Simultaneous painting of three genomes in hexaploid wheat by BAC-FISH

Peng Zhang, Wanlong Li, Bernd Friebe, and Bikram S. Gill

Abstract: Fluorescence in situ hybridization (FISH) is widely used in the physical mapping of genes and chromosome landmarks in plants and animals. Bacterial artificial chromosomes (BACs) contain large inserts, making them amenable for FISH mapping. In our BAC-FISH experiments, we selected 56 restriction fragment length polymorphism (RFLP)-locus-specific BAC clones from the libraries of *Triticum monococcum* and *Aegilops tauschii*, which are the A- and D-genome donors of wheat (*Triticum aestivum*, 2*n* = 6*x* = 42), respectively. The BAC clone 676D4 from the *T. monococcum* library contains a dispersed repeat that preferentially hybridizes to A-genome chromosomes, and two BAC clones, 9I10 and 9M13, from the *Ae. tauschii* library contain a dispersed repeat that preferentially hybridizes to the D-genome chromosomes. These repeats are useful in simultaneously discriminating the three different genomes in hexaploid wheat, and in identifying intergenomic translocations in wheat or between wheat and alien chromosomes. Sequencing results show that both of these repeats are transposable elements, indicating the importance of transposable elements, especially retrotransposons, in the genome evolution of wheat.

Key words: bacterial artificial chromosome (BAC), fluorescence in situ hybridization (FISH), transposable elements (TEs), wheat, *Triticum aestivum*.

Résumé : L'hybridation in situ en fluorescence (FISH) est beaucoup employée pour la cartographie physique de gènes ou de points de repère chromosomiques chez les plantes et les animaux. Les chromosomes bactériens artificiels (BAC) contiennent des inserts de grande taille ce qui les rend utiles pour la cartographie FISH. Dans le présent travail, les auteurs ont sélectionné 56 clones BAC (spécifiques d'un locus RFLP) des banques du *Triticum monococcum* ou de l'*Aegilops tauschii*, les espèces ayant contribué respectivement les génomes A et D du blé (*Triticum aestivum*, 2*n* = 6*x* = 42). Le clone BAC 676D4 de la banque du *T. monococcum* contient une séquence répétitive dispersée qui marque de manière préférentielle les chromosomes du génome A. Les clones BAC 9I10 et 9M13, de la banque de l'*Ae. tauschii*, portent une séquence répétitive dispersée qui marque les chromosomes du génome D. Ces séquences répétitives permettent de distinguer simultanément les trois génomes différents chez le blé hexaploïde ainsi qu'à identifier des translocations intergénomiques chez le blé ou entre le blé et des chromosomes étrangers. Le séquençage a révélé que ces séquences répétitives sont des éléments transposables, ce qui illustre le rôle important de ces éléments, particulièrement les rétrotransposons, dans l'évolution du génome chez le blé.

Mots clés : chromosome bactérien artificiel (BAC), hybridation in situ en fluorescence (FISH), éléments transposables, blé, *Triticum aestivum*.

[Traduit par la Rédaction]

Introduction

Fluorescence in situ hybridization (FISH) is a powerful tool for physical mapping and chromosome identification. Specific clones isolated from cosmid, P1, BAC (bacterial artificial chromosome), or YAC (yeast artificial chromosome) libraries can be used for FISH mapping to generate high-density cytological maps (Lichter et al. 1990). The use of genomic DNA cloned in large-insert vector BACs (Shizuya et al. 1992) as probes in FISH experiments is called BAC-FISH. BAC-FISH has been used to physically map specific DNA sequences and identify individual chromosomes in plants with small genomes, such as rice (Jiang et al. 1995), cotton (Hanson et al. 1995), sorghum (Gomez et al. 1997), *Arabidopsis* (Fransz et al. 2000; Jackson et al. 2000), and potato (Dong et al. 2000). In addition, BAC-FISH is a useful technique for isolating repeated sequences from specific chromosome regions and for identifying molecular cytogenetic makers in plants with large genomes, such as wheat (Zhang et al. 2004).

Simultaneous discrimination of the three different genomes in hexaploid wheat, *Triticum aestivum* L., has been difficult by genomic in situ hybridization (GISH) (Mukai et al. 1993) because of the close affinities among the diploid donor species, *T. urartu* Tumanian ex Gandilyan (Sax 1922; Kihara 1924), *Ae. speltoides* Tausch, and *Ae. tauschii* Coss. (McFadden and Sears 1944, 1946; Kihara 1944). Therefore, methods that can easily differentiate the three genomes unambiguously are required to detect intergenomic translocations between chromosomes belonging to different genomes.

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We have identified two dispersed repetitive DNA sequences that preferentially hybridized to the A- and D-genome chromosomes, respectively. These two repeats can be used as probes in BAC-FISH experiments to simultaneously discriminate the three different genomes and to identify intergenomic translocations in polyploid wheat.

Material and methods

Plant material

Eleven diploid, seven tetraploid, and four hexaploid *Aegilops* species; two diploid, tetraploid, and hexaploid *Triticum* species (Zhang et al. 2004); one 'Langdon' durum disomic substitution line 0236–5–1; and one wheat–rye translocation line RL6144 (T2AS-2RS.2RL) were analyzed (McIntosh et al. 1995). The nomenclature for *Aegilops* and *Triticum* follows that of van Slageren (1994). All materials are maintained by the Wheat Genetics Resource Center at Kansas State University, in Manhattan, Kansas.

