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Amplification of Drug Resistance Genes Flanked by Inversely Repeated IS*1* Elements: Involvement of IS*1*-Promoted DNA Rearrangements before Amplification

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Tn*2653* contains one copy of the *tet* gene and two copies of the *cat* gene derived from plasmid pBR325 and is flanked by inverted repeats of IS*1*. Transposed onto the P1-15 prophage, it confers a chloramphenicol resistance phenotype to the *Escherichia coli* host. Because the prophage is perpetuated as a plasmid at about one copy per host chromosome, the host cell is still tetracycline sensitive even though P1-15 is carrying one copy of the *tet* gene. We isolated P1-15::Tn*2653* mutants conferring a tetracycline resistance phenotype, in which the whole transposon and variable flanking P1-15 DNA segments were amplified. Amplification was most probably preceded by IS*1*-mediated DNA rearrangements which led to long direct repeats containing Tn*2653* sequences and P1-15 DNA. Subsequent recombination events between these direct repeats led to amplification of a segment containing the tetracycline resistance gene in tandem arrays.

Duplication and amplification of drug resistance genes in procaryotes have often been observed in strains which were isolated by selection for increased resistance to antibiotics (reviewed in references 1 and 5). The amplified DNA segments are usually found as tandem arrays. Gene duplication may result from unequal crossing over between homologous sequences flanking the gene(s) undergoing duplication. The same process, i.e., unequal crossing over between duplicated genes, can give rise to higher amplification. Two copies of an insertion (IS) element flanking drug resistance genes as direct repeats often serve as the sequence homology for duplication (5, 12, 22). IS elements are about 0.8- to 1.8-kilobase (kb)-long transposable elements residing on plasmids, bacteriophage genomes, and the chromosomes of many bacteria, and they can cause DNA rearrangements such as insertion, deletion, inversion, and cointegration between two DNA molecules (7, 12, 16). In enteric bacteria, IS*1* is one of the most active IS elements and is found as the flanking element in many transposons. For amplification of drug resistance genes in transposons flanked by direct repeats of IS*1*, recombination between the IS*1* sequences resulting in the first tandem duplication appears to be a rate-limiting step for subsequent amplification (17, 20).

In this paper, we report the amplification of drug resistance genes flanked by inverted repeats of IS*1* and located on the bacteriophage P1-15 genome. Physical analyses of amplified genes indicated the DNA rearrangements promoted by IS*1* have provided direct repeats and that subsequent amplifications have occurred by recombination between these directly repeated sequences. Since DNA rearrangements prior to amplification(s) were different among the different isolates studied, tandemly repeated segments associated with amplified genes were not unique and contained various P1-15 DNA sequences. This is in contrast to the amplification of transposons flanked by directly repeated IS*1* elements, in which the repeated units in different isolates usually correspond to the transposons (17, 20, 22).

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MATERIALS AND METHODS

Bacterial and phage strains and media. *Escherichia coli* K-12 WA921, used as the host throughout, was described previously (9). The bacteriophages P1-15::Tn*2653* WI1 and P1-15::Tn*2653* XII1 are two of the P1-15 derivatives isolated previously (11). Both phages carry the heat-inducible *c*1ts225 mutation (23). Culture conditions used were as described previously (9). When supplemented with antibiotics, the media contained 25 μg of chloramphenicol or 25 μg of tetracycline per ml.

Isolation of P1-15::Tn*2653* derivatives carrying an amplified tetracycline resistance gene. Lysogenic *E. coli* WA921 (P1-15::Tn*2653*) was grown at 30°C overnight to saturation in LB and plated on LACm or LATc plates. The plates were incubated at 30°C. Chloramphenicol-resistant (Cm^r) colonies appeared after 2 days of incubation, whereas tetracycline-resistant (Tc^r) colonies appeared after more than 3 days. The ability to produce infectious phage particles from the Tc^r colonies upon heat induction was examined as described previously (9). From the amplified Tc^r derivatives of P1-15::Tn*2653*, we isolated Cm^r but Tc^s mutants by the plaque center test (9).

Nucleic acid procedures. Preparation of phage DNA, restriction cleavage analyses, and electron microscopy were carried out as described previously (11, 17). Restriction enzymes were obtained from New England BioLabs or Boehringer GmbH and were generally used as recommended by the suppliers.

