Single-site manipulation of tomato chromosomes in vitro and in vivo using Cre-lox site-specific recombination

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Abstract

With the aim of developing new techniques for physical and functional genome analysis, we have introduced the Cre-lox site-specific recombination system into the cultivated tomato (Lycopersicon esculentum). Local transposition of a Ds(lox) transposable element from a T-DNA(lox) on the long arm of chromosome 6 was used to position pairs of lox sites on different closely linked loci. In vitro Cre-lox recombination between chromosomal lox sites and synthetic lox oligonucleotides cleaved the 750 Mb tomato genome with 34 bp specificity to release unique 65 kb and 130 kb fragments of chromosome 6. Parallel in vitro experiments on Saccharomyces cerevisiae chromosomes show the efficiency of cleavage to be 50% per chromosomal lox site at maximum. By expressing the Cre recombinase in tomato under control of a constitutive CaMV 35S promoter, efficient and specific somatic and germinal in planta inversion of the 130 kb fragment is demonstrated. The combined use of in vitro and in vivo recombination on genetically mapped lox sites will provide new possibilities for long range restriction mapping and in vivo manipulation of selected tomato genome segments.

Introduction

Techniques for high-resolution molecular linkage mapping and pulsed-field gel electrophoresis (PFGE) of megabase DNA have opened the way to integration of genetic linkage maps and DNA restriction maps of large eukaryotic genomes. However, the short average size of fragments generated by conventional restriction enzymes usually confines the span of integrated maps to small genome segments, and leaves the upper range of PFGE resolution power (6–9 Mb) largely unexploited. In addition, genetic functions encoded within genomic restriction fragments generally remain unknown until mutagenized.

It would be useful to devise a method that can cleave a genome only at selected pairs of genetic markers in vitro, and that can be used to specifically remove, invert or duplicate the selected genetic interval in a living cell. Physical information about large genetic distances could be obtained from the size of a single mega-restriction fragment, without the need for contig building. Precise in vivo deletion of a specific fragment could map existing gene markers in segmental haploids, while new genetic functions may be uncovered in homozygous deficiency or duplication stocks.

The required technology for such a method can be provided by site-specific recombination systems like Cre-lox, FLP/FRT and R/RS. These systems consist of a specific target DNA sequence (lox (34 bp), FRT (34 bp), RS (31 bp)), and a recombinase (Cre, FLP, R) that is necessary and sufficient to induce crossovers between two target sites (reviewed in [22]). High specificity and a simple two-element design make this class of recombination systems attractive for use in heterologous hosts. Site-specific chromosomal deletions, inversions, duplications and translocations have been induced efficiently with all three systems in a variety of different higher eukaryotes, including plants and mammals (reviewed in [17, 27, 33]). In addition, 34 bp specific cleavage of the 15 Mb Schizosaccharomyces pombe genome by intermolecular in vitro recombin-
tion between artificial chromosomal lox sites and lox oligonucleotides has been demonstrated [28].

We are interested in applying the in vitro and in vivo systems of Cre-lox site-specific recombination for long-range restriction mapping and genome manipulation in the cultivated tomato (Lycopersicon esculentum). Although genetic mapping of this important model and crop species has been intense [37], current techniques for genomic restriction mapping are unlikely to bridge distantly located markers on the genetic map of its relatively large (750 Mb) genome. Functional analysis of the tomato genome has long been restricted to mutagenesis by chemical and physical means, and has more recently been supplemented with heterologous transposons (Ac/Ds) from maize [16, 41]. Introduction and genetic mapping of lox sites in the tomato genome will provide new possibilities to select specific genetic intervals for PFGE restriction mapping using the 34 bp cleavage specificity of the in vitro Cre-lox recombination system and for functional analysis by targeted recombination in vivo.

In this report, the operation of the in vitro and in vivo components of the Cre-lox system in the tomato genome is demonstrated. We have used the natural tendency of short-range transpositions of maize Ds elements to efficiently place lox sites on different closely linked loci on the genetically well mapped tomato chromosome 6 [40]. We demonstrate the in vitro release of unique segments of this chromosome and show that these fragments can be specifically rearranged in planta in a predictable manner.

Materials and methods

DNA constructs

Yeast integrative plasmid pJS210 was constructed by amplification of a 1.1 kb internal ABP1 fragment from Saccharomyces cerevisiae strain AB 1380, with primer sequences 5'-CCCGGATCCGCTCCCTCCATTGCGTACGGGG - 3' and 5'-CGCGGATCCGCTTGTGGTTCGACATCTC-GC-3' and insertion of the BamHI-digested amplification product into the BamHI site of pYIpLac204 [13] that was equipped with lox oligonucleotide cloned into the HindIII site. Plasmid pJS211 was constructed by inserting a 2.2 kb HindIII genomic CRY1 fragment (kindly provided by Dr J. Friesen [14]) into the HindIII site of pYIpLac211 [13], previously equipped with lox oligonucleotide in the EcoRI site.

The plasmid pCEL535 for expression of Cre recombinase in Escherichia coli was obtained from Dr P.J.J. Hooykaas (State University of Leiden, Netherlands) and consisted of the Cre gene containing its own translational start and stop codon, PCR-amplified from Pl lysogenic E. coli and cloned between the EcoRI and SpI sites of pUC19.

The binary vector plasmid pD3 was constructed from the Ds-containing plasmid pTT281 [31], in which the HindIII/Asel fragment containing the GUS gene was replaced by a HindIII/EcoRI fragment containing a CaMV 35S-BAR-nos3' fusion of the BAR gene described in [6] to create the plasmid pCTP103. Into the unique HindIII site of this plasmid, a synthetic oligonucleotide was inserted that contained the lox sequence and a NotI recognition site, to create the plasmid pMH21737. pMH21737 was linearized at its BgII site and inserted into the leader of a CaMV 35S-hygromycin phosphotransferase (HPT)-nos3' gene fusion present on plasmid pMH2057. pMH2057 was constructed by subcloning a tms2 gene from pTT283 [31] into pUC19 as a 3.7 kb Psdl/Sphl fragment. The CaMV 35S-HPT-nos3' fusion, also derived from pTT283, was included in pMH2057 as a 2.2 kb SpI fragment. Insertion of pMH21737 into pMH2057 yielded the plasmid pMH3030. pMH3030 was linearized at its KpnI site and inserted into the KpnI site of the binary vector pCGN1548 [23] that contained a lox oligonucleotide in its BamHI site. The total T-DNA construct was designated D3, the binary vector pD3.

Binary vector plasmid pMH2626 was constructed by first subcloning a 3.4 kb SacI/HindIII fragment of pED23, containing the CaMV 35S-nos3' fusion (kindly provided by Dr D.W. Ow, PGEC, Albany, CA [5]), into pIC19R to obtain pMH303. The fragment was transferred as a 3.4 kb HindIII/Xhol fragment into HindIII/SalI sites of pBin19, and the resulting plasmid was designated pMH2626.

Transgenic yeasts

To construct transgenic S. cerevisiae strains yJS210 and yJS211, plasmids pJS210 and pJS211 were linearized by cleaving the unique BgII or BgIII sites respectively within the cloned yeast genes, and used to transform strain AB 1380 by the LiCl method [2]. yJS212 was constructed by retransformation of yJS211 with plasmid pJS210. Correct single-copy integrations were selected by Southern analysis.