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Nucleolar dominance in triticales: control by unlinked genes

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Hybrid plants and animals often show suppression of activity of ribosomal genes (rDNA) originating from one of the parental or ancestral species. In the wheat × rye amphiploid triticale, containing 28 chromosomes of wheat origin and 14 from rye, rDNA of rye origin (on chromosome 1R) is not normally expressed, while the 1B- and 6B-origin rDNA from wheat shows strong expression. Expression of rDNA can be accurately assessed by the silver staining method, which stains both interphase nucleoli and metaphase rDNA sites that were actively expressed at the previous interphase. We show here that substitution of another rye chromosome, 2R, by a chromosome from hexaploid wheat, 2D (triticale-2D(2R)), prevents suppression of the rye-origin rDNA, and leads to activity of all six major rDNA loci. These results were found in two different triticales and supported by rDNA behaviour in wheat–rye chromosomal addition lines. Models for chromosomal interactions leading to control of rDNA expression are presented.

Key words: gene expression, nucleolar dominance, rDNA, substitution lines, triticale

Introduction

Rapid progress has been made in understanding the regulation and control of expression patterns of organ-specific genes (Goldberg *et al*. 1989, Dillon *et al*. 1993, Higgins 1993, Lu *et al*. 1993). Constitutively expressed genes are in general more difficult to study, although there are clear regulatory mechanisms. In the present paper, we examine the genetic control of nucleolar dominance, an extreme form of gene suppression: in many hybrid organisms, the 18S–5.8S–25S rRNA genes (rDNA) from one parent are often suppressed, while those from the other parent are preferentially expressed (Reeder 1985). Systems involving preferential inactivation of particular rRNA gene loci are found widely in plant and animal hybrids. In itself, the system is of interest because the gene product, rRNA, is important, but the interaction is also a valuable model for studying gene expression because the regulation of individual known loci can be examined directly by cytological methods.

In wheat (*Triticum aestivum* (L.) em. Thell) × rye (*Secale cereale* L.) hybrids and in the artificial amphiploid triticale (× *Triticosecale* Wittmack, $2n = 6x = 42$), nucleolar dominance of wheat is observed, resulting in almost total inactivation of rDNA of rye origin (Thomas & Kaltsikes 1983, Cermeño *et al*. 1984, Lacadena *et al*. 1988). Rye carries a single pair of nucleolar organizing regions (NORs, the site of rRNA genes) on the 1R satellited chromosome, while tetraploid (A and B genomes) and hexaploid (A, B and D genomes) wheat have major rDNA loci on the short arms of satellited chromosomes 6B and 1B, and minor loci on non-satellited chromosomes, 5DS and 1AS, and additional, probably normally unexpressed and perhaps variable loci, on 7DL, 5AL, 1BL, 7DS and 3DS (see Mukai *et al*. 1991, 1993, Jiang & Gill, 1994). In the large majority of cells of the hybrids, the rye NOR is virtually undetectable by silver staining (Cermeño *et al*. 1984, Lacadena *et al*. 1984, Silva *et al*. 1995), a method that reveals interphase nucleoli and stains the metaphase NORs, which were transcribed during the previous interphase (Hubbell 1985, Jimenez *et al*. 1988).

In the present work, we aimed to examine the genetic control of rRNA gene suppression in lines derived from wheat × rye hybrids using molecular cytogenetic methods. These are able to provide insight into interactions between genomes, genes and loci controlling expression.

Materials and methods

Plant material

The hexaploid triticale cultivars, Drira, Juanilho, Lasko, Bacum, Rosner and Rhino, were used. Cultivars Juanilho, Drira and Bacum were supplied by the Estaçao Nacional de Melhoramento de Plantas, Elvas, Portugal; cultivars Lasko (Poznan Plant Breeders, Poland) and Rosner (University of Manitoba, Canada) were obtained from the National Institute of Agricultural Botany, Cambridge, UK; cultivar Rhino,

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Rhino-2D(2R) and associated unpublished data were kindly supplied by Dr W. Pfeiffer, CIMMYT, Mexico. Cultivars Drira, Juanilho and Lasko are AABBRR triticales ($2n = 6x = 42$, with full sets of seven pairs of chromosomes from each of the genomes A, B and R). Cultivar Rosner has a chromosome substitution ($2n = 6x = 42$; AABBRR(2D(2R))(2D(2R)), with chromosome pair 2R from rye substituted by the homoeologous chromosomes 2D from hexaploid wheat; Appels *et al.* 1982). Cultivar Bacum, analysed by *in situ* hybridization during this work, also has a 2D(2R) chromosome substitution. The varieties are not known to be related. Cultivar Rhino is a AABBRR hexaploid triticale; the 2D(2R) substitution line directly derived from the AABBRR line was used in this study and will be referred to throughout the text as Rhino-2D(2R). The hexaploid wheat line (cv. Chinese Spring) with the disomic addition of chromosome pair 1 from rye (cv. Imperial) was also used in these studies (CS + 1R, Driscoll & Sears 1971, supplied by T. E. Miller and S. Reader).

