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Nucleolus organizer competition in *Triticum aestivum* – *Aegilops umbellulata* chromosome addition lines

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Summary. The nucleolar organizer activity of wheat (*Triticum aestivum, AABBDD*) and *Aegilops umbellulata* (UU) chromosomes have been analyzed in the complete set of the chromosome addition lines by using a highly reproducible silver-staining procedure. Chromosomes 1U and 5U produce the partial inactivation of wheat nucleolar organizer chromosomes 6B, 1B and 5D. The chromosomes D and G from *Ae. umbellulata,* which are not SAT-chromosomes, seem to specifically influence the activity of wheat NORs. The predominant status of the U genome with respect to nucleolar competition in the *Triticeae* is confirmed.

Key words: Nucleolar competition – Amphiplasty – Ag-NORs – Addition lines – *Triticum aestivum* – *Aegilops umbellulata*

Introduction

A highly reproducible silver-staining procedure has recently proved useful for analyzing nucleolar activity and competition in both natural species and their hybrid combinations and derivatives in *Triticeae* (Lacadena et al. 1984a, b; Cermeño et al. 1984a, b; Orellana et al. 1984; Santos et al. 1984). Comparative analyses of nucleolar organizer regions (NORs) of somatic metaphase chromosomes made by phase contrast, C-banding and silver staining have demonstrated that the activity of the NORs of certain chromosomes can be suppressed or partially inhibited by the presence of other SAT-chromosomes. The NOR competition is cytologically expressed as amphiplasty: a term proposed to denote morphological changes which occur in chromosomes following interspecific hybridization (Rieger et al. 1976). The secondary constriction of the SAT-chromosome of one of the parental species is missing in the hybrid and the satellite is retracted onto the chromosome arm as a consequence.

It is known that NORs are the sites of rRNA genes. Martini et al. (1982) pointed out why the rRNA gene system is useful for studying the control of gene expression: the products of the rRNA genes are easily purified, gene activity can be assessed cytologically by observing nucleoli and Ag-NORs, and it is not difficult to estimate the amount of rRNA in the cells.

Martini et al. (1982) analyzed the partial inactivation of wheat (*Triticum aestivum*) nucleolus organizers by chromosomes 1U and 5U from *Aegilops umbellulata,* in chromosome addition and substitution lines, by counting nucleoli in root tip cells, measuring the volumes of individual nucleoli and examining metaphase chromosomes for constrictions.

In this paper the NOR activity of wheat and *Aegilops umbellulata* chromosomes in the complete set of chromosome addition lines is analyzed by using the silver procedure developed by Lacadena et al. (1984a).

Materials and methods

Materials

The complete set of *Triticum aestivum* cv. 'Chinese Spring' – *Aegilops umbellulata* chromosome addition lines (A through G) was kindly supplied by Dr. Gordon Kimber (Dept. of Agronomy, Univ. of Missouri-Columbia, USA). The correspondence with the chromosome terminologies used in previous works based on C-banding techniques carried out in the Plant Breeding Institute, Cambridge, UK (Teoh and Hutchinson 1983) and our own data (Cermeño et al. 1984b), as well as in homoeology relationships (Martini et al. 1982), are indicated below:

Chromosome terminology		Remarks
Kimber	P.B.I., Cambridge	
A	F	Telocentric
B	G	1U
C	D	5U
D	E?	Comparison of C-banding patterns is not conclusive
E	C?	Comparison of C-banding patterns is not conclusive
F	A?	
G	B?	

Methods

Seeds were germinated at 20 °C on wet filter paper in Petri dishes. 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 root tips were subsequently fixed in 1 : 3 acetic acid : ethanol.

The silver staining procedure was carried out according to Lacadena et al. (1984a). The C-banding technique has been described by Giráldez et al. (1979).

The comparative analysis of somatic metaphase chromosomes by phase contrast, C-banding and Ag-staining was made according to the following cytogenetic rationale: the nucleolar organizer chromosomes (SAT-chromosomes) were identified both with phase contrast and C-banding from comparisons of the same metaphase cells. On the other hand, silver-stained nucleolar organizer regions (Ag-NORs) – that is to say, the active NORs (see review by Howell 1982) – were also identified, being located in close correspondence with the secondary constrictions as observed previously by phase contrast of the same cells. In other words, with the Ag-staining we can know how many active NORs (Ag-NORs) are present in the cell and with the C-banding technique it is possible to identify which chromosomes (SAT-chromosomes) carry active NORs.