RESULTS

Amplification of the tetracycline resistance gene of Tn*2653*. The transposon Tn*2653* is 5.95 kb long and contains inversely repeated IS*1* elements at its termini (11). It also carries two copies of the chloramphenicol resistance gene as inverted repeats and one copy of the tetracycline resistance gene (Fig. 1). These two genes are derived from the plasmid pBR325 (11, 21). The bacteriophage P1-15 is a phage P1 derivative carrying no IS*1*, and P1-15::Tn*2653* phages are plaque-forming P1-15 derivatives having acquired Tn*2653* (Fig. 1) (11). Tn*2653* is inserted in the *mod*(P15) gene on

FIG. 1. Structures of transposon Tn*2653* and of the bacteriophage P1-15 genome. Transposon Tn*2653* carries two copies of the chloramphenicol resistance gene and one of the tetracycline resistance gene derived from plasmid pBR325 (11, 21). Its transposition activity is mediated by two IS*1* located in inverted orientation at the ends of the transposon. The position and orientation of the chloramphenicol and tetracycline resistance genes (including their promoters) are indicated by the open and solid arrows, respectively. The horizontal arrows under the physical map of Tn*2653* indicate the long inversely repeated sequences in Tn*2653*. Tn*2653* does not contain a *Bgl*II restriction site. Phage P1-15 hybrid 2 is a plaque-forming recombinant between phage P1 and plasmid p15B (18). The solid rectangles in the map represent the 0.6-kb inverted repeats in the invertible C segment (14). The location of the genes for *Eco*P15 restriction and modification is identified by res mod (13); the *c*1 gene mutation 225 resulting in a temperature-sensitive *c*1 repressor is derived from phage P1 (23). Short bars above the map show *Bgl*II restriction sites, and the numbers identify the *Bgl*II fragments (2). The vertical arrows labeled XII1 and WI1 point to the insertion sites of Tn*2653* in P1-15::Tn*2653* XII1 and P1-15::Tn*2653* WI1, respectively.

P1-15::Tn*2653* XII1 (13), whereas P1-15::Tn*2653* WI1 carries Tn*2653* inserted in the region to the left of the *cin* gene encoding the site-specific recombinase for C-segment inversion (14).

The phenotype of both WA921(P1-15::Tn*2653* XII1) and WA921(P1-15::Tn*2653* WI1) is Cm^r Tc^s, because the prophage plasmid P1-15 replicates at about one copy per host chromosome (25), and this copy number of the tetracycline resistance gene is too low to allow the host bacteria to grow on LA plates containing 25 μg of tetracycline per ml (15). When WA921(P1-15::Tn*2653*) was grown to saturation in LB, however, the culture produced Tc^r colonies at a frequency of about 7×10^{-8} per Cm^r colony. Under the same conditions, a culture of WA921(P1-15) would give Tc^r colonies at a frequency of less than 3×10^{-9} per CFU. About half of the Tc^r colonies derived from WA921(P1-15::Tn*2653*) could produce infectious phage particles upon heat induction, indicating that the lysogens contain nondefective P1-15 prophage derivatives. These lysogens may harbor oversized plasmid genomes which, owing to gene amplification, have become longer than the roughly 100-kb packageable into a phage head. Since the DNA in P1 and P1-15 phage particles is circularly permutated and terminally redundant (25), all the regions of a genome are present in populations of phage DNA, even if the genome has become oversized (17). Some of the phage particles induced from such an oversized prophage contain the entire nondefective phage genome and can often produce plaque-forming derivatives upon infection (9, 10, 17).

DNA of phage induced from the Tc^r lysogen was subjected to restriction cleavage analysis (Fig. 2). The DNA of all phage derivatives studied gave restriction patterns characteristic of gene amplification, since some restriction bands were more intense than the others and since some bands were apparently increased in molecular weight. The patterns, however, varied from isolate to isolate. Not only was Tn*2653* amplified, but also variable segments of the P1-15 genome, were amplified. Thus, in 12 independent Tc^r isolates, the amplified segments were all different, even in genomes derived from the same WA921(P1-15::Tn*2653*) strain. In the following sections, we describe the genome structures of four Tc^r derivatives containing gene amplifications, two from P1-15::Tn*2653* WI1 and the other two from P1-15::Tn*2653* XII1.

