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Physical mapping of 5S rDNA reveals a new locus on 3R and unexpected complexity in a rye translocation used in chromosome mapping

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Abstract. Using fluorescence in situ hybridization (FISH) with probe pScT7, three different 5S rDNA loci were detected in the satellite of rye chromosome 1R (*5SDna-R1*) and in the short arms of chromosomes 3R (*5SDna-R3*) and 5R (*5SDna-R2*) respectively. All three loci showed polymorphism for the hybridization signal intensity. In order to determine the localization of these rye 5S rDNA multigene loci with higher precision within the corresponding chromosome arms, the probe pScT7 was physically mapped by FISH in relation to the following five translocations (Wageningen Tester Set): *T850W* (1RS/4RL), *T248W* (1RS/6RS), *T273W* (1RS/5RL), *T305W* (2RS/5RS) and *T240W* (3RS/5RL). Accurate physical maps of the translocation breakpoints had previously been made using electron microscope analysis of spread pachytene synaptonemal complexes of heterozygotes for the different translocations. The results indicate that locus *5SDna-R3* is located between the breakpoint of translocation *T240W* and the telomere, whereas locus *5SDna-R2* is located between the breakpoint of translocation *T305W* and the centromere, the hybridization of probe pScT7 on *T305W* translocated chromosomes demonstrating the complex nature of this translocation. On the other hand, the simultaneous detection of probes pScT7 and pTA71 (18S-5.8S-26S rDNA) with two different fluorochromes, indicated that the breakpoints of translocations *T850W* and *T248W* are located between loci *Nor-R1* and *5SDna-R1*.

Introduction

The increasing improvement of in situ hybridization techniques has revealed its usefulness for mapping DNA sequences directly on metaphase chromosomes. In addition to the ability to generate clear signals from labeled probes, the achievement of precise gene localization with these techniques requires a reference system on the mitotic chromosomes, or the simultaneous hybridization of several probes (Lawrence 1990; Korenberg et al. 1992).

In the Triticeae, using chromosome in situ hybridization, the tandemly repetitive sequences of 18S-5.8S-26S rDNA (Appels et al. 1980; Miller et al. 1983; Mukai et al. 1991; Leitch and Heslop-Harrison 1992), the different families of highly repetitive sequences (Bedbrook et al. 1980; Jones and Flavell 1982a, b; Appels and Moran 1984) and the telomeric sequences (Schwarzacher and Heslop-Harrison 1991), were assigned to the secondary constriction of chromosome 1R, the telomeric or interstitial C-heterochromatin bands and the telomeres, respectively. However, a lower degree of accuracy was achieved in the cytological location of other loci not associated with specific chromosome features such as the 5S rDNA multigene family (Appels et al. 1980; Reddy and Appels 1989; Mukai et al. 1990; Leitch and Heslop-Harrison 1993), the storage protein genes (Clark et al. 1989; Gustafson et al. 1990; Lehfer et al. 1993) and the α-amylase2 gene (Leitch and Heslop-Harrison 1993).

In rye, the structure and organization of 5S and 18S-5.8S-26S rDNA multigene families have been studied in detail (Appels et al. 1980; Reddy and Appels 1989). Using radioactively labeled probes, Appels et al. (1980) mapped the 18S-5.8S-26S multigene family at the secondary constriction, on the short arm of chromosome 1R, and reported the existence of a first locus of 5S rDNA (*5SDna-R1*) located on the satellite of chromosome 1R, close to the nucleolus organizer region (NOR). A second 5S rDNA locus (*5SDna-R2*), difficult to detect in some lines, was later located on the short arm of chromosome 5R by Reddy and Appels (1989).

In the investigation reported here, a third 5S rDNA locus located on chromosome arm 3RS was found using fluorescence in situ hybridization (FISH). The three 5S rDNA loci were physically mapped with respect to five different rye translocations involving chromosomes 1R, 3R and 5R. The position of the breakpoint of each translocation was previously determined by means of electron

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microscope (EM) analysis of spread pachytene synaptonemal complexes (SCs) of heterozygotes. The breakpoints were used as accurate physical reference points in order to reach a higher degree of precision in the physical location of 5S rDNA loci.