BAC clone screening

Altogether, 56 BAC clones from three libraries were used, as described by Zhang et al. (2004), including 29 derived from a *T. monococcum* L. subsp. *monococcum* BAC library (Lijavetzky et al. 1999), 11 derived from an *Ae. tauschii* binary BAC (BIBAC) library (Moullet and Lagudah 1998; Moullet et al. 1999), and 16 from an *Ae. tauschii* BAC library developed within a National Science Foundation project (http://wheat.pw.usda.gov/PhysicalMapping/).

BAC clones were selected from the above libraries by screening high-density BAC filters with genetically mapped restriction fragment length polymorphism (RFLP) markers. Among the 29 *T. monococcum* BAC clones, 27 were previously mapped to the short arms of group 5 chromosomes, and two to chromosome arm 3AL (Li and Gill 2002). Eleven *Ae. tauschii* BIBAC clones were mapped to chromosome arm 1DS (Spielmeyer et al. 2000). The 16 *Ae. tauschii* BAC clones were selected using the pools of RFLP probes that hybridized to group 3, 5, 6, and 7 chromosomes (Zhang et al. 2004).

FISH analysis

The barley clone pHvG38 contains a 900-bp GAA-satellite sequence cloned into the *Sma*I restriction site of the plasmid pUC13 (Pedersen et al. 1996). The GAA-satellite sequence has multiple hybridization sites on the B-genome chromosomes, and some minor sites on A- and D-genome chromosomes. The pHvG38 FISH pattern is similar to the N-banding pattern of wheat (Pedersen et al. 1996). Clone pAs1 contains a 1-kb fragment in the plasmid pUC8, was isolated from *Ae. tauschii* (Rayburn and Gill 1986*a*), and belongs to the *Afa* repeat family. Clone pAs1 permits identification of the D-genome chromosomes (Rayburn and Gill 1986*b*). Using both pHvG38 and pAs1 clones, all 21 chromosomes of hexaploid wheat can be identified (Pedersen and Langridge 1997). Clone pGc1R-1 is a 258-bp fragment of a tandem repetitive element cloned from a wheat – *Ae. speltoides* T2B-2S translocation line and hybridizes to telomeric and subtelomeric regions of most *Ae. speltoides* chromosomes (Nasuda 1999; Friebe et al. 2000). The repeat was inserted into the *Eco*RI/*Mse*I restriction site of the pT-Adv plasmid vector (Clontech, Palo Alto, Calif.). This clone has 98% homology to the 5′-end of the S-genome-specific repetitive element pAesKB52 that was isolated by Anamthawat-Jonsson and Heslop-Harrison (1993).

Probe labeling, FISH, and GISH analyses were conducted according to methods described by Zhang et al. (2001, 2004). One microgram of BAC, plasmid DNA, or total genomic DNA of rye was labeled with biotin-14-dATP using the BioNick Labeling System (Invitrogen Life Technologies, Carlsbad, Calif.), rhodamine-6-dUTP using Rhodamine-Nick Translation Mix (Roche Applied Science, Indianapolis, Ind.), or fluorescein-12-dUTP using nick translation in accordance with the manufacturer's protocols. The hybridization solution (approximately 75% hybridization stringency), containing 50% deionized formamide (Fisher Scientific, Pittsburgh, Penn.), 2× SSC, 10% dextran sulfate (Sigma, St. Louis, Mo.), 0.3 mg/mL of sheared salmon testes DNA (Sigma, St. Louis, MO), and about 1 μg/mL of labeled probes, was denatured by boiling for 5 min. After chilling on ice for 5 min, 30 μL of the denatured hybridization mixture was applied to each slide, and hybridization was performed in a humid chamber at 37 °C overnight. Posthybridization washes were in 2× SSC at 42 °C for 10 min, 50% formamide in 2× SSC at 42 °C for 10 min (approximately 80% washing stringency), and 2× SSC at 42 °C for 10 min. The biotin-labeled probes were detected with fluorescein-avidin DN (Vector Laboratories, Burlingame, Calif.). Chromosomes were counterstained with propidium iodide (Molecular Probes, Eugene, Ore.) in Vectashield (Vector Laboratories, Burlingame, Calif.). The fluorescein- or rhodamine-labeled probes were counterstained with propidium iodide or 4′,6-diamidino-2-phenylindole (Molecular Probes) in Vectashield, respectively.

Shotgun subcloning and DNA sequence analysis

Shotgun subcloning and DNA sequence analysis were conducted as described by Zhang et al. (2004). The TOPO Shotgun Subcloning Kit (Invitrogen Life Technologies) was used for subcloning. Approximately 15 μg of BAC DNA was nebulized. After precipitation and resuspension, the sheared DNA was electrophoresed, and DNA fragments of 500 bp to 1.5 kb were selected. DNA was gel purified, blunt-end repaired, dephosphorylated, and ligated into the pCR4Blunt-TOPO vector. The ligation was transformed into TOP10 *Escherichia coli* using electroporation. The transformation culture was plated out on petri dishes with LB-carbenicillin, isopropyl-1-thio-galactopyranoside, and 5-bromo-4-chloro-3-indolyl-galactopyranoside, and incubated at 37 °C overnight. White colonies were picked and transferred into 384-well microtiter plates. A replicator was used to make a copy of the colonies in the 384-well plate onto N+ Hybond membrane (Amersham Biosciences, Piscataway, N.J.). Bacteria were allowed to grow overnight, lysed, denatured on membrane in 0.4 mol/L NaOH for 5 min, neutralized in 2× SSC for 5 min, and vigorously shaken in 2× SSC for 15 min. Colony-blot hybridization was carried out with the genomic DNA of *T. monococcum* as a probe for BAC 676D4, or with the genomic DNA of *Ae. tauschii* as a probe for BAC 9M13. Colonies that gave strong hybridization signals were selected, and plasmid DNA was isolated for FISH analysis.

