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Map-based cloning in crop plants: tomato as a model system II. Isolation and characterization of a set of overlapping yeast artificial chromosomes encompassing the *jointless* locus

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Abstract A map-based cloning technique for crop plants is being developed using tomato as a model system. The target gene *jointless* is a recessive mutation that completely suppresses the formation of flower and fruit pedicel abscission zones. Previously, the *jointless* locus was mapped to a 3 cM interval between the two molecular markers TG523 and RPD158. Physical mapping of the *jointless* region by pulsed-field gel electrophoresis demonstrated that TG523 and RPD158 reside on a 600 kb *Sma*I fragment. In this study, TG523 was used as a probe to screen a tomato yeast artificial chromosome (YAC) library. Six tomato YAC (TY) clones were isolated, ranging from 220 to 380 kb in size. Genetic mapping of YAC ends demonstrated that this set of overlapping YACs encompasses the *jointless* locus. Two YAC ends, TY159L (L indicates left end) and TY143R (R indicates right end), cosegregate with the *jointless* locus. Only one of the six YACs (TY142) contained single-copy DNA sequences at both ends that could be mapped. The two ends of TY142 were mapped to either side of the *jointless* locus, indicating that TY142 contains a contiguous 285 kb tomato DNA fragment that probably includes the *jointless* locus. Physical mapping of the TY142 clone revealed that TY159L and TY143R reside on a 55 kb *Sal*I fragment. Southern blot hybridization analysis of the DNAs of tomato lines nearly isogenic for the *jointless* mutation has allowed localization of the target locus to a region of less than 50 kb within the TY142 clone.

Key words *Lycopersicon esculentum* · Map-based cloning · YAC physical mapping · Abscission · *jointless*

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Introduction

Abscission is a universal process whereby plants shed organs such as fruit, flowers, and leaves and thus regulate their growth and development. Abscission zones are the regions of plants where abscission occurs. In tomato, two loci have been identified that completely suppress the formation of flower and fruit pedicel abscission zones – the *jointless* mutations *j* and *j-2* (Butler 1936; Rick 1957). In jointed (wild-type) tomato, the abscission zone develops in the middle of the pedicel, which can be easily visualized by the presence of an indentation termed the "joint" (Butler 1936; Wing et al. 1994). Flowers and fruits are shed from jointed plants at the abscission zones, and thus often retain the distal parts of pedicels and calyxes when shed. In tomato plants homozygous for the *jointless* mutation, the development of abscission zones is completely suppressed. Since there is no region at which the plant can shed its flowers or fruit, unfertilized flowers will shrivel and die on the pedicels and fruit will remain on the plant until manually harvested. This latter property is agronomically useful in the tomato processing industry because the final product upon mechanical harvesting is a "stemless" tomato. Therefore, the isolation of the *jointless* gene is of significance for studying the mechanism of plant abscission zone development and modifying flower and fruit abscission in plants.

In our attempt to isolate the *jointless* gene (*j*), a map-based cloning technique is being used. Map-based cloning is a newly developed technique by which a target gene is cloned based only on its phenotype and position on a genetic map (Rommens et al. 1989; Arondel et al. 1992; Giraudat et al. 1992; Chang et al. 1993; Martin et al. 1993). A map-based cloning strategy often includes the following steps (Wing et al. 1994): The target gene is first mapped relative to molecular markers. Second, physical mapping is performed by pulsed-field gel electrophoresis (PFGE) and Southern blot

hybridization with the most closely linked markers to the target gene. This step is important in estimating the feasibility of using map-based cloning to initiate a walk to the target gene. If the closest markers are physically far away from the target gene, more chromosome walking steps and more markers will be required to reach the target locus. The third step is chromosome walking. Although cosmid libraries may be used, a yeast artificial chromosome (YAC) or bacterial artificial chromosome (BAC) library is more practical for long-range chromosome walking in higher eukaryotes. YACs (> 150 kb, e.g., see Martin et al. 1992) and BACs (> 100 kb, Shizuya et al. 1992) generally carry much larger DNA inserts than cosmid clones (< 50 kb). Lastly, once a candidate clone(s) is isolated, gene identification via genetic complementation and/or antisense suppression should follow to demonstrate that the candidate clone truly carries the target gene.

Tomato is an ideal crop plant for developing and establishing a map-based cloning system. Tomato has a medium genome size (1.88–2.07 pg/2C) among crop plants (Arumuganathan and Earle 1991) and a low level of repetitive sequences (Zamir and Tanksley 1988). A high density restriction fragment length polymorphism (RFLP) map has been developed (Tanksley et al. 1992) and a complete YAC library has been constructed (Martin et al. 1992). Transformation is also well developed and is essential for verification of a target gene (McCormick et al. 1986).

In our previous study, we reported the first two steps in the isolation of the *jointless* gene by using a map-based cloning strategy: genetic and physical mapping (Wing et al. 1994). In this study, the next step of map-based cloning of the *jointless* gene is reported: the isolation and characterization of a set of overlapping YACs that encompass the *jointless* locus.