Silver staining

Silver staining was performed on all lines studied following the technique described in Neves *et al.* (1995). Briefly, seeds of each cultivar were germinated in tap water, cold treated for 48 h to synchronize the cells, recovered at 25°C for 26–30 h to maximize metaphase number, and root tips were ice treated for accumulation of condensed metaphases before fixation in FAA [1:1:18 (v / v) formaldehyde (37%)–ethanol (50%)–glacial acetic acid] for 2–3 days at 4°C. The intact root tips were then washed in distilled water to remove the fixative, immersed in a 15% $AgNO_3$ solution (pH 5.5 adjusted with formic acid) overnight at 60°C, washed in distilled water, developed in 1% hydroquinone–10% formaldehyde (1:1) for 5–10 min at room temperature and fixed in freshly prepared photographic fixative. The preparation was made by squashing in 45% acetic acid. The number of nucleoli per interphase cell and the number of silver-stained subterminal metaphase NOR (Ag-ST-NOR) chromosomes were scored for each cultivar. In this study, only the activity of the satellite NORs (present on chromosomes 1B, 6B and 1R) is analysed at metaphase; other loci show little (5D, 1A) or no (7D) expression detectable by silver staining at metaphase (Silva *et al.* 1995), while the terminal heterochromatin of many rye chromosomes also stains with silver. The expression of 5D and 1A NORs can be seen at interphase when they form nucleoli.

In situ hybridization

To analyse the chromosome constitution of the hexaploid triticale cultivars, Lasko and Bacum, non-radioactive fluorescence *in situ* hybridization following Schwarzacher *et al.* (1989) was used with genomic rye DNA cv. Petkus Spring as a probe, in the presence of an excess of unlabelled wheat DNA (Anamthawat-Jónsson *et al.* 1990), to distinguish the parental origin of the chromosomes. Two cloned repetitive sequences, pTa71 (a 9-kb fragment containing the 18S–5.8S–25S rRNA genes and intergenic spacers isolated from wheat, Gerlach & Bedbrook 1979) and pSc119.2 (a 611-bp fragment consisting of a repetitive sequence isolated from rye, Bedbrook *et al.* 1980, subcloned and kindly provided by McIntyre *et al.* 1990), were used to help individual chromosome identification. Both sequences are homologous to wheat and rye DNA.

Results

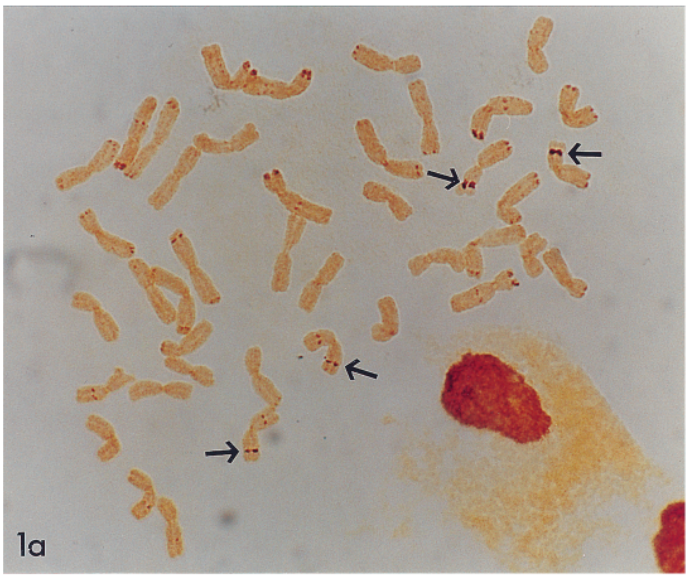
Table 1 summarizes the frequency of interphase nuclei with different numbers of nucleoli (scored after silver staining), and of metaphase cells with different numbers of silver-stained satellite nucleolar organizing regions. Analysis of the nucleolar number by ANOVA (Snedecor & Cochran 1987) showed a very highly significant difference ($P < 0.001$) between cultivars Rosner and Bacum and the three other cultivars in the analysis (Drira, Juanilho and Lasko). Cultivars Rosner and Bacum also showed additional classes of cells with seven and, for Bacum, eight nucleoli, never observed in the other three cultivars in which the maximum number of nucleoli observed was 6. Because of nucleolar fusion during the cell cycle, the maximum number of nucleoli regularly observed is of greater importance than averages. The analysis of metaphase cells with silver-stained subterminal NORs (Ag-ST-NORs) showed that most metaphase cells in cvs Juanilho, Drira and Lasko had four Ag-ST-NORs (86%, Table 1, Figure 1a). In triticale cultivars Bacum and Rosner, six Ag-ST-NORs were observed in 92% and 78% of metaphase cells (Table 1 & Figure 1b).