BAC subclones that generated the same FISH hybridization patterns as the original BAC clones were sequenced.

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Contigs were assembled using phrap version 0.990319 (http://www.phrap.org) and AssemblyLIGN version 1.0.9c (Oxford Molecular, Madison, Wis.) (Brooks et al. 2002). Sequences were then tested for similarities to other known sequences in the public databases using the BLASTn search.

Results

In a previous study (Zhang et al. 2004), we showed that the majority of the BAC clones (40 of 56) hybridized to all wheat chromosomes over their entire length. Three BAC clones did not give a hybridization signal on any wheat chromosome. Three BAC clones gave FISH patterns similar to those of the repetitive DNA families pAs1 and pSc119.2 (Bedbrook et al. 1980; Rayburn and Gill 1986*b*; McIntyre et al. 1990; Mukai et al. 1993), one BAC clone preferentially hybridized to all centromeric regions, one BAC clone preferentially hybridized to all subtelomeric chromosome regions, one BAC clone contained a localized tandem repeat and hybridized to five pairs of D-genome chromosomes in Chinese Spring (CS) wheat, and four BAC clones hybridized only to a proximal region in the long arm of chromosome 4A of CS wheat. Sequencing results showed that the repeats responsible for the above specific hybridization patterns are all transposable elements (Zhang et al. 2004). The FISH patterns of the BAC clones described in this paper are different because they preferentially hybridized to specific genomes in wheat and, thus, are described separately.

Retrotransposon preferentially hybridizing to the A-genome chromosomes

The *T. monococcum* BAC clone 676D4 (147.2 kb) hybridized strongly to 14 chromosomes of CS wheat (Figs. 1*a* and 1*b*). Sequential reprobing of the same metaphase cell with two repetitive DNA sequences, pHvG38 (Pedersen et al. 1996; Pedersen and Langridge 1997) and pAs1 (Rayburn and Gill 1986*a*, 1986*b*), identified all 21 pairs of chromosomes (Fig. 1*c*). These data revealed that BAC 676D4 hybridized strongly to A-genome chromosomes, and painted them over their entire length, except for the distal region of the long arm of chromosome 4A, which is known to have originated from 7BS as the result of a species–specific cyclic translocation involving chromosomes 4A-5A-7B (Naranjo et al. 1987).

BAC 676D4 also painted the A-genome chromosomes strongly in the tetraploid wheats *T. turgidum* L. (data not shown) and *T. timopheevii* (Zhuk.) Zhuk. (Fig. 1*d*). The same translocation was observed in *T. turgidum* and *T. aestivum*, but a different major species–specific cyclic translocation involving chromosomes $6A^{t}$-1G-4G is present in *T. timopheevii* (Gill and Chen 1987; Jiang and Gill 1994), which also was detected in our BAC-FISH (Fig. 1*d*).

Although clone 676D4 appears to be A-genome-specific in polyploid wheat, it hybridized to all diploid *Aegilops* and *Triticum* species tested, including *T. urartu* (AA), *Ae. speltoides* (SS), and *Ae. tauschii* (DD), and also to all the genomes with similar intensities in some tetraploid and hexaploid *Aegilops* species, including *Ae. biuncialis* Vis. ($U^{b}U^{b}M^{b}M^{b}$); *Ae. columnaris* Zhuk. ($U^{c}U^{c}M^{c}M^{c}$); *Ae. crassa* Boiss. ($X^{cr}X^{cr}D^{cr1}D^{cr1}D^{cr2}D^{cr2}$); *Ae. geniculata* Roth ($U^{g}U^{g}M^{g}M^{g}$); *Ae. neglecta* Req. *ex* Bertol. ($U^{n}U^{n}M^{n}M^{n}N^{n}N^{n}$); *Ae. peregrina* Hackel ($S^{p}S^{p}U^{p}U^{p}$); *Ae. triuncialis* L. ($U^{t}U^{t}C^{t}C^{t}$); *Ae. ventricosa* Tausch ($D^{v}D^{v}N^{v}N^{v}$), but not *Ae. juvenalis* (Thell.) Eig ($X^{j}X^{j}D^{j}D^{j}U^{j}U^{j}$); *Ae. cylindrica* Host ($C^{c}C^{c}D^{c}D^{c}$); and *Ae. vavilovii* (Zhuk.) Chennav. ($X^{va}X^{va}D^{va}D^{va}S^{va}S^{va}$) (data not shown). In *Ae. juvenalis*, this clone hybridized to all U^{j}-genome chromosomes with higher intensity than it did to X^{j}- and D^{j}-genome chromosomes. In *Ae. cylindrica*, this clone hybridized preferentially to the C-genome chromosomes. In addition to the dispersed labeling, this clone also produced a localized FISH pattern on the C-genome chromosomes. Chromosomes of different genomes were identified based on their morphology. In *Ae. vavilovii*, this clone hybridized more weakly to 14 of the chromosomes than it did to the remaining ones. This BAC clone also has weak hybridization in rye, barley, and oats (data not shown).

