Evolution of Tn21-related Transposons: Isolation of Tn2425, which Harbours IS161

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(Received 21 August 1984; revised 19 November 1984)

The isolation of two multi-resistance transposons, Tn2425 and Tn1831, and their relation to Tn21 and Tn2424, is described. A 1.7 kb segment present in Tn2424 and Tn2425 was identified as an IS element by *rec*-independent transposition, resulting in a cointegrate structure that carries two direct repeated copies of the IS element. By the isolation of this IS element we demonstrated that transposition is one mechanism leading to sequence variations in Tn21-like structures, especially in the region between the *mer* operon and the *sul* gene.

INTRODUCTION

The transposon Tn21 has been shown to harbour the genes encoding resistance towards mercuric chloride (*mer* gene), sulphonamides (*sul* gene) and streptomycin/spectinomycin (*aadA* gene) as well as the transposition functions *tnpR* (resolvase) and *tnpA* (transposase) (De la Cruz & Grinsted, 1982).

Recently, we described the isolation of the multi-resistance transposons Tn2424 (Meyer *et al.*, 1983) and Tn2411 (Kratz *et al.*, 1983a). Tn2424 mediated resistance to mercuric chloride (Hg^R), sulphonamides (Su^R), streptomycin/spectinomycin (Spec^R) [AAD-(3'')], chloramphenicol (Cm^R) and amikacin (Ami^R) [AAC-(6')], and included the total sequence of Tn21 and two additional DNA segments of 1.7 and 4.0 kb. The latter fragment encoded resistance to amikacin (*aacA* gene) and chloramphenicol (*clm* gene), whereas the smaller one lacked detectable functions (Meyer *et al.*, 1983). Tn2411 was completely included in Tn21 but lacked a 1.45 kb segment (Kratz *et al.*, 1983a).

Tanaka *et al.* (1983) presented a model of the evolution of Tn21-related multi-resistance transposons based on an ancestral mercury transposon Tn2613. Despite their structural relationship, these elements differed in several insertions within the region between the *mer* operon and the *sul* gene.

In order to investigate these sequence variations, we isolated and compared additional Tn21-related elements. Because of their length and the lack of detectable functions some of the inserted fragments are assumed to represent IS elements.

METHODS

The plasmids and bacterial strains used are listed in Table 1. All methods, such as DNA preparation, the transformation procedure, electron microscopy, DNA digestions, and cloning experiments, have been described before (Meyer *et al.*, 1983).

Resistances towards different drugs are abbreviated as follows: Hg^R (mercuric chloride), Su^R (sulphonamides), Sm^R (streptomycin), Spec^R (spectinomycin), Cm^R (chloramphenicol), Tc^R (tetracycline), Ap^R (ampicillin), Km^R (kanamycin), Ami^R (amikacin) and Tp^R (trimethoprim).

The designation of genotypes follows the nomenclature of Bachmann (1983).

Table 1. *Bacterial strains, plasmids and transposons*

Strains	Relevant characteristics*	Reference
<i>Escherichia coli</i> JC2926	<i>rpsL recA thi thr arg his lac</i>	Bachmann (1972)
<i>E. coli</i> SK1592	<i>gal thi ton sbcB15 hsr4 rec+</i>	Kushner (1978)
Plasmids		
pHS1	Tc ^R . Temperature sensitive in replication	Hashimoto & Sekiguchi (1976)
pBP81	Ap ^R Hg ^R . Ligated <i>SalI</i> fragment of pBR322::Tn2424	This paper
pBP812	Ap ^R Hg ^R Tc ^R . Cointegrate between pBP81 and pHS1	This paper
pBP8124	Tc ^R . pHS1::IS161	This paper
pNO1523	Ap ^R . pBR322 derivative harbouring a cloned <i>rpsL</i> (<i>strA</i>) gene	Dean (1981)
pMH2	Tc ^R Cm ^R Hg ^R Su ^R Sm ^R /Spec ^R <i>tra</i> ⁺ . One part of the R plasmid R5	Haas & Davies (1980)
R702	Km ^R Tc ^R Su ^R Sm ^R /Spec ^R Hg ^R <i>tra</i> ⁺	Villarroel <i>et al.</i> (1983)
pUB307	Km ^R Tc ^R <i>tra</i> ⁺	Bennett <i>et al.</i> (1975)
R751	Tp ^R <i>tra</i> ⁺	Meyer & Shapiro (1980)
Transposons		
pACYC184::Tn21	Cm ^R Hg ^R Su ^R Sm ^R /Spec ^R	This paper
pBR322::Tn2424	Ap ^R Hg ^R Su ^R Sm ^R /Spec ^R Cm ^R Am ⁱ ^R	Meyer <i>et al.</i> (1983)
pBR322::Tn2411	Ap ^R Tc ^R Su ^R Sm ^R /Spec ^R	Kratz <i>et al.</i> (1983a)
pNO1523::Tn2425	Ap ^R Hg ^R Su ^R Sm ^R /Spec ^R	This paper
pNO1523::Tn1831	Ap ^R Hg ^R Su ^R Sm ^R /Spec ^R	This paper

