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Association of transgene integration sites with chromosome rearrangements in hexaploid oat

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Abstract Transgene loci in 16 transgenic oat (*Avena sativa* L.) lines produced by microprojectile bombardment were characterized using phenotypic and genotypic segregation, Southern blot analysis, and fluorescence in situ hybridization (FISH). Twenty-five transgene loci were detected; 8 lines exhibited single transgene loci and 8 lines had 2 or 3 loci. Double FISH of the transgene and oat C- and A/D-genome-specific dispersed and clustered repeats showed no preferences in the distribution of transgene loci among the highly heterochromatic C genome and the A/D genomes of hexaploid oat, nor among chromosomes within the genomes. Transgene integration sites were detected at different locations along individual chromosomes, although the majority of transformants had transgenes integrated into subtelomeric and telomeric regions. Transgene integration sites exhibited different levels of structural complexity, ranging from simple integration structures of two apparently contiguous transgene copies to tightly linked clusters of multiple copies of transgenes interspersed with oat DNA. The size of the genomic interspersions observed in these transgene clusters was estimated from FISH results on prometaphase chromosomes to be megabases long, indicating that some transgene loci were significantly larger than previously determined by Southern blot analysis. Overall, 6 of the 25 transgene loci were associated with rearranged chromosomes. These results suggest that particle bombardment-mediated transgene integration may result from and cause chromosomal breakage and rearrangements.

Key words Genetic engineering · Oat (*A. sativa* L.) · Microprojectile bombardment · FISH · Transgene DNA integration

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Introduction

The introduction and manipulation of genes via genetic transformation has become an important approach for the genetic modification and improvement of many crops. Microprojectile bombardment is commonly used for transformation of commercially important species including cereals such as maize, wheat, rice, barley, and oat. Yet little is known about the mechanisms of transgene integration into the plant genome following DNA delivery via microprojectile bombardment. It is likely that during and/or after delivery into the plant cell nucleus, transgene DNA is subjected to intensive structural modifications, such as shearing, nuclease digestion, and ligation, resulting in complex integration patterns (Pawlowski and Somers 1996; Kohli et al. 1999). Transgene copy numbers integrated into the genome may vary from 1 to more than 20; however, multiple copies of transgenes often cosegregate as a single locus. Multiple copies of transgenes within some single loci are interspersed by plant genomic DNA sequences (Kohli et al. 1998; Pawlowski and Somers 1998). While chromosomal localization and characterization of the structures of transgenes have been reported in transgenic plants obtained through T-DNA-mediated transfer (Ambros et al. 1986a, 1986b; Moscone et al. 1996; Mouras et al. 1987; Mouras and Negrutiu 1989; Pawlowski and Somers 1996), fewer studies have been conducted on transgenic

plants produced by microprojectile bombardment (Chen et al. 1999; Pedersen et al. 1997). Pedersen et al. (1997) reported the localization of transgenes on metaphase chromosomes of transgenic barley, wheat, and triticale obtained via microprojectile bombardment using fluorescence in situ hybridization (FISH). One to three transgene integration sites were localized on different chromosomes of these species with a tendency for transgenes to be integrated near chromosome ends.

In the investigation reported here, we characterizated a population of 16 transgenic oat lines produced via microprojectile bombardment using a combination of phenotypic and genotypic segregation analysis, Southern blot hybridization, and FISH. The objectives were to determine the number of transgene loci, their chromosomal localization and distribution among the three genomes of hexaploid oat and to characterize the internal structure of transgene integration sites in each line.

Materials and methods

Plant materials

Allohexaploid oat (*Avena sativa* L., genotype GAF/Park) was used to produce 16 independent transgenic lines using microprojectile bombardment of mature embryo-derived tissue cultures (Torbert et al. 1998) to introduce the plasmid pNGI containing two marker genes, *gusA* and *nptII* (Klein et al. 1989). Plants regenerated from the same transgenic tissue culture (generation T_0), as well as self-pollinated progeny of these plants, were designated as a "transgenic line". Between 25 and 125 T_1 plants from each line were used in phenotypic (GUS-staining) segregation analysis, and 12–25 T_1 plants from each line were analyzed by Southern blot hybridization.

β-Glucuronidase (GUS) staining

GUS staining of kernel endosperm portions was performed as described in Pawlowski et al. (1998).

DNA isolation, blotting, and hybridization

Total genomic DNA was extracted from green leaves as described in Ananiev et al. (1997). Restriction endonuclease *Sca*I, which has only one restriction site in pNGI, and three noncutters, *Bst*EII, *Hpa*I, and *Xho*I, were used to digest genomic DNA. The DNA fragments were separated on 0.8% agarose (*Sca*I) or on 0.35% agarose (noncutters) and blotted to Immobilon nylon membrane (Millipore). DNA transfer, prehybridization, and hybridization of the membranes were performed according to the manufacturer's recommendations. The whole pNGI plasmid was labeled with [^{32}P]-dCTP (Amersham) using a Rediprime labeling kit (Amersham) and used as a probe.