To determine the genome specificity of clone 676D4, we hybridized it to slides that contained a mixture of *T. urartu*, *Ae. speltoides*, and *T. turgidum* nuclei in a single preparation. We cohybridized the S-genome-specific clone pGc1R-1 to distinguish the S- and A-genome chromosomes (Figs. 1*g* and 1*h*). Clone 676D4 hybridized to both *T. urartu* and *Ae. speltoides* chromosomes. Signal intensities on the S-genome chromosomes were weaker than those on the A-genome chromosomes (Fig. 1*g*), but similar to those on the B-genome chromosomes of *T. turgidum* (Fig. 1*h*). The B-genome chromosomes have either a lower amount of or a lower sequence homology to the 676D4 sequence.

BAC-FISH, using clone 676D4 as a probe in combination with clone pAs1 in the Langdon durum disomic substitution line 0236–5–1, identified a whole-arm translocation T3AS·3DL between a 3A and a 3D chromosomes (Fig. 1*e*). BAC-FISH analysis of the wheat–rye translocation stock RL6144 (T2AS-2RS·2RL) mapped the breakpoint in the proximal region of the 2AS arm (Fig. 1*f*) and, thus, confirmed previous results (McIntosh et al. 1995).

Eleven shotgun subclones were selected on the basis of their strong hybridization signals to the colony-hybridization blot, using genomic DNA of *T. monococcum* as a probe. Among these subclones, with sizes ranging from 500 bp to 2 kb, five, including clone pTm4-P1, gave the same FISH hybridization pattern as BAC 676D4. The remaining six subclones did not give any hybridization signal. The five positive subclones were sequenced and assembled into one contig, contig 13 (GenBank accession No. AY375391), which is 3282 bp (Table 1). Sequencing of the positive shotgun subclones revealed that approximately the first 25% of the contig sequence has 73% sequence identity with the long-terminal repeats (LTR) of the *gypsy*-like retrotransposon *Erika* in *T. monococcum* (Wicker et al. 2001), and the remaining 75% of the contig sequence has a 90% sequence identity with the LTR of the *gypsy*-like retrotransposon Sukkula in *T. monococcum* (SanMiguel et al. 2002) (Table 2). This sequence has 10 copies in contig 13 (data not shown).

Retrotransposon preferentially hybridizing to the D-genome chromosomes

Two BAC clones from the *Ae. tauschii* library, 9M13 and 9I10, contain at least two distinct repetitive sequences. One is a localized tandem repeat that hybridized to five pairs of D-genome chromosome in CS wheat, as described by Zhang et al. (2004). The other repetitive sequence is dispersed and

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Fig. 1. (*a–c*) Sequential fluorescence in situ hybridization (FISH) on Chinese Spring (CS) metaphase chromosomes indicating that BAC 676D4 only hybridized to A-genome chromosomes. (*a*) Chromosomes were counterstained with DAPI and fluoresce blue. (*b*) Bacterial artificial chromosome (BAC) DNA was labeled with rhodamine-6-dUTP and visualized with red fluorescence. This BAC strongly painted the seven A-genome chromosomes over their entire length, except for the distal region of the long arm of chromosome 4A, which is known to have originated from 7BS (arrows indicate the translocation breakpoint). (*c*) the B- and D-genome chromosomes were labeled by two repetitive DNA sequence probes, pHvG38 (yellow–green fluorescence) and pAs1 (red fluorescence), respectively. The absence of major pHvG38 and pAs1 FISH sites on the chromosomes painted by BAC 676D4 indicate that these chromosomes belong to the A genome. (*d*) BAC 676D4 also hybridized more strongly to seven pairs of A-genome chromosomes than to the G-genome chromosomes in *Triticum timopheevii*; the FISH procedure was the same as in Fig. 1*b*. Arrows point to the A–G translocation chromosomes. Note that a different species-specific translocation exists in *T. timopheevii* than in *T. aestivum*. (*e*) Detection of an intergenomic translocation involving A- and D-genome chromosomes in a durum substitution line. BAC 676D4 DNA was labeled with biotin-14-dATP, detected with fluorescein-avidin DN, and visualized with yellow–green fluorescence. Clone pAs1 was labeled with rhodamine and cohybridized to identify the D-genome chromosomes. The chromosome with pAs1 sites at both ends is 3D. Twelve complete A-genome chromosomes and the short arm of chromosome 3A hybridized with BAC 676D4 and fluoresce green. The other arm of this chromosome belongs to the long arm of chromosome 3D, as indicated by the pAs1 FISH site. The arrowhead points to the translocation breakpoint of T3AS·3DL, and arrows point to the translocation breakpoint on chromosome 4A. (*f*) Detection of a wheat–rye translocation in stock RL6144. BAC DNA and rye genomic DNA were labeled with rhodamine-6-dUTP and fluorescein-12-dUTP, and visualized with red and yellow–green fluorescence, respectively. Arrowheads point to the translocation breakpoints in proximal region of the 2AS arm, and arrows point to the translocation breakpoint on chromosome 4A. (*g*) and (*h*) FISH pattern of BAC 676D4 and clone pGc1R-1 on mitotic metaphase cells of *Ae speltoides* and *T. urartu* (*g*), and *Ae. speltoides* and *T. turgidum* (*h*), respectively. Figures 1*g* and 1*h* were taken from the same slide with the same exposure time. Clone pGc1R-1 was used to identify *Ae. speltoides* chromosomes. BAC 676D4 DNA and clone pGc1R-1 were labeled with rhodamine-6-dUTP and fluorescein-12-dUTP, and visualized with red and yellow–green fluorescence, respetively: (*g*) The hybridization intensity of BAC 676D4 on *T. urartu* chromosomes (the cell on the right) is stronger than that on *Ae. speltoides* chromosomes (the cell on the left). (*h*) BAC 676D4 hybridized to the A-genome chromosomes more strongly than to the B-genome chromosomes in *T. turgidum* (the cell on the right). The hybridization intensity of BAC 676D4 on *Ae. speltoides* chromosomes (the cell on the left) is similar to that on the B-genome chromosomes in *T. turgidum*. Scale bars equal 10 µm.

