Chromosome Research 1996, **4**, 277–281

In situ localization of yeast artificial chromosome sequences on tomato and potato metaphase chromosomes

Jörg Fuchs, Dorothee-U. Kloos, Martin W. Ganal & Ingo Schubert

Received 22 November 1995; received in revised form 19 February 1996; accepted for publication by J. S. (Pat) Heslop-Harrison 19 February 1996

***In situ* localization of short low- or single-copy sequences is still difficult in plants. One solution to this problem could be the use of large yeast artificial chromosomes (YACs) for fluorescence *in situ* hybridization. Two YACs specific for a single copy marker on the long arm of the NOR-chromosome 2 of tomato (*Lycopersicon esculentum*) were selected. Both probes hybridized exclusively to this chromosome, although one produced a slightly dispersed hybridization signal. Hybridization of these YACs onto potato chromosomes showed a clear single locus on the homoeologous potato chromosome in both cases.**

Key words: chromosome specific repetitive sequence, fluorescence *in situ* hybridization, potato, tomato, yeast artificial chromosome

Introduction

During the last decade, fluorescence *in situ* hybridization (FISH) has been used successfully for cytological mapping of repetitive sequences and multigene families in plant genome analysis. However, the localization of short low- or single-copy sequences is still difficult. At present, reliable *in situ* detection on plant metaphase chromosomes requires a target sequence of at least 10 kb per locus (Ambros *et al.* 1986, Lehfer *et al.* 1993, Leitch & Heslop-Harrison 1993, Fuchs *et al.* 1994a, Jiang & Gill 1994, Fuchs & Schubert 1995). Alternatively, large insert clones such as those available from libraries constructed in yeast (YAC, Burke *et al.* 1987) or bacterial (BAC, Shizuya *et al.* 1992) artificial chromosomes may be used. Such clones contain up to several hundred kb of DNA of which a large proportion is specific for the respective locus in the genome. Thus, they should provide a strong hybridization signal at this locus relative to a weak background signal. However, with increasing genome size such clones contain disproportionally more repeated DNA sequences that hybridize to almost all regions in a genome and hence obscure the locus containing the unique sequences. In genomes that are not too complex (e.g. mammals), the hybridization signals caused by the interspersed sequences can be suppressed by prehybridization with unlabelled genomic DNA or the respective *Cot 1* fraction. However, in organisms with very large genomes such as many plants, the high amount of short dispersed repetitive sequences makes successful *in situ* localization of complex probes impossible even with blocking (Fuchs *et al.* 1994b, 1996).

Recently, BACs were successfully used for *in situ* localization in plants (rice, *Sorghum*, cotton) with a relatively small genome size (1C ~ 1 pg; Woo *et al.* 1994, Jiang *et al.* 1995, Hanson *et al.* 1995). Corresponding data for YACs have not yet been reported.

In this study, we present data on the feasibility of *in situ* hybridization of YACs onto tomato metaphase chromosomes. Tomato provides an ideal subject for testing this because of the availability of a saturated genetic map based on restriction fragment length polymorphism (RFLP) markers for the isolation of probes (Tanksley *et al.* 1992). Furthermore, this genetic map contains several cytogenetic landmarks such as the nucleolus organizer region (NOR), 5S rRNA genes, macrosatellites and subtelomeric repeats suitable for chromosome identification. Finally, a large wealth of data is available on the structure, dispersion pattern, evolutionary conservation and copy number of the major interspersed repeated DNA sequences that can be used to explain hybridization data obtained in such experiments (Ganal *et al.* 1988, Zamir & Tanksley 1988).

Materials and methods

Isolation of chromosome-specific YACs

The YAC library of tomato (Martin *et al.* 1992) was screened by polymerase chain reaction (PCR) with a primer pair derived from a chromosome-specific RFLP marker. For this purpose, the cloned marker CT277 (900 bp), localized on chromosome 2 of the molecular linkage map of the tomato genome (Tanksley *et al.* 1992), was sequenced from both ends with an A.L.F. sequencer (Pharmacia) according to standard procedures. A primer pair for this marker was designed for PCR amplification (CAT CTC ACA GTG TTT TGC AG and GAT GCT GGC

J. Fuchs (corresponding author), D.-U. Kloos, M. W. Ganal and I. Schubert are at the Institut für Pflanzengenetik and Kulturpflanzenforschung, 06466 Gatersleben, Germany. Tel: (+49) 39482 5257; Fax: (+49) 39482 5137.

