karyosomes or micronucleoli, can be observed in the interphase nucleus of many plant species. These bodies are related to the nucleolar chromosomes and mainly consist of different ribonuloproteins (CHAMBERLAND, LAFONTAINE, 1993). Such structures have also been observed in *Arabidopsis* nuclei of meristematic and callus cells as well (MALUSZYNSKA, unpublished).

rDNA activity

The number of rRNA genes in plants is considerably greater than that required to sustain ribosome synthesis. This large number of rRNA genes raises many questions such as: why are there so many genes, how do the genes evolve, which genes are selected to be active from the gene family? The total nucleolus activity in the cell is regulated in response to the need for ribosomes. There are many genes involved in this regulation at the different levels of RNA transcription, RNA processing, protein and 5S RNA accumulation and ribosome formation. The activity of the rRNA genes at each NOR is reflected in the size of the nucleolus and on this basis it is possible to estimate gene activity by cytological observation. This is an advantage which is not available for many other genes and makes this set of genes especially useful for studying the activity of a chromosomal locus and control of its expression.

In species with more than one rDNA locus variability can be observed between nonhomologous NORs. Selective silver staining is used to distinguish between active and inactive rRNA gene clusters while *in situ* hybridization localises all the rDNA sequences. Sequential use of both methods allows us to distinguish active and inactive loci in the same cell (HASTEROK, MALUSZYNSKA, 1998). In species with numerous rDNA loci many of them are non active. For example, in *B. campestris* 10 among 20 chromosomes bear rRNA genes of which only 2 NORs are active, in *Brassica napus* from 14 sites of rDNA only 4 are active. The size of the silver deposit on the NOR is positively correlated to the degree of transcriptional activity of rDNA loci.

The amplification of rDNA repeats has also been reported (LLOYD *et al.*, 1994; SUBRAHMANYAM *et al.*, 1994). During vascular cambium reactivation in *Abies balsamea* the relative rRNA genes content and nucleolar volume increased transiently prior to cambial cell division. This amplification

of the number of rRNA gene copies is probably necessary because of slower rates of transcription and translation in the cold temperature of early spring. The increase in relative content of rDNA and nucleolar activity were delayed in the tree in which reactivation was late (LLOYD *et al.*, 1994).

Greater activity of one rDNA locus compared to another in the same cell (nucleolar dominance) is observed in many species with multiple loci of rRNA genes. The comparison of the activity of rRNA loci in aneuploid and substitution lines of the Chinese Spring variety of hexaploid wheat has shown that the 1B locus is dominant to the 6B which is dominant to the 5D (MARTIN, FLAVELL, 1985). When chromosome 1U from *Aegilops umbellulata* was introduced into wheat its rDNA locus was dominant over all the wheat rRNA loci. This dominance is not due to a larger number of rRNA genes at the locus. The relative activity of rDNA loci is correlated with the number of 135 bp repeats that lie upstream from the gene promoter, the locus with, the greater activity is the one with more these repeats (FLAVELL *et al.*, 1990; 1993).

Chromosomal dominance was also observed in barley. The NOR 6 (1600 of rRNA genes) organizes a larger nucleolus than NOR7 (2 600 of rRNA genes). When both these NORs were combined in one chromosome through translocation, the activity of NOR7 was reduced (ANASTASSOVA-KRISTEVA *et al.*, 1980; SUBRAHMANYAM *et al.*, 1994).

The nucleolar dominance in interspecific hybrids or allopoliploid species is widespread, but the epigenetic phenomenon leading to the formation of a nucleolus around the rRNA genes of only one parent is poorly understood. The nucleolar competition which can occur in interspecific hybrid is called differential amphiplasty. This phenomenon was first described by NAVASHIN (1934) in some interspecific hybrid of *Crepis*. The secondary constriction of the SAT-chromosome of one parental species is lacking in the hybrid, thus the satellite is retracted onto the chromosome and is not distinguishable (WALLACE, LANGRIDGE, 1971). Differential amphiplasty has been reported in natural allopoliploid species such as wheat and artificial amphiploids such as triticale. The behaviour of rye rRNA genes in wheat x rye hybrid has been investigated by several authors. It has been determined that rye rRNA genes in a wheat background show significantly less activity than in diploid rye. The suppression of rye rDNA activity is because the rye NOR locus contains shorter rDNA spacer than those of wheat (LACADENA *et al.*, 1984; GUSTAFSON *et al.*, 1988).