Materials and methods

Plant material. The plant material used in the FISH experiments and in the SC analyses was from different sources: two karyotypically normal inbred lines (J14 and E; received from J. Orellana); five lines homozygous for different reciprocal translocations (*T850W*, *T248W*, *T273W*, *T305W*, and *T240W*) belonging to the Wageningen Tester Set (Sybenga and Wolters 1972), received from J. Sybenga; the progenies from different crosses involving plants of the above lines and cv. Ailés; and a line doubly ditelocentric for chromosomes 1R and 3R, obtained from ditelocentrics received from J. Sybenga.

DNA probes. The clones used in this study were pTA71 (Gerlach and Bedbrook 1979) containing a 9 kb EcoRI fragment of the wheat 18S-5.8S-26S rRNA gene repeat unit, and pScT7 (Lawrence and Appels 1986), which encloses a 462 bp BamHI fragment of the rye 5S rDNA unit.

C-banding and FISH analysis. Root tips from germinating seeds were immersed in tap water at 0° C for 24 h to shorten the chromosomes, and fixed in 1:3 acetic acid:ethanol for 1 to 4 months at 4° C. Chromosome slides were made according to the standard squash procedures for the C-banding technique as well as for FISH experiments. C-banding was performed following the method described by Giraldez et al. (1979). Slides were pretreated prior to FISH with RNAase and pepsin according to Wiegant et al. (1991).

The insert from the pScT7 probe was digested, electrophoresed in agarose, excised from the gel and finally nick translated with digoxigenin 11-dUTP (Boehringer) while the whole pTA71 plasmid probe was directly labeled by nick translation with biotin 16-dUTP (Boehringer) (Pendás et al. 1993a).

Single hybridization and immunological amplification of digoxigenin-labeled probes was performed according to Pendás et al. (1993a, b). Chromosomes were denatured in 70% formamide, 2×SSC for 2.5 min at 80° C and quickly immersed in cold 70% ethanol and ethanol dehydrated. Posthybridization washes were carried out at 37° C in 50% formamide, 2×SSC, and 60° C 2×SSC. (1×SSC is 0.15 M NaCl, 0,015 M sodium citrate.) Double hybridization with biotin+digoxigenin was performed under the same conditions, at a final concentration of each probe of 4 ng/µl.

Simultaneous detection of both signals involved one antibody and two Avidin-FITC (fluorescein isothiocyanate) layers (green signal) for biotin (Pinkel et al. 1986) and three antibodies for digoxigenin detection (Pendás et al. 1993b), the final one being conjugated to TRITC (tetramethylrhodamine β isothiocyanate) (red). Chromosomes were 4′,6-diamidino-2-phenylindole (DAPI) counterstained and identified by the C-banding-like pattern obtained as a result of the chromosomal denaturation (Heng and Tsui 1993). Three overlapping photographs were taken in order to visualize simultaneously biotin (yellow), digoxigenin (red) and DAPI counterstained chromosomes (blue). Visualization and photographs were taken using a Fluophot Nikon microscope and a 400 ASA color slide film.

Synaptonemal complex analysis. The pachytene SCs of plants heterozygous for translocations *T850W*, *T248W*, *T240W*, *T273W* and *T305W* were analyzed following the spreading and staining procedure of de Jong et al. (1989) as modified by Naranjo et al. (1989).

The SCs were studied in the EM, photographed and printed at a final magnification of ×7000. The lengths of the SCs were measured on the photographic prints by means of a digitizing tablet connected to a computer.