In situ hybridization

Root tips from 6- to 10-week-old plants were incubated on filter papers saturated with a solution of 0.05% colchicine, 0.025% 8-hydroxyquinoline, and 2% DMSO for 2 h at room temperature and then fixed in 45% acetic acid. Root apices were squashed on microscope slides in 45% acetic acid. After the cover slips were removed, the slides were dehydrated in absolute alcohol for 10 min, air-dried, and stored at room temperature. The pNGI plasmid (Klein et al. 1989), the oat C-genome-specific clustered (AsC1) and dispersed (AsC2) repeats (Ananiev et al. 1998), and the A/D-genome-specific dispersed repeat AsA1 (Ananiev et al. 1998) were digested with *Sau*3A and used as probes for in situ hybridization. DNA of the transgene was labeled with the indocarbocyanines Cy3-dCTP and Cy3-dUTP (Amersham), and repetitive sequences were labeled with either fluorescein-12-dUTP (Stratagene) or rhodamine-6-dUTP (Boehringer Mannheim) using a Prime-IT Fluor Fluorescence Labeling Kit (Stratagene). Direct FISH was performed according to the protocol provided by the manufacturer of the Prime-IT Fluor Fluorescence Labeling Kit (Stratagene). After hybridization, chromosomes were counterstained and mounted in antifade solution (Vector Laboratories) containing 20 ng/ml of 4,6-diamino-2-phenylindole (DAPI). Preparations were then examined using a Nikon E800 photomicroscope equipped with fluorescent optics. Digital images were collected using a CoolCam liquid-cooled, three-chip color CCD camera (Cool Camera Company). Blue, red, and green images were captured to a 486DX2 personal computer using IMAGE PRO PLUS version 3.0 software (Media Cybernetics) and later combined on the computer utilizing ADOBE PHOTOSHOP version 5.0. Pictures were printed on a Fujix Pictography 3000 digital image printer (Fuji North America). FISH was performed in repetition on 3–10 T_1 plants from each line. To determine transgene loci numbers using FISH, we scored at least 20 high-quality metaphase spreads per line.

Results

Transgene locus number

Phenotypic segregation analysis was conducted to estimate transgene locus numbers in 16 independent transgenic oat lines produced using microprojectile bombardment. The pNGI plasmid that was used for transformation contains the maize AdhI promoter-intron expression cassette fused to *gusA* (Klein et al. 1989), which is expressed in seed of transgenic oat. Endosperm portions of T_1 seeds from single T_0 plants from 15 lines and 2 T_0 plants of line 3812 were stained for GUS activity to determine segregation of the transgene phenotype (Table 1). Segregation ratios not different from 3:1 were observed in 9 lines, indicating the presence of a single functional transgene locus (Table 1). In lines 3803–1, 3806–6, and 3839–4 GUS segregation ratios approximated 15:1, suggesting the presence of 2 independent functional loci. In line 3824–1, GUS staining indicated 3 transgene loci. Segregation in lines 3801–8, 3812–1, 3812–2, and 3830–1 did not fit Mendelian ratios (Table 1).

To further characterize transgene locus numbers, we performed genotypic segregation analysis using Southern blot hybridization. Genomic DNA was digested with *Sca*I or *Bst*EII and hybridized with pNGI as a probe (Fig 1); pNGI has a single *Sca*I site and no *Bst*EII site. Within progeny of lines 3800–1, 3801–8, 3805–7, 3809–1, 3812, 3815–7, 3818–4, 3819–3, 3824–1, and 3830–1 identical transgene hybridization patterns for all transgene-positive plants were observed, indicating the presence of only 1 transgene locus (Table 1). Among 12 plants analyzed in each of lines 3811–3 and 3843–3, 1 and 3 plants, respectively, had hybridization patterns different from the patterns of sibling progeny, suggesting the presence of 2 loci (Table 1). Lines 3803–1, 3806–6,

Table 1 Number of transgene loci and genome of integration in transgenic allohexaploid oat lines detected by segregation analysis using GUS staining and Southern blot hybridization, and FISH

Transgenic line no.	GUS+/GUS- T_1 plants	Number of transgene loci indicated by:			Genome of integration
		GUS staining[a]	Southern	FISH	
3800-1	66/21	1	1 (25)[b]	2	A/D
3801-8	15/10	?	1 (22)	1	A/D
3803-1	116/9	2	2 (12)	2	C, A/D
3805-7	28/10	1	1 (22)	1	A/D
3806-6	35/3	2	2 (15)	2	C, A/D
3809-1	25/14	1	1 (12)	1	C
3811-3	40/10	1	2 (12)	2	C
3812-1	12/13	?	1 (12)	2	A/D
3812-2	23/20	?	1 (12)	2	A/D
3815-7	32/9	1	1 (12)	2	A/D
3818-4	34/11	1	1 (22)	1	C
3819-3	29/12	1	1 (12)	1	C
3824-1	109/3	3	1 (12)	1	C
3830-1	24/18	?	1 (12)	1	A/D
3839-4	91/9	2	1 (12)	2	A/D
3843-3	21/6	1	2 (12)	1	A/D
3849-1	28/15	1	3 (12)	3	A/D

