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Evidence for wheat-rye nucleolar competition (amphiplasty) in triticale by silver-staining procedure

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Summary. Amphiplasty in hexaploid triticale, the artificial amphiploid of tetraploid wheat and diploid rye, is analyzed for the first time using a modified, highly reproducible, silver-staining procedure. A comparative analysis of metaphase somatic cells by phase contrast, C-banding and silver-staining of the hexaploid triticale cv. 'Cachirulo' and its parents, namely, the tetraploid durum wheat cv. 'Enano de Andujar' and the diploid rye cv. 'Petkus' has been made. Two silver-stained nucleolar organizer regions (Ag–NORs) (the chromosome pair 1 R) are observed in all rye plants analyzed, whereas four Ag–NORs (chromosome pairs 1 B and 6 B) are found both in the tetraploid wheat parent and in the triticale. The rye Ag–NORs are absent in the triticale. Since the Ag-staining reaction of NORs can be considered as an indication for genetic activity, the silver procedure can be used to visualize gene functionality at the rDNA sites with conventional light microscopy and, consequently, the modified Ag-staining method described can be very useful in analyzing the amphiplasty phenomenon in natural or artificial hybrid combinations and derivatives in the *Triticum* group and its relatives.

Key words: Amphiplasty – Nucleolar competition – C-banding – rDNA – Nucleolar organizer – Silver staining – Triticale

Introduction

Amphiplasty is the term proposed to denote morphological changes which occur in chromosomes following interspecific hybridization. Changes affecting individual chromosomes of the complement are called differential amphiplasty (Rieger et al. 1976). This phenomenon was first described by Navashin (1928, 1934) in some interspecific hybrids of *Crepis:* the secondary constriction of the SAT-chromosome of one of the parental species is lacking in the hybrid, thus the satellite is retracted onto the chromosome and is not distinguishable. McClintock (1934) – who introduced the term nucleolar organizer – noted that differential amphiplasty was related to the formation of nucleoli in such a way that only chromosomes with a secondary constriction were active in the formation of nucleoli. Later on, Wallace and Langridge (1971) described amphiplasty in several other *Crepis* hybrids and Doerschug et al. 1976) concluded that the nucleolar organizer suppression in *C. capillaris* × *C. dioscorides* hybrids must be at the transcriptional level.

Differential amphiplasty has been reported in many other interspecific hybrids, both in plants (for references see Lange and Jochemsen 1976) and animals (*Xenopus,* Honjo and Reeder 1973; Cassidy and Blackler 1974; *Drosophila,* Durica and Krider 1978; mouse-human hybrid cells, Eliceiri and Green 1969; Bramwell and Handmaker 1971; Marshall et al. 1975; Miller et al. 1976 a, b; Croce et al. 1977; siamang × gibbon hybrid, Shafer et al. cited by Howell 1977). Differential amphiplasty has been also reported both in natural allopolyploid species such as tetraploid and hexaploid *Triticum* (Morrison 1953; Crosby 1957; Darvey and Driscoll 1972) and artificial amphiploids as the triticale (Darvey 1974; Thomas and Kaltsikes 1983).

Nucleolus organizer regions (NORs), which are associated with secondary constrictions of satellite chromosomes, have been shown to be the sites of 18 S and 28 S ribosomal RNA genes in many animal (*Drosophila,* Ritossa and Spiegelman 1965; Henning et al. 1982; *Xenopus,* Wallace and Birnstiel 1966; in a variety of mammalian species, Henderson et al. 1972, 1974) and plant species (*Zea mays,* Phillips et al. 1971; *Vicia faba,* Scheuermann and Knälmann 1975; *Hordeum,* Subrahmanyam and Gerlach 1978; *Triticum,* Flavell and

O'Dell 1976; Gerlach et al. 1980; Miller et al. 1980; Hutchinson and Miller 1982; *Secale*, Miller et al. 1980; *Aegilops*, Hutchinson and Miller 1982).

Silver-staining methods have been developed for the differential staining of the NORs of animal (Howell et al. 1975; Howell and Black 1980; Goodpasture and Bloom 1975; Bloom and Goodpasture 1976) and plant chromosomes (Hizume et al. 1980; Sato et al. 1980). The sites detected by the Ag–As method in nine mammalian species (Goodpasture and Bloom 1975) and in a variety of primates (Tantravahi et al. 1976), as well as in the human using the slightly different Ag–SAT method (Howell et al. 1975), correspond exactly to those obtained by in situ hybridization with rRNA in the same species (Miller et al. 1976a). Warburton and Henderson (1979) found in human cells that the size of the silver-staining regions was positively correlated with the amount of label present after hybridization in situ with rRNA.