Table 1. Results of contig assembly. The subclones of the individual BAC clones that generated the same FISH hybridization patterns as the original BAC clones were sequenced and then assembled into contigs.

BAC Clones	Repeats	GenBank acc. No.	Contig No.	Subclones
676D4	Repeat preferentially hybridizing to the A-genome chromosomes	AY375391	13	4-P1 (pTm4-P1), 4-I24, 4-E24, 4-H24, 4-H1
9M13	Repeat preferentially hybridizing to the D-genome chromosomes	AY375393	6	9-N12, 9-A17
		AY375392	14	9-M24, 9-K21, 9-E21, 9-P18, 9-N11 (pAet9-N11)

Table 2. DNA sequence similarity between the repetitive sequences present in BAC clones and those present in other cereals.

BAC clones	Contigs	Species	Repetitive sequences	Best DNA sequence identity (%)	*E* value	GenBank acc. No.	References
676D4	13	*T. monococcum*	*gypsy*-like retrotransposon, *sukkula*	90	0	AF459639	SanMiguel et al. 2002
		T. monococcum	*gypsy*-like retrotransposon, *Erika*	73	5×10^{-72}	AF326781	Wicker et al. 2001
9M13	6	*T. monococcum*	*gypsy*-like retrotransposon, *Romani*	86	0	AF459088	Yan et al. 2002
	14	*T. monococcum*	*gypsy*-like retrotransposon, *Romani*	87	0	AF459088	Yan et al. 2002
		T. monococcum	centromeric *Tai*I-family repetitive sequence	90	3×10^{-7}	AB077255	M. Kishii and H. Tsujimoto, unpublished data

hybridized strongly to seven pairs of chromosomes in CS wheat (Fig. 2*a*). The dispersed repeat in BAC 9M13 hybridized to all seven pairs of chromosomes in *Ae. tauschii*, but did not hybridize or hybridized with very low intensity to chromosomes in *T. urartu*, *Ae. speltoides*, and *T. turgidum* (data not shown). These data suggest that the seven labeled chromosome pairs in CS wheat belong to the D genome.

Shotgun subcloning of BAC 9M13 was used to separate the two repetitive sequences into different plasmids. Ten subclones, with sizes ranging from 750 bp to 1.3 kb, were