Results and discussion

Physical mapping of the translocation breakpoints

The rye plants used in this study showed a high degree of polymorphism for the amount of telomeric C-heterochromatin. The breakpoints of these translocations are located in the following chromosome arms (de Vries and Sybenga 1976; Sybenga et al. 1990; Alonso-Blanco et al. 1993): the satellite of 1RS and 4RL in translocation *T850W*; the satellite of 1RS and 6RS in *T248W*; 3RS and 5RL in translocation *T240W*; 1RS (between the NOR and the centromere) and 5RL in *T273W*; and 2RS and 5RS in *T305W*. Owing to their differences in size and arm ratio, all translocated chromosomes could be cytologically distinguished from the non-translocated ones, even without C-banding.

The precise localization of the breakpoints of these translocations was previously established by means of SC analysis by Alonso-Blanco et al. (1993) and Alvarez et al. (1994). Table 1 shows the relative lengths of the segments into which the breakpoints divide the chromosomes involved in the different translocations. Figure 1 is a diagram, based on mitotic C-banding and SC analysis, in which the relative positions of these breakpoints are indicated. In summary, the translocations studied divide the satellite of chromosome 1R into three segments: a proximal one between the breakpoint of *T248W* and the NOR which, agreeing with the results of de Jong et al. (1989), represents 35.8% of the satellite; a second one (20.5%) between the breakpoints of *T850W* and *T248W*; and a distal one (43.7%) between the telomere and the breakpoint of *T850W*. On the other hand, chromosome arms 3RS and 5RS are divided into two halves by the breakpoints of *T240W* and *T305W*, respectively.

Identification and physical mapping of 5S rDNA loci

The FISH experiments using the 5S rDNA probe (pScT7) in normal, non-translocated plants, showed a maximum of three pairs of hybridization signals (Fig. 2c). Two of these pairs of signals corresponded to the previously described loci (Appels et al. 1980; Reddy and Appels 1989) *5SDna-R1* and *5SDna-R2* since in plants carrying translocation *T273W*, hybridization signals appeared on both arms of the small translocated chromosome (Fig. 2e), formed by chromosome arms 1RS and 5RS. The third locus was mapped in the short arm of chromosome 3R, in the segment between the breakpoint of translocation *T240W* and the telomere, probably close to the telomeric C-heterochromatin. This assignment could be deduced from FISH experiments using translocation *T240W*, in which the small translocated chromosome (formed by chromosome arms 3RS and 5RS) carried hybridization signals on both arms (Fig. 2f). Also, in plants with telocentric chromomsomes 3RS and 3RL, the hybridization signal appeared in one of the telocentrics (data not shown). Following Reddy and Appels nomenclature (1989), the 5S rDNA locus mapped on 3RS should be referred to as *5SDna-R3*.

Table 1. The relative lengths (%) of the different segments of the chromosomes involved in the translocations studied, derived from electron microscope (EM) analysis of the pachytene synaptonemal complex (SC) formed by the corresponding quadrivalents in heterozygotes

Translocation	Chromosomes involved	Chromosome segment				Number of cells
		Satellite	Telomere (S)-TB	TB-NOR	TB-Telomere (L)	
T240W	3R	–	25.4±1.6	–	74.6±1.6	6
	5R	–	33.8±2.1	–	66.2±2.1	
T248W	1R	–	10.4±1.2	5.8±1.4	83.8±1.3	7
	6R	–	17.9±1.6	–	82.1±1.6	
T273W	1R	13.5±0.6		11.6±2.9	74.9±2.8	5
	5R	–	40.9±1.1	–	59.1±1.1	
T305W	2R	–	47.4±1.8	–	52.6±1.8	5
	5R	–	20.8±1.6	–	79.2±1.6	
T850	1R	–	6.3±0.7	8.1±0.8	85.6±0.6	10
	4R	–	53.1±2	–	46.9±2.0	

Telomere (S), short arm telomere; Telomere (L), long arm telomere; TB, translocation breakpoint; NOR, nucleolar organizing region