[a] Number of loci showing good agreement with Mendelian ratios based on χ^2 tests ($P > 0.1$). Question marks indicate that segregation ratios did not fit Mendelian expectations

[b] In parentheses, number of T_1 plants analyzed by Southern hybridization. To detect segregation between unlinked transgene integration sites with a probability 99% using Southern hybridization analysis, 9 GUS-positive plants need to be analyzed. To detect recombination between two independent loci localized in telomeric or subtelomeric regions of the same chromosome with a probability 95%, 11 GUS-positive plants should be included into the Southern hybridization analysis

and 3839–4 showed three different patterns of hybridization among segregating progeny, clearly indicating the presence of 2 independent transgene loci in each line. Southern blot analysis of progeny from line 3849–1 revealed at least five distinct hybridization patterns, which likely indicated 3 transgene loci.

To investigate the number of transgene integration sites and their chromosomal localization, we conducted FISH on 3 to 10 T_1 plants that showed the most complex Southern hybridization patterns from each line to insure that all transgene loci were visualized. Transgene-specific FISH signals were detected on metaphase chromosomes of all 16 lines. The detection frequency of FISH signals was from 70% to 100% of the chromosome spreads analyzed. The intensity of the signals varied depending on copy number of the transgene at each locus. Plants that were homozygous and hemizygous for the transgene integration sites were detected among T_1 progeny of different lines. Based on our ability to detect FISH signals in line 3830–1, which contained only two transgene copies integrated into a single site based on Southern blot analysis (Fig. 1), it appears that low-copy numbers of transgenes were detected. Because 8 lines exhibited more than 1 transgene integration locus, a total of 25 transgene integration sites were detected among the 16 transgenic lines. The FISH results are summarized in Table 1 and Fig. 2.

Transgene localization

FISH using C- (AsC2) and A/D- (AsA1) genome-specific dispersed repeats allowed us to distinguish between chromosomes of the highly heterochromatic C genome, and the A and D genomes of allohexaploid oat (Fig. 3*a*). We also used a C-genome-specific clustered repetitive sequence (AsC1) to identify all 7 chromosomes of the C genome (Fig. 3*b*). Multiple telomeric translocations between the C- and A/D-genome chromosomes were detected in untransformed oat (Fig. 3*a*) in agreement with previous reports (Jellen et al. 1994; Leggett and Markhand 1995). Transgene integration sites were observed on C-genome chromosomes in 8 instances and on A/D-genome chromosomes in 18 instances (Table 1 and Fig. 2). Based on chromosome morphology, translocations, and specific FISH signals of AsC1, we concluded that transgene integration occurred in 5 different chromosomes of the C genome and in at least 7 different chromosomes of the A and D genomes. Integration sites were observed in different locations along individual chromosomes; however, the majority of transformants exhibited transgene integration into subtelomeric or telomeric regions (Table 1 and Fig. 2). In lines 3800–1, 3801–8, and 3824–1, transgene integration sites were detected at the border of C and A/D-chromosome translocations (Figs. 2 and 4).

Organization of transgene integration loci

Southern hybridization using pNGI as a probe revealed highly polymorphic transgene integration patterns among the transgenic lines with different levels of complexity (Fig. 1). The transgene-hybridizing fragment numbers determined with the noncutting enzyme corresponded to the locus numbers predicted from the segregation and FISH analyses only in lines 3805–7, 3806–6,

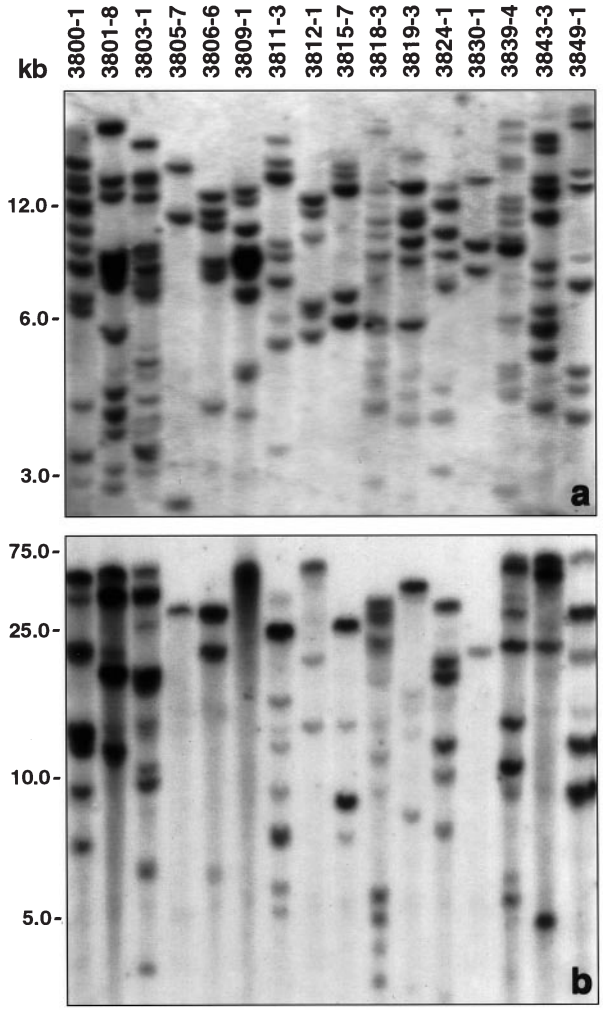

Fig. 1a, b Southern blot hybridization of the pNGI plasmid to genomic DNA from 16 independent transgenic oat lines digested with *Sca*I, which cuts at a single site in pNGI (**a**), and *Bst*EII, which does not cut the plasmid (**b**)