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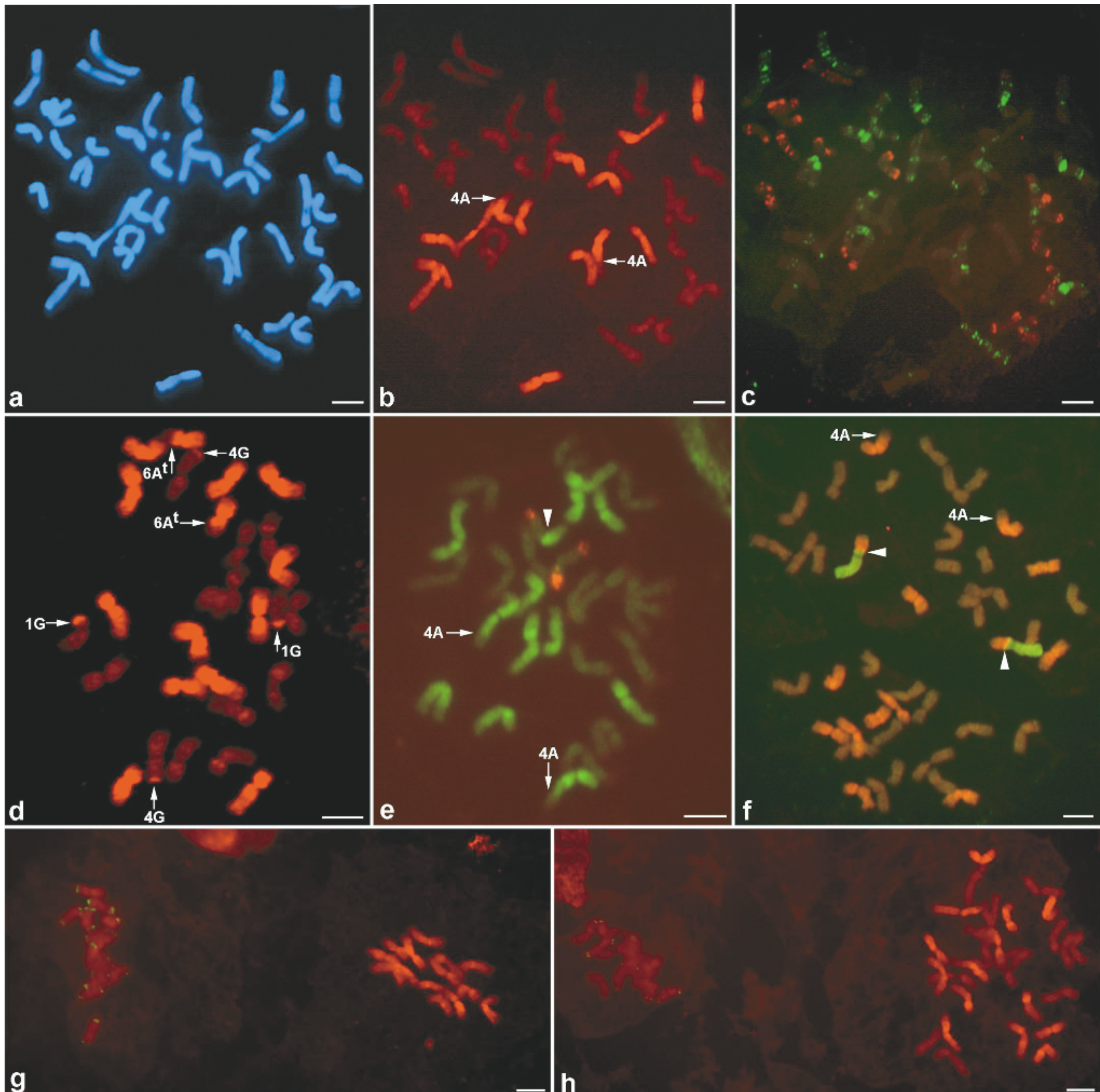

selected that gave strong hybridization signals in the colony-hybridization blot, using the genomic DNA of *Ae. tauschii* as a probe. Among these clones, three contained the tandem repeat and seven contained the dispersed repeat. Using the subclone pAet9-N11, which contained the dispersed repeat as a probe, seven D-genome chromosome pairs were labeled over their entire length in CS wheat. The subclone pAet9-N11 also hybridized to all seven D-genome chromosomes pairs in *Ae. ventricosa* and *Ae. cylindrica* (data not shown).

Subclones containing the dispersed repeat were sequenced and assembled into two contigs, 6 (GenBank acc. No. AY375393) and 14 (GenBank acc. No. AY375392), which are 1531 bp and 1949 bp, respectively (Table 1). Sequencing of these subclones showed that the sequence that is responsible for the D-genome-preferential hybridization pattern has an 87% sequence identity to the LTR of the *gypsy*-like retrotransposon Romani in *T. monococcum* (Yan et al. 2002), and a 90% sequence identity to the centromeric TaiI-family repetitive sequence in *T. monococcum* (Table 2).

Combining the BAC clones 676D4 and 9M13, which contain repeats hybridizing preferentially to the A- and D-genome chromosomes, respectively, as probes in a BAC-FISH experiment allowed an easy and simultaneous discrimination of the three genomes in hexaploid wheat (Figs. 2*b*–2*e*).

Discussion

Highly repetitive sequences can be divided into two types on the basis of their organization and distribution pattern. One type is localized tandem repeats that are arranged in tandem arrays and are present as clusters along chromosomes, such as the tandem repetitive DNA sequences present