Materials and methods

Plant materials, molecular markers, and construction of a tomato YAC library

Lycopersicon esculentum LA624 (*jointless* homozygote *j/j*) and *Lycopersicon pennellii* LA716 (jointed homozygote *J/J*) were kindly provided by Dr. C.M. Rick, Tomato Genetics Stock Center, University of California at Davis, California, USA. The F_2 population segregating for *jointless* was generated from an F_1 cross between LA624 (female) and LA716 (male) (Wing et al. 1994). *L. esculentum* LA3021 (*J/J*) and its near-isogenic line (NIL) LA3023 (*j/j*) were kindly provided by Dr. C. M. Rick, and *L. esculentum* Heinz 1706 (*J/J*) and its NIL Heinz 1706 (*j/j*) were kindly provided by Dr. J. Philouze (1991).

TG523 is a single-copy RFLP marker that has been mapped 1.5(+ / − 2.0) cM from the *jointless* locus and RPD158 is a single-copy random amplified polymorphic DNA (RAPD) marker that has been mapped 1.5 (+ / − 2.0) cM from the *jointless* locus on the other side. The maximum physical distance between the two markers is 600 kb (Wing et al. 1994).

The tomato YAC library used for chromosome walking in this study was constructed from high molecular weight (HMW) DNA of *L. esculentum* cvs. VFNT cherry and Rio Grande-PtoR by Martin et al. (1992). The library was constructed from size-selected partial *Eco*RI digests of tomato HMW DNA ligated into the *Eco*RI site of pYAC4 (Burke et al. 1987). It has an average insert size of 150 kb, and contains approximately 40,000 clones.

Screening of the YAC library

The tomato YAC library was screened according to Martin et al. (1992), using a gel-purified ^{32}P-labeled insert of TG523 as a probe. The YAC colonies that gave hybridization signals were single-colony purified and rescreened by PFGE Southern blot hybridization with the insert of TG523. YACs that were confirmed by hybridization with TG523 were further analyzed. HMW DNA preparation, PFGE, and Southern blotting and hybridization of the YAC clones were performed as described by Wing et al. (1993).

YAC end isolation

Total YAC DNA was isolated according to Sherman et al. (1986). The YAC ends were isolated by plasmid rescue and/or by the inverse polymerase chain reaction (IPCR, Silverman et al. 1989).

For isolating the left end of a YAC by plasmid rescue, total YAC DNA was digested with *Xho*I, which cuts once in the pYAC4 vector near the telomere and potentially once to several times within the tomato DNA insert. After *Xho*I digestion, the DNA was self-ligated with T4 DNA ligase and transformed into competent cells of *Escherichia coli* strain DH5α. The transformants were plated onto LB medium with 50 μg/ml ampicillin. Positive clones were selected by resistance to ampicillin and *Eco*RI (the original insert cloning site) and *Xho*I digestion of the plasmid DNAs.

For YAC end isolation by IPCR, total YAC DNA was digested with *Acc*I, *Hinc*II, *Sal*I, *Bgl*I, and *Ava*II, or *Hae*III, *Hha*I, *Hpa*I, *Nar*I, *Alu*I, and *Nla*IV, respectively, for the right end of the YAC and with *Taq*I, *Hae*III, *Nhe*I, *Rsa*I, and *Eco*RV, respectively, for the left end of the YAC. The restriction fragments of the DNA were self-ligated with T4 DNA ligase and the circularized DNA fragments were used as templates for IPCR. The IPCR reaction consisted of a 50 μl mixture containing 10 mM TRIS-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 mg/ml gelatin, 200 μM dNTPs, 0.8 μM primers, 10 ng template, and 1 unit AmpliTaq DNA Polymerase (Cetus). The mixture was first denatured at 94° C for 4 min in a DNA thermal cycler (Perkin-Elmer Cetus). Subsequently, 30 cycles of reactions were performed at 94° C, 1 min 30 s for denaturation; 60 °C, 2 min for annealing; and 72°, 3 min for primer extension. In the last cycle, the primer extension was extended for an additional 7 min. The sequences of the pair of primers for the left ends are: 5′-GTAG-CCAAGTTGGTTTAAGGC-3′ and 5′-AGGACGGGTGTGG-TCGCCATGATCGCG-3′, which are specific to the regions of pYAC4 near and on the 5′ side of the *Eco*RI cloning site. The sequences of the pairs of primers for the right ends are 5′-CTGGGAAGTGAATGGAGACATA-3′ and 5′-AGGAG-TCGCAATAAGGGAGAG-3′ when *Acc*I, *Hinc*II, *Sal*I, *Bgl*I, and *Ava*II were used to generate the IPCR template, and 5′-CTGGGAAGTGAATGGAGACATA-3′ and 5′-GACTTGCAAG-TTGAAATATTTCTTTCAAGC-3′ when *Hae*III, *Hha*I, *Hpa*I, *Nar*I, *Alu*I, and *Nla*IV were used to generate the IPCR template. These sequences are specific to the regions of pYAC4 near and on the 3′ side of the *Eco*RI cloning site. The IPCR products were purified and digested with *Eco*RI. The IPCR products that gave single bands on a gel and could be cleaved by *Eco*RI as assayed by agarose gel electrophoresis were cloned into the plasmid pCRII using the TA Cloning Kit (InVitrogen, USA). In cases where more than one IPCR product was generated from different enzyme digests for a single YAC end and met the above criteria, only one IPCR product was cloned.

