Physical and genetic analysis of IS110, a transposable element of Streptomyces coelicolor A3(2)

Keith F. Chater, Celia J. Bruton, Stephen G. Foster*, and Ivan Tobek**
John Innes Institute, Colney Lane, Norwich NR4 7UH, Great Britain

Summary. On at least three independent occasions a 1.6 kb segment of Streptomyces coelicolor DNA was detected in apparently the same location in an attP-deleted derivative of the temperate phage φC31 that carried a selectable viomycin resistance gene. This sequence (termed IS110) allowed integration of the phage (giving viomycin-resistant transductants) at homologous sequences (detected by Southern hybridisation) at several locations in the S. coelicolor genome. The inserted prophages facilitated genetic mapping of two IS110 copies in the chromosomal linkage map. A third copy did not exhibit simple segregation with chromosomal markers, and there appeared to be a frequent DNA rearrangement close to this copy. Some variation in the number of copies of IS110 and their location has taken place in the pedigree of S. coelicolor derivatives. IS110 did not hybridise to any known S. coelicolor plasmid, nor to any of several other IS-like elements previously described in other Streptomyces plasmids or phages. It hybridised strongly to DNA from only a small minority of other Streptomyces species and was absent from S. lividans, a close relative of S. coelicolor.

Insertion sequences (IS elements) are phenotypically cryptic DNA elements, of about 1.5 kb or less, able to transpose into new locations in the DNA present in a cell, either within or between replicons (Iida et al. 1983). They have mainly been studied in E. coli K12, which contains several different types of IS, some in several copies. It may reasonably be supposed that IS elements are widespread among prokaryotes, and that some new kinds will be found particularly when organisms taxonomically far from E. coli are analysed. However, very few examples of IS elements in such prokaryotes have been described: we are aware only of those found in the archaeabacterium Halobacterium halobium (e.g. Das Sarma et al. 1983). Studies of new IS elements will be best pursued in species in which there are well-established genetics and endogenous cloning systems. This is the case for Streptomyces coelicolor A3(2) (see the reviews by Hopwood and Chater 1984 and Chater and Hopwood 1984). In this paper, we describe the transposition of an IS element from the S. coelicolor genome into derivatives of a temperate phage (φC31: Lomovskaya et al. 1980), and the use of one of the resulting phages to analyse, physically and genetically, the locations of copies of the element in the S. coelicolor genome.

The new insertion sequence, IS110, was observed at first by chance, and then deliberately, after its transposition into cloning vectors derived from the temperate Streptomyces phage φC31. The vectors (KC400 and KC404: Harris et al. 1983) contain tph, a viomycin resistance gene, for vector selection and are deleted for their attP site. We have previously shown that DNA cloned into these vectors can direct their integration into the homologous regions of recipient genomes (Chater and Bruton 1983): in the absence of significant homology between the genomes of the phages and the recipient, integration (giving viomycin-resistant lysogens) does not occur.

We discovered three clonally independent derivatives (KC407, from KC404, and KC410 and KC411, from KC400) of these vectors that had spontaneously acquired the ability to lysogenise S. coelicolor (strain J1501, a φC31 (=Pgl-) derivative (Chater et al. 1982) was used throughout except where specified). In spot transduction tests (Chater et al. 1982) KC407, KC410 and KC411 gave lysogens at a frequency comparable to that observed in other (unpublished) experiments for integration directed by a cloned S. coelicolor DNA fragment of about 3–4 kb. This was particularly clear in KC411, which arose during the in vitro insertion of a 1.1 kb PstI to BglII internal segment of the S. coelicolor glycerol utilisation operon (gly) into KC400. Phages carrying this segment could integrate into the gly operon, generating a glycerol-sensitive (Gyl+) phenotype (Seno et al. 1984). The gly segment was present in KC411, but only 31% of KC411 transductants were Gyl+, 69% being Gyl+ due (as we show in detail with KC407) to integration elsewhere in the genome via the IS110 copy spontaneously acquired by KC411.