Tc^r derivatives of P1-15::Tn*2653* WI1. P1-15::Tn*2653* WI-5A and P1-15::Tn*2653* WI-6A are Tc^r derivatives of P1-15::Tn*2653* WI1. Restriction cleavage analysis of P1-15::Tn*2653* WI-5A DNA with *Bgl*II revealed that not only Tn*2653* but also the P1-15 segment, including *Bgl*II-9, *Bgl*II-10, and parts of *Bgl*II-2 and *Bgl*II-11, have undergone amplification (Fig. 1 and 2). Amplification was also observed by electron microscopy of single-stranded P1-15::Tn*2653* WI-5A DNA (Fig. 3A). The tandem repeats consisted of Tn*2653* and an adjacent, approximately 8.5-kb-long DNA segment. Since the integration site of the transposon in P1-15::Tn*2653* WI1 is about 1.3 kb to the left of the invertible C segment, the electron micrograph presented in Fig. 3A also indicates that the amplified P1-15 DNA segment must be to the left of the insertion site of Tn*2653* in the parental P1-15::Tn*2653* WI1 genome. Although part of *Bgl*II-2 underwent amplification, *Bgl*II-2 itself in P1-15::Tn*2653* WI-5A remained intact (Fig. 2). The simplest interpretation of these results is that a circular molecule consisting of the 8.5-kb P1-15 DNA segment and Tn*2653* had formed by transpositional excision (10) and had reintegrated, probably by homologous recombination, into a P1-15::Tn*2653* WI1 sister genome, thus resulting in a tandem duplication. Subsequent

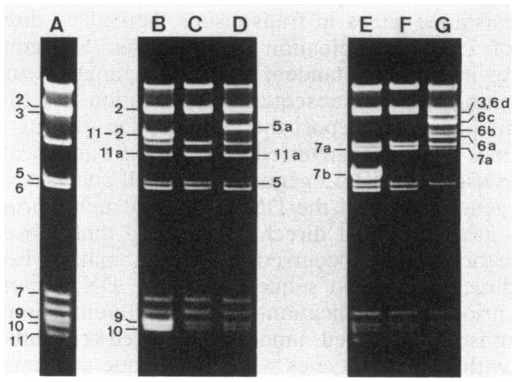

FIG. 2. Amplification of Tn*2653* and P1-15 DNA segments demonstrated by restriction fragment analysis. Phage DNA was digested with *Bgl*II, and electrophoresis was performed in 0.8% agarose gels. Lanes: A, P1-15 DNA; B, P1-15::Tn*2653* WI-5A DNA; C, P1-15::Tn*2653* WI1 DNA; D, P1-15::Tn*2653* WI-6A DNA; E, P1-15::Tn*2653* XII-7A DNA; F, P1-15::Tn*2653* XII1 DNA; G, P1-15::Tn*2653* XII-8A DNA. The relevant fragments indicated correspond to the *Bgl*II fragments in Fig. 1, 4, and 5. *Bgl*II-12 is too small to be seen in the gel.