YAC end mapping

The YAC ends were mapped in an F_2 population of 100 plants segregating for *jointless* developed in our previous study (Wing et al. 1994) by standard RFLP mapping techniques. Plant DNAs used for RFLP analysis were isolated according to Bernatzky and Tanksley (1986). The DNAs were digested with *Bst*NI, *Dra*I, *Eco*RI, *Eco*RV, *Hae*III, and *Hind*III, fractionated on 0.8% agarose gels, blotted onto Hybond-N + membranes (Amersham, USA), and hybridized with the ^{32}P-labeled inserts of the YAC end clones as previously described (Wing et al. 1994). Linkage analysis was performed using version 1.0 of the Mapmaker program for Macintosh computers (Lander et al. 1987) using the Kosambi mapping function with a L.O.D. score of 3.0. Standard errors were calculated according to the maximum likelihood method of Allard (1956).

YAC overlap analysis

Total YAC DNA was slot-blotted onto Hybond-N + membrane (Amersham, USA) and hybridized with the gel-purified ^{32}P-labeled inserts of the YAC end clones. Overlap analysis of the YACs was based on the hybridization of the YAC DNAs with the inserts of the YAC end clones.

Restriction site mapping of TY142

The restriction sites of TY142 were mapped as described by Burke et al. (1987). The main modification was that the YAC insert ends were used as probes and HMW DNA of TY142 was prepared in agarose microbeads (Wing et al. 1993). TY142 was streaked on a plate lacking uracil and tryptophan and grown for 2–3 days at 30° C. Cells from a single colony were inoculated into 5 ml of YPD broth (1% yeast extract, 2% peptone, and 2% dextrose) and grown overnight at 30° C with shaking. 0.5 ml of the overnight culture was inoculated into 200 ml of YPD broth and grown for 16–18 h at 30° C with shaking. The cells were harvested by centrifugation at 4000 *g* for 10 min, washed twice in 50 mM EDTA, pH 8.0, resuspended in 5 ml of SCE buffer (1 M sorbitol, 0.1 M sodium citrate, 0.06 M EDTA, final pH 7.0 adjusted with KOH) plus 100 mM β-mercaptoethanol and 1 mg/ml Zymolyase-100T (ICN Biomedicals, USA), and incubated at 37° C for 5 min. The cells were mixed with 5 ml of liquified 1% low melting point agarose in SCE, prewarmed at 42° C in a 500 ml flask. 20 ml of light mineral oil, prewarmed to 42° C, was added immediately and the contents of the flask were shaken vigorously for 2–3 s and then poured into a 500 ml beaker containing 150 ml of ice-cold SCE, which was vigorously swirled with a magnetic stir bar. The contents were swirled for 5 min and agarose microbeads formed. The microbeads were pelleted by centrifugation at 500 *g* for 15 min. From the 200 ml overnight culture, approximately 10 ml of microbeads were usually obtained, containing 2–5 µg HMW DNA per 100 µl of microbeads. To remove the yeast cell walls, the microbeads were resuspended in SCE plus 100 mM β-mercaptoethanol and incubated overnight at 37° C with gentle shaking. The microbeads were pelleted as above, resuspended in 0.5 M EDTA, pH 9.0, 10 mM TRIS-HCl, pH 8.0, 1% sodium lauryl sarcosinate, and 0.1 mg/ml proteinase K, and incubated overnight at 50° C with gentle shaking. The microbeads were pelleted, washed once in 0.5 M EDTA, pH 9.0, for an hour at 50° C, once in 0.05 M EDTA, pH 8.0, for another hour at 4° C, and stored in 0.05 EDTA, pH 8.0, at 4° C. The HMW DNA in microbeads could be stored in 0.05 EDTA, pH 8.0, at 4° C for a year without significant degradation.

Before use, the microbeads were washed three times in TE (10 mM TRIS-HCl, 1 mM EDTA, pH 8.0) plus 0.1 mM phenylmethyl sulfonyl fluoride (PMSF) and three times in TE on ice, 1 h each. The beads were then equilibrated with the appropriate restriction enzyme buffer for 2 h on ice, with one change after 1 h. The buffer was replaced by a suitable volume of fresh buffer supplied with a suitable amount of *Sma*I or *Sal*I and the beads were incubated on ice for 30 min. The digestion reactions were then incubated in a 37° C water bath. The reaction was stopped by adding 1/10 volume 0.5 M EDTA, pH 8.0. The partial digestions were obtained by decreasing amounts of the enzyme and 20 min of incubation and the complete digestions were obtained by adding a suitable amount of the enzyme and 3 h of incubation. The digested DNAs were fractionated by PFGE, blotted, and hybridized with the inserts of the left and right end clones of TY142, respectively, as described by Wing et al. (1993).

Physical mapping of TY142

The same Southern filter of TY142 DNA used for restriction site mapping was hybridized with the ^{32}P-labeled inserts of mapped single-copy YAC end clones RPD158 and TG523, respectively, with removal of probes from the blot between hybridizations. The position of a marker on TY142 was determined by the size of the hybridizing band in a complete digest and the number of hybridizing bands in partial digests. For example, if a YAC has two sites for a restriction enzyme the hybridization pattern would give three bands (including the entire YAC band) on a partially digested DNA blot when hybridized with a probe from either terminal fragment. However, when hybridized with a probe from the middle fragment the hybridization pattern would give four bands (including the YAC band) on the same Southern blot.