CHEN and co-workers (1996), on the basis of molecular analysis of rDNA have shown nucleolar dominance in *Brassica* genus. In *B. carinata* there is a dominance of rRNA genes from *B. nigra* over genes of *B. oleracea*. In both natural and synthetic *B. napus* the rRNA genes of *B. campestris* dominate over genes of *B. oleracea*. In cytogenetic analysis of *Brassica* species we did

not observe such dominance. In all allotetraploid species the number of active rDNA loci was equal to the sum of active loci in ancestral species. In *B. oleracea*, 2 out of 4 loci of rRNA genes were active; in *B. campestris* 2 out of 10 loci were active and in *B. napus* 4 loci were active. Similar results were obtained for the hybrid *B. oleracea* x *B. campestris* — a synthetic rapeseed (MALUSZYNSKA, HASTEROK, 1998; HASTEROK, unpublished).

The difference between results of molecular analysis and cytogenetic observations can be due to different developmental stages of tissues used for analysis. The DNA for molecular analysis was isolated from leaves, from differentiated tissue, while chromosomal analysis was done in root meristematic cells of seedlings. This explanation can be supported by observing different NOR activity in meristematic cells of different root types of *Allium cepa*. In primary roots obtained from germinated seeds, 4 strong Ag-NORs were detected while in cells of adventitious roots, from bulbs, only one pair of rRNA genes loci was active (HASTEROK, MALUSZYNSKA, 1998). Other investigations have shown a different rDNA expression during plant development. In normal wheat and rye plants the rDNA genes are active at least up to late gametogenesis but in wheat x rye F_1 hybrids inactivation of the rye rDNA occurs during embryogenesis, 4-5 days after pollination (NEVES *et al.*, 1995). Similar results were obtained for triticale embryo development. In normal triticale plants rye-origin rDNA is inactive. During microsporgenesis the inactivation undergoes meiotic reprogramming which leads to the expression of rye rRNA genes in pollen grain (SILVA *et al.*, 1995).

DNA methylation in rRNA genes

There is substantial evidence that the activity of some genes is correlated with modifications of specific DNA bases, among which the most frequent is the methylation of cytosine. Up to 20–30% of the cytosine is methylated in nuclear plant genomes (RICHARDS, 1997). It has been shown that methylation of the cytosine influences the activity of rDNA in many animals and plants. In wheat, rRNA gene expression depends on rDNA methylation and on the structure of the intergenic region between 18S and 25S in the rDNA repeat unit. FLAVELL *et al.* (1993) postulated that more actively transcribed wheat rDNA loci have longer rDNA intergenic spacer and a higher number of genes with more unmethylated cytosine sites. Longer rDNA intregenic spacer can provide more binding sites for proteins which protect against methylation and for factor allowing transcription. Sites where proteins are not bound become methylated and genes without regulatory proteins are condense into hetero-

chromatin and the genes are thus removed from nucleolus formation. The authors propose a model to explain the differential expression of rRNA genes.