To test the hypothesis that the chromosome constitution of cv. Bacum was directly correlated with the unusual expression of rye rRNA genes in a triticale, nucleolar activity was studied in a hexaploid wheat line with the disomic addition of 1R chromosomes (CS + 1R; $2n = 6x + 2 = 44$). To evaluate any genotypic influence of the wheat progenitor genome on rye origin NOR expression, nucleolar activity was studied through silver staining in cv. Rhino and Rhino-2D(2R) (Table 1). AABBRR Rhino showed only wheat origin NOR expression in the great majority of cells (75%), as occurred in cultivars Drira, Juanilho and Lasko. Rhino-2D(2R) showed 1R NOR activity in more than 85% of cells, and the ANOVA test indicates that this distribution is similar to the ones observed in cultivars Bacum and Rosner. After silver staining, the CS + 1R line showed a high frequency of metaphase cells with five or six Ag-ST-NORs (68%, Table 1); this distribution was significantly different from the ones observed in both AABBRR and 2D(2R) substitution triticales.

Double target fluorescence *in situ* hybridization was used to allocate rDNA loci observed by silver staining to the different genomes in metaphase cells of cultivars Lasko and Bacum using rye genomic DNA and the wheat rDNA unit as probes. When probed with rye genomic DNA, triticale Lasko showed a balanced amphiploid chromosome constitution with 14 chromosomes of rye origin and 28 chromosomes of wheat origin (Figure 2). Six major rDNA sites were identified on satellite chromosomes 1B and 6B of wheat origin and on chromosomes 1R (Figure 2). *In situ* hybridization on triticale Bacum revealed 12 chromosomes of rye origin and 30 chromosomes of wheat origin (Figure 3a). rDNA hybridization confirmed the identification of two pairs of major NORs on wheat chromosomes

Table 1. Frequencies of interphase and metaphase cells with different numbers of nucleoli and silver-stained, subterminal satellite NORs (Ag-ST-NORs) in triticale cultivars and a wheat-1R addition line

