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ORIGINAL PAPER

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Telomere-homologous sequences occur near the centromeres of many tomato chromosomes

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Abstract Several bacteriophage lambda clones containing *i*nterstitial *t*elomere *r*epeats (ITR) were isolated from a library of tomato genomic DNA by plaque hybridization with the cloned *Arabidopsis thaliana* telomere repeat. Restriction fragments lacking highly repetitive DNA were identified and used as probes to map 14 of the 20 lambda clones. All of these markers mapped near the centromere on eight of the twelve tomato chromosomes. The exact centromere location of chromosomes 7 and 9 has recently been determined, and all ITR clones that localize to these two chromosomes map to the marker clusters known to contain the centromere. High-resolution mapping of one of these markers showed cosegregation of the telomere repeat with the marker cluster closest to the centromere in over 9000 meiotic products. We propose that the map location of interstitial telomere clones may reflect specific sequence interchanges between telomeric and centromeric regions and may provide an expedient means of localizing centromere positions.

Key words High-resolution mapping · Karyotype evolution · Restriction fragment length polymorphism (RFLP) · *Zycopersicon*

Introduction

Telomere repeats are simple sequences that occur predominantly at the ends of eukaryotic chromosomes, where they are arranged in tandem to form large uninterrupted blocks. They are believed to play an important role in protecting the chromosome ends from degradation and are essential for the telomerase-mediated replication of chromosome ends (Biessmann and Mason 1994). The molecular organization of tomato telomeres has been investigated by Ganal et al. (1991). All chromosome ends carry terminal telomere repeats. A block of the 162-bp subtelomeric repeat (TGRI) is localized within a few hundred kb of the terminal telomere repeats on 20 of the 24 chromosome ends. The telomere repeat sequence of tomato has been cloned and sequenced (Ganal et al. 1991). Its sequence [TTT (T/A)GGG] is nearly identical to the *Arabidopsis* consensus sequence [TTTAGGG]. In situ hybridization experiments with tomato chromosomes showed that most of the telomere repeats are located at the chromosome ends, but at least one interstitial telomere site is predicted from in situ results and from the presence of a Bal31-resistant telomere-homologous sequence (Ganal et al. 1991).

Interstitial telomere repeat sequences have been localized by in situ and genetic analysis near the centromeres of several higher eukaryotes (Meyne et al. 1990; Richards et al. 1991; Alfenito and Birchler 1993), where they are thought to have been relocated as a result of Robertsonian fusion or chromosome arm inversion events during karyotype development. We have investigated this phenomenon in tomato and made use of the results to generate centromere-proximal molecular markers in tomato, which should be useful for the mapping and characterization of tomato centromeres.

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Materials and methods

Lambda library screen

A lambda library that had been constructed from partially digested tomato genomic DNA (Sugita et al. 1987) was screened with the

cloned *Arabidopsis* telomere repeat sequence pAtT4 (Richards and Ausubel 1988). Plating of phage and transfer of the plaques to HybondN$^+$ membrane were as described (Sambrook et al. 1989). Plasmid pAtT4 was radiolabeled by random priming. Blots were hybridized at 55° C and washed in 2×SSC, 1% SDS at 55° C. Positive lambda plaques were isolated and purified to homogeneity in secondary and tertiary screens. DNA was isolated from these purified plaques as described (Sambrook et al. 1989).

Mapping of lambda clones

The tomato mapping population consisting of 62 F_2 plants from a cross between *L. esculentum* and *L. pennellii* (Tanksley et al. 1992) was used to determine map locations of the telomere lambda clones. Single-copy or low-copy-number restriction fragments of 15 telomere-homologous lambda clones were identified by sequentially probing Southern blots of digested lambda DNA with the radiolabeled telomere probe and radiolabeled total tomato genomic DNA at 65° C. Insert-derived restriction fragments which did not give a signal with either probe were extracted from agarose gels and used as probes on mapping filters. ITR-1a (chromosome 9) was mapped at high resolution in four additional populations representing over 9000 meiotic products.