Restriction analysis of DNA from KC407, KC410 and KC411 revealed, in all three cases, an insertion of 1.6 kb of additional DNA within 0.5 kb of the righthand cohesive end. This region is close to, but not within, the previously defined major dispensable region of the φC31 genome (Chater et al. 1981). The sequence was designated IS110 (from a block of IS numbers issued by Dr. E.M. Lederberg of the Plasmid Reference Center). Within the limits of restriction analysis (perhaps 10–20 bp) the IS110 insertions...
were in the same location and had the same orientation. The most detailed mapping was with KC407 (Fig. 1).

Southern hybridisation using 32P-labelled KC407 DNA as probe was done with restriction digests of J1501 DNA and DNA of S. lividans 66 (the usual host for most φC31 propagation: Lomovskaya et al. 1972) (results not shown). Hybridisation was observed only to J1501 DNA. With SstII (which gave a 1.3 kb internal fragment with IS110 in KC407) a single strong band of 1.3 kb was seen. This showed that IS110 originated from S. coelicolor (not from the propagation host S. lividans). Moreover, the absence of any other strongly-hybridising SstII bands in J1501 DNA confirmed that the insertion in KC407 DNA did not involve replacement of vector DNA with the insertion of significantly more than 1.6 kb of J1501 DNA.

With SphI and PstI digests, several strongly hybridising bands were seen, suggesting that S. coelicolor contained several copies of IS110. This was further analysed using the isolated 1.3 kb SstII internal fragment of IS110 as a 32P-labelled probe to digested J1501 DNA (Fig. 2). Two enzymes, KpnI and SphI, which cut IS110 only once (close to one end), gave three hybridising bands, whereas four bands were detected in digests with BamHI or PstI, neither of which cuts within IS110. Further experiments (given below) suggested that there may in fact be three IS110 copies in J1501, one of which is located in a region subject to a frequent DNA rearrangement.

Southern analysis of DNA from nine independent J1501 (KC407) lysogens showed that prophage integration could occur into any of the host’s IS110 copies (Fig. 3). Arbitrarily designating the PstI-generated hybridising bands of J1501 DNA as A, B, C and D (Fig. 3), six insertions were obtained into A, two into B, and one into C+D. This verified that the various copies of IS110 were highly conserved in sequence (recombination being very sensitive to minor sequence divergence). Insertion into C+D could have resulted from formation of a double lysogen. However, this should be a very low probability event, and it seems more likely that C and D are both manifestations of a single copy of IS110 in a DNA region subject to a high frequency, reversible rearrangement (one possibility is shown in Fig. 4).

The inserted prophages in examples of each of the three classes of lysogens were genetically mapped in the J1501 genome by conventional conjugal crosses (Hopwood 1967) using viomycin resistance as a marker (Fig. 5). IS110-A was located at about 10 o’clock on the linkage map, between proA and pabA, and IS110-B is the 3 o’clock silent region between argA and cysD. However, IS110-(C, D) did not segregate as a typical chromosomal marker (data not shown). It gave different apparent map locations in replications of the same cross, or when alternative selections were applied, or with different crosses. Viomycin-resistant and -sensitive recombinants were both frequent, in contrast to the situation for the non-localisable unstable chloramphenicol resistance determinant of S. coelicolor (Freeman et al. 1977). Either IS110-(C, D) is not on the chromosome or, as suggested earlier, it is subject to some kind of instability.

S. coelicolor A3(2) is known to contain a variety of mobile genetic elements: the well-defined autonomous plasmid SCP2 (Bibb et al. 1977); two transmissible plasmids, SCP1 and SLP4, unisolable as intact molecules (Hopwood et al. 1973, 1983); an integrated plasmid, SLP1, able to transfer into S. lividans and reside there as an autonomous CCC DNA molecule or integrated at a preferred site (Omer and Cohen 1984) in the S. lividans chromosome (Bibb et al. 1981), and a low-abundance 2.6 kb “mini-circle” with some transposon-like properties (D.J. Lydiate and H. Ikeda, personal communication). The 1.3 kb SstII fragment of IS110 did not hybridise to isolated SCP2, SLP1 or 2.6 kb minicircle, or to total DNA of S. lividans containing either SLP4 or integrated SLP1, or to total DNA of S. parvulus containing SCP1. Thus the unusual behavior of the IS110-(C, D) region was not due to linkage of IS110 with any of these