As the silver procedures can be used to visualize gene activity at the rDNA sites (NORs) with conventional light microscopy, Ag-staining methods could be used to study amphiplasty. However, this kind of analysis has been only used in mouse-human hybrid cells (Miller et al. 1976a, b; Croce et al. 1977). In plant material, only the phenomenon of "nucleolar dominance" has been analyzed using silver staining of the NORs in barley translocation lines (Anastassova-Kristeva et al. 1977; Nicoloff et al. 1979).

In this paper amphiplasty in hexaploid triticale, the artificial amphiploid between tetraploid durum wheat and diploid rye, is analyzed for the first time using a modified, highly reproducible, Ag-staining method.

Material and methods

Material

Ten plants of the hexaploid triticale cv. 'Cachirulo' (2n = 6x = 42, genome constitution AABBRR) obtained by Sánchez-Monge (1969) were analyzed as well as 5 plants of its wheat parent, *Triticum turgidum durum* cv. 'Enano de Andujar' (2n = 4x = 28, genome constitution AABB), and 5 plants of its rye parent, *Secale cereale* cv. 'Petkus' (2n = 2x = 14, genome constitution RR).

Methods

In order to obtain mitotic metaphase cells, seeds were germinated on moistened filter paper in Petri dishes at 20 °C. When the primary roots were 1 cm long they were excised and immersed in tap water at 0 °C for 36–48 h to shorten the chromosomes. The tips were subsequently fixed in acetic alcohol 1 : 3.

The C-banding procedure was carried out according to the method of Giraldez et al. (1979).

The silver-staining method for nucleolar organizer regions is based on that of Hizume et al. (1980) with the following modification:

1) The fixed material was squashed in a drop of 45% acetic acid and after removing the coverslides, slides were air dried.

2) The slides were covered with acetic alcohol 1 : 3 and flame-dried.

3) 1 g of $AgNO_3$ was dissolved in 1 ml of a solution of 0.02 g of sodium citrate ($C_6H_5Na_3O_7$–$2H_2O$) in 500 ml of distilled water adjusted to pH 3 with formic acid. One or two small drops of this fresh solution were added onto each slide and coverglasses were floated on the liquid.

4) The slides were placed in a moisture chamber in a stove at 55–60 °C for one-half to several hours. During the course of the staining the intensity of the reaction was monitored under a microscope.

5) When the chromosome arms were stained yellow to light brown, the slides were rinsed thoroughly in distilled water and air dried, They were then soaked in xylene and mounted in DePex.

Results

The nucleolar organizer chromosomes were identified in the three species analyzed both with phase contrast (Fig. 1 a, c, e) and C-banding (Fig. 1 b, d, f). On the other hand, silver-stained nucleolar organizer regions (Ag–NORs) were also clearly identified (Fig. 2 b, d, f), being located in close correspondence with the secondary constrictions as observed by phase contrast (Fig. 2 a, c, e).

The results obtained are shown in Table 1. Two Ag–NORs were observed in all (46) metaphase cells analyzed in the 5 rye plants (Fig. 2 b). Four Ag–NORs were observed in all metaphase cells analyzed in both the 5 wheat plants (Fig. 2 d) and the 10 triticale plants (Fig. 2 f) (a total of 50 and 61 cells, respectively).

The number of nucleoli observed at interphase cells are also shown. Contingency tests indicated that the distributions found in each species are homogeneous and, consequently, individual data can be brought together. A strong tendency of nucleoli to fuse at interphase is deduced.