Plant material		Genome constitution	Frequencies of interphases with different numbers of nucleoli								No. of scored interphase cells	Frequencies of metaphases with different numbers of Ag-ST-NORs					No. of scored metaphase cells
			1	2	3	4	5	6	7	8		2	3	4	5	6	
Triticale × *Triticosecale*	cv. Drira	AABBRR $6x=2n=42$	19.58	35.01	32.93	11.79	0.17	0.52	0	0	577	0	8.49	85.62	2.62	3.27	153
	cv. Juanilho	AABBRR $6x=2n=42$	17.69	40.70	29.20	11.89	0.52	0	0	0	2103	1.82	8.49	86.06	2.42	1.21	165
	cv. Lasko	AABBRR $6x=2n=42$	18.87	37.20	32.07	10.51	0.27	1.08	0	0	371	0	6.38	88.30	4.26	1.06	94
	cv. Rhino	AABBRR $6x=2n=42$	13.00	47.69	26.30	10.98	1.45	0.58	0	0	346	0	8.69	65.22	21.74	4.35	46
	cv. Bacum	AABBRR(2R(2D)) $6x=2n=42$	12.89	23.02	34.07	26.70	2.03	0.55	0.37	0.37	543	0	0	4.41	2.94	92.65	65
	cv. Rosner	AABBRR(2R(2D)) $6x=2n=42$	4.71	27.36	46.33	19.76	0.79	0.79	0.26	0	764	0	0	12.50	9.38	78.12	32
	cv. Rhino-2R(2D)	AABBRR(2R(2D)) $6x=2n=42$	13.79	48.20	28.85	8.46	0.46	0.12	0	0.12	863	0	2.86	12.86	30.00	54.28	70
Wheat–rye addition line	CS + 1R	AABBDD + 1RR $2n=44$	19.31	36.34	33.33	8.93	1.64	0.36	0.09	0	1098	2.44	7.32	29.27	21.95	39.02	41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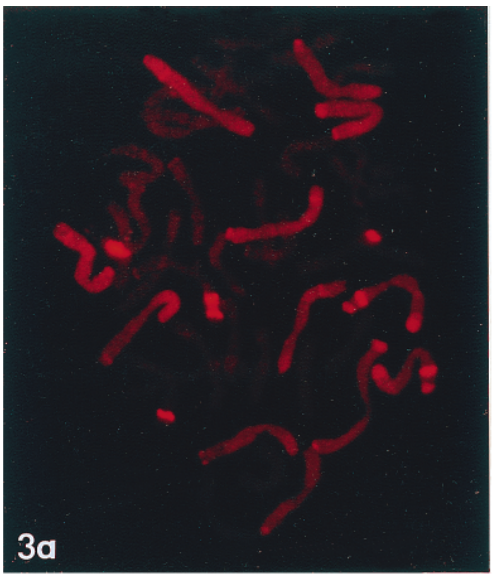
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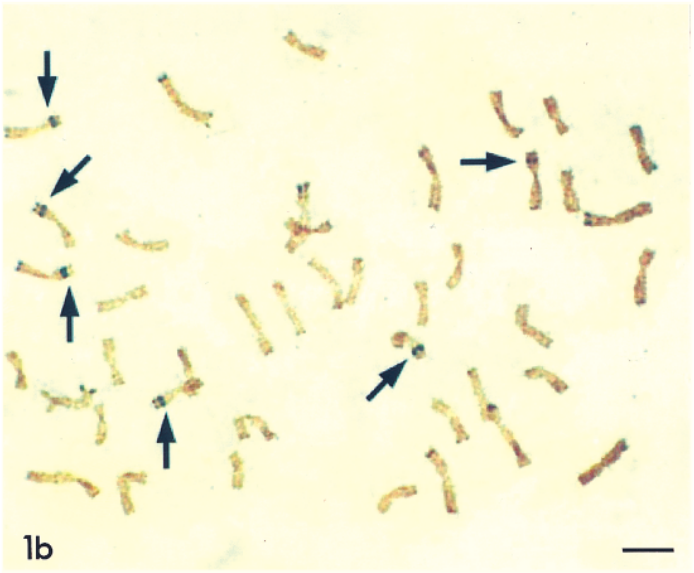
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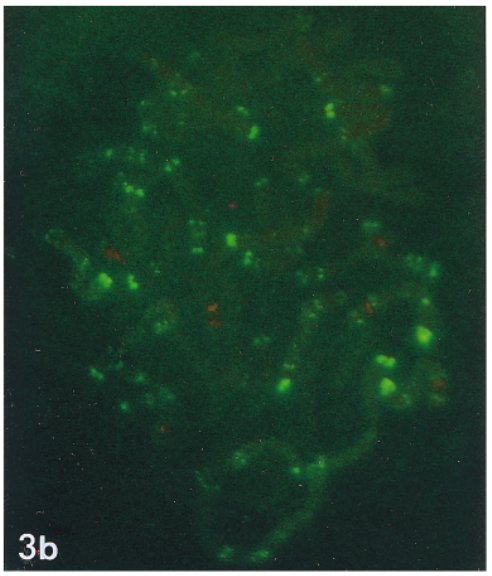
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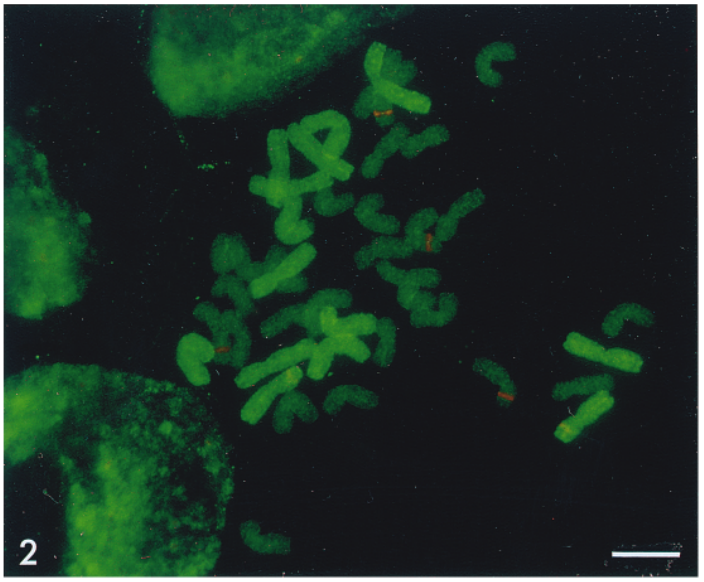
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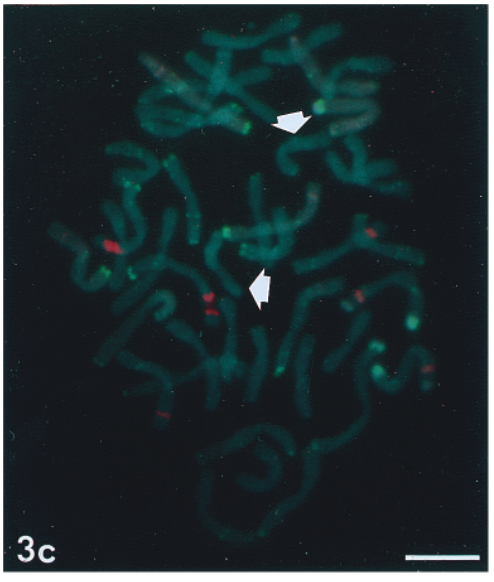
3c