The first two authors have equally contributed to this work and both should be considered as first authors.

ATT GTT GCT TC). Screening of the YAC library was done according to Green & Olson (1990) with some modifications: the YAC library consisting of more than 36 000 clones was organized in 380 DNA pools containing 96 single yeast clones each. In the first step DNA pools were screened by PCR with the above-mentioned CT277-specific primer pair and amplified fragments were analysed by separation on 1% agarose gels. The 96 yeast clones of those DNA pools that revealed marker-specific fragments were subjected directly to a second PCR step. For lysis of cells, 5 µl of a 1 mg/ml solution of zymolyase 100 T (ICN) was added to 5 µl of the glycerol stock cultures and incubated for 30 min at 37°C. Then, PCR was run in a total volume of 50 µl. After this second PCR step, single positive clones were isolated on selective media and intact yeast chromosomes were extracted according to Carle & Olson (1985). The size of the respective YACs was determined by pulsed-field gel electrophoresis (PFGE), blotting and hybridization with the marker. YAC DNA was isolated from agarose (low-melting point) gels after PFGE by adsorption to glass milk (Vogelstein & Gillespie, 1979) with a Gene Clean Kit (Bio 101).

In situ hybridization

Metaphase spreads of tomato (cv. Moneymaker) and a dihaploid potato (BP89/22, kindly provided by Dr Schuchmann, Firma Bioplant, Ebstorf) were made on glass slides from root tips of seedlings grown on wet filter paper, treated on 0.05% colchicine for 2 h, fixed in 3:1 ethanol–acetic acid and digested in 1% pectinase and 1% cellulase (in 0.01 M citric acid–sodium citrate buffer, pH 4.5–4.8) for 30 min at 37°C. After squashing of meristems in 45% acetic acid, slides were used immediately for FISH or stored in glycerol at 4°C.

YAC DNA was labelled with Biotin-16-dUTP either by PCR (Fuchs *et al.* 1994b) after amplification with degenerated primers according to Pich *et al.* (1994) or directly by nick translation using a kit (Amersham) according to the instructions of the manufacturer. FISH was performed as described by Fuchs & Schubert (1995). Metaphases were evaluated using a Zeiss Axioskop with appropriate filter combinations. Fluorochrome images were captured separately with a cooled CCD camera (Photometrics), pseudocoloured and merged and in some cases processed on the computer (Adope Photoshop, Gene Join). Complete images were printed on a Phaser II SDX (Tektronix).

Results and discussion

YAC isolation and characterization

For the purpose of testing the feasibility of *in situ* hybridization with entire YACs on tomato chromosomes, we have focused on chromosome 2. This chromosome contains the NOR with the ribosome RNA genes on the short arm. Therefore, chromosome 2 is easily identifiable, for instance, by propidium iodide staining. For the isolation of YACs, we have chosen CT277 as a marker that is approximately in the centre of the genetic map of the long arm of chromosome 2. After partial sequencing of CT277, oligonucleotide primers were designed and used for the screening of a library of YACs as described in the Materials and Methods. Three YAC clones (YAC 117.A05 = 200 kb, YAC 153.B12 = 370 kb, YAC 610.H01 = 180 kb) out of approximately five genome equivalents were identified with these primers. Unfortunately, the largest YAC clone co-migrated with a yeast chromosome on pulsed-field gels and thus could not be purified from yeast DNA. Because of this, the *in situ* hybridizations were performed with only two clones (YAC 117 and YAC 610). Preliminary data on these two YACs indicate that they most probably extend into opposite directions from the CT277 site (data not shown).

In situ hybridization on tomato chromosomes

For *in situ* hybridizations, the respective YAC clones were purified on preparative pulsed-field gels and excised from such gels. *In situ* hybridizations with biotin-labelled YAC DNA without any blocking with genomic DNA revealed clear signals on 60% of 40 inspected metaphase plates in both cases. Signals were always associated with the NOR-carrying chromosome of tomato. No other chromosome besides chromosome 2 was reproducibly labelled with either of the two YACs. This indicates the absence of repetitive sequences dispersed along all chromosomes from both YACs. However, the signals obtained with the two YACs were slightly different. While YAC 117 resulted in two distinct signals on both chromatids of chromosome 2 (Figure 1f), YAC 610 produced dispersed hybridization signals and apparently labelled the entire chromosome 2 (Figure 1a). After image processing by empirical changing of brightness and contrast of the signal image, only the two most intensive signal dots remained visible at a similar chromosomal position as for YAC 117 (Figure 1b). The interspersed hybridization pattern of YAC 610 on chromosome 2 is probably caused by repeated DNA sequences in the region that is contained in YAC 610 and which are predominantly located on this chromosome of tomato.

