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The influence of B chromosomes on rDNA organization in rye interphase nuclei

M. Delgado, L. Morais-Cecílio, N. Neves, R. N. Jones & W. Viegas

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Patterns of rye rDNA organization in interphase nuclei were studied through the use of *in situ* hybridization in spreads of root meristem cells from plants with and without B chromosomes (Bs). In cells from plants without Bs each rDNA locus is organized as a single perinucleolar knob of condensed chromatin with decondensed chromatin inside the nucleolus. In plants with Bs there is a marked modification of the pattern, found in more than 23% of nuclei, which involves several regions of condensed chromatin interspersed with decondensed chromatin inside the nucleolus. This B-induced alteration in rDNA interphase organization suggests a change in expression of the rRNA genes located on the A chromosomes probably related to the reduction in nuclear RNA observed previously in plants with Bs. The influence of the Bs on the expression of A chromosome genes, through rearrangement of interphase chromatin, could provide the basis of an explanation for some of the known phenotypic effects of B chromosomes in rye.

Key words: B chromosomes, interphase chromatin, rDNA organization, rye

Introduction

In the cereals rRNA genes are present in many thousands of copies assembled in tandem arrays in one or a few loci (nucleolar organizing regions—NORs). In rye there is one pair of NORs in the short arms of chromosome 1 (Schlegel *et al.* 1986). The control of transcriptional activity of rRNA genes can involve the suppression of expression of the whole locus, as often occurs in the 1R NOR of rye in the presence of wheat genomes (reviewed in Viegas *et al.* 1994), or the inactivation of genes within the individual loci (Leitch *et al.* 1992). It has been shown by *in situ* hybridization in interphase nuclei that the pattern of expression of rDNA varies between species (Leitch & Heslop-Harrison 1993). In rye the gene sequences at the distal (telomeric) end of the locus, which are the ones dispersed inside the nucleolus, are the ones most often expressed, while the genes at the proximal end of the locus remain condensed at the periphery of the nucleolus (Leitch *et al.* 1992).

An interesting aspect of this variation in the pattern of chromatin organization, involving the rDNA, is to study the way in which the patterns may alter in response to nucleotype changes. In rye, and in many other species for that matter, this aspect can be studied through observations on the nucleoli of plants with and without supernumerary B chromosomes (Bs). The Bs in rye have recently been reviewed by Jones & Puertas (1993), and details on their wider significance in other species can be found in Jones & Rees (1982). Bs are additional and non-essential chromosomes that are present in some individuals of a population and absent from others. They have irregular modes of inheritance, and in rye they display mitotic drive based on non-disjunction at first mitosis in the male and female gametophytes. This drive promotes and maintains their spread in populations despite the presence of harmful phenotypic effects of varying severity (Jones 1991). Phenotypic effects of Bs in rye have been observed at different levels of analysis: at the nuclear level phenotypic effects are known to cause reductions in the levels of nuclear proteins and nuclear RNA (Kirk & Jones 1970) and at the endophenotypic level phenotypic effects on physiological and reproductive fitness have been observed (Jones & Puertas 1993). The rye Bs are lacking in major gene loci, except that is for the genetic element that controls the non-disjunction process. This element has been localized to the distal end of the long arm of the B, and its activity appears to be mediated by DNA methylation (Neves *et al.* 1992). Otherwise it is

M. Delgado (corresponding author), L. Morais-Cecílio, N. Neves and W. Viegas are at Departamento de Botânica e Engenharia Biológia, Instituto Superior de Agronomia, Tapada da Ajuda, P-1399 Lisboa Codex, Portugal. Tel: (+351) 1 363 8161; Fax: (+351) 1 363 5031. R.N. Jones is at The Institute of Biological Sciences, University of Wales Aberystwyth, Sir George Stapledon Building, Aberystwyth SY23 3DD, Wales UK. Email: rnj@aber.ac.uk.

assumed that the genetic activity of the rye Bs is through their quantitative effects on A chromosome genes, which could involve effects on interphase chromatin.