1B and 6B and a third pair located on chromosome 1R (Figure 3a). To identify the wheat–rye chromosome substitution involved in cv. Bacum, morphological analysis of the chromosomes was complemented with *in situ* hybridization and analysis of the distribution of the repetitive DNA sequence pSc119.2. By comparison with wheat (cv. Chinese Spring, Mukai *et al*. 1993) and rye (cv. Petkus Spring, N. Neves & J. S. Heslop-Harrison, unpublished results) pSc119.2 banded karyotypes, the analysis showed that rye chromosome pair 2R was missing and homoeologous pair 2D from wheat was present (Figure 3b & c).

Discussion

Analysis of nucleolar activity in triticale cultivars Drira, Juanilho and Lasko showed only four major silver-positive NORs on wheat chromosomes 1B and 6B (Table 1 & Figure 1a), supporting previous observations (Cermeño *et al*. 1984, 1987, Lacadena *et al*. 1984, 1988) and confirming the suppression of expression of rye-origin rDNA in triticale as a result of nucleolar dominance and genomic interactions in AABBRR cultivars. In contrast, cultivars Bacum and Rosner showed six well-defined silver-positive NORs (Table 1 & Figure 1b). *In situ* hybridization showed that cv. Bacum, like Rosner, had 12 rye origin chromosomes and 30 wheat origin chromosomes, with the loss of chromosome pair 2R and gain of pair 2D (Figure 3). The increase of the frequency of interphase cells with six nucleoli and the appearance of two additional classes of cells with seven and eight nucleoli observed in these cultivars confirmed the expression of all major rDNA loci present (1B, 6B and 1R), contrasting with the reduced activity of 1R rRNA loci detected in the three AABBRR cultivars analysed. The result indicates that the expression of ribosomal genes from rye origin, usually silent in AABBRR triticales, is mediated by the 2D(2R) chromosome substitution.

To demonstrate any effect of the wheat genome on rye origin rRNA gene expression, nucleolar activity was studied in cv. Rhino and a 2D(2R) substitution line produced by chromosome manipulation of AABBRR cv. Rhino (Table 1). Whereas AABBRR Rhino showed the same pattern of 1R rDNA suppression as the other AABBRR triticale lines, in Rhino-2D(2R) rye rRNA genes were found to be active, indicating that the 2D(2R) substitution prevents suppression of rye origin rDNA expression.

The influence of the 2D(2R) chromosome substitution could be ascribed either to the presence of 2D or to the absence of 2R. In octoploid triticales ($2n = 8x = 56$; genome constitution AABBDDRR), where chromosomes 2D and 2R are both present, rDNA of rye origin is not active (Cermeño *et al*. 1987). Therefore, the suppression of the amphiplasty phenomenon in 2D(2R) hexaploid triticales is associated with the absence of chromosome 2R. This suggestion is supported by the detection of five or six Ag-ST-NORs in 68% of metaphase cells in the hexaploid wheat line with a disomic addition of 1R chromosomes (Table 1), indicating that non-nucleolar chromosomes from rye directly influence rye origin rDNA expression in triticales. The significant difference between the distribution of metaphase cells with different number of Ag-ST-NORs in CS + 1R and the 2D(2R) triticale lines suggests a secondary influence, perhaps species-specific transcription factors, located on rye chromosomes other than 1R or 2R.