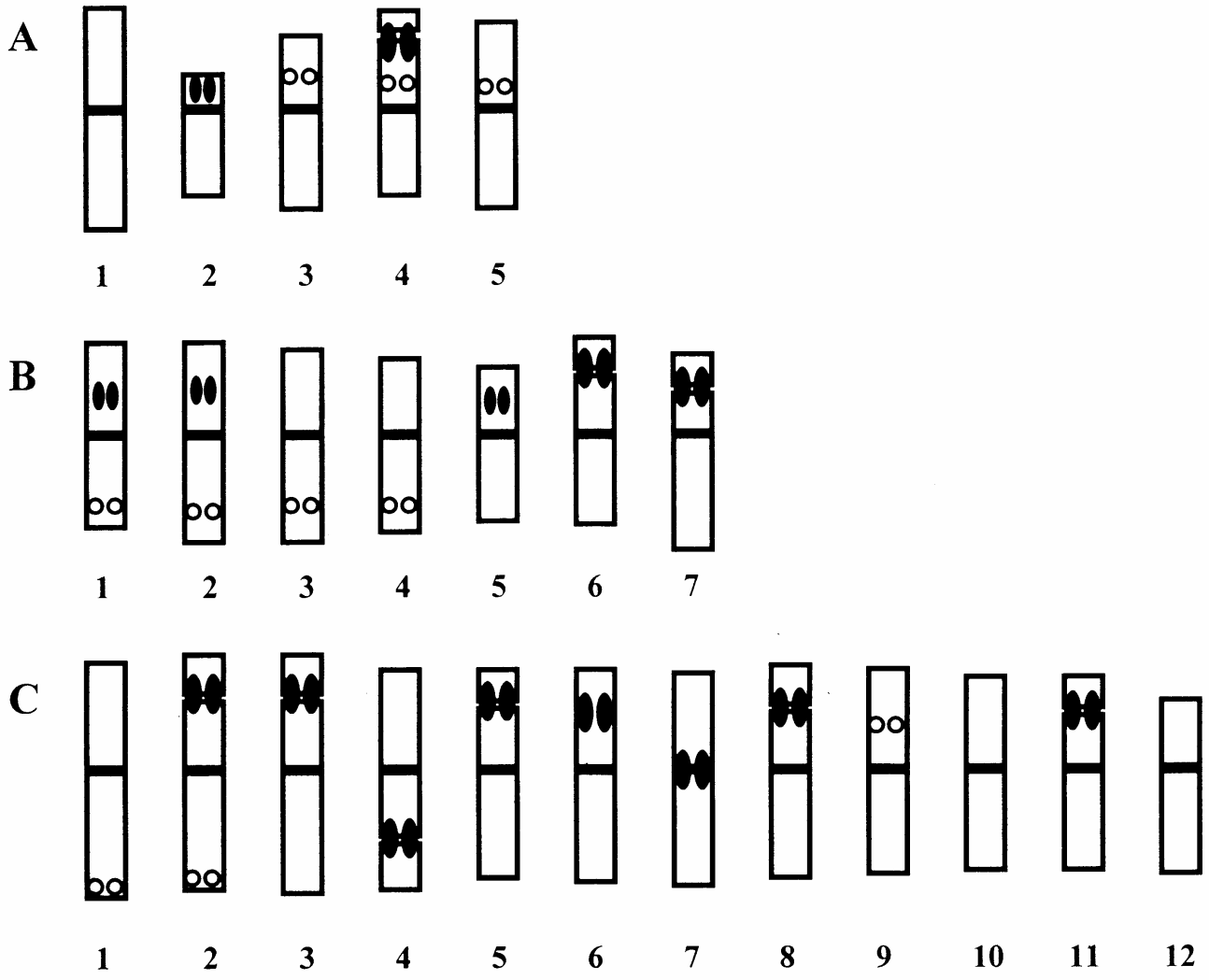
The drug 5-azacytidine (5-AC), a structurally modified cytidine analogue, when incorporated into a newly synthesized DNA strand leads to its undermethylation and a subsequent gene activation. 5-AC is known to cause activation of silent genes, decondensation of chromatin, and affects the time and level of DNA synthesis. The presence of 5-AC during germination of wheat-rye hybrid allowed the expression of the nucleolus organizer region belonging to the rye genome which is normally inactive in a wheat background (VIEIRA *et al.*, 1990). When hybrids were treated with 5-AC shortly after pollination the activity of rye origin rDNA loci was present in the resulting seedlings (NEVES *et al.*, 1995). This strongly suggests that by unmethylation of cytosines the phenomenon of differential expression and nucleolar dominance are removed.

rDNA as a chromosome marker

NOR-chromosomes can be distinguished easier than many other chromosomes in the complement even in very altered karyotypes of long term callus culture. It was reported that secondary constriction is a common breakage point in the chromosome. In several investigations of tissue cultures of *Crepis*, the NOR chromosomes were most frequently involved in the different chromosomal rearrangements (SACRISTAN, 1971; ASHMORE, GOULD, 1981; MALUSZYNSKA, 1990).

The 5S and 18S-5.8S-26S gene loci provide useful chromosome markers to examine genome evolution or chromosome variation in tissue culture *in vitro*. DNA-DNA *in situ* hybridization has become the method of choice for localizing these sequences, especially where multiple loci cannot be differentiated by restriction fragment length polymorphism. Simultaneous *in situ* hybridization of two rDNA probes, 5S rDNA (pTa794) and main rDNA (pTa71) allow identification of each arm of each *Hordeum* chromosome (LEITCH, HESLOP-HARRISON, 1993), *Arabidopsis*, (MALUSZYNSKA, unpublished; MURATA *et al.*, 1997) or *Pinus* (DOUDRICK, 1996) (Fig. 4). Comparative mapping of rRNA genes on chromosome 1 and 5 within *Triticeae* has shown multiple and complex evolutionary rearrangement of the chromosome arms (CASTILHO, HESLOP-HARRISON, 1995).

rDNA is the first chromosome marker to be used in an investigation of nucleus architecture (HESLOP-HARRISON, BENNETT, 1990; ROWLINS,



Legend:

●● - 18S-5.8S-25S rRNA loci

oo - 5S rRNA loci

Fig. 4. Localization of 18S-5.8S-25S and 5S rDNA on the chromosomes of A — *Arabidopsis thaliana*, B — *Hordeum vulgare*, C — *Pinus elliottii* (from: MURATA *et al.*, 1997; LEITCH, HESLOP-HARRISON, 1993; DOUDRICK, 1996; modified)

SHAW, 1990; LEITCH *et al.*, 1992; JORDAN *et al.*, 1992; LEITCH, HESLOP-HARRISON, 1993). The position of individual or pair of chromosomes in interphase nuclei is one of the most difficult problems in genome structure investigation. Whether chromosomes lie in random positions with respect to each other or have special compartments is still an open question. One such compartment is the nucleolus, a well defined structure with a well defined function, in which many copies of the rRNA gene are transcribed, where the 45S primary transcript is processed and combined with the 5S transcripts. The nucleolus is therefore an ideal system for studying transcrip-

tion, transcript processing and transport. It is the only structure in the interphase nucleus where gene expression can be investigated at both the molecular and cytogenetic level.