Discussion

The silver procedure can be used to visualize gene activity at the rDNA sites with conventional light microscopy (Howell 1977). According to Miller et al. (1976a, b), the staining reaction is an indicator of genetic activity because in mouse-human somatic hybrid cells only those NORs functionally active during the preceding interphase are stained by silver. The Ag-staining of the NORs of prematurely condensed chromosomes from human cells showing different degrees of rRNA-gene activity, observed by Schmiady et al. (1979), clearly indicated a close correlation between the positive Ag-staining of NORs and the activity of rRNA genes, thus confirming the findings of Miller et al. (1976a, b). Schwarzacher et al. (1978) stated that the

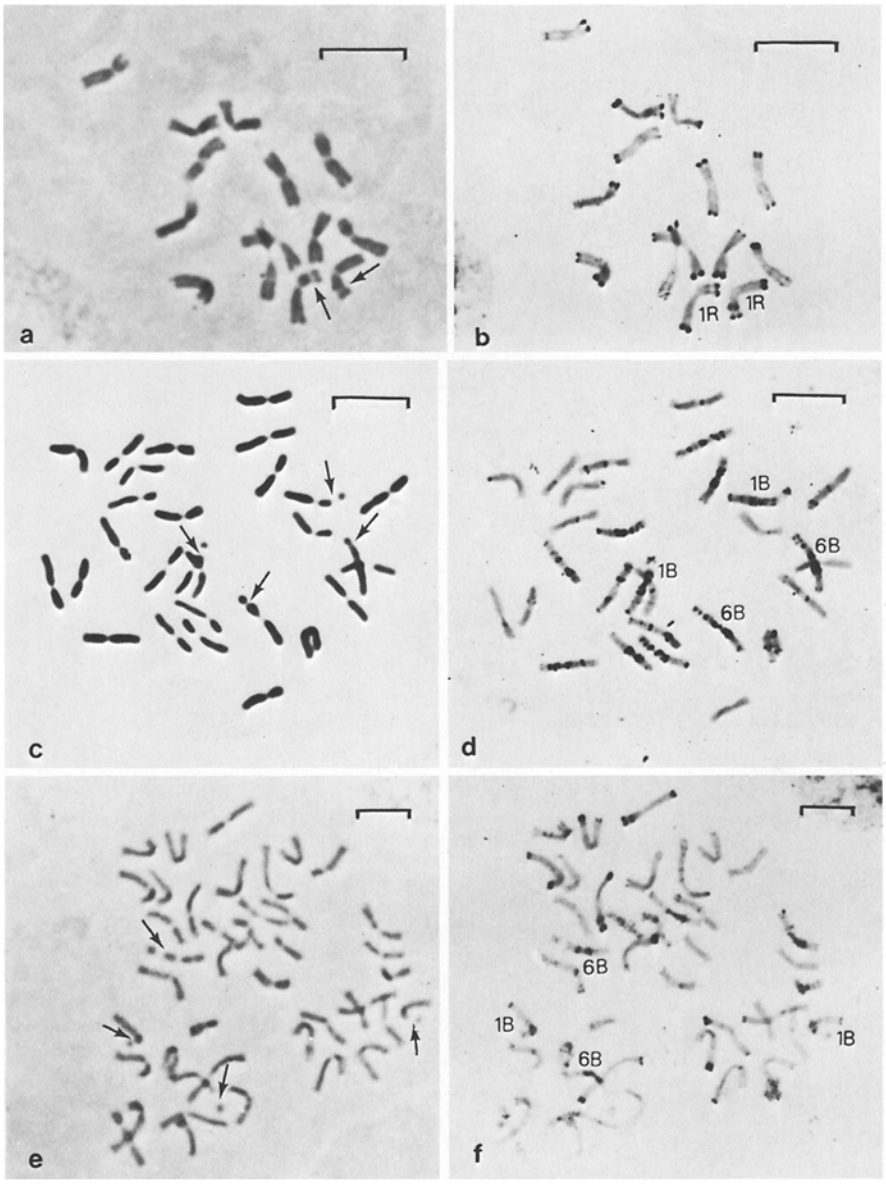

Fig. 1 a–f. Phase contrast and C-banded somatic metaphase cells of the hexaploid triticale cv. 'Cachirulo' and its parental species: diploid rye cv. 'Petkus' and tetraploid durum wheat cv. 'Enano de Andujar'. **a** and **b**, 'Petkus' rye, phase contrast and C-banding of the same cell; **c** and **d**, 'Enano de Andujar' tetraploid wheat, phase contrast and C-banding of the same cell; **e** and **f**, 'Cachirulo' hexaploid triticale, phase contrast and C-banding of the same cell. *Arrows* indicate the secondary constrictions. Bars represent 10 μm

most likely interpretation is that Ag-stainable material is a component of ribonucleic protein accumulating around active NORs. In mitosis some of this material remains at the NORs, whereas in the first division of meiosis it is completely removed before diakinesis. Their electron micrographs of human cells reveal that the Ag-stainable substance is located on the outside of the NORs or around them but not in the chromosomes themselves. This is in agreement with the cytochemical tests made by Howell (1977) in cricket oocyte chromosomes which revealed that the silver binds neither to the rDNA nor transcribed rRNA but rather to proteins which rapidly associate with the freshly-transcribed rRNA.