Results

YAC library screening

The *jointless* locus was previously mapped to an interval of 3.0 (+ / − 1.9) cM between the two RFLP markers TG523 and RPD158, 1.5 (+ / − 2.0) cM from each marker (Wing et al. 1994). Physical mapping of the *jointless* region showed that both markers hybridize to a common 600 kb *Sma*I restriction fragment (Wing et al. 1994), demonstrating that in the region of chromosome 11 containing the *jointless* locus, the maximum average ratio of kilobase pairs to centimorgans is 200 kb/CM. These results demonstrated the feasibility of initiating a chromosome walk to the *jointless* locus using the most closely linked marker(s) to screen a tomato YAC library. In this study, TG523 was used as a probe to screen 40,000 clones from an ordered tomato YAC library (Martin et al. 1992) by colony hybridization. Figure 1A shows a contour clamped homogeneous electric field (CHEF) gel used to resolve the YACs from the seven positive clones isolated in the primary screen. The approximate sizes of the seven YACs range from 220 to 380 kb. Figure 1B shows an autoradiograph of a Southern filter from the gel shown in Fig. 1A hybridized with TG523. Six of the seven YAC clones hybridized with TG523. The ratio of physical to genetic distances described above suggests the possibility that one or more of these clones may contain the *jointless* locus. However, one or two additional steps of chromosome walking may be required to reach

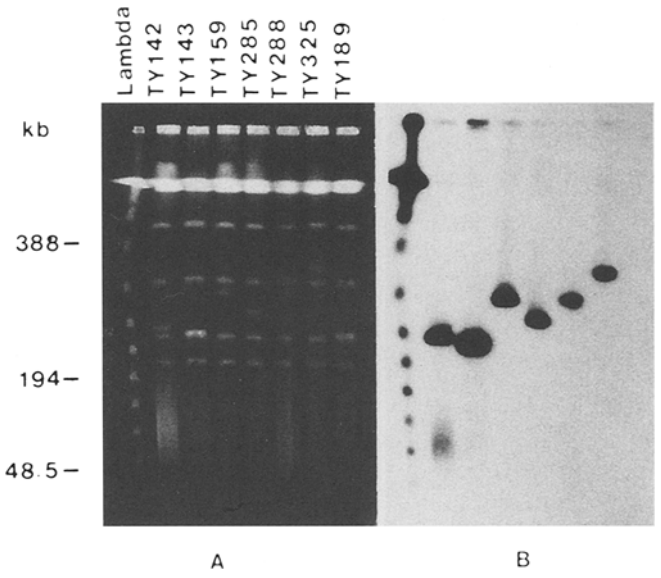

Fig. 1A, B Pulsed-field gel electrophoresis (PFGE) analysis of tomato yeast artificial chromosomes (YACs) isolated by colony hybridization of the YAC library with TG523. **A** The YACs on the ethidium bromide-stained PFGE gel. The bands common to all lanes are *Saccharomyces cerevisiae* chromosomes and the unique, or higher density bands, if a YAC comigrates with a yeast chromosome, in each lane indicate a YAC. **B** The YACs on the PFGE blot from the gel shown in **A** hybridized with TG523. Note that TY189 did not hybridize with TG523 and thus was not used for further analysis. The total high molecular weight (HMW) DNAs of the YACs were subjected to contour clamped homogeneous electric field electrophoresis (CHEF) in a 1% agarose gel, 0.5 × TBE (1 × is 89 mM TRIS-borate, 2 mM EDTA), using the MJ Research Programmable Power Inverter for 40 h at 150 V, 35 s pulse, and 11° C

the *jointless* locus. Tomato YAC (TY) 142, TY143, and TY159 contained insert DNAs from tomato cv. VFNT cherry (jointed homozygote) and TY285, TY288, and TY325 contained insert DNAs from tomato cv. Rio Grande-PtoR (*j-2* homozygote).

YAC end isolation

To determine the maximum distance covered by the first step of a chromosome walk toward the *jointless* locus and to isolate suitable markers for the next step of the chromosome walk, the ends of the six YACs were isolated and used for genetic mapping and YAC overlap analysis. Two methods were used to isolate the YAC ends: (1) IPCR (Silverman et al. 1989) and (2) plasmid rescue. IPCR is applicable to either end of a YAC, however plasmid rescue can only be used to isolate the left ends of YACs when pYAC4 (Burke et al. 1987) is used as the vector for YAC library construction.

To isolate the left ends of the six YACs by plasmid rescue, total YAC DNA was digested, self-ligated and transformed into *E. coli*. Positive clones were selected by resistance to ampicillin and digestion of the plasmid DNA with *Eco*RI (YAC cloning site) and *Xho*I (restriction site for plasmid rescue). Of the six YAC left ends, four, TY285L (L indicates left end), TY142L, TY159L, and TY143L, were cloned by this technique. For the remaining two left ends, ampicillin-resistant colonies were obtained but the DNAs of the clones could not be digested by the restriction enzymes *Eco*RI and *Xho*I, and thus were not analyzed further.