Nucleoli were observed at interphase by silver-staining. Contingency tests indicated that the distributions of the number of nucleoli found in the different plants of each chromosome addition line were homogeneous and, consequently, individual data have been pooled. The maximum numbers of nucleoli found are useful for infering the actual number of active NORs present in the cell although not all of them can be detected as Ag-NORs by the silver-staining procedure (Cermeño et al. 1984a).

Results and discussion

The number of Ag-NORs and nucleoli observed, respectively, at somatic metaphase and interphase cells are shown in Table 1.

From the results obtained, two main conclusions can be drawn: firstly, SAT-chromosomes from *Ae. umbellulata* (1U and 5U) produce a partial inactivation of

Table 1. Silver-stained nucleolar organizer regions (Ag-NORs) and number of nucleoli observed, respectively, at metaphase and interphase in root tip cells of common wheat, *Triticum aestivum* cv. 'Chinese Spring' (genome constitution *AABBDD*), *Aegilops umbellulata* (*UU*) and their chromosome addition lines

Material	No. of plants	No. of metaphase Ag-NORs cells scored	No. of nucleoli at interphase								Total no. of cells	Remarks on nucleolar competition: active NORs[d]
			1	2	3	4	5	6	7	8		
Triticum aestivum[a] cv. 'Chinese Spring' (CS)	10	39 4 2 6	196	378	316	143	20	2	–	–	1,055	6B > 1B ≫ 5D
Aegilops umbellulata[b]	5	36 4	153	292	64	12	–	–	–	–	521	1U > 5U
T. aestivum–Ae. umbellulata addition lines[c]												
CS+(A) (A)	5	39 4	123	542	286	102	13	–	–	–	1,066	6B > 1B ≫ ≫ 5D
CS+(B) (B)	5	20 2 3 3	159	473	327	133	23	4	–	–	1,119	1U > 6B ≫ 1B
CS+(C) (C)	5	15 2 8 3	69	301	374	249	99	17	7	–	1,110	5U > 6B ≫ 1B ≫ 5D
CS+(D) (D)	5	40 3	174	567	298	106	19	1	–	–	1,165	1B ≫ 6B ≫ 5D
CS+(E) (E)	5	23 4	113	538	277	87	12	2	–	–	1,029	6B > 1B ≫ 5D
CS+(F) (F)	5	30 4	87	525	348	90	4	–	–	–	1,054	6B > 1B ≫ 5D
CS+(G) (G)	5	28 4 10 5 1 6	49	291	405	255	97	16	–	–	1,113	6B > 1B > 5D

[a] Data taken from Cermeño et al. (1984a)
[b] Data taken from Cermeño et al. (1984b)
[c] *Ae. umbellulata* chromosome terminology according to Kimber. See "Materials" section in the text
[d] The symbols > and ≫ refer to the different sizes of the Ag-Nors which are positively correlated with the activity of the rDNA genes