Sequence analysis

Lambda DNA fragments were subcloned into pBluescript vector (Stratagene) and sequenced with an ABD 373A DNA Sequencer at the Analytical and Synthesis Facility of Cornell University using the M13FWD and M13REV primers.

Trisomic dosage analysis

The chromosomal arm location of ITR-1a (chromosome 9) was determined by assessing the signal strength of ITR-1a bands relative to control markers of known arm location in complementary trisomic lines (2N + 9S·9S and 2N + 9L·12L) (Frary et al. 1996). Southern blots prepared with *DraI*-digested genomic DNA from trisomic lines and diploid control plants from the same seedlot were sequentially probed with ITR-1a and the control markers. The hybridization signal was recorded on Phosphor Imager screens (Molecular Dynamics Sunnyvale, Calif.) and quantified using the volume integration procedure of ImageQuant.

Results and discussion

Isolation and mapping of lambda clones with telomere homology

Lambda plaques representing an estimated 2.7 genome equivalents were screened with the telomere repeat sequence of *A. thaliana* (Richards and Ausubel 1988), and twenty clones that hybridized to the repeat were purified to homogeneity. Low-copy-number restriction fragments of fourteen lambda clones could be mapped onto the existing high-density tomato RFLP map. Six of the probes (43%) hybridized to DNA from the *L. esculentum* parent but not the *L. pennellii* parent and thus had to be mapped as dominant markers. This is unusual, since other single-copy probes characterized thus far in tomato hybridize to DNA from both parents (our unpublished results). The absence of homologous sequences in *L. pennellii* for so many ITR-proximal sequences may result from rapid evolution of centromere-proximal sequences.

Figure 1 shows the map locations of the 14 telomere-homologous sequences that could be mapped with adjacent single-copy or low-copy-number sequences. None of the fourteen clones mapped to a chromosome end. This was expected, as the library, which was constructed from partially digested genomic DNA (Sugita et al. 1987), is unlikely to contain portions of the large uninterrupted blocks of telomere repeats located at the chromosome ends. Eight of the lambda clones mapped to three clusters containing four, two and two clones each. Restriction analysis of the co-localizing clones showed that most share common restriction fragments but are not identical, indicating that they may be overlapping clones.

All ITR clones map near the centromere of the eight different chromosomes (3, 4, 5, 7, 8, 9, 11 and 12) on which they were localized. The centromeres of two chromosomes (7 and 9) have been placed very precisely on the molecular map (Frary et al. 1996), and the ITR clones located on these chromosomes map exactly to the marker clusters (TG166 and CD3, respectively) known to contain the centromere. The centromere of chromosome 3 has been located on the integrated molecular-classical map in the interval between the markers TG66 and TG246 (Koornneef et al. 1993; Van der Biezen et al. 1994), the same interval to which ITR-14a maps. The centromeres of chromosomes 5, 9, 11 and 12 presumably lie just below the breakpoints of the paracentric short arm inversions that differentiate potato and tomato (Tanksley et al. 1992). The interstitial telomere repeats found on those chromosomes map very close to each inversion point. Lastly, morphological markers flanking the centromeres of chromosomes 4, 8 and 11 have been placed on the molecular map, thus defining an interval on the molecular map that contains the centromere. Again, the ITR clones from these chromosomes all map within these intervals.

Eleven of the fourteen interstitial telomere sequences were localized to five of the six shortest chromosomes (8, 5, 9, 11, 12). This group also contains the four chromosomes whose short (5, 9, 11, 12) arm is inverted relative to potato (Tanksley et al. 1992).