According to our observations (Table 1, Figs. 1 and 2), amphiplasty in hexaploid triticale cv. 'Cachirulo' has been demonstrated using a silver-staining method: the nucleolar organizer chromosomes from the rye parent do not show any kind of positive Ag-staining. In other words, the expression of rye NORs is suppressed by the presence of the wheat genomes. As far as we know this is the first time in which such a phenomenon is cytologically demonstrated, although the occurrence of amphiplasty in the hexaploid triticale cv. 'Rosner' was

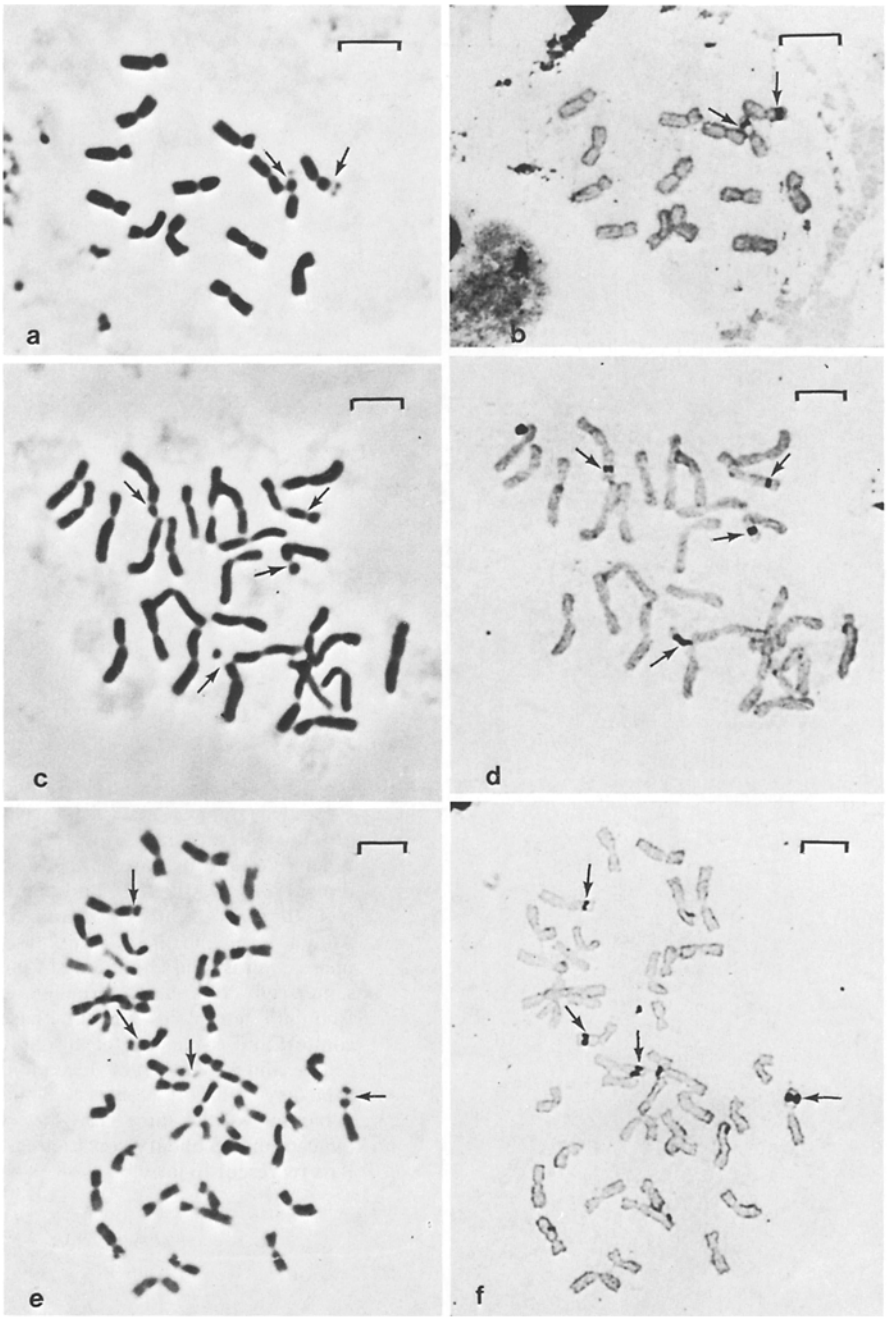

Fig. 2a–f. Phase contrast and Ag-stained somatic metaphase cells of the hexaploid triticale cv. 'Cachirulo' and its parental species: diploid rye cv. 'Petkus' and tetraploid durum wheat cv. 'Enano de Andujar'. **a** and **b**, 'Petkus rye', phase contrast and Ag-NORs of the same cell; **c** and **d**, 'Enano de Andujar' tetraploid wheat, phase contrast and Ag–NORs of the same cell; **e** and **f**, 'Cachirulo' hexaploid triticale, phase contrast and Ag–NORs of the same cell. *Arrows* indicate the NORs. Bars represent 10 μm

suggested by Darvey (1974) indicating that chromosomes 1B and 6B are the active pairs of nucleolar chromosomes. However, he surprisingly stated that "although three different rye constrictions were visible, there was no nucleolar activity of any of the rye chromosomes". Shkutina (1966) also suggested that the elimination of the rye genome from octoploid triticale was due to the "inactivation of the rye nucleoli". Later she stated that "mostly, only one wheat nucleolus functions in the triticales analyzed" (Shkutina and Khvostova 1971).