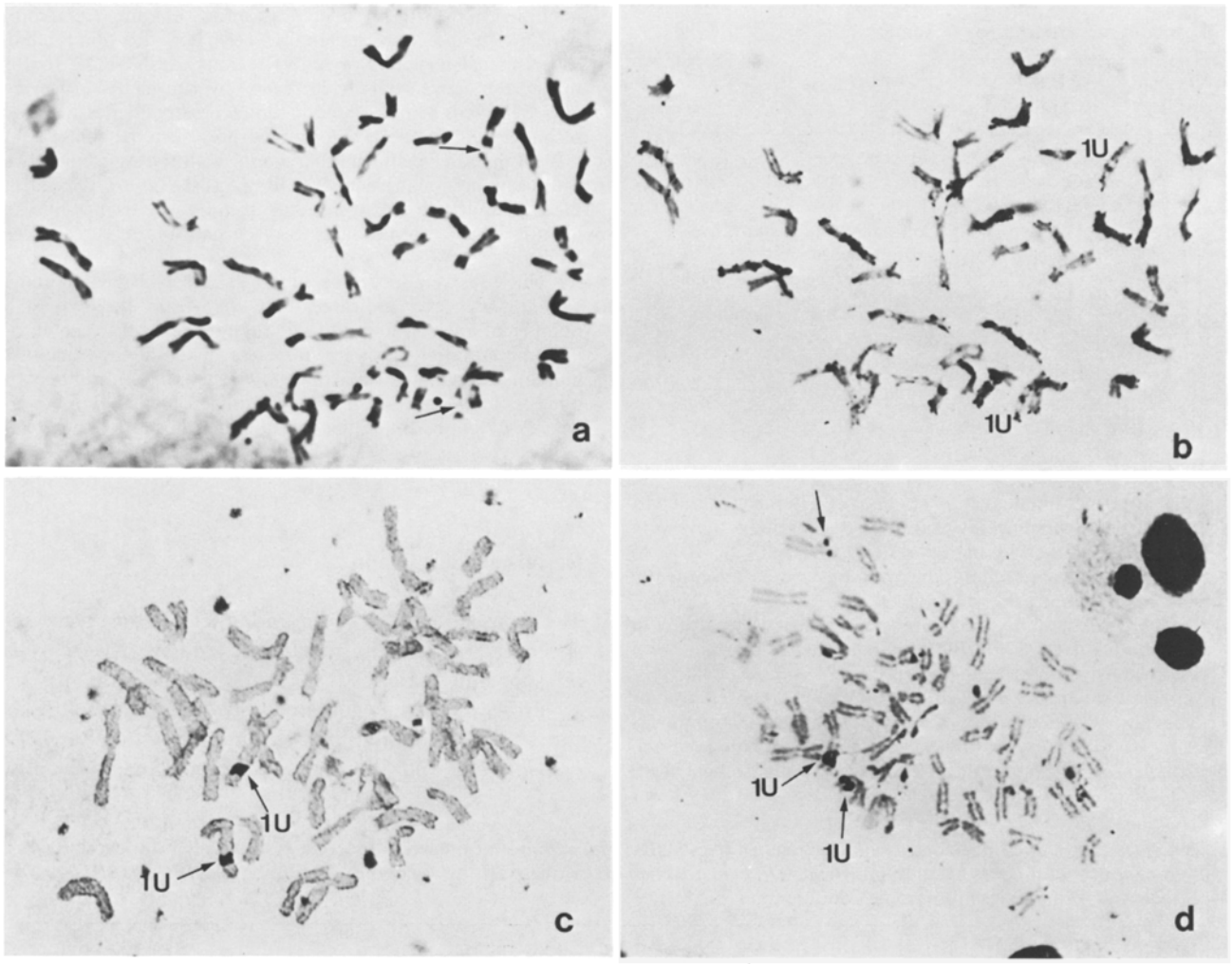

Fig. 1a–d. Somatic metaphase cells of *Triticum aestivum* cv. 'Chinese Spring' + *Aegilops umbellulata* 1U 1U chromosome addition line. Partial view of the same cell observed unstained by phase contrast (**a**) and C-banding (**b**). The C-banding pattern allows the satellited chromosomes to be identfied as the 1U chromosomes. (**c**) Two Ag-NORs belonging to the 1U chromosome pair. (**d**) Three Ag-NORs; the small one belongs to a wheat chromosome

wheat nucleolus organizer chromosomes (6B, 1B and 5D) as previously pointed out by Martini et al. (1982) (Figs. 1 and 2); secondly, the other five chromosomes from *Ae. umbellulata* added to the wheat chromosome complement do not drastically change the behaviour of the wheat nucleolus organizers either in number of observed Ag-NORs (Fig. 3) nor in distribution of the number of nucleoli found at interphase (Cermeño et al. 1984a). However, some detailed accounts should be given.

In chromosome addition line CS+(B)(B) – which corresponds to the *umbellulata* 1U chromosome – two or three Ag-NORs are observed corresponding, respectively, to the pair of chromosomes 1U and to this pair plus the 6B chromosome. That is to say, the Ag-NORs of chromosomes 1B are not detected although the maximum number of nucleoli found at interphase indicates that there is a certain degree of activity in the nucleolar organizer region of chromosome 1B.

In chromosome addition line CS+(C)(C), corresponding to the 5U chromosome, the wheat nucleolus organizers are partially inactivated by the presence of the *umbellulata* chromosome. Ag-NORs are detected on the 5U chromosome pair and only on one 6B chromosome. However, as in the preceding case, the maximum number (seven) of nucleoli found (Fig. 4) indicates that even the NOR of chromosome 5D can be active.