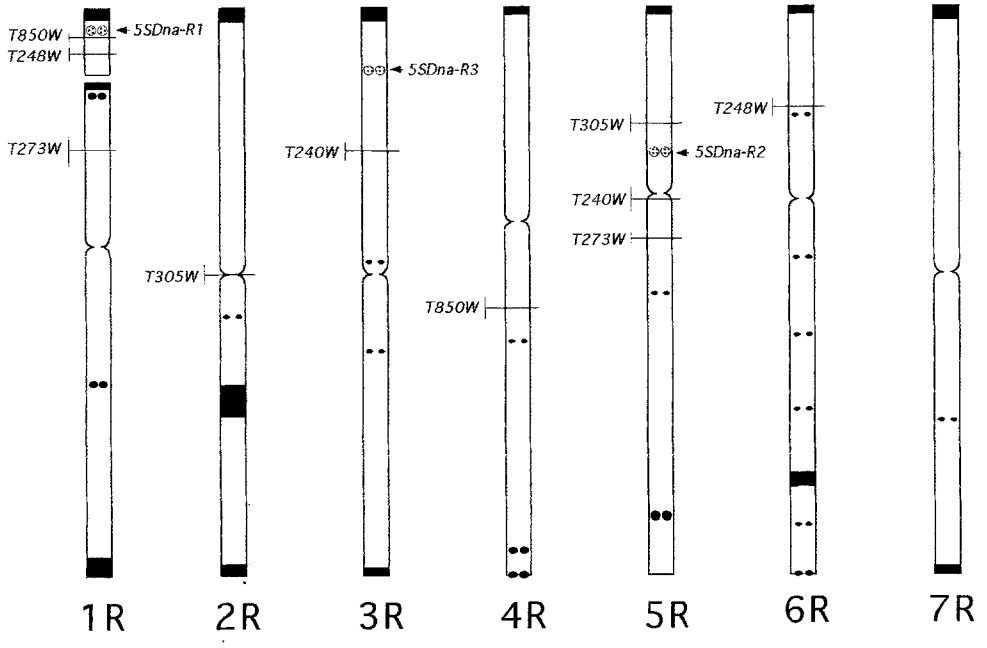

Fig. 1. The physical map (within relative chromosome positions) of the breakpoints of the five translocations analyzed, based on SC measurements (standard errors are indicated by *vertical bars*), indicating the localization of 5S rDNA loci mapped by fluorescence in situ hybridization (FISH)

Concerning the localization of locus *5SDna-R1* within the satellite, the in situ hybridization of probe pScT7 on metaphases of plants with translocations *T850W* and *T248W* (which have their breakpoints in the satellite) showed hybridization signals on the translocated chromosomes without the satellite, the translocated chromosomes carrying the satellite not being labeled (Fig. 2a, d). Therefore, *5SDna-R1* is located distally with respect to both translocation breakpoints. Since the breakpoint of *T850W* is located near the middle of the satellite, distal to the breakpoint of *T248W*, it can be concluded that *5SDna-R1* is physically located in the distal half of the satellite (Fig. 1), a position equivalent to the one occupying the corresponding 5S rRNA homoeolocus in chromosome 1B (Kota et al. 1993).

These results show a discrepancy with the results of Rogowsky et al. (1993) who, using wheat-rye recombinants, physically located the storage protein locus *Sec1* (*Gli-R1*) between *5SDna-R1* and the telomere, and the conclusions of Sybenga et al. (1990) who, using heterozygotes for translocation *T850W*, found a recombination frequency of 2.2% between *Sec1* and this translocation, and assuming that chiasmata were not formed in the segment of the satellite distal to the breakpoint, located *Sec1* (*Gli-R1*) between this translocation and the NOR. Our own meiotic observations of heterozygotes for translocation *T850W* indicate that chiasmata do form in the distal segment of the satellite (21.3% of metaphase I cells having at least one chiasma), supporting the marker order: 1RS telomere...*Sec1*(*Gli-*