References

ANASTASSOVA-KRISTEVA M., RIEGER R., KUNZEL G., NICOLOFF H., HAGBERG A. 1980. Further evidence on "nucleolar dominance" in barley translocation lines. Barley Genet. Newsl., **10**: 3—6.

APPELS R., GERLACH W. L., DENNIS E. S., SWIFT H., PEACOCK W. J. 1980. Molecular and chromosomal organization of DNA sequences coding for the ribosomal RNAs in cereals. Chromosoma, **78**: 293—311.

APPELS R., BAUM B. R., CLARKE B. C. 1992. The 5S DNA units of bread wheat (*Triticum aestivum*). Plant Syst. Evol., **183**: 183—194.

APPELS R., DVORAK J. 1982. The wheat ribosomal DNA spacer region: Its structure and variation in populations and among species. Theor. Appl. Genet., **63**: 337—348.

ASHMORE S. E., GOULD A. R. 1981. Karyotype evolution in a tumour derived plant tissue culture analysed by Giemsa C-banding. Protoplasma, **106**: 297—308.

BABOLA M. S., SMITH D. E., KLEIN S. A. 1992. Five major nuclear ribosomal repeats represent a large and variable fraction of the genomic DNA of *Picea rubens* and *P. mariana*. Molecular Biology and Evol., **9**: 125—137.

BALDWIN B. G., SANDERSON M. J., PORTER J. M., WOJCIECHOWSKI M. F., CAMPBELL C. S., DONOGHUE M. J. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. Ann. Missouri Bot. Gard., **82**: 247—277.

BAUM B. R., APPELS R. 1992. Evolutionary change at the *5S Dna* loci of species in the Triticeae. Plant Syst. Evol., **183**: 195—208.

BAUM B. R., JOHNSON D. A. 1994. The molecular diversity of the 5S rRNA gene in barley (*Hordeum vulgare*). Genome, **37**: 992—998.

BENNETT M. D., LEITCH I. J. 1995. Nuclear DNA amounts in angiosperms. Annals of Botany, **76**: 113-176.

BENSLIMANE A., HARTMANN C., De-BUYSER J., HENRY Y., PICARD E., RODE A. 1988. Ribosomal DNA as a convenient probe to follow segregation and possible divergency from expected homozygosity after haploidization of an androgenetic process. Theor. Appl. Genet., **75**: 389—396.

BIRNSTIEL M. L., WALLACE H., SIRLIN J. L., FISCHBERG M. 1966. Localization of the ribosomal DNA components in the nucleolar organizer region of *Xenopus laevis*. Natl. Cancer Inst. Monogram, **23**: 431—447.

BLOOM S. E., GOODPASTURE C. 1976. An improved technique for selective silver staining of nucleolar organizer regions in human chromosomes. Hum. Genet., **34**: 199—206.

BORISJUK N. V., DAVIDJUK Y. M., KOSTISHIN S. S., MIROSHNICHENCO G. P., VELASCO R., HEMLEBEN V., VOLKOV R. A. 1997. Structural analysis of rDNA in the genus *Nicotiana*. Plant Mol. Biol., **35**: 655—660.

BREIMAN A., FELSENBURG T., GALUN E. 1987. Nor loci analysis in progenies of plants regenerated from the scutellar callus of bread-wheat. A molecular approach to evaluate somaclonal variation. Theor. Appl. Genet., **73**: 827—831.

BRETTELL R. I. S., PALLOTTA M. A., GUSTAVSON J. P., APPELS R. 1986. Variation at the *Nor* loci in triticale derived from tissue culture. Theor. Appl. Genet., **71**: 637—643.

BROWN G. R., AMARASINGHE V., KISS G., CARLSON J. E. 1993. Preliminary karyotype and chromosomal localization of ribosomal DNA sites in white spruce using fluorescence *in situ* hybridization. Genome, **36**: 310—316.

CASTILHO A., HESLOP-HARRISON J. S. 1995. Physical mapping of 5S and 18-5.8S-25S rDNA and repetitive DNA sequences in *Aegilops umbellulata*. Genome, **38**: 91-96.