Although in the two addition lines in which the *umbellulata* nucleolus organizer chromosomes are added the distributions of the number of nucleoli at interphase do not differ from that of the parental

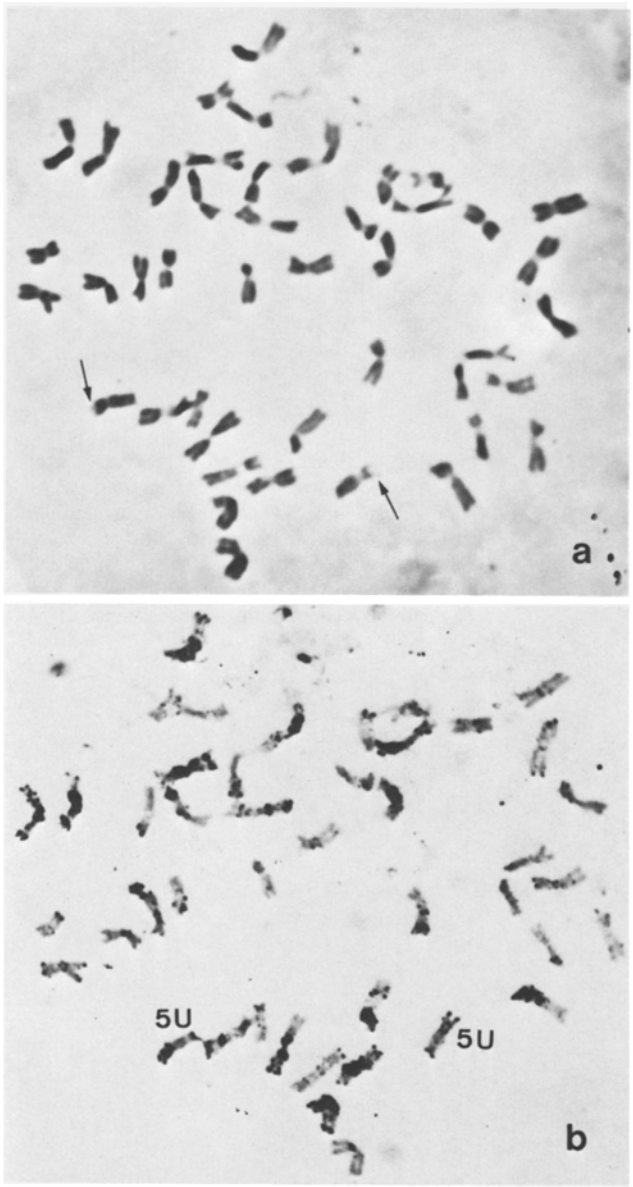

Fig. 2a, b. Somatic metaphase cells of *Triticum aestivum* cv. 'Chinese Spring' + *Aegilops umbellulata* 5U 5U chromosome addition line. The same cell observed unstained by phase contrast (**a**) and C-banding (**b**). The two SAT-chromosomes are identified as the 5U pair

wheat, their repressive effects on wheat NORs are cytologically expressed (amphiplasty) as the almost total disappearance of the secondary constrictions of the 6B and 1B chromosomes as observed by phase contrast and the corresponding absence of Ag-NORs. This behaviour agrees with the observations made by Martini et al. (1982) who also found a major discontinuity in the sizes of the nucleoli between cells carrying 1U chromosomes and 'Chinese Spring' wheat cells: in the addition line they observed two large(macro-)nucleoli and 1–4 small(micro)nucleoli. Plants of the 5U chromosome addition line showed one or two macronucleoli and up to four micronucleoli, but these small nucleoli were larger than the micronucleoli of the 1U chromosome addition line. These researchers concluded that the effect of the 5U chromosome on the formation of wheat nucleoli is similar to that of the 1U chromosomes but that inactivation of wheat NORs is less marked. We have also found the formation of micronucleoli (Fig. 4).

In addition line CS+(D)(D) the results obtained suggest that the *umbellulata* chromosome, which is not a SAT-chromosome, seems to specifically influence the NOR activity of chromosome 6B of wheat since only three Ag-NORs are observed and the analysis by C-banding technique in 25 cells clearly demonstrated that the secondary constrictions are visible on the two 1B chromosomes but only on one 6B chromosome. However, the distribution of the number of nucleoli at interphase is similar to that found in the parental wheat. These observations are in agreement with previous works of other authors which suggested that the genetic control of nucleolar activity in common wheat appears to be rather complex (Mohan and Flavell 1974; Viegas and Mello-Sampayo 1975; Flavell and O'Dell 1976, 1979; Flavell and Martini 1982).