3809–1, and 3830–1 (Fig. 1*b*). In the remaining 12 lines, the numbers of transgene-hybridizing fragments exceeded the numbers of transgene loci determined from phenotypic and genotypic segregation analyses. These results indicated that the majority of transgene loci in this population consisted of multiple transgene copies interspersed with oat DNA, as previously described for transgenic oat (Pawlowski and Somers 1998).

Transgene integration sites were detected as single FISH signals on both chromatids of metaphase chromosomes (Figs. 2 and 5*a,c,e*). however, on less condensed metaphase chromosomes of lines 3801–8, 3818–4, and 3839–4, we sometimes observed 2, closely linked integration sites (data not shown). Moreover, FISH on prophase and prometaphase chromosomes showed that at least 9 out of the 25 transgene loci appeared to be clusters of 2 or 3 integration sites separated with fragments of oat DNA (Fig. 5*b,d,f*, and data not shown). These results provided visual evidence that some transgene loci

Fig. 2 Distribution and localization of transgene integration sites (*red*) among the C (*green*, painted with AsC2) and A/D (*blue*, DAPI) genomes in 16 transgenic oat lines ▶

Fig. 3a-f Direct FISH of pNGI and oat C- and A/D-genome-specific repeats to metaphase chromosomes of the oat wild-type GAF/Park and three transgenic oat lines. Transgene-specific FISH signals are indicated with *arrows*. Chromosomes were counterstained with DAPI. **a** Double FISH of the A/D-genome-specific dispersed AsA1 repeat (*red*) and the C-genome-specific dispersed AsC2 repeat (green) to GAF/Park chromosomes. **b** FISH of the C-genome-specific clustered repeat AsC1 (*red*) to GAF/Park chromosomes. **c** Double FISH of pNGI (*red*) and AsC2 (*green*) in line 3815–7. Transgene integration sites were detected on two arms of the same rearranged chromosome. **d, e** Double FISH of pNGI and AsC2 in lines 3812–1 (**d**) and 3812–2 (**e**). Transgenes are detected on both arms of normal (line 3812–1) and diminutive (line 3812–2) chromosomes. Southern analyses using *Sca*I are shown as *insets*. **f** Double FISH of pNGI and AsC2 in line 3824–1. A translocated chromosome is indicated with the *box*. *Bar*: 10 μm ▶

Fig. 4a–c Localization of the transgene in the borders of C-A/D translocations in lines 3800–1 (**a**), 3801–8 (**b**), and 3824–1 (**c**). DAPI-counterstained A/D genome chromatin is *blue*, and chromatin painted with the C-genome-specific repeat AsC2 is *green*. Transgenes are indicated with *arrows*. *Bar:* 10 μm ▶

were organized as clusters of transgenes interspersed with oat DNA. Considering that the size of an average oat chromosome is about 600 megabases and that the distance between transgenes within a cluster appears to be greater than 1% of the total prometaphase chromosome length (Fig. 5*f*), we estimated that the intervening oat DNA is at least a few megabases long, indicating that the size of some transgene clusters may exceed several megabases.

Association of the transgene integration sites with chromosomal aberrations

In lines 3800–1, 3812, and 3815–7, transgene integration sites were detected on two arms of the same A/D chromosomes (Fig. 2). In line 3800–1, the transgene integration site on the short arm was detected on the border of a translocation with a large C-genome chromosome fragment (Figs. 2 and 5*a*). All translocated A/D-genome chromosomes in the parental genotype GAF/Park carried C-genome translocations only on their long arms (Figs. 3*a*), which indicates that this chromosome is rearranged. Two transgene integration sites also were detected on an A/D-genome chromosome in 3812–1 and on a small, diminutive A/D chromosome in 3812–2 (Figs. 2 and 3*d,e*). Both lines were regenerated from the same transgenic tissue culture. An additional transgene hybridization fragment was observed in Southern analysis of regenerant 3812–1 compared to 3812–2 when DNA was digested with *Sca*I (Fig. 3*d,e*). From the similarities in Southern blot and FISH patterns it appears that the diminutive chromosome detected in 3812–2 was likely the product of breakage of the chromosome observed in 3812–1. This observation indicates that in this line chromosome breakage and possible loss of an acentric fragment in line 3812–2 occurred after transgene integration. In line