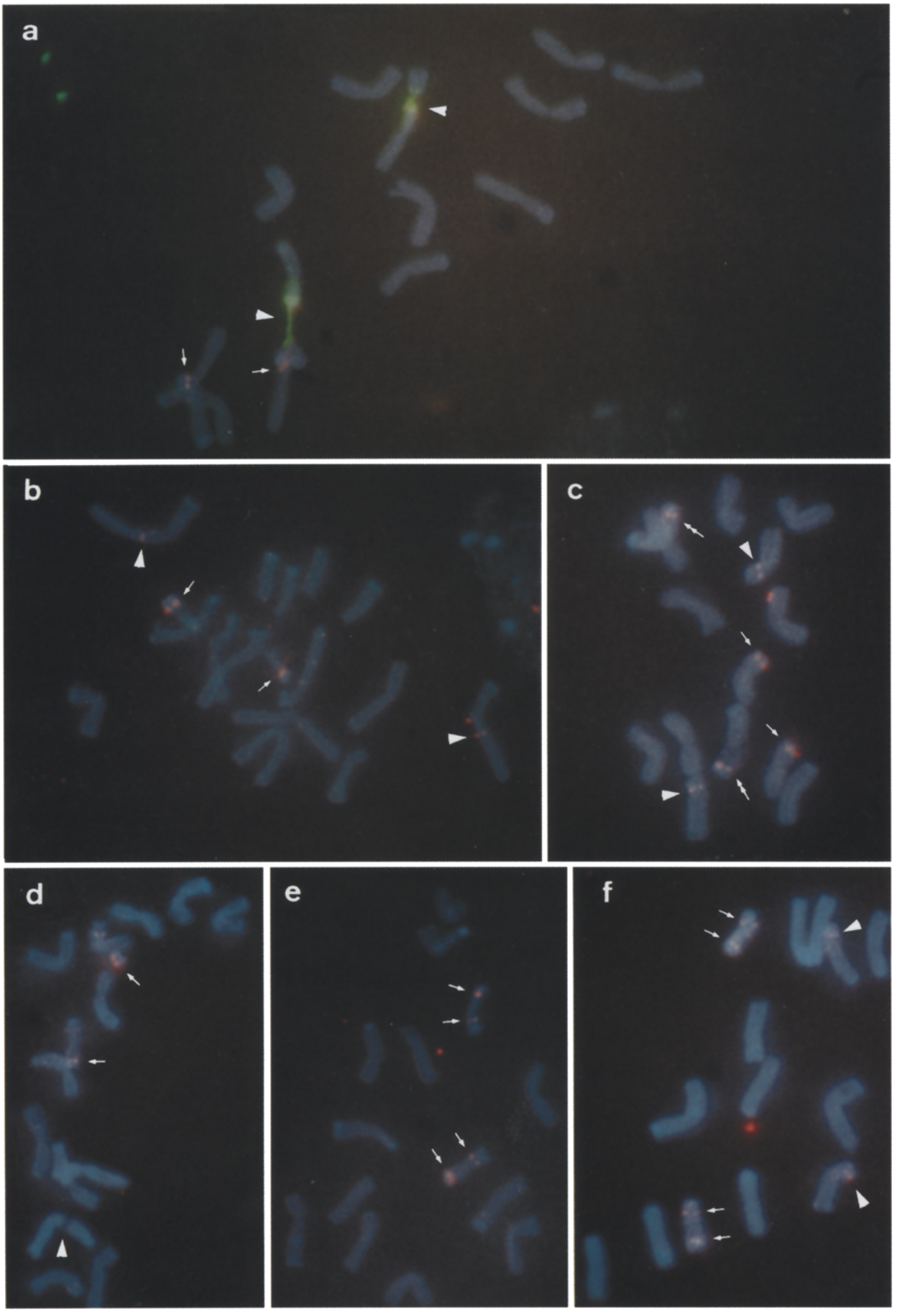

Fig. 2a–f

R1)...5SDna-R1...T850W; similar to that found for the corresponding homeoloci in the D-genome (Lagudah et al. 1991).