* See Methods for abbreviations.

RESULTS

Isolation and characterization of Tn2425 and Tn1831

The IncP1 plasmid R702 harbours a transposon of about 15.5 kb, designated Tn1831 (Villarroel *et al.*, 1983). The IncFII plasmid pMH2 has been demonstrated to share extensive homology to R100, from which Tn21 originated (Haas & Davies, 1980). Therefore, we tested plasmids R702 and pMH2 for transposition of Tn21-related elements.

Transposition experiments were done in *Escherichia coli* JC2926 (*recA*), with pNO1523 as the recipient plasmid. This replicon consists of pBR322 carrying a cloned *rpsL* gene, the chromosomal gene encoding the ribosomal S12 protein. This *rpsL* gene is dominant to its chromosomal mutation in streptomycin resistant mutants and leads to phenotypic streptomycin sensitivity. Thus, the insertion of a transposable element into the *rpsL* region of pNO1523 is detectable by selection for the activation of the chromosomal streptomycin resistance.

DNA of *E. coli* JC2926 harbouring pMH2/pNO1523 or R702/pNO1523 was used to transform *E. coli* JC2926 with selection for ampicillin and spectinomycin resistance, and for high levels of streptomycin resistance (1 mg ml⁻¹). The resulting transformants were replicated for tetracycline, chloramphenicol and kanamycin sensitivity.

Representative clones of pNO1523::Tn1831 and pNO1523::Tn2425 (Tn21-related transposons from pMH2), which occurred with frequencies between 10⁻⁵ to 10⁻⁶, and pA-CYC-184::Tn21, pBR322::Tn2424 and pBR322::Tn2411 were subjected to restriction and heteroduplex analysis (Fig. 1). Tn21 was completely included in Tn2425, which was itself homologous to Tn2424 except that it lacked the insertion of the amikacin and chloramphenicol resistance genes (Fig. 1). Tn2425 included all the restriction sites present in Tn21, and, in addition, an insertion of about 1.7 kb between the *mer* and the *sul* genes (designated insertion loop 3). Insertion loop 3 of Tn2425 corresponded to the 1.7 kb fragment detected in heteroduplexes between Tn2424 and Tn21 (Meyer *et al.*, 1983). Tn1831 differed from Tn21 in the lack of a 2.8 kb fragment (loop 2 in Fig. 1) at a distance of about 4.3 kb from the left inverted repeat. Furthermore, one *Bam*HI restriction site present in all the Tn21-like elements was not detectable in Tn1831.

Additional transposition experiments revealed that both Tn1831 and Tn2425 were transferable to other plasmids, such as pUB307 or R751.

Fig. 1. Restriction maps and schematic structures of several *Tn21*-related elements. The maps of *Tn2424*, *Tn21* and *Tn2411* are taken from Meyer *et al.* (1983) and Kratz *et al.* (1983a). The closed boxes at the ends of the structures indicate the inverted repeats. The insertion loops (1–3) correspond to the insertions detected in different heteroduplexes. Their lengths were measured to be 1.45 kb (loop 1), 2.8 kb (loop 2) and 1.7 kb (loop 3). All transposons have been reduced to their common backbone structure.

Fig. 2. Isolation procedure of IS161. *E. coli* JC2926 harbouring pHS1 and pBP81 was selected at 42 °C for ampicillin and tetracycline resistance. The cointegrate structure pBP812 was resolved either in *recA* or *rec+* *E. coli* strains by recombination involving the two direct repeats of IS161. (The arrows indicate the orientation of the IS elements in the plasmids.) The letters correspond to the following restriction sites: H, *Hind*III; S, *Sma*I; E, *Eco*RI.