CAMPELL B. C., SONG Y., POSCH T. E., CULLIS C. A., TOWN C. D. 1992. Sequence and organization of 5S ribosomal RNA-ecoding genes of *Arabidopsis thaliana*. Gene, **112**: 225—228.

CHAMBERLAND H., LAFONTAINE J. G. 1993. Localization of snRNPs antigens in nucleolus-associated bodies: study of plant interphase nuclei by confocal and electron microscopy. Chromosoma, **102**: 220—226.

CHEN Z. J., PIKAARD C. S. 1996. Nucleolar dominance in *Arabidopsis* and *Brassica* polyploids is due to reversible, genotypebiased rRNA gene silencing enforced by DNA methylation. Abstracts, 7th International Conference on Arabidopsis Research, UEA, Norwich, S89.

CUADRADO A., CEOLONI C., JOUVE N. 1995. Variation in highly repetitive DNA composition of heterochromatin in rye studied by fluorescence *in situ* hybridization. Genome, **38**: 1061—1069.

DOUDRICK R. L. 1996. Genetic recombinational and physical linkage analysis on slash pine. Unifying Plant Genomes. HESLOP-HARRISON J. S. (Ed.). SEB, Cambridge: 53—60.

DOUDRICK R. L., HESLOP-HARRISON J. S., NELSON C. D., SCHMIDT T., NANCE W. L., SCHWARZACHER T. 1995. Karyotype of slash pine (*Pinus elliottii* var. *elliottii*) using patterns of fluorescence *in situ* hybridization and fluorochrome banding. J. Hered, **86**: 286—296.

FLAVELL R. B. 1986. The structure and control of expression of ribosomal RNA genes. Oxford Surveys of Plant Molecular & Cell Biology, **3**: 251—274.

FLAVELL R. B., SARDANA R., JACKSON S., O'DELL M. 1990. The molecular basis of variation affecting gene expression: evidence from studies on the ribosomal RNA gene loci of wheat. In: Gene Manipulation in Plant Improvement II. GUSTAFSON J. P. (Ed.). Plenum Press, New York: 419—430.

FLAVELL R. B., SMITH D. B. 1974. Variation in nucleolar organiser rRNA gene multiplicity in wheat and rye. Chromosoma, **47**: 327-334.

FLAVELL R. B., O'DELL M., SARDANA R., JACKSON S. 1993. Regulatory DNA of ribosomal RNA genes and control of nucleolus organizer activity in wheat. Crop Sci., **33**: 889—894.

FLAVELL R. B., O'DELL M. 1979. The genetic control of nucleolus formation in wheat. Chromosoma, **71**: 135—152.

FRIEBE B., JIANG J., GILL B. 1995. Detection of 5S rDNA and other repeated DNA on supernumerary B chromosomes of Triticum species (*Poaceae*). Plant Syst. Evol., **196**: 131—139.

FUNAKI K., MATSUI S., SASAKI M. 1975. Localization of nucleolar organizers in animal and plant chromosomes by means of an improved N-banding technique. Chromosoma, **49**: 357—370.

GALASSO I., SCHMIDT T., PIGNONE D., HESLOP-HARRISON J. S. 1995. The molecular cytogenetics of *Vigna unguiculata* (L.) WALP: the physical organization and characterization of 18-5.8S-25S rRNA genes, 5S rRNA genes, telomere-like sequences, and a family of centromeric repetitive DNA sequences. Theor. Appl. Genet., **91**: 928—935.

GERLACH W. L., BEDBROOK J. R. 1979. Cloning and characterization of ribosomal RNA gene from wheat and barley. Nucl. Acids Res., **7**: 1869—1885.

GOLDSBOURGH P. B., ELLIS T. H. N., CULLIS C. A. 1981. Organization of the 5SRNA genes in flax. Nucleic. Acids Res., **9**: 5895—5903.

GUSTAFSON J. P., DERA A. R., PETROVIC S. 1988. Expression of modified rye ribosomal RNA genes in wheat. Proc. Natl. Acad. Sci. USA, **85**: 3943—3945.

HANSON R. E., ISLAM-FARIDI M. N., PERCIVAL E. A., CRANE C. F., Ji Y., McKNIGHT T. D., STELLY D. M., PRICE H. J. 1996. Distribution of 5S and 18S-28S rDNA loci in a tetraploid cotton (*Gossypium hirsutum* L.) and its putative diploid ancestors. Chromosoma, **105**: 55—61.

HASTEROK R., MALUSZYNSKA J. 1998. Sequential silver staining and FISH — their use to distinguish active from inactive rDNA loci. Plant Cytogenetics. MALUSZYNSKA J. (Ed.). Univ. of Silesia Press: 168—172.