The IPCR technique was employed to isolate the remaining YAC ends, plus the four left ends isolated by plasmid rescue. Total YAC DNA was digested, self-ligated, and amplified by PCR. To eliminate any PCR amplification artifacts, all IPCR products were tested for their ability to be digested by *Eco*RI (the original insert cloning site) and the restriction enzyme used to generate the circular IPCR template. In total, 60 IPCR reactions were performed, however, only 41 (68%) produced visible amounts of products on agarose gels. While all 41 IPCR products showed single bands, only 24 (59%) could be cleaved with *Eco*RI and the restriction enzyme used to generate the circular IPCR template. All of the YAC ends were isolated by IPCR except for TY159R (R indicates right end) for which no IPCR product was digestible with *Eco*RI. A single IPCR product was selected according to the criteria described above for each of the 11 YAC ends and was cloned for genetic analysis.

To further verify the criteria used to select a IPCR product for YAC end cloning as described above, the hybridization patterns to tomato genomic DNA of the IPCR products from YAC ends TY285L, TY142L, TY159L, and TY143L were compared with those of the corresponding left ends isolated by plasmid rescue. In every case, both the IPCR product and the insert of its corresponding rescued plasmid produced identical hybridization patterns (data not shown).

Genetic mapping of the YAC ends

In order to detect RFLPs for genetic mapping of the YAC ends, total DNA was isolated from the parents of the F_2 population used for mapping *jointless* (Wing et al. 1994), digested with six different restriction enzymes, fractionated, blotted and hybridized with the YAC ends isolated above. Of the 11 cloned YAC ends, 8 hybridized as low or single-copy sequences and 3 hybridized as repetitive elements (see Fig. 2B). Seven of the remaining 8 single-copy YAC ends produced RFLPs between the parents and one, TY285R, did not. Only a single YAC, TY142, had single-copy sequences at both ends.

To map the seven single copy YAC ends that produced RFLPs, Southern blots, which were prepared from the DNA of 100 plants of the F_2 mapping population used to map *jointless* in our previous study (Wing et al. 1994), were hybridized with inserts of the YAC end

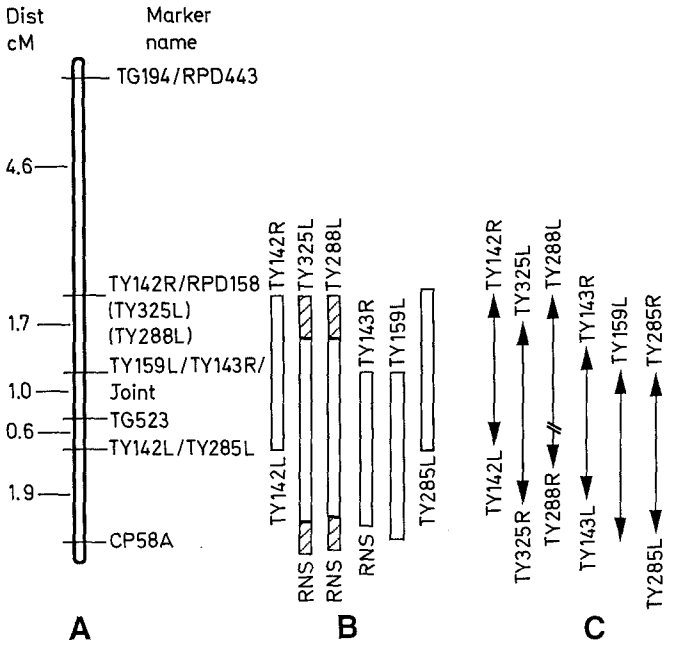

Fig. 2A–C The genetic map of the *jointless* region with YAC ends (**A**), the approximate positions of the YACs on the genetic map estimated from the genetic mapping of the YAC ends (**B**), and the relationship of the YACs determined by overlap analysis. The *hatched bars* at the ends of TY325 and TY288 in **B** indicate that TY325L and TY288L could be mapped between TY142R and TY159L but their exact genetic positions could not be determined. In **C**, the symbol "\\" indicates that clone TY288 is a chimeric YAC

clones. The resultant genetic map relative to the *jointless* locus is shown in Fig. 2A. The log likelihood of this genetic map is > 1000-fold higher than those of any other maps. TY325L and TY288L cosegregate and were mapped between TY142R and TY159L. The genetic distances of TY325L and TY288L relative to TY142R and TY159L could not be determined. TY159L and TY143R cosegregate with the *jointless* locus. TY142 was the only YAC both of whose ends could be mapped. TY142L cosegregates with TY285L and was mapped 1.6 cM from the *jointless* locus on one side and TY142R cosegregates with RPD158 and was mapped 1.7 cM from the *jointless* locus on the other side. This result suggests that TY142 contains the *jointless* locus.

Since TY142 is 285 kb long and both ends of it were mapped 3.3 cM apart, the correspondence of genetic distance to physical distance between the two ends of TY142 could be estimated to be 86 kb/cM. Since we previously demonstrated that the *jointless* locus mapped between RFLP markers TG523 and RPD158, which share a common 600 kb *Sma*I fragment (Wing et al. 1994), we predicted that the right end of TY142 should also reside on the same 600 kb *Sma*I fragment. Thus TY142R was hybridized to the same Southern filter used previously for physical mapping of the *jointless* locus (Wing et al. 1994). As predicted, TY142R hybridized to the same 600 kb *Sma*I fragment as did TG523 and RPD158 (data not shown).