Cointegrate formation after transposition of insertion loop 3

The ease of transposition of the different insertion loops present in the region between the *mer* operon and the *sul* gene was investigated using the left part of Tn2424, as this included the corresponding sequence, and all three insertion loops, of Tn2425.

The plasmid pBR322::Tn2424 was digested with *Sa*I and the resulting fragments were ligated with T4 DNA ligase. After transformation we screened for Ap^R Hg^R Cm^S Spec^S Tc^S colonies. One clone, carrying a self ligated *Sa*I fragment, was designated pBP81. This plasmid consisted of one part of pBR322 and the left side of Tn2424, including the *mer* genes and the three insertion loops.

As has been depicted in several models for transposition (Muster & Shapiro, 1981; Galas and Chandler, 1981), the transposition of an IS element results in a cointegrate structure between the donor and the recipient replicons. pHS1 (Tc^R and temperature sensitive in replication) was used as the recipient plasmid during cointegrate formation with the donor pBP81 (Fig. 2). *E. coli* JC2926 (*recA*), transformed with both plasmids, was grown at 42 °C selecting for ampicillin and tetracycline resistance. As pHS1 is unable to replicate at 42 °C, only cointegrates formed between pHS1 and pBP81 were able to mediate this phenotype at this temperature. Corresponding clones were detected at a frequency of about 10⁻⁷ (in relation to Ap^R Tc^R colonies at 37 °C) and purified at 42 °C with selection for Ap^R Tc^R colonies. The DNA of six clones was used to transform *E. coli* JC2926 and the plasmids of the resulting transformants were analysed by endonuclease digestion.

Of the six pHS1/pBP81 cointegrates that were analysed, one was probably due to the transposition of IS102 (Machida *et al.*, 1982) from pHS1, while the other five were formed via insertion loop 3, now designated IS161 (in agreement with the Plasmid Reference Center, Stanford University, Calif., USA). Restriction patterns revealed that IS161 had transposed into different regions of pHS1 in each cointegrate.

One representative IS161-mediated cointegrate, pBP812, was used for further experiments. The restriction profile of this plasmid in relation to pHS1 and pBP81 is shown in Figs 2 and 3. IS161 is located on the third *Eco*RI fragment of pBP81 and carries a *Sma*I restriction site. From the transposition models mentioned above, one would expect a cointegrate structure with

Fig. 3.

Fig. 4.

Fig. 3. Restriction analysis of pBP81, pHS1 and pBP812, a cointegrate formed between pHS1 and pBP81 via *IS161*. The lane representing *SmaI* digested pBP812 apparently indicates the duplication of *IS161*, which carries one restriction site for *SmaI*. In pBP812 the *SmaI* fragments of pBP81 (donor plasmid) remained unchanged, whereas the *SmaI* fragment of pHS1 (9.2 kb) forms two distinct fragments, with sizes of about 4.4 and 6.5 kb, due to the insertion of *IS161*. The small *SmaI* restriction fragment of pHS1 (0.2 kb) is not visible in this gel (1% agarose).

Fig. 4. Restriction analysis of pBP8124 (pHS1 :: *IS161*) and pHS1. *SmaI*/*EcoRI* digestion of pBP8124 generates two fragments of about 4.6 kb that are unresolved owing to their similar size. *SmaI*/*HindIII* digestion results in a fragment that corresponds to the internal part of *IS161*.

duplicated IS elements (each carrying a *SmaI* restriction site) that flank the ends of the donor plasmid (pBP81) as direct repeated copies (Fig. 2). The results shown in Fig. 3 support this assumption, as one additional *SmaI* restriction site was detected in pBP812 and both *SmaI* fragments of the donor plasmid remained unchanged.

Cointegrate resolution in *rec⁺* and *recA* *E. coli* strains

The cointegrate plasmid pBP812 was used to transform *E. coli* SK1592 (*rec⁺*). As the two copies of the IS element are directly repeated, the cointegrate can undergo homologous recombination and thus be resolved into the original donor and recipient plasmids, each carrying one copy of the IS element. The DNA of a transformant clone *E. coli* SK1592(pBP812) was prepared, and was used to transform *E. coli* JC2926. Transformants were selected for

Fig. 5. Heteroduplex formed between pHS1 and pBP8124 (pHS1::IS161). Measurement of different structures resulted in a length of 1.7 kb for IS161 (arrowed) using single stranded ϕ X174 DNA (5375 kb) as an internal length standard. Magnification $\times 67000$.

tetracycline resistance and replicated for ampicillin sensitivity (loss of pBP81). Four clones out of 500 showed this phenotype.