In situ hybridization with tomato YACs onto potato chromosomes

From genetic mapping, it is known that the chromosomes of tomato and potato are co-linear, except for a small number of inversions. More than 99% of all single-copy tomato probes cross-hybridize under standard hybridization conditions (Tanksley *et al.* 1992). In addition, repeated DNA sequences of tomato, including the major tandemly (TGRI) and interspersed repeated (TGRII) sequences are absent from the potato genome (Ganal *et al.* 1988, Zamir & Tanksley 1988). Thus, we have tested whether it is possible to use the tomato YACs as probes for *in situ* hybridizations onto potato chromosomes and whether by this method, a clearer hybridization signal for YAC 610 is obtainable. As chromosomes 2 of tomato and potato are absolutely co-linear based on current data and both contain the

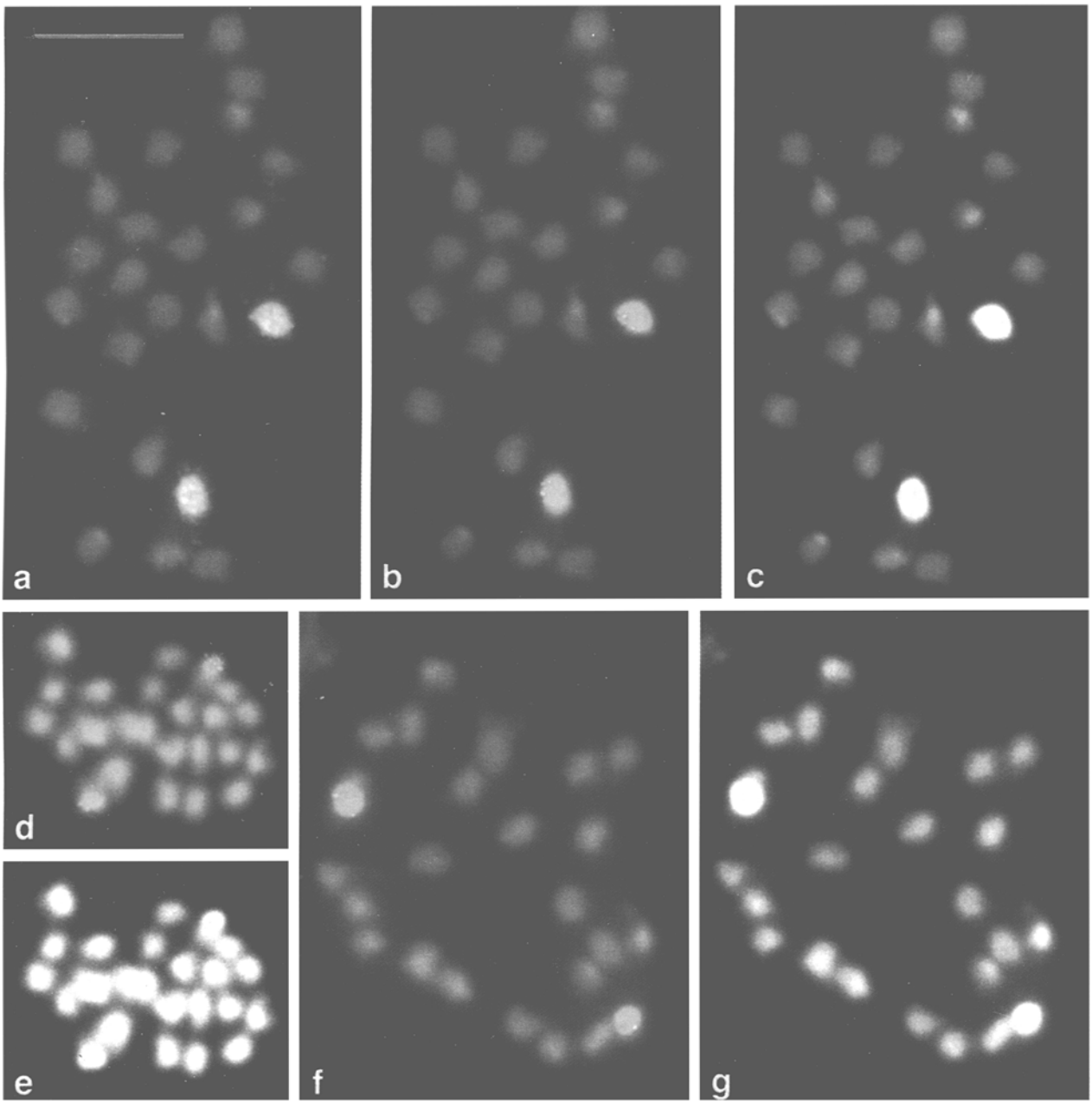

Figure 1. Physical mapping of YACs 117 and 610, both specific for chromosome 2, on metaphase chromosomes of tomato and potato via fluorescence *in situ* hybridization. Biotinylated YAC DNA was detected with streptavidin–FITC and chromosomes were counterstained using propidium iodide (PI). Scale bar = 10 μm. **a-c** YAC 610 hybridized onto tomato chromosomes. The hybridization pattern reveals dense signals along the entire chromosome 2 (**a**). The same metaphase after image processing shows reduction of signals to the two most intensive spots on each chromosome (**b**). The same metaphase after PI staining, the GC-rich NOR on chromosomes 2 reveal the brightest fluorescence (**c**). **d–e** The same YAC hybridized onto homoeologous chromosomes of potato resulted in distinct dots on each satellite chromosome (**d**). The

NOR on the short arm, this chromosome should readily be identified in a dihaploid potato.

Hybridization with both YACs on potato chromosomes indeed yielded the expected hybridization signals exclusively on the NOR-carrying chromosome 2 of potato. Interestingly, YAC 610, which gave dispersed hybridization signals on chromosome 2 of tomato, resulted in a very distinct signal in more than 40% of the 25 inspected metaphase plates (Figure 1d). This supports the assumption that the dispersed labelling of chromosome 2 of tomato was caused by an interspersed repeated DNA element that does not cross-hybridize to the potato genome. As in tomato, YAC 117 labelled the same potato chromosome in about 25% of the inspected metaphase plates at a single location on chromosome 2.

Conclusions