Evidence that the architecture of interphase chromatin is related to gene expression has already been provided (Bennett 1984, Manuelidis & Borden 1988). In this study we have analysed the influence of the rye B chromosome on the physical organization of the A chromosome rDNA loci.

Materials and methods

Plant material

Root tips were obtained from seedlings of five strains of *Secale cereale* L.: cultivars Imperial and Centeio do Alto, which lack Bs, and populations from Transbaikal (Siberia), Yakutia (Far East Russia) and JNK (Japan) all of which carry Bs. The Bs in rye have a standard form, known as the standard fragment B, and which on cytological criteria at least is uniform throughout the range of distribution of rye (Lima De Faria 1963). There is no reason to believe that the heterogeneous material used in these experiments will affect the generality of the conclusions reached. Seeds were germinated on moist filter papers at 25°C for 48 h, followed by 24 h at 4°C and 24.5 h at 25°C. Roots were excised and immersed in ice-cold water for 24 h to induce c-metaphases. Fixation was in ethanol–acetic acid (3:1, v/v). For each rye strain at least two distinct root tips were used.

in situ hybridization

Cells were spread by enzymatic digestion, using the meristematic apex with the root cap removed and following the procedure of Schwarzacher *et al.* (1989). The *in situ* procedure was modified from Leitch *et al.* (1991), using the DNA probe pTa71 and a specific probe for the rye B chromosomes. The pTa71 is a 9 kb *Eco*RI fragment of the ribosomal DNA from wheat (*Triticum aestivum*), containing the 5.8S, 18S, 25S and non-transcribed spacer sequences, and was kindly supplied by R.B. Flavell and M. O'Dell. This probe was labelled with biotin-11-dUTP by nick translation and detected with the streptavidin–Cy3 conjugate. The B-specific probe from rye is an *Eco*RI fragment corresponding to part of a specific rye B chromosome family of highly repeated sequences, D1100 (Sandery *et al.* 1990): it hybridizes with the telomeric heterochromatin at the end of the long arm of the B (Blunden *et al.* 1993). The D1100 probe was direct labelled with FITC (fluorescein isothiocyanate isomer I). DNA was counterstained with DAPI (4′,6-diamino-2-phenylindole dihydrochloride), and cytological observations were made on cells at metaphase and interphase using fluorescence microscopy.

Results

Metaphase

In B-containing material the number of Bs in root meristem cells was determined directly by chromosome counting (Table 1) and confirmed by *in situ* hybridisation with the B-specific probe (Figure 1a & b). No hybridization signals were detected on any of the A chromosomes. Labelling with pTa71 showed two sites of hybridization on metaphase chromosomes, corresponding to the location of the NORs in chromosome 1R. No rRNA loci were detected in any of the B chromosomes (Figure 1c).

Interphase

In the majority of interphase nuclei pTa71 hybridizes to two well-defined sites in perinucleolar positions. Fainter labelling shows fine traces of signal connected to the strongest sites and extending into the nucleolus (Figure

Table 1. Frequency (%) of interphase nuclei with intranucleolar condensed rDNA chromatin in different rye strains with or without B chromosomes

Rye strain	No. of Bs	Percentage of nuclei with condensed intranucleolar rDNA[a]	No. of interphase cells
Non-carrying Bs			
Imperial	—	6	200
C. Alto	—	5	100
Carrying Bs			
Yakutia	0	5	200
	2	28	100
Transbaikal	3	23	100
	4	33	100
JNK	3	23	100

[a]No significant variance was observed between root tips from the same strain.