Since all six YAC clones were isolated by hybridization to TG523, and at least one end was mapped for each YAC, we were able to estimate the positions of the YACs on the genetic map of the *jointless* region (Fig. 2B). All three YAC ends containing repeated elements TY143L, TY288R, and TY325R, reside in approximately the same region.

YAC overlap analysis

To confirm the relationship among the YACs as determined by genetic mapping of the YAC ends, total YAC DNAs were slot-blotted and hybridized with the inserts of all 11 cloned YAC ends plus RPD158. RPD158 did not hybridize to any YAC even though it cosegregates with TY142R as described above, indicating that it does not reside on TY142. Of the 11 YAC end probes, 10 gave the relationship of the YACs shown in Fig. 2C and thus agreed with the YAC end genetic mapping (Fig. 2B). One YAC end, TY288R, which contains repetitive elements, only hybridized to the DNA of TY288, which conflicts with the results shown in Fig. 2C. This result may imply that clone TY288 is a chimera.

Physical mapping of TY142

The above genetic and physical mapping results indicate that TY142 contains a contiguous 285 kb DNA fragment and is thus the best candidate YAC to contain the *jointless* locus. To confirm these results, TY142 was physically mapped. First, a two-enzyme restriction site map was constructed for TY142. HMW DNA of TY142 was partially and completely digested with *Sma*I and *Sal*I, respectively, fractionated, blotted, and hybridized with TY142L and TY142R, respectively. Identical restriction site maps for TY142 were obtained when TY142L and TY142R were used as probes, separately. Figure 3 shows the restriction site map of TY142 using TY142R as a probe.

The genetic mapping of the YAC ends showed that all seven single-copy YAC ends, TG523, and RPD158 seem to be localized within TY142 (Fig. 2A) and overlap analysis of the YACs showed that six of the seven YAC ends and TG523 hybridized to TY142 (Fig. 2C). To determine whether the genetic positions of the YAC ends surrounding the *jointless* locus on TY142 corresponded to their actual physical positions, these markers were hybridized to the PFGE blot of TY142 used for the restriction site mapping. The results are shown in Fig. 3. All the markers tested were also physically mapped to TY142 except for TY285L and RPD158, neither of which hybridized to the DNA of TY142. This result agreed with that of YAC overlap

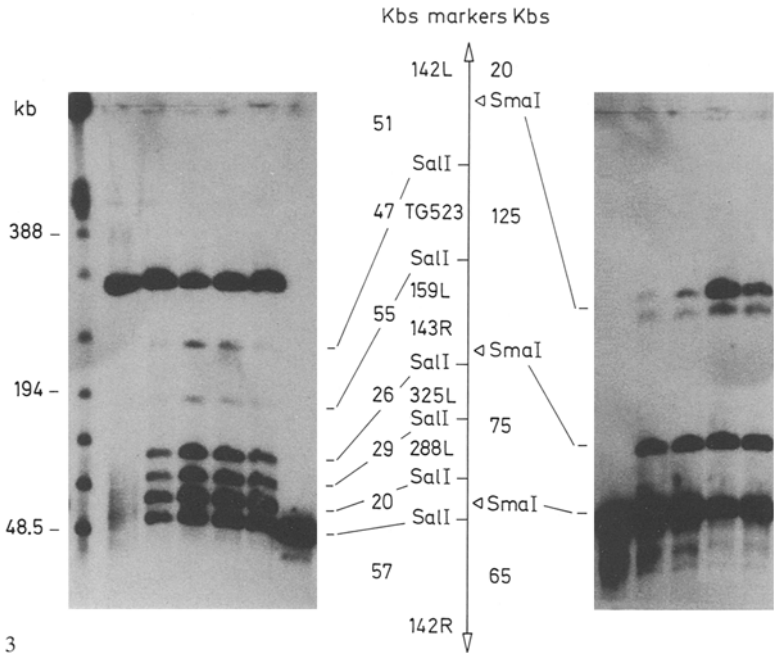

Fig. 3 Restriction site map of TY142 which includes the *jointless* locus. The autoradiographs, showing *Sal*I (left) and *Sma*I (right) digests hybridized to TY142R, were from a single gel blot cut along the lambda ladder (left most lane of the *Sal*I digested DNA blot). The second lane of the *Sal*I digest from left is uncut TY142. The DNA of TY142 was subjected to CHEF electrophoresis in a 1% agarose gel, 0.5 × TBE, using the MJ Research Programmable Power Inverter at 120 V, 30 s pulse, and 8° C for 58 h. 142L is TY142L, 142R is TY142R, and so on

analysis (Fig. 2C). TY325L and TY288L both hybridized to the 75 kb *Sma*I fragment but TY325L hybridized to the 29 kb *Sal*I fragment and TY288L hybridized to the 26 kb *Sal*I fragment in the complete digests of TY142 HMW DNA. Therefore, TY325L and TY288L were physically mapped to two different *Sal*I fragments of TY142. In the complete digests of TY142, TY159L and TY143R both hybridized to the 55 kb *Sal*I fragment, which was located in the middle of TY142. TG523 hybridized to the 47 kb *Sal*I fragment located next to the 55 kb *Sal*I fragment. Both of these *Sal*I fragments share a 125 kb *Sma*I fragment. In addition, TG523 gave fewer bands than TY159L or TY143R when they hybridized to the partially *Sal*I digested DNA of TY142. These results confirmed that the genetic mapping data for the YAC ends correspond with the physical mapping of TY142.

