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Short Communication

A Functional Map of Plasmid ColE1

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Summary. Examination of the properties of ColE1 derivatives containing either deletions or insertions of transposable genetic elements, has enabled a functional map of plasmid ColE1 to be constructed.

ColE1, one of the smallest plasmid replicons studied in procaryotes, has been used extensively in studies of plasmid DNA replication (Tomizowa et al., 1977), gene expression (Tyler and Sherratt, 1975), and as a vector for the cloning of DNA fragments after in vitro genetic manipulation (Hershfield et al., 1974; Clarke and Carbon, 1976). Here we present some new data and collate results from elsewhere that allow a functional map of ColE1 to be constructed (Fig. 1).

We have been using transposons as 'genetic mutagens' to obtain insertion mutations in ColE1 and other plasmids (Dougan and Sherratt, 1977a, b). Such insertion mutations have distinguishable phenotypes and can be mapped by heteroduplex electron microscopy or by agarose gel electrophoresis after digestion with specific restriction endonucleases. In addition to studies of twelve ColE1: Tn*1* plasmids reported previously (Dougan and Sherratt, 1977a), we have now characterised a further seventeen transposon insertions into ColE1, including nine insertions of Tn*3* (Ap^R and about 85% homologous to Tn*1*) that were originally isolated and mapped by So et al. (1975), seven insertions of Tn*501* (Hg^R) (Stanisich et al., 1977) and one insertion of Tn*7* (Tp^R Sm^R) (Barth et al., 1977).

All transposon insertions that result in a failure to produce normal amounts of biologically active colicin E1 map between 0.84 and 0.92. Insertions into ColE1 at the site of cleavage by restriction endonuclease *Eco*RI also fail to produce detectable amounts of active colicin E1 (Hershfield et al., 1974). The colicin E1 protein itself has a molecular weight of 56,000 daltons, therefore approximately 27% of the ColE1 genome (4.2×10^6 daltons) is required to encode for this structural gene. Heffron et al. (1977) have shown that DNA sequences required for expression of immunity must lie between 0.02 and 0.08. We have isolated

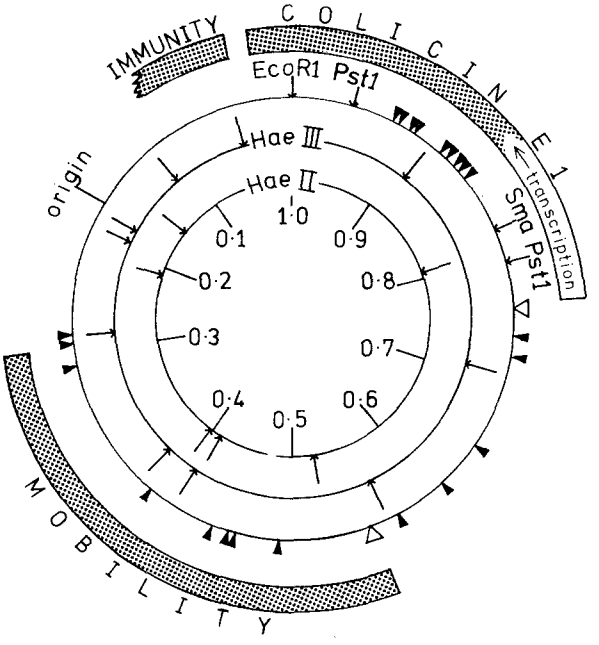

Fig. 1. Map of ColE1. Numbers represent the proportion of the ColE1 genome measured anticlockwise from the single *Eco*RI restriction site. The cleavage sites of restriction endonucleases are indicated by arrows (→). *Hae*II, *Hae*III, *Eco*RI, *Sma*I, and *Pst*I restriction endonuclease sites are included (Tomizawa et al., 1977; Oka and Takanami, 1976; Hershfield et al., 1974; Armstrong et al., 1977; Selker et al., 1977). The sites of known Tn*1* and Tn*3* insertions are represented by solid triangles (◀) on the outer circle; sites of Tn*501* insertions are shown by open triangles (◁). All transposon insertions were mapped by heteroduplex analysis as described previously (Dougan and Sherratt, 1977). ColE1 and ColE1::Tn DNA were mixed, denatured, and reannealed to form a proportion of heteroduplex molecules (see Fig. 2). In these, a single-stranded loop of unpaired transposon DNA projects from the site of insertion. RSF2124 (So et al., 1975) has been widely used as a cloning vehicle. It is a Mob^+ ColE1::Tn3 with the transposon inserted at 0.27 (shown here)