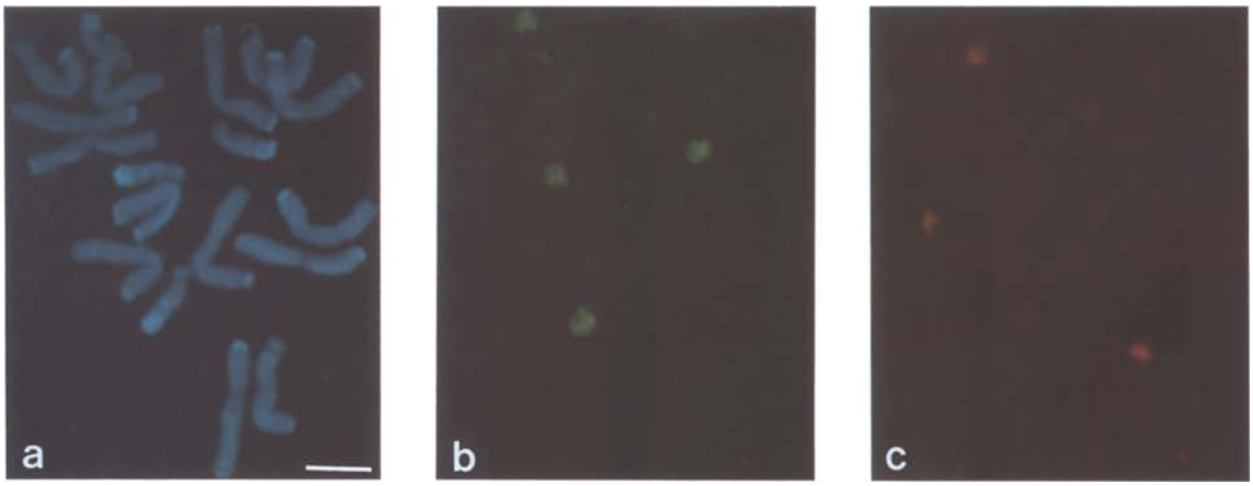

Figure 1. Root tip cell of Transbaikal rye with four B chromosomes. **a** DAPI staining for DNA. **b & c** Simultaneous *in situ* hybridization with a biotin-labelled rDNA probe (pTa71) detected with streptavidin–Cy3 (red) and a rye B-specific DNA D1100 probe direct labelled with FITC (yellow). **b** The sites of hybridization with the specific probe for the telomeric end of the long arm of the B identify a cell with four Bs. **c** The sites of hybridization of pTa71 corresponding to the location of rRNA genes. Bar =10 μm.