HEMLEBEN V., BACHMANN K., PRICE H. L. 1978. Ribosomal gene numbers in *Microseridinae* (*Composite: Cichorieae*). Experientia, **34**: 1452.

HEMLEBEN V., WERTS D. 1988. Sequence organization and putative regulatory elements in the 5S rRNA genes of two higher plants (*Vigna radiata* and *Matthiola incana*). Gene, **62**: 165—169.

HERNANDEZ-VERDUN D., ROUSSEL P., GAUTIER T. 1993. Nucleolar proteins during mitosis. In: Chromosomes Today. SUMNER A. T., CHANDLEY A. C. (Eds.). Chapman & Hall, London: 79—90.

HESLOP-HARRISON J. S., BENNETT M. D. 1990. Nuclear architecture in plants. Trends in Genet., **6**: 401—405.

HIZUME M., SATO S., TANAKA A. 1980. A highly reproducible method of nucleolus organizer regions staining in plants. Stain Technology, **55**: 87—90.

HIZUME M. 1992. Exact location of rRNA genes in *Vicia faba* chromosomes. Cytologia, **57**: 471—475.

HIZUME M. 1993. Chromosomal localization of 5S rRNA genes in *Vicia faba* and *Crepis capillaris*. Cytologia, **58**: 417—421.

INGLE J., TIMMIS J. N., SINCLAIR J. 1975. The relationship between satellite deoxyribonucleic acid, ribosomal RNA gene redundancy and genome size in plants. Plant Physiol., **55**: 496—501.

JIANG J., GILL S. 1994. New 18S-26S ribosomal RNA gene loci: chromosomal landmarks for the evolution of polyploid wheats. Chromosoma, **103**: 179—185.

JORDAN E. G., MARTIN G., BENNETT M. D., FLAVELL R. B. 1982. Nucleolar fusion in wheat. J. Cell Sci., **56**: 485—495.

JORDAN E. G., ZATSEPINA O. V., SHAW P. J. 1992. Widely dispersed DNA within plant and animal nucleoli visualised by 3-D fluorescence microscopy. Chromosoma, **101**: 478—482.

LACADENA J. R., CERMENO M. C., ORELLANO J., SANTOS J. L. 1984. Evidence for wheat-rye nucleolar competition (amphiplasty) in triticale by silver-staining procedure. Theor. Appl. Genet., **67**: 207—213.

LANDSMANN J., UHRIG H. 1985. Somaclonal variation in *Solanum tuberosum* detected at the molecular level. Theor. Appl. Genet., **71**: 500—505.

LAPITAN N. L. V., GANAL M. W., TANKSLEY S. D. 1991. Organization of the 5S ribosomal RNA genes in the genome of tomato. Genome, **34**: 509—514.

LEITCH A. R., MOSGOLLER W., SHI M., HESLOP-HARRISON J. S. 1992. Different patterns of rDNA organization at interphase in nuclei of wheat and rye. J. Cell Sci., **101**: 751—757.

LEITCH A. R., HESLOP-HARRISON J. S. 1993. Ribosomal RNA gene expression and localization in cereales. Chromosomes Today, **11**: 91—100.

LEITCH A. R., SCHWARZACHER T., JACKSON D., LEITCH I. J. 1994. *In situ* hybridization. BIOS Scientific, Oxford.

LEITCH I. J., HESLOP-HARRISON J. S. 1993. Physical mapping for four sites of 5S rDNA sequences and one side of the alfa-amylase gene in barley (*Hordeum vulgare*). Genome, **36**: 517—523.

LINARES C., GONZALEZ J., FERRER E., FOMINAYA A. 1996. The use of double fluorescence *in situ* hybridization to physically map the position of 5S rDNA genes in relation to the chromosomal location of 18S-5.8S-26S rDNA and a C genome specific DNA sequence in the genus *Avena*. Genome, **39**: 535—542.

LLOYD A. D., MELLEROWICZ E. J., CHOW C. H., RIDING R. T., LITTLE C. H. A. 1994. Fluctuations in ribosomal RNA gene content and nucleolar activity in the cambial region of *Abies balsamea* (*Pinaceae*) shoots during reactivation. American Journ. of Botany, **81**: 1384—1389.

LOPEZ-LEON M. D., NEVES N., SCHWARZACHER T., HESLOP-HARRISON J. S., HEWITT G. M., CAMACHO J. P. M. 1994. Possible origin of B chromosome deduced from its DNA composition using double FISH technique. Chromosome Research, **2**: 87—92.

LUBARETZ O., FUCHS J., AHNE R., MEISTER A., SCHUBERT I. 1996. Karyotyping of three *Pinaceae* species via fluorescent *in situ* hybridization and computer-aided chromosome analysis. Theor. Appl. Genet., **92**: 411—416.