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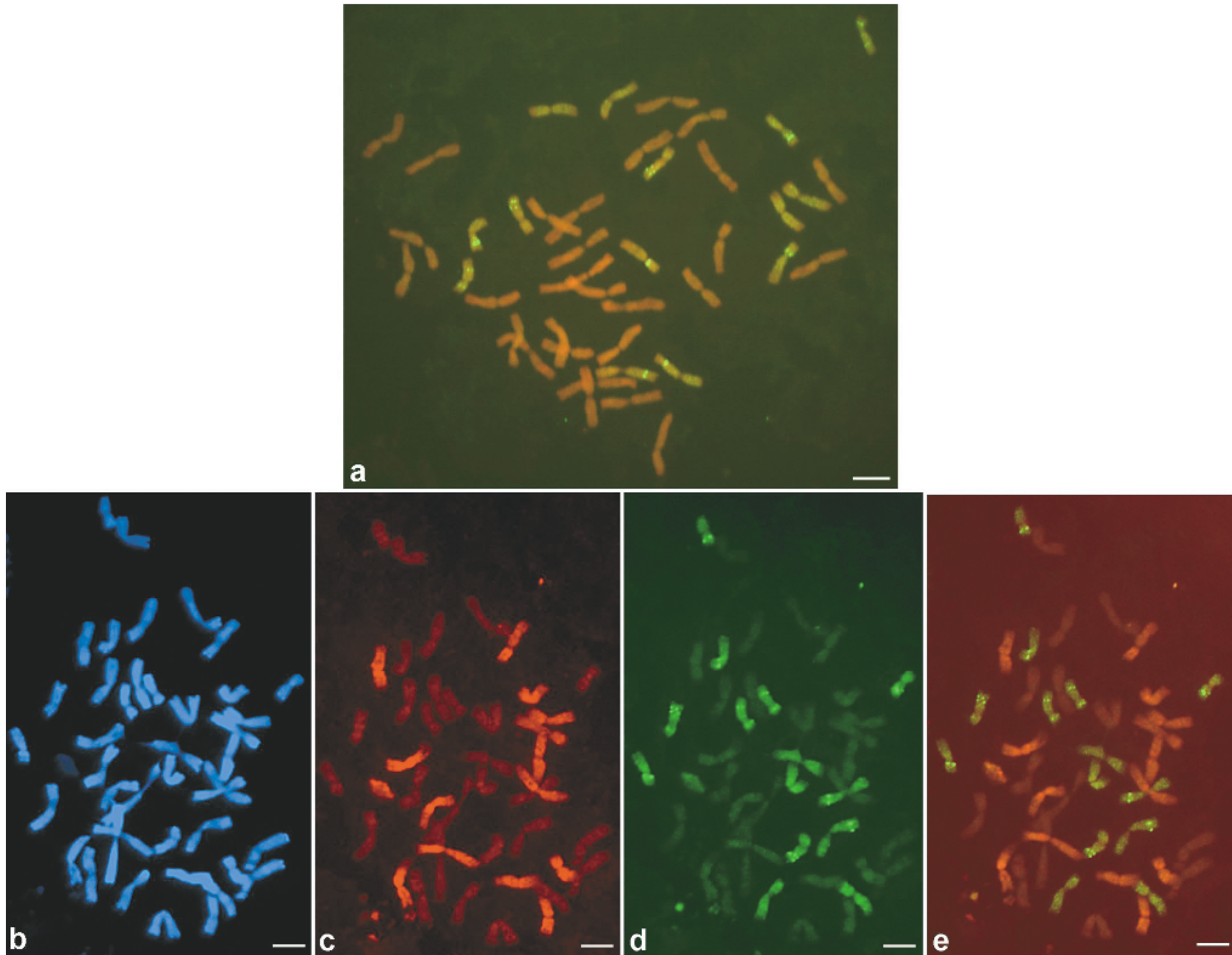
Fig. 2. (*a*) The FISH pattern of BAC 9M13 on CS wheat: BAC DNA was labeled with biotin-14-dATP, detected with fluorescein-avidin DN, and visualized with yellow–green fluorescence. Chromosomes were counterstained with propidium iodide and fluoresce red. The tandem repeat present in BAC 9M13 hybridized to five pairs of D-genome chromosomes, whereas the dispersed repeat hybridized to the entire length of all seven pairs of D-genome chromosomes. (*b–e*) Combining the two BAC clones, 676D4 and 9M13, as probes allows simultaneous discrimination of the three genomes in CS wheat. (*b*) Chromosomes were counterstained with DAPI and fluoresce blue. (*c*) BAC 676D4 was labeled with rhodamine-6-dUTP and 14 A-genome chromosomes were visualized with red fluorescence. (*d*) BAC 9M13 was labeled with biotin-14-dATP, detected with fluorescein-avidin DN, and 14 D-genome chromosomes were visualized with green fluorescence. (*e*) Merged image from (*c*) and (*d*): 14 A-genome chromosomes fluoresced red, 14 D-genome chromosomes fluoresced yellow–green, and 14 unlabeled B-genome chromosomes were brown. Scale bars equal 10 µm.



in BAC clones 9M13 (Figs. 2*a*, 2*d*, and 2*e*) and 9I10. In the Triticeae tribe, localized tandem repeated sequences are useful as markers for chromosome identification (Bedbrook et al. 1980; Appels et al. 1981; Jones and Flavell 1982; Rayburn and Gill 1986*b*; McIntyre et al. 1990; Mukai et al. 1992; Anamthawat-Jonsson and Heslop-Harrison 1993).

The second type of repetitive sequences is dispersed repeats that are interspersed with unrelated repeated or unique DNA sequences (Flavell 1986). Dispersed repeats can be found at a few chromosomal regions, or distributed throughout an entire genome, such as the dispersed repetitive DNA sequence present in BAC clones 676D4, 9M13, and 9I10 that preferentially hybridized to the A- (Figs. 1*b*, 1*d*–1*h*, 2*c*, and 2*e*) and D-genome chromosomes (Figs. 2*a*, 2*d*, and 2*e*). Dispersed repetitive sequences can be used to identify parental genomes in interspecific hybrids (Hutchinson and Lonsdale 1982; Appels and Moran 1984; McIntyre et al. 1990; Anamthawat-Jonsson and Heslop-Harrison 1993; Vershinin et al. 1994) and to study plant genome evolution (Dvorák and Zhang 1992). The dispersed repetitive DNA sequence present in BAC clone 676D4 that preferentially hybridized to the A-genome chromosomes was successfully used to detect translocations involving A-genome chromosomes, such as a whole-arm translocation involving A- and D-genome chromosomes in hexaploid wheat (Fig. 1*e*) and a translocation between an A-genome chromosome and an alien rye chromosome (Fig. 1*f*). Therefore, this repeat is useful for determining intergenomic translocations. The identification of a dispersed repeat that preferentially hybridized to the D-genome chromosomes will further expand this applica-

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tion. As shown in this study, BAC-FISH, using these two dispersed repeats as probes, provides a simpler technique for simultaneously differentiating chromosomes belonging to three genomes in hexaploid wheat (Figs. 2*b*–2*e*) than GISH (Mukai et al. 1993), where the ratio between the blocking DNA and probe DNA is critical and difficult to adjust, especially for very closely related genomes. One of the reasons that our painting method using these two dispersed repeats as probes is more successful than GISH is due to the selection of genomic dispersed repeats over a mixture of specific- and nonspecific-repeats in GISH.