Approximate location of the *jointless* locus on TY142

Since *jointless* arose as a spontaneous mutation in a field of cultivated tomatoes (Butler 1936), we would not expect to detect a difference between *jointless* and wild (*JOINTED*) type plants at a gross DNA level unless the mutation resulted from an insertion, deletion, or rearrangement. Genetic analysis reveals that two YAC ends, TY159L and TY143R, cosegregate with the *jointless* phenotype and the physical mapping of TY142 shows that they reside on a 55 kb *Sal*I fragment. To obtain a clue as to where the *jointless* locus resides on TY142, DNA was isolated from two pairs of independently developed NILs for the *jointless* locus, LA3021 (*J*/*J*) vs NIL LA3023 (*j*/*j*) and Heinz 1706 (*J*/*J*) vs NIL Heinz 1706 (*j*/*j*) (Philouze 1991). DNA was digested with *Alu*I, *Bst*NI, *Dra*I, *Eco*RI, *Hae*III, and *Hind*III, electrophoresed, blotted, and hybridized with TY159L, TY143R, TY288L, TY325L, TG523, TY142L, and TY142R, respectively. Of all the markers tested along the length of TY142, only a single marker – TY143R – was shown to be polymorphic between the two NILs of each pair and the polymorphisms were identical between these two pairs of NILs. Of the six restriction enzymes tested with TY143R, three, *Bst*NI, *Hae*III, and *Hind*III, were shown to be polymorphic. In each instance, the hybridized band in the *J*/*J* lines was always 1.1 kb larger than that in the *j*/*j* lines. Figure 4 shows the RFLP pattern for the DNAs of the NILs digested with *Bst*NI and hybridized with the above markers. The approximate position of the *jointless* locus on TY142 determined from the above result is shown in Fig. 4 (solid black bar). Genetically, TY143R cosegregates with TY159L and the *jointless* locus and is 1.0 cM from TG523, corresponding to 86 kb (86 kb/cM in the *jointless* region). Physically, TY143R shares a 55 kb *Sal*I fragment with TY159L and TY143R. Furthermore TY159L share a (55 + 47) kb fragment with TG523 and a (55 + 26) kb fragment with TY325L. This implies that the "introgressed" fragment carrying the *jointless* locus is contained within an approximately 50 kb fragment (solid black bar in Fig. 4) of TY142.

Discussion

TY142 contains the *jointless* locus

Genetic mapping of the YAC ends revealed that the set of overlapping YACs isolated encompasses the *jointless*

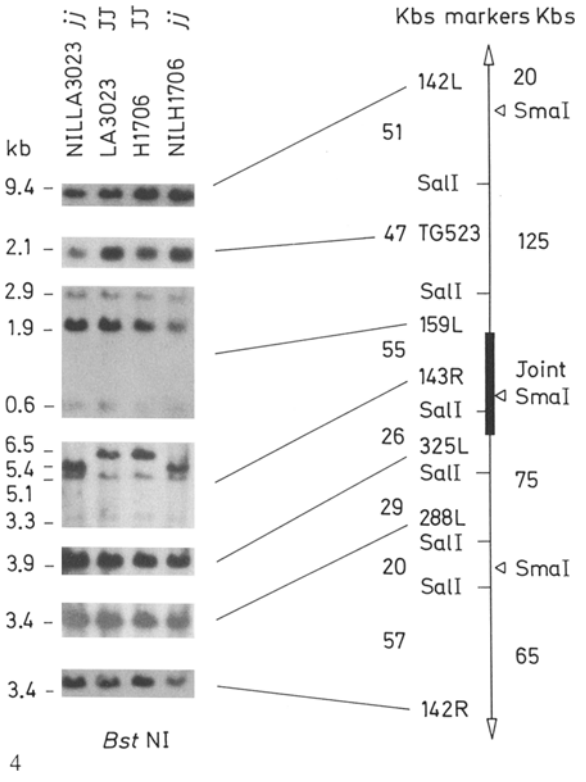

Fig. 4 *Bst*NI restriction fragments of the DNAs from the two pairs of NILS for *jointless* that hybridize with TY142L, TG523, TY159L, TY143R, TY325L, TY288L, and TY142R, respectively. Note that TY143R is the only probe that gave restriction fragment length polymorphisms (RFLPs) between the *j/j* lines and the *J/J* lines. The *solid black bar* indicates the position of "introgression" of alien chromatin carrying the *jointless* locus

locus. In addition, TY142 appears to contain a contiguous piece of tomato DNA, the ends of which reside on either side of the *jointless* locus. Thus it appears that TY142 contains the *jointless* locus. To confirm the genetic mapping of the YAC ends, the YAC ends were physically mapped within TY142. In every case, the physical placement of the YAC ends corresponded with their previously determined genetic positions. Furthermore, the positions of TY288L and TY325L, which were genetically mapped somewhere between TY142R and TY143R/TY159L, could be ordered by physical mapping. The physical mapping of TY142 supports the contention that the *jointless* locus lies within TY142 as determined genetically.