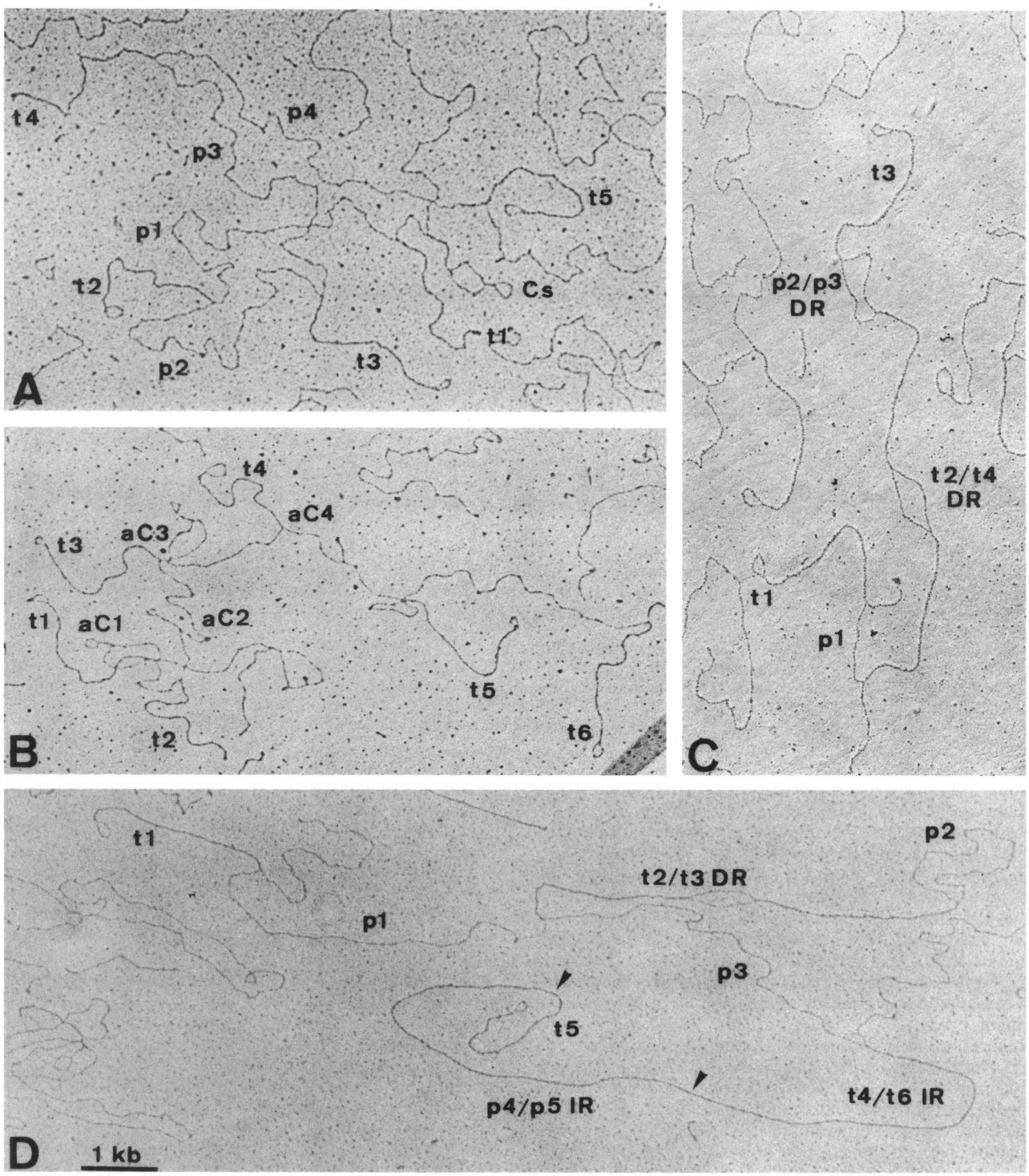

FIG. 3. Electron micrographs of snap-back structures observed in single-stranded phage DNA of (A) P1-15::Tn*2653* WI-5A; (B) P1-15::Tn*2653* WI-6A; (C) P1-15::Tn*2653* XII-7A, and (D) P1-15::Tn*2653* XII-8A. The DNA molecule shown in panel A displays the C-segment snap-back (Cs) and five copies of Tn*2653* (t1 to t5) separated by the amplified P1-15 DNA segment (p1 to p4). The molecule in panel B shows six copies of Tn*2653* (t1 to t6) and the amplified P1-15 segment, including part of the C-segment (aC1 to aC4). The amplified units of P1-15 DNA contained the C-segments flanked by a short inverted repeats (see Fig. 4), some of which (e.g., aC2 and aC4) failed to form a comlete snap-back structure. In panel C, the molecule contains four copies of Tn*2653* (t1 to t4). Two of them, t2/t4, are certainly present as direct repeats (DR) because the central unique sequence (containing the tetracycline resistance gene) has not annealed. It also contains three copies of the amplified P1-15 DNA segment (p1 to p3). The molecule shown in panel D carries six copies of Tn*2653* (t1 to t6) separated by the amplified segment of P1-15 DNA (p1 to p5). The long snap-back is interpreted to contain two Tn*2653* (t4/t6) and 2 P1-15 DNA segments (p4/p5) in inverted orientation (IR) flanking another copy of Tn*2653* (t5). The approximate junctions between these DNA segments are marked by arrowheads. This long snap-back thus corresponds to the long inverted repeats of P1-15::Tn*2653* XII-8A(II) in Fig. 5. The bar indicates the length of 1kb of DNA. All four phage DNA preparations analyzed represented mixtures of oligomeric forms of the amplified segments.