The presence of a single pair (1R) of silver-stained NORs in the rye parent (Fig. 2b) agrees both with the karyotypical and the in situ hybridization data previously known (Miller

Table 1. Silver-stained nucleolar organizer regions (Ag–NORs) and nucleoli visualized, respectively, at somatic metaphase and interphase cells in the hexaploid triticale cv. 'Cachirulo' (2n = 6x = 42, genome constitution AABBRR) and its two parental species, namely, the tetraploid wheat (*Triticum turgidum durum*) cv. 'Enano de Andujar' (2n = 4x = 28, AABB) and the diploid rye (*Secale cereale*) cv. 'Petkus' (2n = 2x = 14, RR)

Species	Plant	No. of metaphases	Ag–NORs	No. of nucleoli at interphase[a]				Total
				1	2	3	4	
Rye parent	P-1	2	2	80	27	–	–	107
	P-2	7	2	73	27	–	–	100
Secale cereale	P-3	2	2	80	20	–	–	100
cv. 'Petkus'	P-4	13	2	81	26	–	–	107
	P-5	22	2	103	16	–	–	119
Total		46	2	417	116	–	–	533
Wheat parent	EA-1	7	4	25	44	35	5	109
	EA-2	8	4	21	46	31	2	100
T. turgidum durum	EA-3	14	4	21	49	31	4	105
cv. 'Enano de Andujar'	EA-4	10	4	26	43	26	5	100
	EA-5	11	4	26	52	30	2	110
Total		50	4	119	234	153	18	524
Hexaploid triticale cv. 'Cachirulo'	CH- 1	8	4	7	55	39	10	111
	CH- 2	6	4	7	55	33	10	105
	CH- 3	9	4	5	50	34	11	100
	CH- 4	4	4	9	37	47	7	100
	CH- 5	7	4	12	55	38	6	111
	CH- 6	2	4	10	43	41	8	102
	CH- 7	6	4	14	46	30	10	100
	CH- 8	7	4	13	47	32	8	100
	CH- 9	3	4	22	48	30	5	105
	CH-10	9	4	14	49	32	5	100
Total		61	4	113	485	356	80	1034