The physical mapping of TY142 showed that TY142 contains three *Sma*I and six *Sal*I sites. This appears to disagree with our earlier (Wing et al. 1993b) and present work demonstrating that TG523, TY142R, and RPD158 hydridize to a common 600 kb *Sma*I fragment and several uncommon *Sal*I fragments. Since tomato cv VFNT Cherry was used as the DNA source for both TY142 construction (Martin et al. 1992) and physical mapping of the *jointless* region (Wing et al. 1994), the presence of additional *Sma*I sites within TY142 cannot be explained by a difference in DNA source. A probable explanation is that *Sma*I is methylation sensitive and some of the *Sma*I sites might be methylated in tomato but not in S. *cerevisiae*.

Genetic mapping is a rough measure of a genome because the correspondence between genetic distance and physical distance varies greatly in different genome regions (Ganal et al. 1989; Wing et al. 1994). Therefore, physical mapping is often necessary for establishing the feasibility of using chromosome walking strategies for gene cloning and identification. The genetic mapping of the YAC ends, TG523, and RPD158 in the present study showed that TY285L with TY142L, RPD158 with TY142R, TY325L with TY288L, and TY159L with TY143R cosegregate (Fig. 2A). The physical mapping of the markers relative to TY142 indicates that TY285L and RPD158 are not in TY142, and TY325L and TY288L hybridize to different *Sal*I fragments (Fig. 3). TY159L and TY143R both reside on a 55 kb *Sal*I fragment of TY142 and also cosegregate with the *jointless* locus. This result is compatible with the genetic localization of the *jointless* locus.

The *jointless* locus is located in an approximately 50 kb fragment of TY142

The two ends of TY142 map on either side of the *jointless* locus, TY159L and TY143R cosegregate with the *jointless* locus, and TY325L, TY288L, and TG523 are closely linked to the jointless locus (see Figs. 2A, 3). When these markers were used as probes for hybridization to DNAs of two pairs of independent NILs for *jointless*, TY143R was the only marker found to be polymorphic between the two NILs of each pair, and the TY143R restriction patterns for three of the six restriction enzymes tested were also polymorphic. In each instance, the polymorphisms were always identical between the two pairs of independent NILs: the band in *J/J* lines is 1.1 kb larger than that in *j/j* lines (Fig. 4). The two pairs of NILs for *jointless* used in this study are phenotypically quite different and were developed by backcrossing to a recurrent parent in France (Heinz 1706 and NIL Heinz 1706; Philouze 1991) and in the USA (LA3021 and NIL LA3023) independently. The above result most probably indicates the location of "introgression" from the *jointless* donor parent to the recurrent parent.

Further investigations are required to determine the mechanism of mutation from the *jointed* to the *jointless* tomato. Deletion, insertion, rearrangement, or point mutation (base change) are all possible candidates. Southern analysis of the DNA of the NILs for the *jointless* locus suggests that the *jointless* mutation may be the result of DNA deletion from wild-type plants because three of the six restriction enzymes tested showed a polymorphism between *j/j* and *J/J* lines and in each instance, the TY143R-hybridized bands were always 1.1 kb larger in *J/J* lines than in *j/j* lines.

YAC end mapping and YAC overlap analysis both provide guidance for chromosome walking

The results from genetic mapping of the YAC ends agreed with those of overlap analysis for chromosome walking. This indicates that both YAC end mapping and overlap analysis are effective for guiding chromosome walking. Genetic mapping of YAC ends by the RFLP technique for chromosome walking has a major advantage over the overlap analysis, i.e., the orientation and position of a chromosome walk relative to the target gene can be immediately determined. It is not affected by chimeric YACs, which can easily lead chromosome walking in the wrong direction when YAC overlap analysis is used for guiding chromosome walking. However, the effectiveness of the method is often limited by availability of a mapping population and the level of polymorphism between the parents of the population. The latter limitation of genetic mapping is revealed by the failure to map TY285L in this study. It was also fortunate that most of the YAC ends in this study are single copy sequences because it is difficult to map repeated elements by standard RFLP analysis.

The overlap analysis for chromosome walking does not require a mapping population and polymorphism between its parents, but it is often affected by chimeric YACs, which have been discovered in many YAC libraries (for example, see Martin et al. 1992). In the present study, TY288R only hybridized to the DNA of TY288 among the six YACs from the *jointless* region. However, TY288R should also hybridize to TY325, TY285, TY143, and TY159 according to the results of cross-hybridization of the remaining YAC ends (Fig. 2C). The absence of hybridization of TY288R with the DNAs of the four YACs may indicate that TY288 is a chimeric YAC.

Gene identification – the final step

Genetic and physical mapping experiments indicate that the *jointless* locus is contained in tomato YAC 142. The next step in our map-based cloning strategy is to demonstrate biologically that we have indeed cloned the *jointless* locus. Experiments are in progress to genetically complement the *jointless* mutation by transformation with subclones of TY142 (containing the wild-type DNA sequence of the *jointless* locus). In addition, TY142, TY159L, and TY143R are being used as probes to screen cDNA libraries constructed from developing floral meristems. The isolation of cDNA clones using YAC DNA as probes has recently been used successfully to clone the Omega-3 FAD gene from *Arabidopsis* (Arondel et al. 1992) and the *Pto* disease resistance gene from tomato (Martin et al. 1993).

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