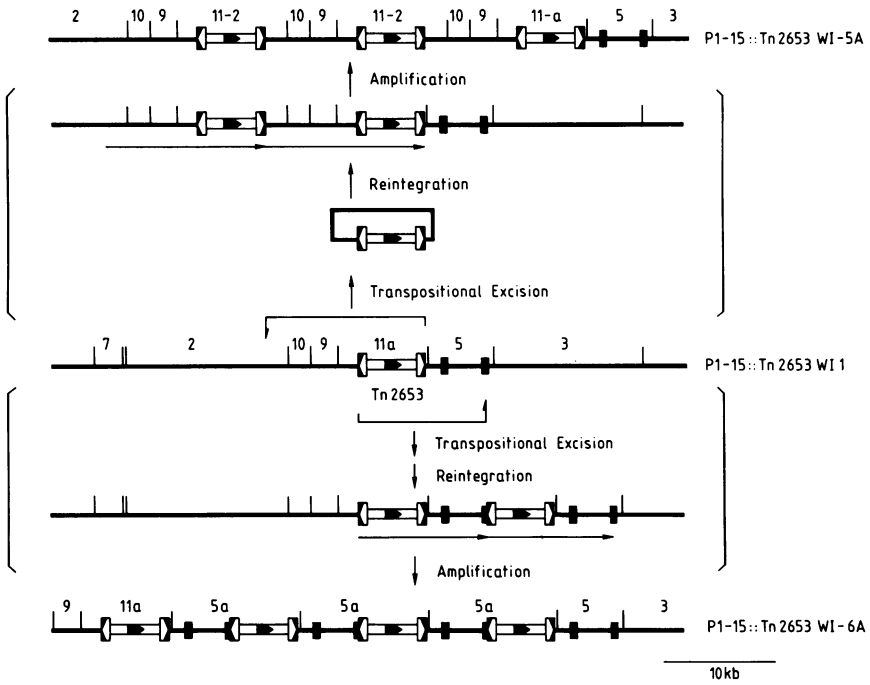

FIG. 4. Diagrams of the amplified part of the genomes of P1-15::Tn*2653* WI1 derivatives and possible DNA rearrangements leading to amplification. The P1-15 region is drawn in the same way as in Fig. 1, while Tn*2653* is shown in a simplified form represented by the flanking IS*1* sequences and the central tetracycline resistance gene (solid arrow). The orientation of Tn*2653* is indicated by the orientation of the tetracycline resistance gene. For the production of P1-15::Tn*2653* WI-5A, the right IS*1* in Tn*2653* is postulated to have mediated transpositional excision of a circular DNA molecule containing Tn*2653* and a P1-15 DNA segment (10). Integration of this circular molecule into another copy of the P1-15::Tn*2653* WI1 genome results in a P1-15::Tn*2653* derivative carrying long direct repeats consisting of Tn*2653* and the integrated P1-15 segment, as indicated by the horizontal arrows under the map. Further homologous recombination between the long direct repeats would yield the genome of P1-15::Tn*2653* WI-5A. Similar processes are assumed to form P1-15::Tn*2653* WI-6A. The number of repeats drawn was chosen arbitrarily to explain the basic structure but does not indicate a unique level of amplification.

unequal crossing over at the direct repeats resulted in the amplified genome of P1-15::Tn*2653* WI-5A (Fig. 4).

Restriction patterns obtained by digestions with *Bam*HI, *Eco*RI, *Hin*dIII, and *Pst*I, as well as by double digestions with these enzymes (data not shown), are consistent with this interpretation. Moreover, the genome structures of three independently isolated Cm^r Tc^s derivatives of P1-15::Tn*2653* WI-5A were identical to that of the parental phage P1-15::Tn*2653* WI1. Thus, the formation of these Cm^r Tc^s revertants must have occurred by intramolecular homologous recombination leading to the deletion of the amplified segment.

Restriction analysis and electron-microscopic studies on P1-15::Tn*2653* WI-6A DNA revealed that its amplified segment includes the 1.3-kb DNA sequence to the left of the C segment, a major part (3.8 kb) of the C segment, and Tn*2653* (Fig. 2, 3B, and 4). Formation of P1-15::Tn*2653* WI-6A most probably involved the excision of a circular molecule comprising the 5.1-kb P1-15 DNA segment, including the C region and Tn*2653*, and its subsequent reintegration into another P1-15::Tn*2653* WI1 genome, resulting in a tandem duplication (Fig. 4). The duplicated part thus contains two different inverted repeats originating from Tn*2653* and from the C segment. Unequal recombination between the long repeated sequence led to further amplification. Analogous to the situation with P1-15::Tn*2653* WI-5A, all Cm^r Tc^s derivatives of P1-15::Tn*2653* WI-6A were identical in genome structure to the ancestral P1-15::Tn*2653* WI1.