In the addition line CS+(G)(G), in which the added *umbellulata* chromosome is not a SAT-chromosome, 4, 5 or 6 Ag-NORs are observed. This indicates that the NORs of wheat chromosomes 6B, 1B and 5D are active. Furthermore, it is worth mentioning that the distribution of the number of nucleoli at interphase clearly shows a deviation towards the classes with higher numbers of nucleoli (4, 5 or 6) in comparison with the nucleolar distribution found in the parental wheat. Consequently, a certain influence of chromosome G from *Ae. umbellulata* on the wheat nucleolus organizer activity might be inferred.

The repressive effect of the SAT-chromosomes from *Ae. umbellulata* on the nucleolar organizer activity of the wheat chromosomes confirms the predominant status of the U genome observed in the genus *Aegilops* (Cermeño et al. 1984b) and in relation to the rye nucleolus organizer (Cermeño and Lacadena 1985).

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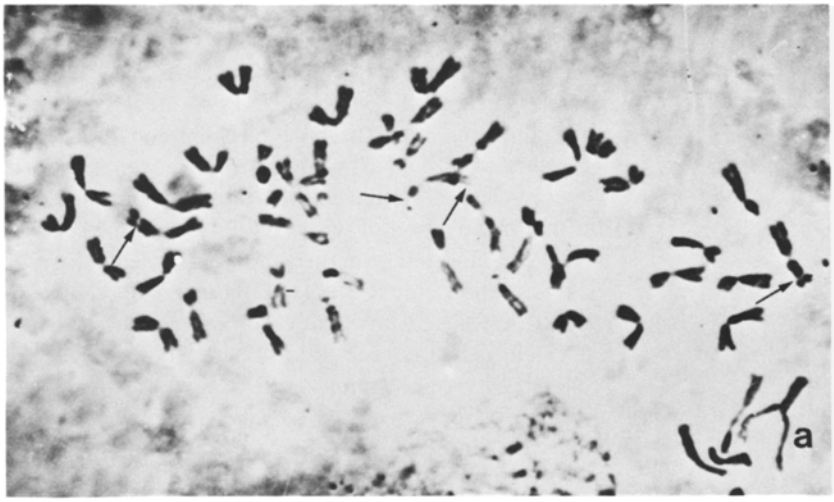
References

Cermeño MC, Lacadena JR (1985) Nucleolar organizer competition in several *Aegilops*-rye hybrids. Can J Genet Cytol (in press)

Cermeño MC, Orellana J, Santos JL, Lacadena JR (1984a) Nucleolar organizer activity in wheat, rye and derivatives analyzed by a silver-staining procedure. Chromosoma 89:370–376



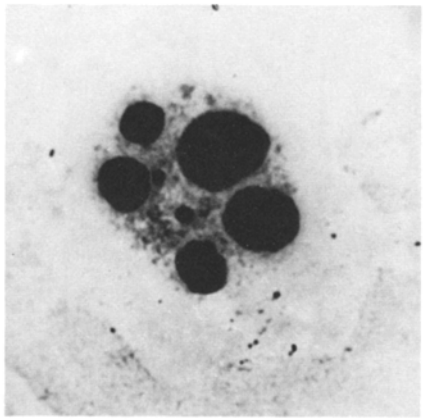
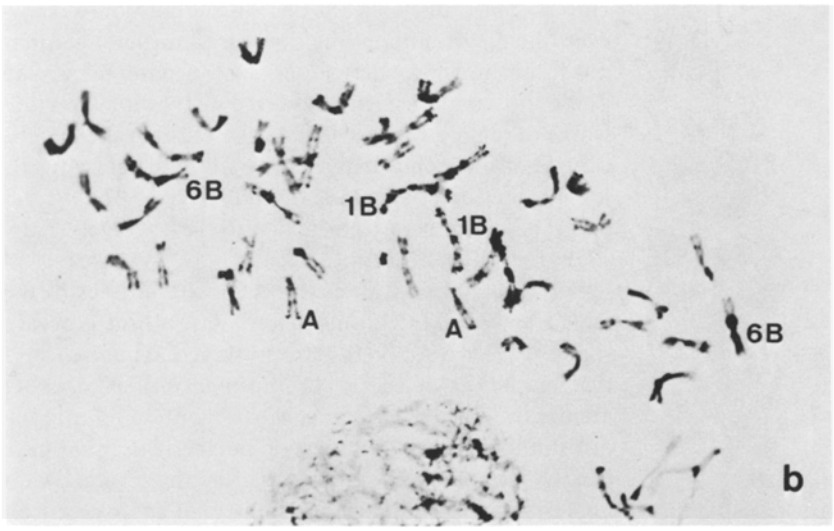
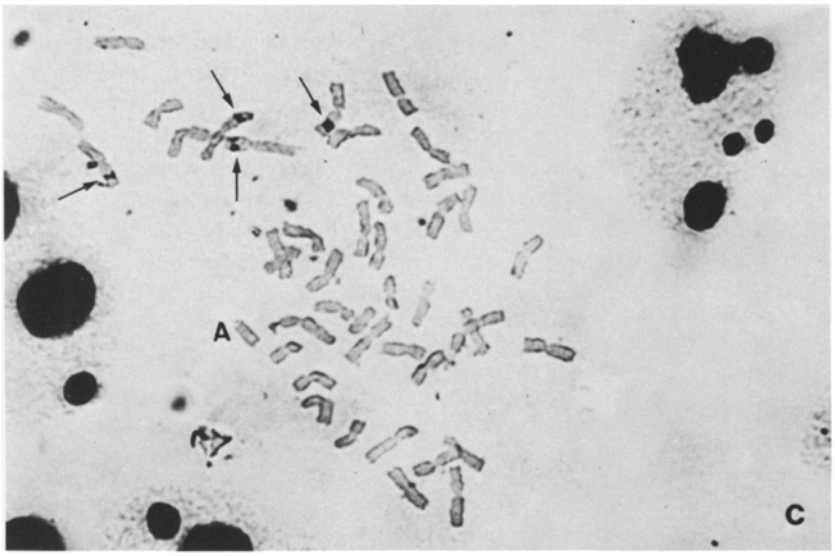