High-resolution mapping of ITR-1a

Due to the supression of genetic recombination near tomato centromeres, many markers map near the centromere in our mapping population (Tanksley et al. 1992). In an attempt to break the linkage between clone ITR-1a and the centromere of chromosome 9, we examined several large populations representing a total

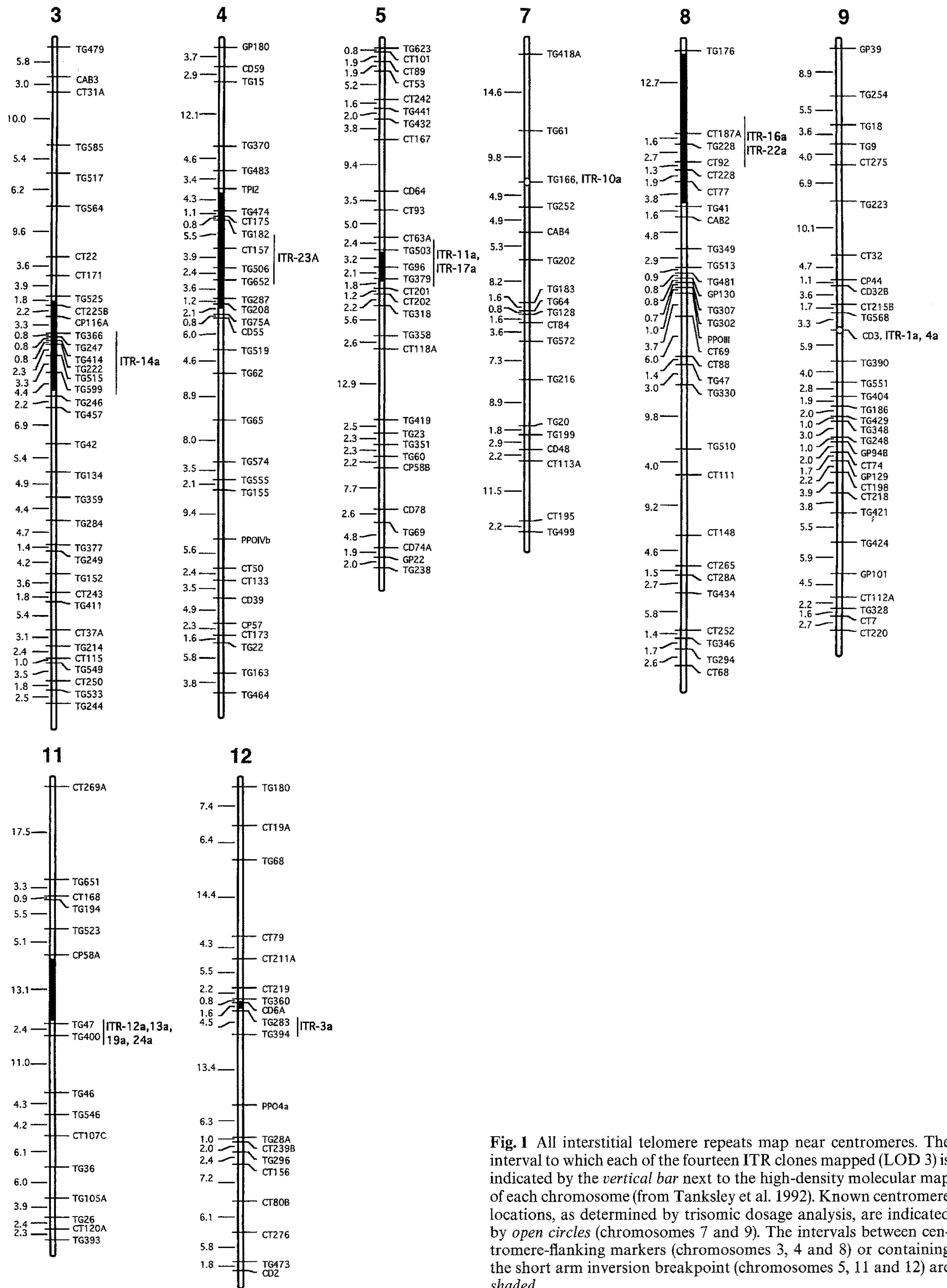

Fig. 1 All interstitial telomere repeats map near centromeres. The interval to which each of the fourteen ITR clones mapped (LOD 3) is indicated by the *vertical bar* next to the high-density molecular map of each chromosome (from Tanksley et al. 1992). Known centromere locations, as determined by trisomic dosage analysis, are indicated by *open circles* (chromosomes 7 and 9). The intervals between centromere-flanking markers (chromosomes 3, 4 and 8) or containing the short arm inversion breakpoint (chromosomes 5, 11 and 12) are *shaded*