Tc^r derivatives of P1-15::Tn*2653* XII1. P1-15::Tn*2653* XII1 [or P1-15*mod*(P15)403::Tn*2653*] carries Tn*2653* inserted in *Bgl*II-7 encoding the *mod*(P15) gene for the recognition-modification subunit of the type III restriction enzyme *Eco*P15 (13) (Fig. 1). We studied two of its Tc^r derivatives, P1-15::Tn*2653* XII-7A and P1-15::Tn*2653* XII-8A. As in the previous two cases, P1-15::Tn*2653* XII-7A carries an amplified DNA segment consisting of Tn*2653* and a 1-kb segment of P1-15 covering *Bgl*II-12 and parts of *Bgl*II-7 and *Bgl*II-2. It still contains one copy of intact *Bgl*II fragments 2 and 7a (Fig. 2 and 5). Snap-back structures observed in the electron microscopy also showed tandem amplification of Tn*2653* and a 1-kb segment of P1-15 DNA (Fig. 3C). Thus, the processes which lead to a DNA amplification in P1-15::Tn*2653* XII-7A appear to be very similar to those in the two P1-15::Tn*2653* WI1 derivatives described above (Fig. 5).

Restriction analysis of P1-15::Tn*2653* XII-8A DNA revealed that both Tn*2653* and a 5.8-kb P1-15 DNA segment consisting of parts of *Bgl*II-6 and *Bgl*II-7 had undergone amplification. Contrary to the other three cases, however, one *Bgl*II fragment (*Bgl*II-6) had disappeared. Instead of *Bgl*II-6, two new fragments, 6a and 6b, arose together with amplified fragment 6c (Fig. 2). The intensity of fragment 6a was as strong as that of the other P1-15 fragments, whereas fragments 6b and 7a had approximately half the intensity of *Bgl*II-6a. The restriction cleavage pattern is best explained by assuming that this phage DNA preparation contains a mixture of at least two differently organized genomes represented by P1-15::Tn*2653* XII-8A(I) and P1-15::Tn*2653* XII-8A(II) in Fig. 5. The first step in their formation is postulated to be an intramolecular replicative transposition of Tn*2653* (7, 12), resulting in P1-15 derivative with two copies of