On the other hand, as described by Mukai et al. (1991) for the wheat chromosomes bearing satellites, the in situ hybridization of the 18S-5.8S-26S rDNA probe (pTA71) on normal plants showed two intense hybridization signals located at both ends of the secondary constriction, joined by a faint signal crossing this constriction (Fig. 2a). The hybridization of this probe to metaphases of plants homozygous for translocation *T248W* (Fig. 2a) revealed that, although the breakpoint of this translocation is close to the secondary constriction, it does not break the cluster of 18S-5.8S-26S rDNA copies located in the satellite.

The results obtained in the in situ hybridization with probe pScT7 on plants carrying translocation *T305W* are doubly interesting. In regard to the mapping of *5SDna-R2*, the corresponding hybridization signal appeared in the long translocated chromosome (Fig. 2b), which has the telomere constitution 2RS-5RL. This indicates that locus *5SDna-R2* is physically located between the centromere of chromosome 5R and the breakpoint of *T305W* (Fig. 1). On the other hand, from the detailed analysis of the within chromosome position of the hybridization signal (Fig. 2b) it can be concluded that *5SDna-R2* is located on the arm of the long rearranged chromosome having the 5RL telomere. This indicates that this arm contains segments from both chromosome arms 5RS and 5RL, and reveals the unexpectedly complex nature of translocation *T305W*.

Fig. 2. a Simultaneous FISH of probes pScT7 (5S rDNA; *red*) and pTA71 (18S-5.8S-26S rDNA; *green*) to metaphase chromosomes of a plant homozygous for *T248W*, showing the localization of *5SDna-R1* (*arrows*) in the translocated chromosome not bearing the NOR (locus Nor-R1), to which probe pTA71 has hybridized (*arrowheads*). **b–f** FISH of probe pScT7. **b** Metaphase chromosomes of a plant homozygous for *T305W*, homozygous for telocentrics 1RS, 1RL and heterozygous for telocentrics 3RS and 3RL. The hybridization signals corresponding to *5SDna-R1* (*arrows*) are in the telocentric chromosomes 1RS, and the ones corresponding to *5SDna-R2* (*arrowheads*) are in the long translocated chromosomes (in the chromosome arms having the 5RL telomere). **c** Metaphase chromosomes of a nontranslocated rye plant of inbred line J14, showing the fluorescence signal corresponding to loci *5SDna-R1* (*arrows*), *5SDna-R2* (*arrowheads*) and *5SDna-R3* (*doubleheaded arrows*). **d** Metaphase chromosomes of a plant heterozygous for *T850W*. The hybridization signals corresponding to locus *5SDna-R1* (*arrows*) appear in the normal chromosome 1R and in the translocated chromosome not bearing the satellite. The translocated chromosome carrying the satellite is indicated by an *arrowhead*. **e** Metaphase chromosomes of a homozygote for *T273W* in which the hybridization signals (*arrows*) corresponding to loci *5SDna-R1* and *5SDna-R2* appear in the same translocated chromosome (short). In these plants, hybridization at locus *5SDna-R3* was undetectable. **f** Metaphase chromosomes of a homozygote for *T240W*. The hybridization signals (*arrows*) corresponding to loci *5SDna-R2* and *5SDna-R3* appear in the two arms of the short translocated chromosome. The hybridization signals corresponding to locus *5SDna-R1* are also indicated (*arrowheads*)