There are a number of models for the control of rDNA expression (Jordan 1991, Flavell *et al*. 1992, Leitch *et al*. 1992, Sardana *et al*. 1993). A model including the effect of the unlinked regulatory locus revealed here (Figure 4a) shows the simple interaction between gene(s) on chromosome 2 and the NOR on chromosome 1 in rye. It is unlikely that the 1R/2R system operates only in hybrid situations, and this regulatory system probably has a role in rye in modulating rRNA gene expression throughout plant development. The model suggests that a 1R product causes the 2R locus to down-regulate the 1R NOR. In wheat (Figure 4b), a parallel system is presumably present, supported by the presence of differences in relative NOR activities in nullisomic–tetrasomic lines (Cermeño *et al*. 1987). X^w indicates an unknown, wheat origin chromosome(s) with a homologous effect to the 2R chromosome. Where the wheat and rye genomes interact (Figure 4c), chromosome 2R suppresses 1R activity, an action induced by wheat chromosomes 1B and 6B. The wheat regulatory chromosomes (X^w)

Figure 1. Triticale root tip metaphase cells showing silver staining of satellite (subterminal) NORs (arrows) active at the previous interphase. **a** Cv. Lasko, four Ag-ST-NORs. **b** Cv. Bacum, six Ag-ST-NORs. Scale bar = 10 μm.
Figure 2. Root tip metaphase cell of triticale cv. Lasko after *in situ* hybridization, probed with rye total genomic DNA, digoxigenin labelled and detected with anti-digoxigenin–FITC, showing 14 chromosomes of rye origin in light green and 28 chromosomes of wheat origin in dark green; the wheat rDNA, directly labelled with Texas red, hybridizes with six major rDNA sites, but does not show which of the six loci are active. Scale bar = 10 μm.
Figure 3. Root tip metaphase cell of triticale cv. Bacum after *in situ* hybridization. **a** Probed with rye total genomic DNA from rye, and wheat rDNA, both labelled with Texas red, showing 12 chromosomes of rye origin and 30 chromosomes of wheat origin, along with six major rDNA sites, including two on chromosomes 1R. **b** Probed with a repetitive sequence of rye origin, pSc119.2, labelled with digoxigenin and detected with anti-digoxigenin–FITC, to enable chromosome identification. **c** Triple bandpass filter micrograph showing DAPI staining (blue), major pSc119.2 sites (blue-green) and rDNA sites (red). The 2D chromosome pair is arrowed. Scale bar = 10 μm.