[a] Contingency tests of the distributions of the number of nucleoli observed in each plant are not significant intraspecies

et al. 1980). On the other hand, our results show (Fig. 2 d) that silver-stained NORs are only distinguishable on chromosomes 1 B and 6 B of 'Enano de Andujar' tetraploid wheat. This is in agreement with previous data on mitotic karyotypes of tetraploid wheat (Giorgi and Bozzini 1969 a; Bozzini and Giorgi 1969; Hutchinson and Miller 1982). On the other hand, the Ag–NOR pattern obtained in the 'Cachirulo' triticale (Fig. 2 f) clearly indicates that rye NORs are not stained by the silver procedure and, consequently, one can infer that rDNA genes of rye chromosomes have not been functionally active during the preceding interphase (Miller et al. 1976 a, b; Schmiady et al. 1979). So, the amphiplasty observed in triticale can be atributable to the lack of expression of the rye rDNA genes by influence of wheat chromosomes. In fact, the existence of a genetic control of nucleolus formation in wheat is known (Darvey and Driscoll 1972; Viegas and Mello-Sampayo 1975; Flavell and O'Dell 1979) as is even the influence of chromosomes of different species (Flavell and Smith 1974; Martini et al. 1982). The latter situation has been also reported in mouse-human hybrid cells (Miller et al. 1967 a, b; Croce et al. 1977).

The nucleolar competition found in the polyploid species of the genus *Triticum*, which can be considered as an expression of a natural amphiplasty, presents some amazing characteristics. It is well established from both karyotypical and molecular analyses of the nucleolar organizer chromosomal constitution of the A, B and D genomes, namely, the chromosomes 1 A and 5 A (Crosby 1957; Miller et al. 1983). 1 B and 6 B (Crosby 1957; Riley et al. 1958; Hutchinson and Miller 1982) and 5 D (Giorgi and Bozzini 1969 b; Hutchinson and Miller 1982). All tetraploid wheats, with the possible exception of *T. carthlicum*, show two pairs (1 B and 6 B) of nucleolar organizer chromosomes (Hutchinson and Miller 1982), whereas hexaploid wheat, *T. aestivum*, shows two "strong" (1 B and 6 B) and two "weak" (1 A and 5 D) nucleolar organizer chromosomes (Crosby 1957; Longwell and Svihla 1960; Bhowal 1972; Miller et al. 1980). The reason why chromosome 1 A is functionally active (although weakly) in *T. aestivum* (AABBDD) but not in *T. turgidum* (AABB) remains unknown. In the hexaploid triticale (AABBRR) analyzed in this work, the presence of the R genome does not change the Ag–NOR pattern of the tetraploid wheat parent.

Finally, a very short coment on the nucleolar fusion. Our results (Table 1) are in agreement with observations previously made in wheat by other authors (Darvey and Driscoll 1972; Viegas and Mello-Sampayo 1975; Flavell and O'Dell 1979). The nucleolar coalescence is probably a consequence of the closed vicinity of the NORs in the nuclear space.

References

Anastassova-Kristeva M, Nicoloff H, Künzel G, Rieger R (1977) Nucleolus formation in structurally reconstructed barley karyotypes with six satellite chromosomes. Chromosoma 62:103–109

Bhowal JG (1972) Nucleolar chromosomes in wheat. Z Pflanzenzücht 68:253–257

Bloom SE, Goodpasture C (1976) An improved technique for selective silver staining of nucleolar regions in human chromosomes. Hum Genet 34:199–206

Bozzini A, Giorgi B (1969) Karyotype analysis in *Triticum*. 2. Analysis of *T. araraticum* Jakubz. and *T. timopheevi* Zhuk. and their relationships with other tetraploid wheats. Caryologia 22:260–268

Bramwell ME, Handmaker SD (1971) Ribosomal RNA synthesis in human-mouse hybrid cells. Biochim Biophys Acta 232:580–583

Cassidy DM, Blacker AW (1974) Repression of nucleolar organizer activity in an interspecific hybrid of the genus *Xenopus*. Dev Biol 41:84–96

Croce CM, Talavera A, Basilico C, Miller OJ (1977) Suppression of production of mouse 28 S ribosomal RNA in mouse-human hybrids segregating mouse-chromosomes. Proc Natl Acad Sci USA 74:694–697

Crosby AR (1957) Nucleolar activity of lagging chromosomes in wheat. Am J Bot 44:813–822

Darvey NL (1974) Nucleolar behaviour in hexaploid triticale. EWAC Newsletter 4:69–70

Darvey NL, Driscoll CJ (1972) Nucleolar behaviour in *Triticum*. Chromosoma 36:131–139

Doerschug EB, Miksche JB, Stern H (1976) DNA variation and ribosomal-DNA constancy in two *Crepis* species and the interspecific hybrid exhibiting nucleolar-organizer suppression. Heredity 37:441–450