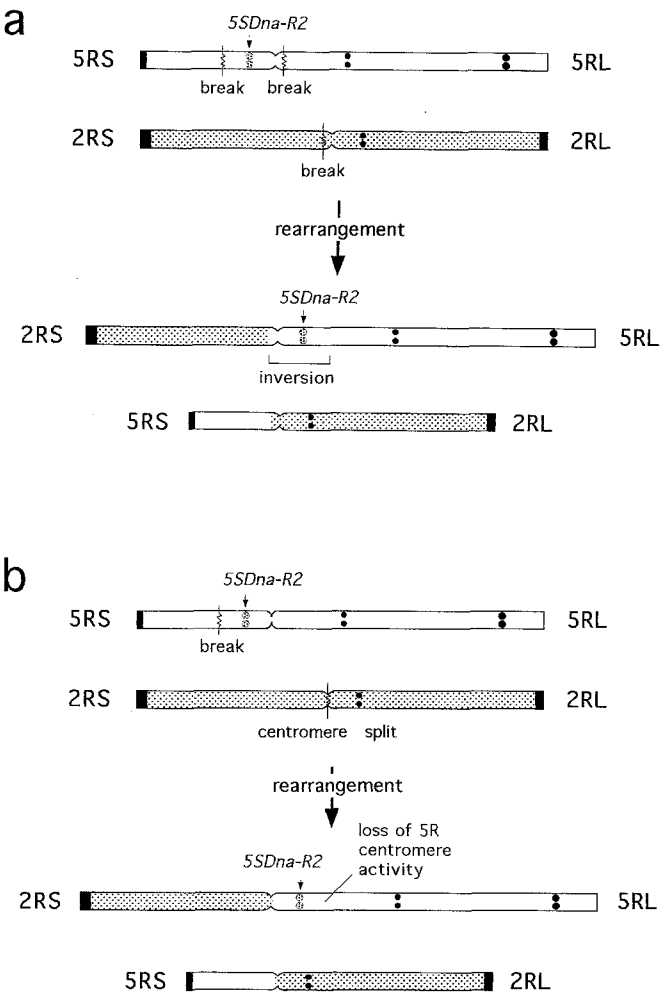

Fig. 3a, b. Diagram illustrating two possibilities for the constitution of translocation *T305W* as deduced from the combined study of the pachytene SC in heterozygotes (mapping the breakpoints in the short arm of chromosome 5R and at or near the centromere of chromosome 2R) and the FISH of probe pScT7 (mapping locus *5SDna-R2* in chromosome arm 5RS of nontranslocated chromosomes and in the arm of the long translocated chromosome having the 5RL telomere). The possible origins of these two alternatives are also indicated

The breakpoints of *T305W* map close to the centromere of chromosome 2R and near the middle of the short arm of chromosome 5R (Fig. 1). A first possibility for the constitution of this chromosome rearrangement could be the existence of a pericentric inversion involving chromosome 5R (for which a third break in chromosome arm 5RL is needed), in addition to the translocation involving chromosome arms 5RS and 2RS (Fig. 3a). However, from the analysis of the pachytene SC formed by translocation *T305W* quadrivalents, no evidence of other chromosomal rearrangement in addition to the interchange itself could be deduced. Therefore, the possibility of *T305W* being formed after the centromere split of chromosome 2R and the breakage of 5RS, followed by a loss of 5R centromere activity in the rearranged chromosomes (Fig. 3b), could also be considered.

Polymorphism at 5S rDNA loci

In FISH experiments using the 5S rDNA probe, variation in number and intensity of the hybridization signals was practically absent among metaphases within the same genotype. Nevertheless, wide between-genotype variation in hybridization signal intensity was observed for all three 5S rDNA loci, the highest differences being present at locus *5SDna-R3*, which became undetectable in many lines (line E and lines homozygous for translocations *T248W*, *T850W*, *T273W* and *T305W*) (Fig. 2a–f). Locus *5SDna-R1* showed the smallest between-genotype differences, whereas variation ranging from faint to strong signal was observed at locus *5SDna-R2*. The permanence of the parental hybridization signal intensity at the 5S rDNA loci in the progenies of different crosses between plants carrying morphologically distinguishable chromosomes (translocations involving chromosomes 1R, 3R and/or 5R, as well as 1R and 3R telocentrics), demonstrated that differences between genotypes were not technical artifacts.

The low and high variation in the number of 5S rDNA tandemly repeated units found by Reddy and Appels (1989) at *5SDna-R1* and *5SDna-R2*, respectively, could account for the polymorphism observed in our study. This correlation suggests that the variation in the number of 5S rDNA units at *5SDna-R3* is, at least, as high as that found at *5SDna-R2*.

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