of over 9000 meiotic products. These populations consisted of: 269 F_2 plants from a *L. esculentum* × *L. cheesmannii* cross (Yen et al. 1995), ca. 1600 F_2 plants from a *L. esculentum* × *L. pennellii* cross, another 1600 F_2 plants from a *L. esculentum* × *L. pimpinellifolium*-cross (Frary et al. 1996), and a backcross population of 2112 individuals from an intraspecific *L. peruvianum* cross (K. Pillen et al., in preparation). The *L. esculentum* × *L. cheesmannii* population was chosen because it had been reported to show a higher recombination frequency in the centromere 9 region than our standard mapping population.

The map with the highest resolution was that constructed from the large *L. peruvianum* backcross (Fig. 2 and Pillen et al., in preparation). On this map the centromere-containing marker cluster (Frary et al. 1996) is resolved into a number of individual markers and smaller clusters, and the centromere has been localized to a 0.05-cM interval between R12 and the TG207 marker cluster. The linkage of ITR-1a and the TG207 cluster remained unbroken in all of the individuals examined, indicating that ITR-1a is one of the most centromere-proximal markers. The TG207 cluster is separated by one recombinant from R-12 (short arm) and by another recombinant from TG 291 (long arm). Only one additional recombinant between R12 and TG291 was isolated in the other populations. It occurred in the *L. esculentum* × *L. cheesmannii* population and lies between R12 and the TG207 cluster.

Sequence analysis

In order to confirm that the lambda clones that had been isolated based on hybridization with the

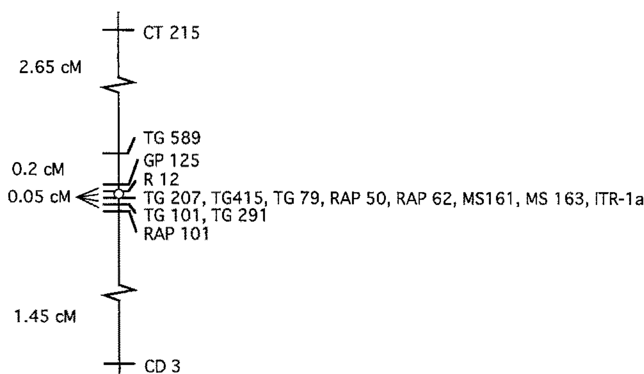

Fig. 2 ITR-1a co-segregates with the centromere of chromosome 9 in over 9000 meiotic products. This high-resolution map of the centromere region of chromosome 9 was constructed using a backcross population (2000 plants) from an intraspecific *L. peruvianum* cross (Pillen et al., in preparation). ITR-1a was mapped in four different populations representing a total of over 9000 meiotic products. No individual was found in which the linkage to the centromere-flanking long arm marker cluster, which was reduced to eight markers, was broken. The location of the centromere is indicated by a *circle*

Arabidopsis telomere repeat actually contain telomere repeat sequences, *Sau*3AI restriction fragments with homology to the *Arabidopsis* telomere sequence were identified by Southern hybridization, subcloned and sequenced. Analysis of a DNA fragment of ITR-24 (ITR-24b) revealed recognizable intact and degenerate telomere repeats arranged in tandem (Fig 3). Another sequence (ITR-1b) contains thirteen interspersed intact telomere repeats arranged head-to-head (data not shown).

ITR-1a lies on the long arm of chromosome 9

To test whether the interstitial telomere repeat sequences on chromosome 9 were placed near the centromere as a result of the inversion of the short arm that differentiates tomato from potato, the arm location of ITR-1a was determined by measuring its dose in two complementary trisomics for chromosome 9, 2N + 9s·9s and 2N + 9L·12L (Table 1). ITR-1a dosage increases in 2N + 9L·12L plants but not in 2N + 9S·9S plants, indicating that this marker is located on the long arm or in the centromere of chromosome 9. This suggests that ITR-1a was not relocated near the centromere of chromosome 9 as a direct result of the arm inversion that differentiates tomato and potato, but may instead be a relic of an earlier event in karyotype evolution.