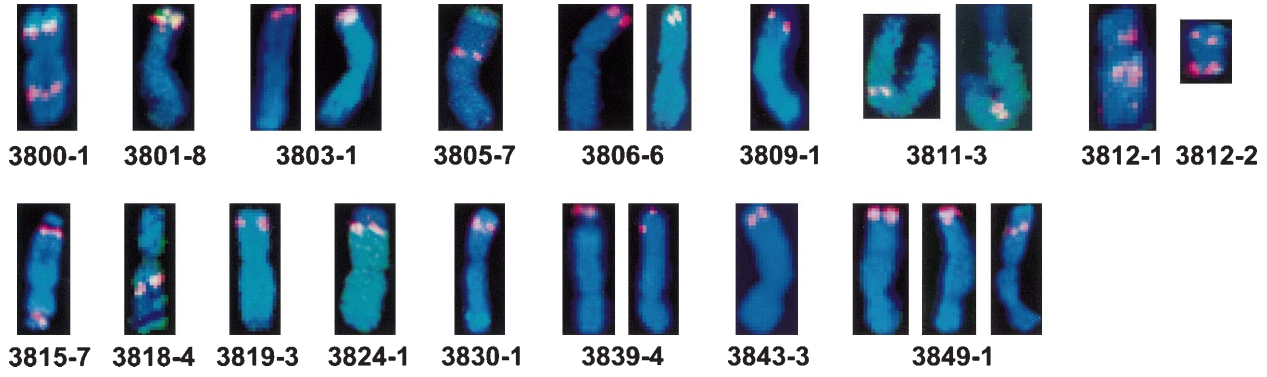

Fig. 2 (Legend see page 875)

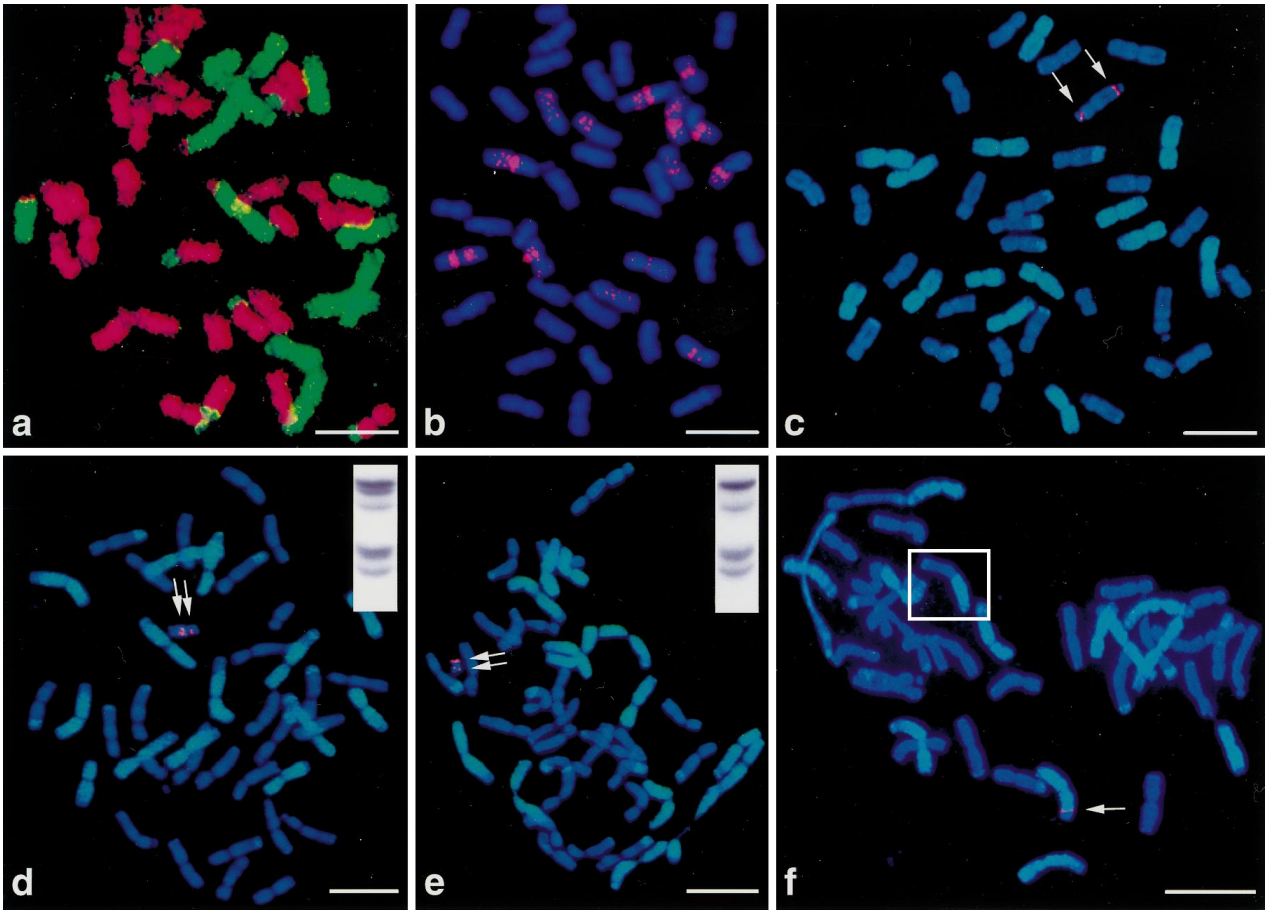

Fig. 3 (Legend see page 875)