MALUSZYNSKA J. 1990. B chromosomes of *Crepis capillaris* (L.) WALLER. *in vivo* and *in vitro*. Univ. of Silesia Press, Katowice.

MALUSZYNSKA J., HASTEROK R. 1997. Molecular cytogenetic studies in *Brassicaceae* family. Current Topics in Plant Cytogenetics Related to Plant Improvement. LELLEY T. (Ed.). Tulln. WUV — Wien: 250—257.

MALUSZYNSKA J., HESLOP-HARRISON J. S. 1991. Localization of tandemly repeated DNA sequences in *Arabidopsis thaliana*. Plant J., **1**: 159—166.

MALUSZYNSKA J., HESLOP-HARRISON J. S. 1992. Molecular cytogenetics of *Arabidopsis*. In: EMBO/EEC Advanced Practical Laboratory Course on Arabidopsis Molecular Genetics. Anonymous Max-Planck-Institut für Züchtungsforschung, Cologne: 10—18.

MALUSZYNSKA J., HESLOP-HARRISON J. S. 1993. Physical mapping of rDNA loci in *Brassica* species. Genome, **36**: 774—781.

MALUSZYNSKA J., SCHWEIZER D. 1989. Ribosomal RNA genes in B chromosomes of *Crepis capillaris* detected by non-radioactive *in situ* hybridization. Heredity, **62**: 59—65.

MARTEL E., RICROCH A., SARR A. 1996. Assessment of genome organization among diploid species ($2n = 2x = 14$) belonging to primary and tertiary gene pools of pearl millet using fluorescent *in situ* hybridization with rDNA probes. Genome, **39**: 680—687.

MARTINI G., FLAVELL R. 1985. The control of nucleolus volume in wheat, a genetic study at three developmental stages. Heredity, **54**: 111—120.

McCLINTOCK B. 1934. The relation of a particular chromosomal element to the development of the nucleoli in *Zea mays*. Z. Zellforsch. Mikr. Anat., **21**: 294—328.

McMULLEN M. D., PHILLIPS R. L., RUBENSTEIN I. 1991. Molecular analysis of the nucleolus organizer region in mize. In: Chromosome Engineering in Plants: Genetics, Breeding, Evolution. GUPTA P. K., TSUCHIYA T. (Eds.). Elsevier, Amsterdam—Oxford—New York: 561—576.

MEDINA F. J., CERDIDO A., FERNANDEZ-GOMEZ M. E. 1995. Components of the nucleolar processing complex (pre-rRNA, fibrillarin and nucleolin) colocalize during mitosis and are incorporated to daughter cell nucleoli. Exp. Cell Res., **221**: 111—125.

MORAN G. F., SMITH D., BELL J. C., APPELS R. 1992. The 5S RNA genes in *Pinus radiata* and the spacer region as a probe for relationships between *Pinus* species. Plant Syst. Evol., **183**: 209—221.

MURATA M., HESLOP-HARRISON J. S., MOTOYOSHI F. 1997. Physical mapping of the 5S ribosomal RNA genes in *Arabidopsis thaliana* by multi-color fluorescence *in situ* hybridization with cosmid clones. Plant J., **12**: 31—37.

NAVASHIN M. 1934. Chromosome alterations caused by hybridization and their bearing upon ceration general genetic problems. Cytologia, **5**: 169—203.

NEVES N., HESLOP-HARRISON J. S., VIEGAS W. 1995. rRNA gene activity and control of expression mediated by methylation and imprinting during embryo development in wheat x rye hybrids. Theor. Appl. Genet., **91**: 529—533.

POLONCO C., PEREZ de la VEGA M. 1995. Length polymorphism in the ribosomal DNA intergenic spacer of rye and slender wild oats. J. Hered., **86**: 402—407.

PRUITT R. E., MEYEROWITZ E. M. 1986. Characterization of the genome of *Arabidopsis thaliana*. J. Mol. Biol., **187**: 169—183.

RASKA I., DUNDR M. 1993. Compartmentalization of the cell nucleus: case of the nucleolus. Chromosomes Today, **11**: 101—119.

RAWLINS D. J., SHAW P. J. 1990. Three dimensional organization of ribosomal DNA in interphase nuclei of *Pisum sativum* by *in situ* hybridization and optical tomography. Chromosoma, **99**: 143—151.

RICHARDS E. J. 1997. DNA methylation and plant development. Trends in Genet., **13**: 319—323.

RITOSSA F. M., SPIEGELMAN S. 1965. Localization of DNA complementary to ribosomal RNA in the nucleolus organizer region of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA, **82**: 7010—7014.