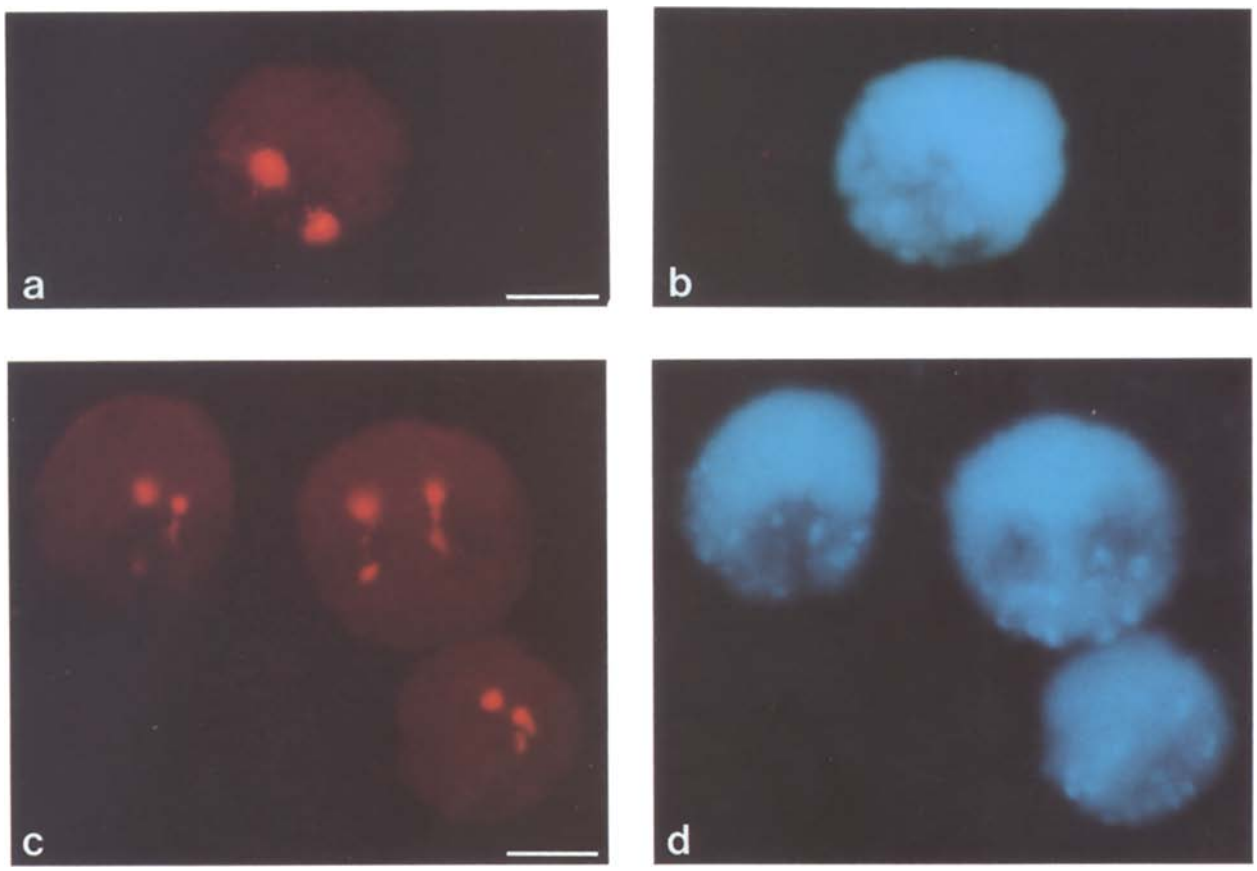

Figure 2. Interphase nuclei after *in situ* hybridization with biotin-labelled rDNA probe (pTa71), detected with streptavidin–Cy3 (red). **a** *S. cereale* cv. Imperial, showing the perinucleolar sites of hybridization and very faint traces of labelled chromatin inside the nucleolus. **c** Transbaikal rye with 3Bs showing hybridized spots inside the nucleolus, as well as the perinucleolar sites and the traces of intranucleolar chromatin. **b & d** the same nuclei as in a and c, respectively, with DAPI staining for DNA. Bar = 10 μm.

2a & b). In some nuclei bright sites of labelling can be seen inside the nucleolus, often joined to less brightly fluorescent traces (Figure 2c & d). The frequency of nuclei with bright intranucleolar hybridization spots differs markedly in plants with and without Bs (Table 1). In the 0B cultivars this frequency varies between 5% and 6%; in contrast, in the +B plants, at least 23% of nuclei in the Transbaikal and JNK rye with 3Bs and a maximum of 33% of nuclei in Transbaikal with 4Bs have these bright spots. In 2B and 0B individuals screened out from the B-carrying material from Yakutia, the frequency of nuclei with intranucleolar condensed rDNA was 28% and 5% respectively; thus in the 0B individuals the frequency is identical to that observed in plants from cultivars that lack Bs. The observation on plants with and plants without Bs from within the same Yakutia cultivar acts as an internal control and eliminates any possible concerns about heterogeneity of the experimental material.

Discussion

The results presented here show that, in 0B plants from both strains with and without Bs, the physical organization of the rDNA loci of the majority of the interphase nuclei is similar to that described for the rye cultivar Petkus Spring by Leitch *et al.* (1992). In rye each rDNA locus is organized as one perinucleolar knob of condensed non-transcribed chromatin connected to decondensed active chromatin extending into the nucleolus. We consider that the low frequency (5–6%) of nuclei with condensed rDNA chromatin inside the nucleolus in 0B cells does not represent a significant deviation from the pattern of rDNA expression proposed for rye by Leitch *et al.* (1992).