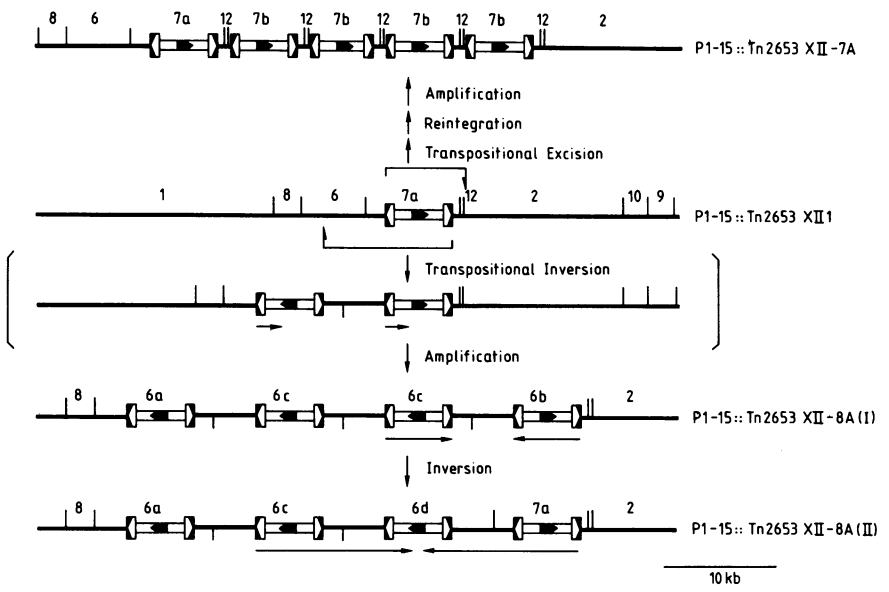

FIG. 5. Diagrams of the amplified part of the genomes of P1-15::Tn*2653* XII1 derivatives and possible DNA rearrangements leading to amplification. The processes involved in the formation of P1-15::Tn*2653* XII-7A are believed to be similar to those described in the legend to Fig. 4. For the formation of P1-15::Tn*2653* XII-8A, a Tn*2653*-mediated transpositional inversion is postulated to have occurred. The resulting genome carries two copies of Tn*2653* in inverted orientation, and the P1-15 segment flanked by Tn*2653* is also inverted, as indicated by the *Bgl*II site in the segment. Recombination between the direct repeats (indicated by the horizontal arrows under the map) in the inversely repeated Tn*2653* elements would yield the amplified structure P1-15::Tn*2653* XII-8A(I). Further recombination between the two neighboring inverted repeats of Tn*2653*, indicated by the horizontal arrows, would result in P1-15::Tn*2653* XII-8A(II). In the latter genome, the long inverted repeats which gave rise to the snap-back structure shown in Fig. 3D are identified by the long horizontal arrows.

inversely repeated Tn*2653* and concomitant inversion of the P1-15 DNA segment in between (as indicated by the *Bgl*II site). Alternatively, intermolecular conservative transposition of Tn*2653* and subsequent recombination between the inverted copies of Tn*2653* would also yield the same structure. Duplication by homologous recombination between direct repeats within the inversely repeated Tn*2653* (as indicated by the horizontal arrows in Fig. 5) and subsequent amplification would then result in the genome structure of P1-15::Tn*2653* XII-8A(I). P1-15::Tn*2653* XII-8A(II) could emerge by recombination between inverted repeats of Tn*2653* (as indicated by the horizontal arrows). The newly formed 6d fragment of *Bgl*II was interpreted to comigrate with *Bgl*II-3 in Fig. 2. *Bgl*II fragments 6b and 7a are present in about the same amount, suggesting that both P1-15::Tn*2653* XII-8A(I) and P1-15::Tn*2653* XII-8A(II) are present in approximately equimolar amounts. Restriction patterns with *Bam*HI, *Eco*RI, *Hin*dIII, and *Pst*I (data not shown) are consistent with this interpretation. P1-15::Tn*2653* XII-8A(II) carries long inverted repeats of about 6.7 kb (horizontal arrows in Fig. 5). Indeed, such long inverted repeats were visualized in the electron microscope and are shown in Fig. 3D. This electron micrograph also shows that the DNA molecule contains five copies of amplified P1-15 segments and six copies of Tn*2653* and that at least two copies of Tn*2653* adjacent to the 6.7-kb inverted repeats are in direct orientation.

Electron-microscopic studies also documented that DNA preparations from all four Tc^r P1-15::Tn*2653* derivatives examined contained mixtures of oligomers of the amplified segments with a variable number of repeated units. The results indicate that lysogens in a single Tc^r colony contain P1-15::Tn*2653* prophages with Tc^r genes amplified to variable degrees. We had previously made similar observations for DNA of P1 derivatives carrying amplified Cm^r transposons flanked by direct repeats of IS*1* (17).

DISCUSSION

Amplification of drug resistance genes in enteric bacteria, observed after selection for increased resistance to antibiotics, was shown to be a result of *rec*-dependent recombination between homologous sequences flanking the genes (1, 5, 8, 17, 20). Directly repeated IS elements at both ends of drug resistance transposons often serve this purpose. Amplified segments derived from such transposons usually consist of the drug resistance genes and one copy of the IS sequence. Their amplified segment is thus constant, independent of the subclone selected, and it is also independent of the site in the genome at which the tansposon is carried. In contrast, amplified segments derived from transposons flanked by inverted repeats of IS*1* differ from one isolate to the other, because a DNA rearrangement providing direct repeats is a prerequisite for amplification by subsequent homologous recombination. In all four cases studied here, DNA rearrangements prior to amplification were promoted by IS*1*. DNA rearrangements mediated by IS*1* could also occur before amplification of genes in transposons flanked by directly repeated IS*1* sequences. Although such amplifications might be rarer than amplification by homologous recombination between direct repeats of IS*1*, they have been detected previously (17, 22). Since transposable elements are generally able to mediate DNA rearrangements (7, 12, 16), not only IS*1* but also other elements could provide flanking direct repeats through DNA rearrangements. It is conceivable that any selective gene(s) being or having be-

come adjacent to one copy of a transposable element could undergo amplification through element-mediated DNA rearrangements.