Restriction data (Figs 2 and 4) and electron microscope heteroduplex analysis (Fig. 5) revealed that these four clones carried pHS1::IS161 (pBP8124). Furthermore, the restriction data indicated the location and orientation of IS161. The *Sma*I and *Hind*III sites internal to IS161 are separated by a fragment of about 1 kb (Fig. 4, lane 5). No restriction sites for *Bgl*II, *Eco*RI, *Pst*I, *Bam*HI, *Sal*I or *Pvu*II were detectable in IS161. Length measurements of heteroduplexes (53 molecules) formed between pHS1 and pBP8124 verified the size of IS161 to be 1.7 kb.

Resolution of the cointegrate pBP812 in a *recA* background was determined by isolation of the DNA from *E. coli* JC2926 followed by *Pst*I digestion and transformation with selection for Tc^R transformants. As pHS1 and the IS element contain no *Pst*I restriction site, only pBP81 and the cointegrate pBP812 were cleaved, whereas pHS1::IS161 remained intact. Transformation with selection for tetracycline resistance resulted in a frequency of about 10^{-4} (Tc^R transformants obtained with *Pst*I-digested versus undigested pBP812). The resulting clones harboured a plasmid, indistinguishable from pBP8124 from the *rec*⁺ strain.

In additional transposition experiments, using pBP8124 as donor plasmid, we isolated cointegrates formed with pBP84-1 or R751.

DISCUSSION

Previously described Tn21-related transposable elements (Meyer *et al.*, 1983; Kratz *et al.*, 1983a,b; Tanaka *et al.*, 1983) differed (i) in the region adjacent to the *aadA* gene, due to insertions or substitutions of different resistance genes, and (ii) in several short sequences in the

Fig. 6. Evolutionary relationships of Tn21-related transposons.

region between the *mer* and the *sul* genes. The latter fact was supported by the isolation of Tn1831 and Tn2425; the former has one less insertion loop, and the latter one more insertion loop, than Tn21.

With the isolation of IS161, we have defined one of the sequences that distinguishes Tn21 and Tn2424, which is also the sequence that distinguishes Tn21 and Tn2425, and have shown it to behave as an IS element. Comparison of the length and the restriction pattern revealed that IS161 is distinct from IS1-5, IS8, IS10, IS15, IS50, IS51, IS102 or IS903 (Comai & Kosuge, 1983; Depicker *et al.*, 1980; Kleckner, 1981; Labigne-Roussel & Courvalin, 1983). Despite the fact that we have not demonstrated the transposition of other insertion-like sequences from Tn21-related structures, it seems reasonable to suggest that the transposition of IS elements into the left part of Tn21-like elements is a likely cause of sequence variations in this group of related transposons.

Finally, summarizing all the data on the numerous Tn21-like elements, Fig. 6 represents a reasonable scheme of several mechanisms involved in the evolutionary relation of these structures. From previous work of Tanaka *et al.* (1983), Tn21 seems to have evolved from an ancestral mercury-resistance transposon such as Tn2613. Transposition of IS161 into Tn21 resulted in the structure of Tn2425. By the insertion of the genes encoding chloramphenicol and amikacin resistance at the predicted recombinational 'hot spots' adjacent to the *aadA* gene (Schmidt, 1984), Tn2425 yielded the structure of Tn2424. Deletion of a 1.45 kb or a 2.8 kb fragment from Tn21 resulted in the structure of Tn2411 or Tn1831, respectively. Involving the mentioned recombinational 'hot spots', the *aadA* gene in Tn2411 was substituted by an *oxa* gene to yield Tn2410 (Kratz *et al.*, 1983a). The same sequences seem to participate in the insertion of genes into Tn21 as demonstrated by the structure of Tn2603 (Yamamoto *et al.*, 1983).

This work was supported by a grant of the Deutsche Forschungsgemeinschaft to B. Wiedemann.

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