In this paper, we have shown that YACs in the size range of approximately 200 kb can be used for *in situ* hybridization on tomato metaphase chromosomes even without the need for blocking repeated DNA sequences. The specificity of the signal was confirmed by the selection of YACs that label the cytogenetically identifiable chromosome 2. Thus YACs provide a tool to localize low- or single-copy genes in plants in the same way as BACs do in *Sorghum*, rice and cotton (Woo *et al.* 1994, Jiang *et al.* 1995, Hanson *et al.* 1995). These three species have relatively small genomes (1C ~ 1 pg, for review of angiosperm DNA contents see Bennett & Leitch 1995) and hence a relatively low amount of repeated DNA sequences. For larger, more complex genomes (> 5 pg, e.g. wheat, barley, field bean), however, this will be much more difficult owing to the much higher number of repeated sequences on large insert clones and the fact that it has not yet been possible to suppress sufficiently these disperse repetitive elements by competition with genomic DNA (Fuchs *et al.* 1996).

Furthermore, this study demonstrates that large insert clones of tomato can be used in *in situ* hybridizations with closely related species (here potato). In fact, the low evolutionary conservation of repeated DNA sequences allows a more precise localization on homoeologous chromosomes as shown for one YAC. This might be a solution for the analysis of organisms with large genomes in the way that large insert clones from closely related species are used for *in situ* hybridization, provided the single-copy sequences on the respective YACs are not too short and too distant from each other to yield reproducible signals.

A set of selected YACs of tomato could be used by simultaneous differential labelling for the individualization of single chromosome pairs that are not distinguishable by other means. The simultaneous hybridization of YACs from a given organism onto a closely related species might allow the study of chromosomal rearrangements that occur during speciation as well as synteny relationships. In the same way, the use of YACs as probes for *in situ* hybridization could allow the isolation of specific chromosomes by chromosome sorting (Pich *et al.* 1995). Finally, the use of whole YAC *in situ* hybridization will open the door for the cytogenetic analysis of chromosomal landmarks such as centromeres in the way that large centromere-associated YAC clones can now be analysed, whether they identify all centromeres of a given plant.

Acknowledgements

We thank Barabara Hildebrandt for skilful technical assistance and Professor R. Rieger for critically reading this manuscript. Part of this research was funded by a grant from the German–Israeli Foundation for Research and Development (GIF) to M. W. G. (G-342-028.12/94).