Amplification of the r-determinant (r-det) flanked by direct repeats of IS*1* on the R plasmid NR1 in *Proteus mirabilis* is one of the best-studied examples of gene amplification in procaryotes (20, 22). In *E. coli*, however, not the entire r-det but only some of its deletion derivatives can undergo amplification (8. 17). The results of these two studies suggest that such derivatives always lack a particular region, which includes the *tnpR* resolvase gene of Tn*21* residing within the r-det (3). It is thus possible that the site-specific resolution system of Tn*21* prevents r-det from undergoing amplification in *E. coli* by destabilizing nascent amplifications.

Edlund and Normark (4) found that recombination between direct repeats of 12 base pairs could result in tandem duplication and further amplification at the *ampC* locus of the *E. coli* chromosome. Since IS*1* carries 23-base-pair imperfect terminal inverted repeats including 8-base-pair perfect repeats (19), the tetracycline and chloramphenicol resistance genes in Tn*2653* are flanked by two sets of 23-base-pair imperfect direct repeats of the IS*1* termini. Recombination between such short homologies within inversely repeated IS*1* elements on Tn*2653* would lead to amplification of the tetracycline and chloramphenicol resistance genes without concomitant amplification of a segment of P1-15 DNA. Since all 12 P1-15 derivatives studied have both the drug resistance genes and a segment of P1-15 DNA amplified, amplification by recombination between the short homologies seems to be too rare to be detected.

Froehlich et al. (6) have recently characterized defective P1ApCm prophages, P1dR, which underwent amplification of drug resistance genes associated with IS*1*-mediated DNA rearrangements. Their findings may be relevant to our observations that roughly half of the rare Tc^r lysogens of WA921(P1-15::Tn*2653*) obtained in our study failed to produce infectious phage particles upon heat induction. Although we did not examine them, it is conceivable that some of these defective Tc^r lysogens harbored P1-15::Tn*2653* derivatives similar to P1dR (6). We also expect to find high-copy-number mutants of the P1-15::Tn*2653* prophages in Tc^r lysogens producing infectious phages (24). However, all Tc^r mutants studied contained P1-15::Tn*2653* prophages with an amplified tetracycline resistance gene.

IS*1* appears to proceed by both conservative and replicative transposition mechanisms (7, 12, 16). Since a number of different pathways involving IS-mediated DNA rearrangements can result in the same genomic structure (10, 11, 12, 20), each pathway suggested to result in the amplified structures in Fig. 4 and 5 represents one of several possibilities. For example, the formation of circular DNA molecules with P1-15 segments and Tn*2653* in transpositional excision might have resulted from one of the following processes: intramolecular replicative transposition of IS*1* or Tn*2653* or intermolecular transposition of IS*1* or Tn*2653* by either a conservative or a replicative mechanism and subsequent homologous recombination between the direct repeats of IS*1* or Tn*2653*. For the intermolecular transposition, the transposed IS*1* could have originated from either Tn*2653* or the host chromosome. Of the four P1-15::Tn*2653* derivatives studied in detail, three appear to involve such transpositional excision of circular molecules and their subsequent reintegration into another copy of P1-15::Tn*2653* (Fig. 4 and 5). Excision and reintegration of circular DNA molecules containing a drug resistance gene and being formed by homologous recombination between direct repeats of IS*1* have previously been postulated as intermediate steps in the amplification process (20, 22). Transpositional excision and reintegration of the circular molecules could occur intramolecularly, immediately after the replication fork has passed the segment to be excised, into the nascent sister DNA molecule. Alternatively, the copy number of the P1-15 plasmid may have increased transiently during selection of Tc^r lysogens, and such excision and reintegration could occur intermolecularly, although the P1-15 prophage usually replicates at a low number of copies per cell (25).

Although the events described are rare, they show that transposons flanked by inversely repeated IS elements can undergo gene amplification. Amplification depends on a characteristic property of transposable elements: their tendency to mediate DNA rearrangements. The frequency of the two-step process required is, however, high enough to be of relevance both for functional adaptation of a microbial population to changing environments and for biological evolution.

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