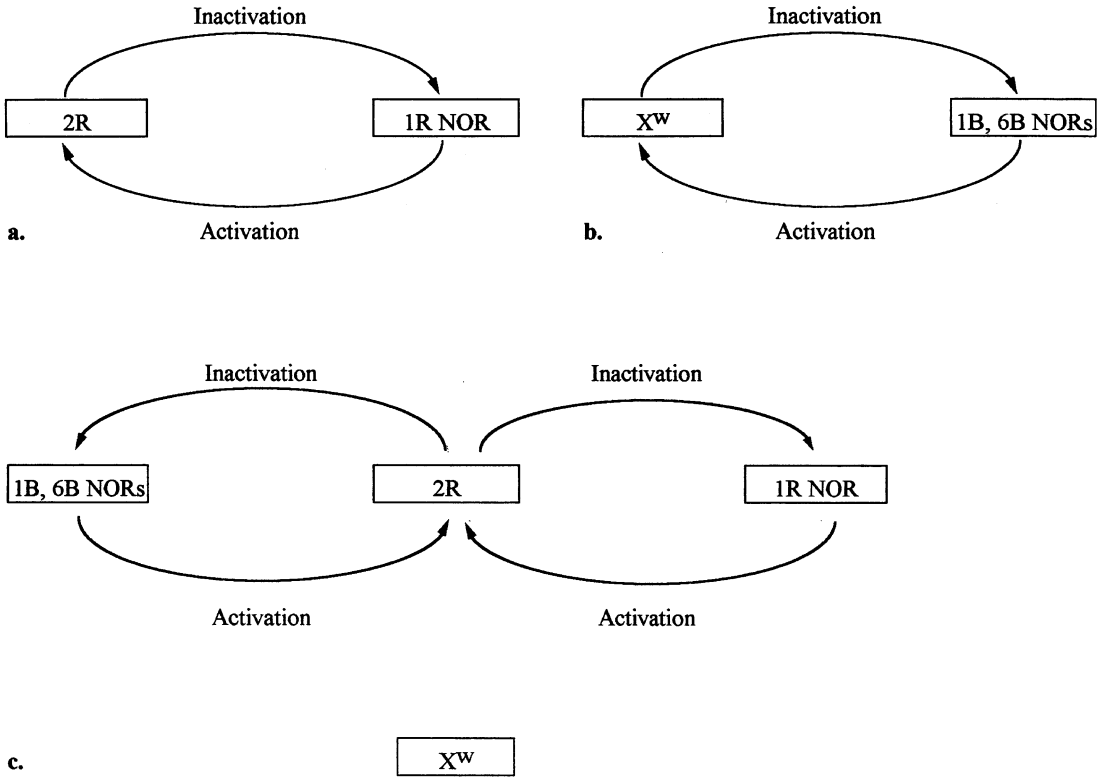

Figure 4. A possible model of interactions between NORs and controlling loci. **a** & **b** The controls acting in rye and wheat respectively, where X^w represents an unknown wheat chromosome with a homologous effect to 2R. In the amphiploid triticale (**c**), wheat origin NORs interact with loci on chromosomes 2R leading to suppression of 1R NOR loci (see Discussion).

equivalent to 2R have little suppressing effect on the 1R NOR, seen in the 2R-deficient lines.

What is the nature of the proposed inactivator of the NOR loci and the activator of the regulatory loci? Although the data are correlative, it seems possible that DNA methylation is ultimately involved in 1R NOR suppression (Neves *et al.* 1995). Sardana *et al.* (1993) have suggested that the number of subrepeats within the intergenic spacer region of the NOR locus is critical to regulation of NOR activity, and propose that there is binding of a protein preventing methylation, thus potentiating expression of the locus. Our data show a negative effect, in which the 2R product inactivates the 1R locus, so the product could be a locus-specific methylase, or a molecule binding to the promoter subrepeats. Whichever, the protein must be more active on the rye NOR locus than on the wheat loci. The allocation to chromosome 2R of gene(s) controlling wheat nucleolar dominance will allow better comprehension of this process through the simultaneous analysis of the methylation pattern of rDNA intergenic sequences and the quantification of global nucleolar activity in distinct addition and substitution genotypes. Schubert & Künzel (1990) examined nucleolar dominance in translocation lines of barley involving the two major NOR-bearing chromosomes. Treatment with the drug azacytidine, to reduce methylation levels, followed by silver staining and *in situ* hybridization as here, showed that hypermethylation alone did not lead to suppression of one NOR when both were translocated to the same chromosome. In parallel experiments with a second line in which the NOR was split by a translocation, the authors suggest a correlation of expression with methylation pattern, leading to unstable expression of the rRNA genes. Thus, modulation of methylation may be one of several mechanisms of rRNA gene modulation.

Triticales released as agricultural varieties, including those studied here, have varied genomic constitutions, and lines with different chromosomes may perform equally well in the field. The complexity of genetic interactions between wheat and rye origin genes, revealed here, indicates that there may be considerable buffering of gene expression, and several genes, perhaps from unlinked loci, may need to be substituted to obtain optimum expression of a locus. This has implications for the use of transformation, in which a single locus may be inserted but turned off. The results also have implications in mapping of traits, since mapping the rye NOR locus by its expression in the hybrid would give a mixed chromosomal location on chromosomes 1R and 2R. It is of course well recog-

nized that different species may have different limiting steps along a single pathway so that quantitative traits like height or protein quantity may map at different sites in the different genomes.

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References

Anamthawat-Jónsson K, Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS (1990) Discrimination between closely related *Triticeae* species using genomic DNA as probe. *Theor Appl Genet* **63**: 337–348.

Appels R, Gustafson JP, May CE (1982) Structural variation in the heterochromatin of rye chromosomes in triticale. *Theor Appl Genet* **63**: 235–244.

Bedbrook JR, Jones J, O'Dell M, Thompson RD, Flavell RB (1980) Molecular characterization of telomeric heterochromatin in *Secale* species. *Cell* **19**: 545–560.