Our interpretations about the physical organization of chromatin in relation to its state of condensation are based on work in rye and in other species. In wheat a distinct pattern of physical organization of rDNA showed most nuclei to have decondensed regions interspersed with condensed chromatin, which was considered to be transcriptionally inactive (Leitch *et al.* 1992). Rawlins & Shaw (1990) showed that in meristematic cells of the pea the rDNA condensation pattern consisted of perinucleolar knobs of non-transcribed chromatin together with intranucleolar spots. It was also shown in this species that organization of the rDNA depended upon the cell type (Shaw *et al.* 1993). In the more differentiated cells the intranucleolar spots of condensed chromatin, which were presumed to be less transcriptionally active, were larger, and the nucleoli themselves were smaller. In the present work the procedure used to prepare cell spreads was intended to minimize the presence of differentiated cells and to standardize their presence in all of the preparations. When the results are viewed in relation to the established findings in rye, wheat and pea, it seems reasonable to conclude that the large number of nuclei with condensed chromatin found in the +B cells reflects an influence of the rye Bs on the organization and expression of the rDNA chromatin in nucleoli.

The significant increase in the number of cells with condensed rDNA found inside the nucleoli in the +B plants cannot be explained by the presence of additional rRNA loci located in the Bs themselves, since no labelling with the pTa71 probe was found on the Bs of any of the populations studied in this work. In contrast, Flavell & Rimpau (1975) reported the presence of rRNA genes in the Bs of Transbaikal rye on the basis of DNA–DNA hybridization studies and established a correlation between the number of Bs and an increase in the number of rRNA genes (estimated at 650 per B). However, these results were obtained using rye Bs in a wheat background and were not substantiated for rye Bs in other strains of rye. Other work based on *in situ* hybridization studies with rDNA probes has also failed to find any rRNA genes on the rye Bs: Niwa & Tsujimoto (1992) used six different diploid strains of rye, and Cuadrado & Jouve (1994) used Bs of a Portuguese strain of rye probed with pTa71 and pTa794. The present work uses rye Bs within rye genotypes, and there is thus no evidence whatsoever that any additional rRNA genes could have been contributed by the B chromosomes. It also seems unlikely that the patterns of expression can be accounted for by chance differences in the A chromosome sets, since in the 0B Yakutia rye the frequency of nuclei with intranucleolar rDNA condensation was identical to that in plants from the other non-carriers of B chromosomes.

The observations described here lead us to hypothesize that some rRNA gene sequences normally available for expression may become inactivated in the presence of B chromosomes in rye. Although proof of this theory is required, the idea is compatible with many aspects of the known effects of Bs on the phenotype. An increase in the number of Bs progressively reduces plant vigour (Jones & Puertas 1993), and there are also clear-cut effects on the nuclear phenotype in terms of reduced levels of nuclear proteins and RNA (Kirk & Jones 1970). The changes in the levels of these nuclear components were determined cytochemically, and in view of the magnitude of the changes there is every reason to suppose that the RNA differences can be attributed to the ribosomal fraction (Jones & Rees 1982).

To speculate further, it is possible to imagine that the presence of the extra B chromosomes could affect other aspects of the interphase disposition and territorial arrangement of the As. Evidence that nuclear architecture is related to gene expression comes from several studies in plants and animals. Bennett (1984) showed that in hybrids between certain grass species the location of the rRNA genes correlated with their activity, and Manuelidis & Borden (1988) proved that patterns of chromosome organization varied in different tissues in humans. Heslop-Harrison (1990) reported that in hybrid cereals the parental genomes are spatially separated, with the genome disposed to the periphery of the

nucleus being preferentially expressed. In this case the differences in disposition and expression were also related to DNA methylation and to aspects of genome imprinting. If it is the case that the rye Bs alter nuclear architecture and A chromosome disposition, which is not unlikely considering that each B contributes about 5% extra nuclear DNA, then a possible basis exists for interpreting and for further studying the manifold effects of Bs on the development and physiology of rye and other B-carrying species. B chromosomes could also provide us with a model system for studying the relationship between nuclear architecture and the genetic activity of chromatin in a more general context.

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