References

Ambros PF, Matzke MA, Matzke AJM (1986) Detection of a 17 kb unique sequence (T-DNA) in plant chromosomes by *in situ* hybridization. *Chromosoma* **94**: 11–18.

Bennett M D, Leitch I J (1995) Nuclear DNA amounts in angiosperms. *Annals Bot* **76**: 113–176.

Burke D T, Carle G F, Olson M V (1987) Cloning of large segments of exogenous DNA into yeast by means of artificial chromosome vectors. *Science* **236**: 806–812.

Carle G F, Olson M V (1985) An electophoretic karyotype for yeast. *Proc Natl Acad Sci USA* **82**: 3756–3760.

Fuchs J, Schubert I (1995) Localization of seed protein genes on metaphase chromosomes of *Vicia faba* via fluorescence *in situ* hybridization *Chrom Res* **3**: 94–100.

Fuchs J, Joos S, Lichter P, Schubert I (1994a) Localization of vicilin genes on field bean chromosome II by fluorescent *in situ* hybridization. *J Heredity* **85**: 487–488.

Fuchs J, Pich U, Meister A, Schubert I (1994b) Differentiation of field bean heterochromatin by *in situ* hybridization with a repeated *FokI* sequence. *Chrom Res* **2**: 25–28.

Fuchs J, Houben A, Brandes A, Schubert I (1996) Chromosome 'painting' in plants—a feasible technique? *Chromosoma* **104**: 315–320.

Ganal M W, Lapitan N L V, Tanksley S D (1988) A molecular and cytogenetic survey of major repeated DNA sequences in tomato (*Lycopersicon esculentum*) *Mol Gen Genet* **213**: 262–268.

Green E D, Olson M V (1990) Systematic screening of yeast artificial libraries by the use of the polymerase chain reaction. *Proc Natl Acad Sci USA* **87**: 1213–1217.

Hanson R E, Zwick M S, Choi S *et al.* (1995) Fluorescent *in situ* hybridization of a bacterial artificial chromosome. *Genome* **38**: 646–651.

Jiang J, Gill B S (1994) Nonisotopic *in situ* hybridization and plant genome mapping: the first 10 years. *Genome* **37**: 717–725.

Jiang J, Gill B S, Wang G-L, Ronald P C, Ward D C (1995) Metaphase and interphase fluorescence *in situ* hybridization mapping of the rice genome with bacterial artificial chromosomes. *Proc Natl Acad Sci USA* **92**: 4487–4491.

Lehfer H, Busch W, Martin R, Herrmann R G (1993) Localization of the B-hordein locus on barley chromosomes using fluorescence *in situ* hybridization. *Chromosoma* **102**: 428–432.

Leitch I J, Heslop-Harrison J S (1993) Physical mapping of four sites of 5S rDNA sequences and one site of the α-amylase-2 gene in barley (*Hordeum vulgare*). *Genome* **36**: 517–523.

Martin, G B, Ganal M W, Tanksley S D (1992) Construction of a yeast artificial chromosome library of tomato and identification of cloned segments linked to two disease resistance loci. *Mol Gen Genet* **233**: 25–32.

Pich U, Houben A, Fuchs J, Meister A, Schubert I (1994) Utility of DNA amplified by degenerate oligonucleotide-primed PCR (DOP-PCR) from the total genome and defined chromosomal regions of field bean. *Mol Gen Genet* **243**: 173–177.

Pich U, Meister A, Macas J *et al.* (1995) Primed *in situ* labelling facilitates flow sorting of similar sized chromosomes. *Plant J* **7**: 1039–1044.

Shizuya H, Birren B, Kin U-J *et al.* (1992) Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Escherichia coli* using an F-factor-based vector. *Proc Natl Acad Sci USA* **89**: 8794–8797.

Tanksley S D, Ganal M W, Prince J P *et al.* (1992) High density molecular linkage maps of the tomato and potato genomes. *Genetics* **132**: 1141–1160.

Vogelstein B, Gillespie D (1979) Preparation and analytical purification of DNA from agarose. *Proc Natl Acad Sci USA* **76**: 615–619.

Woo S-S, Jiang J, Gill B S, Paterson A H, Wing R A (1994) Construction and characterization of a bacterial artificial chromosome library of *Sorghum bicolor*. *Nucleic Acids Res* **22**: 4922–4931.

Zamir D, Tanksley SD (1988) Tomato genome is comprised largely of fast-evolving low copy-number sequences. *Mol Gen Genet* **213**: 254–261.