Durica DJ, Krider HM (1978) Studies on the ribosomal RNA cistrons in interspecific *Drosophila* hybrids. 2. Heterochromatic regions mediating nucleolar dominance. Genetics 89:37–64

Eliceiri GL, Green H (1969) Ribosomal RNA synthesis in human-mouse hybrid cells. J Mol Biol 41:253–260

Flavell RB, O'Dell M (1976) Ribosomal RNA genes on homoeologous chromosomes of groups 5 and 6 in hexaploid wheat. Heredity 37:377–385

Flavell RB, O'Dell M (1979) The genetic control of nucleolus formation in wheat. Chromosoma 71:135–152

Flavell RB, Smith DP (1974) The role of homeologous group 1 chromosomes in the control of rRNA genes in wheat. Biochem Genet 12:271–279

Gerlach WL, Miller TE, Flavell RB (1980) The nucleolus organizers of diploid wheats revealed by in situ hybridization. Theor Appl Genet 58:97–100

Giorgi B, Bozzini A (1969a) Karyotype analysis in *Triticum*. 1. Analysis of *Triticum turgidum* (L) Thell. and some related tetraploid wheats. Caryologia 22:249–259

Giorgi B, Bozzini A (1969b) Karyotype analysis in *Triticum*. 3. Analysis of the presumed diploid progenitors of polyploid wheats. Caryologia 22:279–288

Giráldez R, Cermeño MC, Orellana J (1979) Comparison of C-banding pattern in the chromosomes of inbred lines and open pollinated varieties of rye, *Secale cereale* L. Z Pflanzenzücht 83:40–48

Goodpasture C, Bloom SE (1975) Visualization of nucleolar organizer regions in mammalian chromosomes using silver staining. Chromosoma 53:37–50

Henderson AS, Warburton D, Atwood KC (1972) Localization of ribosomal DNA in the human chromosome complement. Proc Natl Acad Sci USA 69:3394–3398

Henderson AS, Warburton D, Atwood KC (1974) Localization of rDNA in the chromosome complement of the Rhesus (*Macaca mulatta*). Chromosoma 44:367–370

Hennig W, Vogt P, Jacob G, Siegmund I (1982) Nucleolus organizer regions in *Drosophila* species of the repleta group. Chromosoma 87:279–292

Hizume M, Sato S, Tanaka A (1980) A highly reproducible method of nucleolus organizer regions staining in plants. Stain Technol 55:87–90

Honjo T, Reeder RH (1973) Preferential transcription of *Xenopus laevis* ribosomal RNA in interspecies hybrids between *X. laevis* and *X. mulleri*. J Mol Biol 80:217–228

Howell WM (1977) Visualization of ribosomal gene activity: silver stains proteins associated with rRNA transcribed from oocyte chromosomes. Chromosoma 62:361–367

Howell WM, Black BA (1980) Controlled silver-staining of nucleolus organizer regions with a protective colloidal developed: a 1-step method. Experientia 36:1014

Howell WM, Denton TE, Diamond JR (1975) Differential staining of the satellite regions of human acrocentric chromosomes. Experientia 31:260–262

Hutchinson J, Miller TE (1982) The nucleolar organisers of tetraploid and hexaploid wheats revealed by in situ hybridisation. Theor Appl Genet 61:285–288

Lange W, Jochemsen G (1976) Karyotypes, nucleoli, and amphiplasty in hybrids between *Hordeum vulgare* L and *Hordeum bulbosum* L. Genetica 46:217–233

Longwell A, Svihla G (1960) Specific chromosomal control of the nucleolus and of the cytoplasm in wheat. Exp Cell Res 20:294–312

Marshall CJ, Handmaker SD, Bramwell ME (1975) Synthesis of ribosomal RNA in syncaryons and heterocaryons formed between human and rodent cells. J Cell Sci 17:307–325

Martini G, O'Dell M, Flavell RB (1982) Partial inactivation of wheat nucleolus organisers by the nucleolus organiser chromosomes from *Aegilops umbellulata*. Chromosoma 84:687–700

McClintock B (1934) The relation of a particular chromosomal element to the development of the nucleoli in *Zea mays*. Z Zellforsch Mikrosk Anat 21:294–328