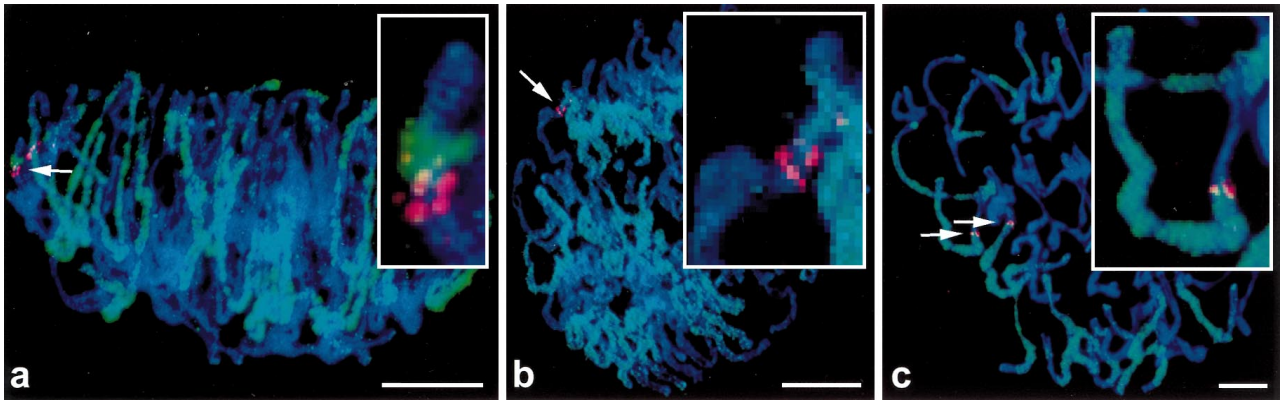

Fig. 4 (Legend see page 875)