Fig. 4. Seven nucleoli observed at interphase in the *Triticum aestivum* + *Ae. umbellulata* 5U 5U chromosome addition line. Major discontinuities in the sizes of the nucleoli (macro- and micronucleoli) are observed. The large nucleoli supposedly correspond to the activity of 5U NORs





◀ **Fig. 3 a–c.** Somatic metaphase cells of *Triticum aestivum* cv. 'Chinese Spring' + *Aegilops umbellulata* (A) (A) chromosome addition line. Partial view of the same cell observed unstained by phase contrast (**a**) and C-banding (**b**). The C-banding pattern allows the SAT-chromosomes to be identified as 6B and 1B. (**c**) Four Ag-NORs corresponding to the pairs 6B and 1B. This cell belongs to a monosomic addition line. Only one A chromosome from *Ae. umbellulata* is present

Cermeño MC, Orellana J, Santos JL, Lacadena JR (1984b) Nucleolar activity and competition (amphiplasty) in the genus *Aegilops*. Heredity 53:603–611

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, Martini G (1982) The genetic control of nucleolus information with special reference to common bread wheat. In: Jordan WG, Cullis CA (eds) The nucleolus. Cambridge University Press, Cambridge, pp 113–128

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

Howell WM (1982) Selective staining of nucleolus organizer regions (NORs). Cell Nucleus 11:89–142

Lacadena JR, Cermeño MC, Orellana J, Santos JL (1984a) Evidence for wheat-rye nucleolar competition (amphiplasty) in triticale by silver-staining procedure. Theor Appl Genet 67:207–213

Lacadena JR, Cermeño MC, Orellana J, Santos JL (1984b) Analysis of nucleolar activity in *Agropyron elongatum*, its amphiploid with *Triticum aestivum* and the chromosome addition lines. Theor Appl Genet 68:75–80

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

Mohan J, Flavell RB (1974) Ribosomal RNA cistron multiplicity and nucleolus organisers in hexaploid wheat. Genetics 76:33–44

Orellana J, Santos JL, Lacadena JR, Cermeño MC (1984) Nucleolar competition analysis in *Aegilops ventricosa* and its amphiploids with tetraploid wheats and diploid rye by the silver-staining procedure. Can J Genet Cytol 26:34–39

Rieger R, Michaelis A, Green MM (1976) Glossary of genetics and cytogenetics: classical and molecular, 4th edn. Springer, Berlin Heidelberg New York, 647 pp

Santos JL, Lacadena JR, Cermeño MC, Orellana J (1984) Nucleolar organiser activity in wheat-barley chromosome addition lines. Heredity 52:425–429

Teoh SB, Hutchinson J (1983) Interspecific variation in C-banded chromosomes of diploid *Aegilops* species. Theor Appl Genet 65:31–40

Viegas WS, Mello-Sampayo T (1975) Nucleolar organization in the genus *Triticum*. Broteria, Ser Cienc Nat 44:121–133