Miller DA, Dev VG, Tantravahi R, Miller OJ (1976a) Suppression of human nucleolus organizer activity in mouse-human somatic hybrid cells. Exp Cell Res 101:235–243

Miller OJ, Miller DA, Dev VG, Tantravahi R, Croce CM (1976b) Expression of human and suppression of mouse nucleolar organizer activity in mouse-human somatic cell hybrids. Proc Natl Acad Sci USA 73:4531–4535

Miller TE, Gerlach WL, Flavell RB (1980) Nucleolus organizer variation in wheat and rye revealed by in situ hybridization. Heredity 45:377–382

Miller TE, Hutchinson J, Reader SM (1983) The identification of the nucleolus organiser chromosomes of diploid wheat. Theor Appl Genet 65:145–147

Morrison JW (1953) Chromosome behavior in wheat monosomics. Heredity 7:203–217

Navashin MS (1928) Amphiplastie- eine neue karyologische Erscheinung. Proc Int Conf Genet 5:1148–1152

Navashin MS (1934) Chromosome alterations caused by hybridization and their bearing upon certain general genetic problems. Cytologia 5:169–203

Nicoloff H, Anastassova-Kristeva M, Rieger R, Künzel G (1979) "Nucleolar dominance" as observed in barley

translocation lines with specifically reconstructed SAT chromosomes. Theor Appl Genet 55:247–251
Phillips RL, Kleese RA, Wang SS (1971) The nucleolus organizer region of maize (*Zea mays* L): chromosomal site of DNA complementary to ribosomal RNA. Chromosoma 36:79–88
Rieger R, Michaelis A, Green MM (1976) Glossary of genetics and cytogenetics: classical and molecular. Springer, Berlin Heidelberg New York, p 647
Riley R, Unrau J, Chapman V (1958) Evidence on the origin of the B genome of wheat. J Hered 49:91–98
Ritossa FM, Spiegelman S (1965) Localization of DNA complementary to ribosomal RNA in the nucleolus organizer region of *Drosophila melanogaster.* Proc Natl Acad Sci USA 53:737–745
Sato S, Hizume M, Kawamura S (1980) Relationship between secondary constrictions and nucleolus organizing regions in *Allium sativum* chromosomes. Protoplasma 105:77–85
Scheuermann W, Knälmann M (1975) Localization of ribosomal cistrons in metaphase chromosomes of *Vicia faba* (L). Exp Cell Res 90:463
Schmiady H, Münke M, Sperling K (1979) Ag-staining of nucleolus organizer regions on human prematurely condensed chromosomes from cells with different ribosomal RNA gene activity. Exp Cell Res 121:425–428
Shkutina FM (1966) The number and size of nucleoli in wheat-rye amphidiploids (2n = 56). Genetika 6:107–112
Shkutina FM, Khvostova VV (1971) Cytological investigation of Triticale. Theor Appl Genet 41:109–119
Subrahmanyam MC, Gerlach W (1978) Cytological localization of rRNA cistrons in *Hordeum:* a test for the non-expression of the nucleolar organizer region in interspecific hybrids. Barley Genet Newslett 8:99–100
Swarzacher HG, Mikelsaar AV, 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
Tantravahi R, Miller DA, Dev VG, Miller OJ (1976) Detection of nucleolus organizer regions in chromosomes of human, chimpanzee, gorilla, orang-utan and gibbon. Chromosoma 56:15–27
Thomas JB, Kaltsikes PJ (1983) Effect of chromosomes 1 B and 6 B on nucleolus formation in hexaploid triticale. Can J Genet Cytol 25:292–297
Viegas WS, Mello-Sampayo T (1975) Nucleolar organization in the genus *Triticum.* Broteria 44:121–133
Wallace H, Birnstiel ML (1966) Ribosomal cistrons and the nucleolar organizer. Biochim Biophys Acta 144:295–310
Wallace H, Langridge WHR (1971) Differential amphiplasty and the control of ribosomal RNA synthesis. Heredity 27:1–13
Warburton D, Henderson AS (1979) Sequential silver staining and hybridization in situ on nucleolus organizer regions in human cells. Cytogenet Cell Genet 24:168–175

Note added in proof

Thomas and Kaltsikes (1983) have recently demonstrated that nucleolus organizers present on wheat chromosomes 1B and 6B to be solely responsible for nucleolus formation in hexaploid triticale.