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

Cermeño MC, Friebe B, Zeller FJ, Krolow KD (1987) Nucleolar competition in different (A/B)(A/B)RR and DDRR tetraploid triticales. *Heredity* **58**: 1–4.

Dillon N, Antoniou M, DeBoer E *et al*. (1993) Regulation of the human B-globulin expression domains. In: Heslop-Harrison JS, Flavell RB, eds. *The Chromosome*. Oxford: BIOS Scientific, pp 193–206.

Driscoll CJ, Sears ER (1971) Individual addition of the chromosomes of 'Imperial' rye to wheat. *Agron Abst* 6.

Flavell RB, O'Dell M, Sardana R, Jackson S (1992) Regulatory DNA of ribosomal RNA genes and the control of nucleolus organizer activity in wheat. *Crop Sci* **85**: 889–894.

Gerlach WL, Bedbrook JR (1979) Cloning and characterization of ribosomal RNA genes from wheat and barley. *Nucleic Acid Res* **7**: 1869–1885.

Goldberg RB, Barker SJ, Perez-Grau L (1989) Regulation of gene expression during plant embryogenesis. *Cell* **56**: 149–160.

Higgins CF (1993) The bacterial chromosome: DNA topology, chromatin structure and gene expression. In: Heslop-Harrison JS, Flavell RB, eds. *The Chromosome*. Oxford: BIOS Scientific, pp 193–206.

Hubell HR (1985) Silver staining as an indicator of active ribosomal genes. *Stain Technol* **60**: 285–294.

Jiang J, Gill BS (1994) New 18S.26S ribosomal RNA gene loci: chromosomal landmarks for the evolution of polyploid wheats. *Chromosoma* **103**: 179–185.

Jimenez R, Burgos M, Diaz de el Guardia R (1988) A study of the Ag-staining significance in mitotic NORs. *Heredity* **60**: 125–127.

Jordan EG (1991) Interpreting nucleolar structure: where are the transcribing genes? *J Cell Sci* **98**: 437–442.

Lacadena JR, Cermeño MC, Orellana J, Santos JL (1984) 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 (1988) Nucleolar competition in *Triticeae*. *Kew Chromosome Conference* **III**: 151–165.

Leitch AR, Mosgoller W, Shi M, Heslop-Harrison JS (1992) Different patterns of rDNA organization at interphase nuclei of wheat and rye. *J Cell Sci* **101**: 751–757.

Lu Q, Wallrath LL, Elgin SCR (1993) *Drosophila* P-element-mediated germ line transformation to examine chromatin structure and expression of in vitro modified genes. *Methods Mol Genet* **1**: 333–357.

McIntyre CL, Pereira S, Moran LB, Appels R (1990) New *Secale cereale* (rye) DNA derivatives for the detection of rye chromosome segments in wheat. *Genome* **33**: 635–640.

Mukai Y, Endo TR, Gill BS (1991) Physical mapping of the 18S.26S rRNA multigene family in common wheat. *Chromosoma* **100**: 71–78.

Mukai Y, Nakahara Y, Yamamoto M (1993) Simultaneous discrimination of the three genomes in hexaploid wheat by multicolor fluorescence *in situ* hybridization using total genomic and highly repeated probes. *Genome* **36**: 489–494.

Neves N, Heslop-Harrison JS, Viegas W (1995) rRNA genes activity and control of expression mediated by methylation and imprinting during embryo development in wheat × rye hybrids. *Theor Appl Genet* **91**: 529–533.

Reeder HR (1985) Mechanisms of nucleolar dominance in animals and plants. *J Cell Biol* **101**: 2013–2016.

Sardana R, O'Dell M, Flavell RB (1993) Correlation between the size of the intergenic regulatory region, the status of cytosine methylation of rRNA genes and nucleolar expression in wheat. *Mol Gen Genet* **236**: 155–162.

Schubert I, Künzel G (1990) Position-dependent NOR activity in barley. *Chromosoma* **99**: 352–359.

Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS (1989) *In situ* localization of parental genomes in a wide hybrid. *Ann Bot* **64**: 315–324.

Silva M, Queiroz A, Neves N *et al*. (1995) Reprogramming of rye rDNA in triticale during microsporogenesis. *Chrom Res* **3**: 492–496.

Snedecor GW, Cochran WG (1987) *Statistical Methods*. 7th edn. Ames, Iowa: Iowa State University Press.

Thomas J, Kaltsikes PJ (1983) Effect of chromosomes 1B and 6B on nucleolus formation in hexaploid triticales. *Can J Genet Cytol* **25**: 292–297.