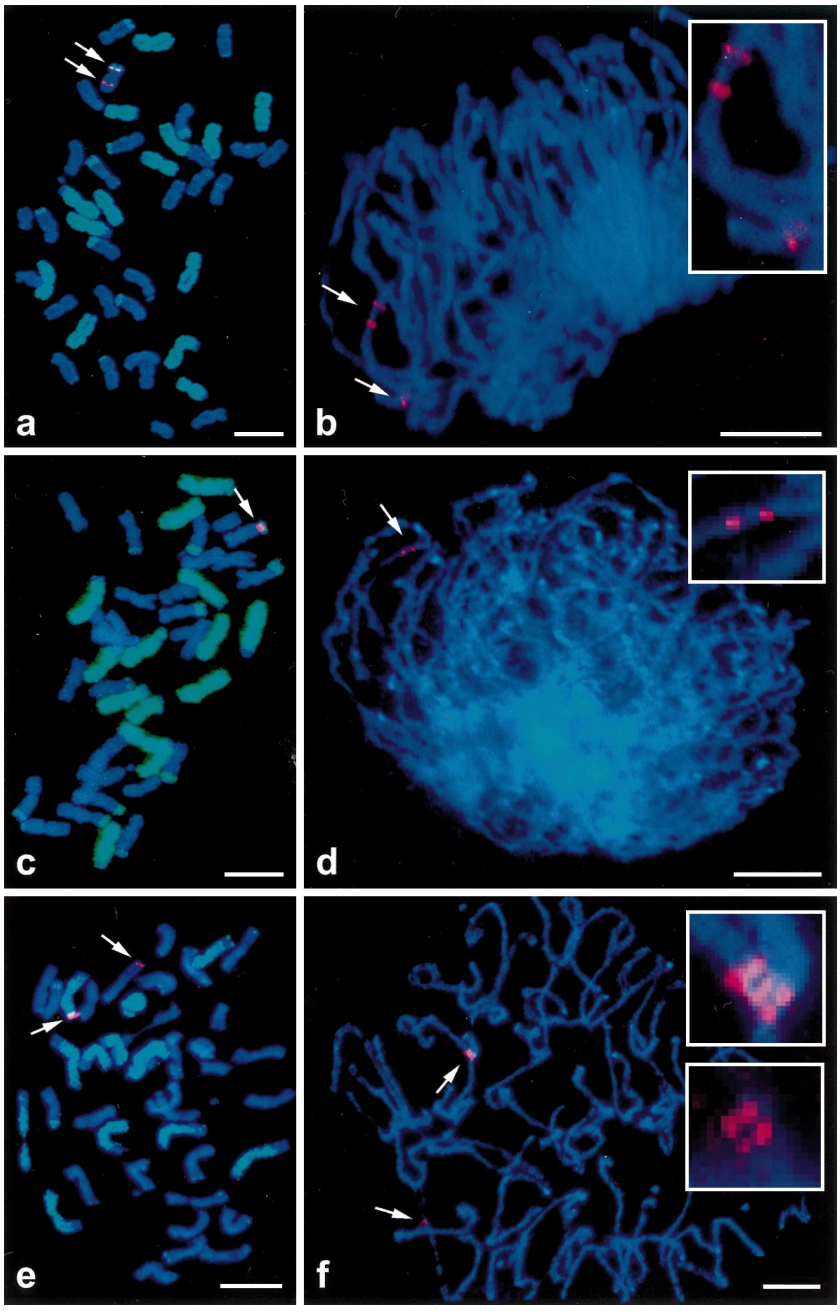

Fig. 5 FISH of the pNGI (*red*) and AsC2 (*green*) on metaphase, prophase and prometaphase chromosomes of transgenic oat lines: 3800–1 (**a, b**), 3801–8 (**c, d**), and 3803–1 (**e, f**). Transgene integration sites are indicated with *arrows*. Chromatin was counterstained with DAPI. *Bar:* 10 µm

3815–7, 2 transgene loci were localized on an A/D genome chromosome with two satellites, indicating that this chromosome was also probably the result of a translocation because there is no such chromosome in oat (Figs. 2 and 3*c*). Line 3815–7 also was monosomic. Recombination between the trangene integration sites on the same chromosome was not observed in the progeny of these 3 lines using either phenotypic segregation, Southern analysis, or FISH (Table 1).

Chromosome counts showed that T_1 plants of line 3824–1 had either 41 or 42 chromosomes, indicating that this line, like 3815–7, also was a segregating monosomic. FISH using the C-genome-specific dispersed repeat, AsC2, to chromosomes of several T_1 plants of line 3824–1 demonstrated that 2 chromosomes (1 from the C- and 1 from A/D-genome) were translocated, resulting in 1 chromosome. However, the transgene locus in this line was detected on another C-genome chromosome (Fig. 3*f*).

Discussion

The combination of phenotypic and genotypic progeny tests, Southern blot analyses, and FISH increased the accuracy of determining integration site numbers and improved our understanding of the internal structure of transgene loci generated via microprojectile bombard-

ment in oat. Transgenic plants produced by microprojectile bombardment usually exhibit multiple copies of transgenes integrated into a single genomic site (Chen et al. 1998; Kohli et al. 1998; Pawlowski and Somers 1996, 1998). In the present study, we observed 8 out of 16 lines that exhibited more than one transgene integration site. In part, this increase in the frequency of multiple transgene locus events was due to our use of FISH, which enabled the detection of 2 transgene loci on single chromosome that did not recombine in 3 lines, 3800–1, 3812, and 3815–7. In 7 other lines there was correspondence between the transgene locus numbers determined using the three analyses. However, in lines 3801–8, 3811–3, 3824–1, 3830–1, 3839–4, and 3849–1 phenotypic segregation either exhibited deficiencies in GUS-positive progeny within a line or underestimated transgene locus numbers. In lines 3801–8, 3830–1, and 3849–1, transgene silencing likely accounted for segregation distortion of the transgene phenotype, as previously described for transgenic oat (Pawlowski et al. 1998). Line 3811–3 appeared to have only one active *gus*A locus based on phenotypic segregation. In contrast, line 3824–1 exhibited a single transgene locus based on Southern analysis and FISH but segregated for GUS activity in a manner suggesting multiple loci. Since this line is monosomic, transgene segregation distortion likely was caused by a chromosomal aberration, possibly involving the homolog of the chromosome carrying the transgene.

Simultaneous FISH of the transgene and the C- or A/D-genome-specific repeats showed that there were no preferences for integration of the transgene into the highly heterochromatic C genome or the A and D genomes of hexaploid oat. Our data also showed that integration occurred randomly among chromosomes of both the C and A/D genomes. Integration sites were detected at different locations along individual chromosomes. However, in 18 out of 25 integration events, transgene integration occurred in telomeric and subtelomeric regions. Distal chromosomal areas are enriched with coding DNA sequences in most plants, whereas heterochromatic regions are mainly located around the centromeres (Gill et al. 1993, 1996). Transgene integration into heterochromatic regions may be expected to result in poor or unstable transgene expression (Iglesias et al. 1997; Jakowitsch et al. 1999). As a result, the transgenic cells would not be selected or regenerated into plants. We propose that transgene integration occurs randomly throughout the oat genome. However, tissue culture selection of transgenic cells, which is employed in most transformation systems, would result in the recovery of transgenic plants with functional transgene loci primarily located in these gene-rich regions.

Evidence for interspersion of the transgene copies with fragments of oat DNA within transgene loci was observed in 12 lines from Southern blot analysis. The size of transgene loci estimated from the sum of the length of the multiple transgene-hybridizing fragments observed in Southern blots using noncutting enzymes ranged from approximately 20 kb to greater than 250 kb, which is in agreement with previous results for oat (Pawlowski and Somers 1998). However, Southern blot analysis did not allow determination of the size of the oat genomic DNA fragments interspersing the tightly linked transgene copies. Our FISH results showed that these interspersions may be extremely large. At least 9 out of 25 transgene loci were visualized in FISH of prometaphase chromosomes to be organized as clusters of multiple transgene fragments interspersed with fragments of oat DNA that appeared to be a few megabases long. This indicates that the size of some transgene clusters may exceed several megabases, which is significantly greater than previously estimated (Pawlowski and Somers 1998).