Interstitial telomere repeats as centromere markers

To date, the centromeres of only chromosomes 7 and 9 have been localized precisely on the molecular map of tomato (Frary et al. 1996). Based on the results presented above, we conclude that the interstitial telomere repeats are preferentially located near centromeres. In

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  1  TGCATCTCTT TTTTTTCTTG CTTGGTTTGT TTTTGCTCTT GGGGGTTAGG
 51  GTTAGGGTTC GTGTTAGAGA TATGGTTAAG GCTAGGGTTT AGGGTTCTTT
101  TTAGGTTTTG GTTTTGGTGT TTAGAATTTT TGGTTTGAAA TCTATAGTTT
151  GGGTTTATTG CTTGGGTTTT AGTATTTTTG GCTTGGGGTT TAGGTTTTAG
201  GGTTAGGGTT AGGGTTAGGG TTTAGGTTAG GGTTTGGATT AGGGTTAGGG
251  TTTAGTTTTT TTAGGTTTTA TGGTTTGTGT TAAGGATTTG AGGTTTAAAA
301  TCTATGGTCT AGTGATAAAT GTCTTGGTTT TCGTATTTTT TACTACTTAG
351  GGTTTAGGGT TTAGGTTTAG GGTTAGGGTT TAGGGTTTTT GAGGTTTCTA
401  AGTTTAGGTT TTAGTATTTT AGCTTTAACA TCAGTGGTTA AATGTTAAAT
451  GTTTGGGTTT TGGTATTTTG GGCTTCGAGT TTAGGGTTTA TGGTAGGGTT
501  AGGGTGTAGG TTGTTTTTTA GGTTTTAATG TTTGGGGTGA TGGATTTGAG
551  TTGTAATATC TACGTTTTAT TGTTTGAACT TAAGGGTTTT GGTATTTTGG
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Fig. 3 Nucleotide sequence of ITR-24b.Telomere repeats (TTTAGGG, TTAAGGG and TTAGGG) are *underlined*

Table 1 Trisomic dosage analysis localizes ITR-1A to the long arm of chromosome 9.

Genetic stock	Signal intensity relative to: 9S Marker CT18			9L Marker CT220		
	Expected ratio if probe lies on long arm	Observed		Expected ratio if probe lies on short arm	Observed	
		CT220 (long arm)	ITR-1a (unknown)		CT215 A (short arm)	ITR-1a (unknown)
Diploid	1.0	1.00	1.05	1.0	1.06	1.04
Diploid	1.0	1.00	0.96	1.0	0.94	0.96
2N + 9L12L	1.5	1.33	1.36	0.67	0.74	1.01
2N + 9L12L	1.5	1.35	1.41	0.67	0.65	1.03
Diploid	1.0	1.02	0.95	1.0	1.0	0.9
Diploid	1.0	0.98	1.05	1.0	1.0	1.1
2N + 9S9S	0.5	0.51	0.62	2.0	1.8	1.2
2N + 9S9S	0.5	0.47	0.57	2.0	1.7	1.2

*Dra*I-digested genomic DNA isolated from diploid and trisomic seedlings from the same trisomic seed batch was electrophoresed on an agarose gel and blotted onto HybondN$^+$. The resulting blot was probed with radiolabeled RFLP markers and exposed overnight to a Phosphor Imager screen. The signal intensity of each band was quantitated using the volume integration program of ImageQuant. Dosage of ITR-1a relative to the diploid marker increases in $2N + 9L \cdot 12L$ plants, but not in $2N + 9S \cdot 9S$ plants, indicating that this marker lies on the long arm of chromosome 9

addition, we have shown by high-resolution mapping that the clone ITR-1a lies in the marker cluster immediately adjacent to the centromere of chromosome 9. This indicates that these new markers may be useful in more precisely determining the map positions of additional tomato centromeres without having to perform the difficult and laborious marker dosage analysis in trisomics.