RODE A., HARTMANN C., BENSLIMANE A., PICARD E., QUETIER F. 1987. Gametoclonal variation detected in the nuclear ribosomal DNA from doubled haploid lines of a spring wheat (*Triticum aestivum* L., cv. 'Cesar'). Theor. Appl. Genet., **74**: 31—37.

SACRISTAN M. D. 1971. Karyotypic changes in callus cultures from haploid and diploid plants of *Crepis capillaris* (L.) WALLER. Chromosoma, **33**: 273—293.

SCHEER U., THIRY M., GOESSENS G. 1993. Structure, function and assembly of the nucleolus. Trends Cell Biol., **3**: 236—241.

SCHMIDT T., SCHWARZACHER T., HESLOP-HARRISON J. S. 1994. Physical mapping of rRNA genes by fluorescent *in situ* hybridization and structural analysis of 5S rRNA and intergenic spacer sequences in sugar beet (*Beta vulgaris*). Theor. Appl. Genet., **88**: 629—636.

SCHNEEBERGER R. G., CULLIS C. A. 1992. Intraspecific 5S rRNA gene variation in flax *Linum usitatissimum* (*Linaceae*). Plant Syst. Evol., **183**: 265—280.

SCHWARZACHER H. G., MIKELSAAR A. V., SCHNEDL W. 1978. The nature of the Ag-staining of nucleolus organizer regions: electron- and light-microscopic studies on human cells in interphase, mitosis and meiosis. Cytogenet. Cell Genet., **20**: 24—39.

SCHWEIZER D., AMBROS P. 1994. Chromosome banding. Stain combination for specific regions. In: Vol. 29 Chromosome Analysis Protocols. GOSDEN J. R. (Ed.). Humana Press Inc. Totowa, NJ: 97-112.

SHAW P. J., JORDAN E. G. 1995. The nucleolus. Annu. Rev. Cell Dev. Biol., **11**: 93—121.

SHI L., ZHU T., KEIM P. 1996. Ribosomal RNA genes in soyabean and common bean: chromosomal organization, expression, and evolution. Theor. Appl. Genet., **93**: 136—141.

SILVA M., QUEIROZ A., NEVES N., BARAO A., CASTILHO A., MORAIS-CECILIO L., VIEGAS W. 1995. Reprogramming of rye rDNA in triticale during microsporogenesis. Chromosome Research, **3**: 492—496.

SUBRAHMANYAM N. C., BRYNGELSSON T., HAGBERG P., HAGBERG A. 1994. Differential amplification of rDNA repeats in barley translocation and duplication lines: role of a specific segment. Hereditas, **121**: 157—170.

VARSANYI-BREINER A., GUSELLA J. F., KEYS C., HOUSMAN D. E., SULLIVAN D., BRISSON N., VERMA D. P. S. 1979. The organization of a nuclear DNA sequence from a higher plant. Molecular cloning and characterisation of soyabean ribosomal DNA. Gene, **7**: 317—334.

VIEIRA R., QUEIROZ A., MORAIS L., BARAO A., MELLO-SAMPAYO T., VIEGAS W. S. 1990. 1R chromosome nucleolus organizer region activation by 5-azacytidine in wheat x rye hybrids. Genome, **33**: 707—712.

WALLACE H., LANGRIDGE W. H. R. 1971. Differential amphiplasty and the control of ribosomal RNA synthesis. Heredity, **27**: 1—13.

XU J., EARLE E. D. 1994. Direct and sensitive fluorescence *in situ* hybridization of 45S rDNA on tomato chromosomes. Genome, **37**: 1062—1065.

XU J., EARLE E. D. 1996. High resolution physical mapping of 45S (5.8S, 18S and 25S) rDNA gene loci in the tomato genome using a combination of karyotyping and FISH of pachytene chromosomes. Chromosoma, **104**: 545—550.

YAKURA K., KATO A., TANIFUJI S. 1983. Structural organisation of ribosomal DNA in four *Trillium* species and *Paris verticillata*. Plant and Cell Physiol., **24**: 1231—1240.

YAKURA K., TANIFUJI S. 1983. Molecular cloning and restriction analysis of EcoR1 fragments of *Vicia faba* rDNA. Plant and Cell Physiol., **24**: 1327—1330.

ZATSEPINA O. V., DUDNIC O. A., TODOROV I. T., THIRY M., SPRING H., TRENDELENBURG M. F. 1997. Experimental induction of prenucleolar bodies (PNBs) in interphase cells: interphase PNBs show similar characteristics as those typically observed at telophase of mitosis in untreated cells. Chromosoma, **105**: 418—430.

Spring Symposium
Cieszyn 19—22 May 1997

PLANT CYTOGENETICS

Edited by

Jolanta Maluszynska

WYDAWNICTWO UNIWERSYTETU ŚLĄSKIEGO
KATOWICE 1998