In the present study, at least 6 transgene integration sites were associated with rearranged chromosomes. Various chromosomal aberrations resulting from chromosomal breakage are observed in plants regenerated from tissue culture (McCoy et al. 1982; Olhoft and Phillips 1998). However, the probability that 6 transgene integration sites were associated with 4 rearranged chromosomes in these oat lines is much higher than expected for random transgene integration and random tissue culture-induced chromosome alterations. In lines 3800–1, 3812, and 3815–7, transgene integration sites were detected on different arms of the same chromosomes. Recombination between these transgene integration sites was not observed in the progeny of these 3 lines. For line 3812, the lack of recombination can probably be explained by the transgene localization in subcentromeric regions, which are known to have reduced crossing-over. However, the transgene integration sites in the remaining 2 lines were located far from centromeres and, therefore, were likely unlinked. The absence of recombination between these transgene integration sites was probably the result of the chromosomal rearrangements observed in these chromosomes. We suggest that particle bombardment and/or the process of transgene integration into the plant genome may lead to host DNA breakage and result in chromosomal aberrations. It also seems possible that the internal structure of tightly linked clusters of interspersed transgenes may lead to DNA breakage.

Transgene integration sites of lines 3800–1, 3801–8, and 3824–1 were detected on the borders of C- and A/D-chromosome translocations. The total number of translocations in these 3 transgenic lines appeared to be the same as in wild-type oat plants, suggesting that they existed before transgene integration. However, in line 3800–1, the transgene integration site occurred at the translocation border in an aberrant chromosome arm as previously mentioned. Moreover, the transgene locus in line 3824–1 segregated in a manner suggesting that it may be monosomic. While it is impossible based on these results to conclude whether these transgene integration either occurred at preexisting translocation borders or created the translocations in these lines, the association of the transgene with 2 out of the 3 apparently aberrant chromosomes suggests the latter possibility.

Two different models have been recently proposed for genomic integration of transgenes introduced by micro-

projectile bombardment. Kohli et al. (1998) hypothesized that the structure of integration loci results from a two-phase process. The "preintegration" phase occurs extrachromosomally and involves the ligation of transgene fragments into complex multicopy arrays of transgenes, which do not contain plant genomic sequences. Recombination at specific sequences within the transgene has also been detected in integrated transgenes in rice (Kohli et al. 1999). These transgene arrays are integrated into the genome at a single site, which initiates a "hot spot" that facilitates integration of other transgene molecules at the same locus. The subsequent integration of other transgene sequences near the initial integration site may result in plant DNA separating the transgenes in a tightly linked cluster (Kohli et al. 1998). Pawlowski and Somers (1998) proposed that integration of transgene DNA at a cluster of active replications forks may result in the interspersion of the integrated transgenes with host DNA.

The results of the present study suggest an alternative model that would account for the three major observations: (1) clustered organization of transgenes in single loci, (2) interspersion of transgenes within clusters with megabase-long DNA fragments, and (3) association of transgene integration loci with rearranged chromosomes. These observations indicate that damage to the host genome may have been overlooked in the previous two models for transgene integration. Delivery of DNA-coated microprojectiles to the nuclei of oat tissue culture cells would likely cause extensive chromosomal DNA breakage. Thus, an extrachromosomal pool of whole and sheared plasmid transgene DNA mixed with host DNA fragments of variable length, including subchromosome fragments, would be created. Random preintegration ligation of DNA fragments in this extrachromosomal pool would produce interspersed structures containing transgene and host DNA. Integration of the ligation products into single or multiple sites in the genome may then occur through a break-repair mechanism (Gorbunova and Levy 1997, 1999; Kohli et al. 1999). The proposed model would explain all features currently reported for the structure of transgene integration loci produced via microprojectile bombardment.

Integration of large transgene clusters with apparent chromosomal rearrangements would likely create a significant mutational load for the transgenic cell. Cells with multiple independent integrations of these structures may be unable to survive or retain morphogenetic potential. Consequently, transformants with single integration loci would be predominantly recovered. Polyploids with two or more sets of homologous chromosomes can tolerate more severe aberrations than diploids (Sears 1996). We believe polyploidy may also buffer against the deleterious effects of transgene integration loci and, therefore, transgenic allohexaploid oats with more than 1 transgenic locus are more frequently isolated compared to diploid rice (Kohli et al. 1998). The genetic buffering of the oat genome may also explain the more complex organization of transgene loci in oat compared to the smaller size of transgene integration loci reported in rice (Kohli et al. 1998). Many crop plants are polyploid or have duplicated regions in their genomes. Transformation of these crops using microprojectile bombardment also may result in large clusters of transgenes interspersed with genomic DNA that are associated with chromosome rearrangements.

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