BAC 676D4 hybridized more strongly to the A-genome chromosomes than to the B- and D-genome chromosomes in hexaploid wheat (Figs. 1*b*, 2*c*, and 2*e*). This sequence has high homology to the LTR of *gypsy*-like retrotransposon *Sukkula* and *Erika* from *T. monococcum*. The dispersed sequence present in BACs 9M13 and 9I10 strongly hybridized to the seven pairs of D-genome chromosomes in hexaploid wheat, but did not hybridize, or hybridized with very low intensity, to the A- and B-genome chromosomes (Figs. 2*a*, 2*d*, and 2*e*). This dispersed repeat strongly hybridized to all seven pairs of chromosomes in *Ae. tauschii*, but did not hybridize, or did so with very low intensity, to chromosomes of *T. urartu* and *Ae. speltoides*. This sequence has high homology with the LTR of the *gypsy*-like retrotransposon *Romani* and the centromeric *Tai*I-family repetitive sequence from *T. monococcum*.

A large proportion of plant genomes consists of repetitive families, many of which are likely to be, or to have evolved from, retrotransposons (Grandbastien 1992; Bennetzen 1993; SanMiguel and Bennetzen 1998). A number of DNA transposable elements and retroelements have been identified in different grass genomes (Kumar and Bennetzen 1999; Wicker et al. 2001, 2003; SanMiguel et al. 2002; TREP database (http://wheat.pw.usda.gov/ITMI/Repeats/index.shtml), as can be seen from the sequencing data of the BAC clones in this study. Transposons are generally dispersed along plant chromosomes, but may associate with particular chromosome regions. Their widespread chromosomal dispersion suggests that transposable elements played a major role in chromosome evolution and had tremendous effects on genome structure and gene function. The relative abilities of plant species, during evolution, to inactivate or remove these elements, or to tolerate their presence, have created unique populations of retrotransposon elements in each plant genome (Bennetzen 2000).

Retrotransposon activity can change the overall genome structure. Because of their high copy numbers and dispersal throughout plant genomes, retrotransposons serve as sites of unequal or ectopic recombination. Ectopic recombination between retrotransposons of the same family that are in direct orientation on the same chromosome can cause reciprocal duplications and deletions, whereas unequal recombination between elements on the same chromosome that are in opposite orientation will cause a chromosomal inversion of the sequences between the two retrotransposons. Ectopic recombination between two elements on different chromosomes can cause reciprocal translocations. Changes such as large inversions and translocations can contribute to reproductive isolation and subsequent speciation. Therefore, although most transposable elements of plants are inactive at any given time, occasional activity can have enormous effects.

BAC 676D4 hybridized to the diploid progenitor species *T. urartu*, *Ae. speltoides*, and *Ae. tauschii*. However, this BAC apparently did not hybridize to the D-genome chromosomes in the derived synthetic wheat (data not shown). These results suggested that this genome-preferential hybridization pattern might be caused by sequence elimination from the D-genome chromosomes in synthetic wheat rather than changes in the chromosomal structure or the accessibility to the probe in different genomes of wheat (Zhang et al. 2003). In the above experiments, BAC 676D4 was hybridized to different species in different preparations, so their relative hybridization intensity was difficult to estimate. By analyzing the hybridization intensity of BAC 676D4 on a mixture of *T. urartu*, *Ae. speltoides*, and *T. turgidum* nuclei in a single preparation (Figs. 1*g* and 1*h*) we have shown that the preferential hybridization of BAC 676D4 to the A-genome chromosomes in hexaploid wheat is not caused by sequence elimination of this repeat during the polyploidization process.

The preferential hybridization pattern of the repetitive sequence in BAC 676D4 to the A-genome chromosomes in hexaploid wheat may be caused by higher copy numbers of this sequence in the A-genome chromosomes thanin the B- and D-genome chromosomes, or a higher level of homology of this sequence to A-genome chromosomes than to B- and D-genome chromosomes. The same reasons may account for the preferential hybridization of the repetitive sequence in BAC 9M13 to D-genome chromosomes in hexaploid wheat.

Our study showed that the BAC-FISH technique allows the rapid isolation of repeated sequences that preferentially hybridize to chromosomes belonging to only one genome in wheat and allowed us to study the distribution of different repetitive sequences in the wheat genome. Thus, BAC-FISH is a powerful technique for identifying molecular cytogenetic markers in plants with large genomes. Furthermore, the genome-specific BAC clones 676D4 and 9M13, in combination with probes pAs1 and pHvG38, allow simultaneous detection of genomic affinity and cytogenetic identification of all 21 chromosomes of hexaploid wheat, including the detection of intergenomic translocations, if any, in one experiment.

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