Interstitial telomere repeats as records of karyotype development

The presence of telomere repeat sequences near so many of the tomato centromeres raises the question of how they were placed there and what, if any, function they perform. Among the plausible mechanisms leading to the placement of telomere sequences near centromeres are illegitimate centromere-telomere recombination and ancient chromosome fusions or arm inversions. Examples of the latter have been documented in other species. Two inverted blocks of telomere repeats located on the long arm of human chromosome 2 mark the site of an ancient telomere-to-telomere fusion that led to the reduction of chromosome number from 24 to 23 between the great apes and humans (Ijdo et al. 1991). Similarly, the Indian muntjac ($2n = 6$ or 7) has a dramatically reduced chromosome number compared to the closely related Chinese muntjac ($2n = 46$) and shows many interstitial hybridization sites on fluorescence in situ hybridization using the human telomere repeat (Lee et al. 1993). The tomato karyotype differs from that of potato by five arm inversions (Tanksley et al. 1992), and from the more distantly related pepper genome by at least 15 smaller inversions and translocations (Tanksley et al. 1988; Prince et al. 1993). Thus the tomato genome has undergone many rearrangements to reach its current configuration, providing multiple opportunities for ITRs to localize near centromeres. The localization of interstitial telomere repeats may allow the reconstruction of tomato karyotype evolution, and the relative abundance of ITRs on the shorter chromosomes may be significant.

The strategy pursued here, namely isolation of ITR clones from a lambda library and mapping of a low-copy-number restriction fragment of the lambda clone, required that the repeats were clonable, and may thus have selected for degenerate repeats and very short blocks of telomere repeats. These repeats may only represent a subset, presumably the most ancient, of all interstitial telomere repeats. Fluorescence in situ hybridization may be useful for the identification and mapping of additional ITRs that evolved more recently, since it is well suited for detecting long stretches of uninterrupted repeats. One interstitial telomere repeat has already been detected by in situ hybridization on an unidentified tomato chromosome (Ganal et al. 1991).

A complementary record of tomato karyotype development may be provided by the map locations of interstitial copies of the subtelomeric repeat TGRI. TGRI is a rapidly evolving repeat that has been found only in *Lycopersicon* species and in the closely related *Solanum lycopersicoides* (Ganal et al. 1988). A related repeat (74% similarity) was recently identified in *S. brevidens*, but neither repeat has been detected in other *Solanum* species (Ganal et al. 1988; Preiszner et al. 1994) In situ hybridization with TGRI to tomato chromosomes has revealed at least seven interstitial sites, including two at the centromeres of chromosomes 2 and

3 (Lapitan et al. 1989). We detected no TGRI repeats on a 300-kb YAC containing the inverted telomere repeat sequence of ITR-1b.

Are centromere-proximal telomere repeats part of functional centromeres?

Although the placement of telomere repeats near the centromere may have been fortuitous, their association with the centromere may have acquired functional significance. A maize B chromosome-specific repeat element that was mapped to the centromere by deletion analysis and in situ hybridization contains long arrays of degenerate telomere repeats that flank a single copy of the maize knob sequence (Alfenito and Birchler 1993). The knob sequence occurs primarily in heterochromatic knobs on maize chromosomes, which can act as neocentromeres in the presence of the abnormal chromosome 10 (Peacock et al. 1981). In light of this information it is noteworthy that a DNA sequence flanking ITR-1a shows a stretch of limited homology (17/19 bp) with the K″ element of *Schizosaccharomyces pombe*, which is one of two essential components of the functional *S. pombe* centromere (Baum et al. 1994). The fact that ITR-1a is not present on the 9S · 9S chromosome of trisomic tomato and therefore not required for the function of centromere 9 does not diminish the significance of the homology, since the 9S · 9S chromosome does not necessarily contain the entire chromosome 9 centromere. Immunostaining of stretched animal and plant chromosomes with centromere-specific antibodies indicates that the centromeres of higher eukaryotes apparently consist of repeated subunits (Zinkowski et al. 1991), presumably allowing portions of the centromere to be deleted without loss of function.

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