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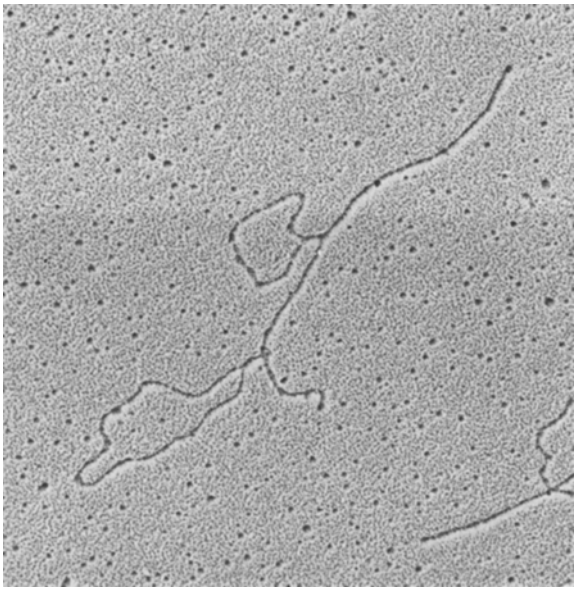

Fig. 2. Electron micrograph showing a heteroduplex between two ColE1::Tn Plasmids. The electron micrograph shows a heteroduplex between a ColE1::Tn*3* (map position 0.29) and a ColE1::Tn*501* (map position 0.56). The linear-molecules were produced using *Sma*I cleavage. These particular inserts are situated at the respective ends of the mobility region of ColE1, which appears as a double-stranded region between the two transposon loops. One can clearly see the terminal inverted repeat sequences as double-stranded stems and the size difference between the two transposons

a ColE1::Tn*501* plasmid which produces active colicin E1 and has the site of transposon insertion at 0.76. From this data the colicin E1 structural gene can be located across the *Eco*RI site somewhere between 0.76 and 0.08. Work by Meagher et al. (1977) using minicells has identified a polypeptide fragment (molecular weight 52,000) of the colicin E1 molecule, produced after insertion of DNA sequences at the *Eco*RI site. We have observed production of a polypeptide which could be a colicin fragment of molecular weight 32,000 daltons in minicells containing ColE1 plasmids with insertions of Tn*1* or Tn*3* at 0.90 (Dougan and Sherratt, 1977a and unpublished). These results give a position and orientation for the colicin E1 structural gene: it must be encoded between 0.77–0.78 and 0.04–0.05 with transcription and translation anticlockwise on the map. Interestingly, of eleven transposition insertions into the colicin E1 structural gene examined, all map within a small region of the gene between 0.84 and 0.92. The reason for this is unknown at present.

We have previously described the properties of a number of ColE1::Tn*1* plasmids which have lost the ability to be mobilised during conjugation by a conjugative plasmid (Dougan and Sherratt, 1977a; Warren and Sherratt, 1977). We are now in a position to define a region on ColE1 in which all insertions result in the production of plasmids which can no longer be mobilised (Mob^-). Only insertions that map between 0.28 and 0.56 produce a Mob^- phenotype. Insertions that map elsewhere are phenotypically Mob^+. One end of this 'mobility' region is defined by two Mob^+ Tn*3* insertions at 0.27 and a Tn*3* insertion at 0.29 which is Mob^-. All insertions that map between this Mob^- Tn*3* insertion and a Tn*501* insertion at 0.56 are Mob^-. A Tn*1* insertion at 0.56 is Mob^+. All the phenotypically Mob^- ColE1 transposon-containing derivatives we have tested are complemented in trans for their mobility deficiency (Warren and Sherratt, 1977) and exhibit a reduction in the level of plasmid protein/DNA relaxation complex (Clewell and Helinski, 1969). This correlates well with data published for ColE1::Tn*3* plasmids isolated by Inselberg (1977). This strengthens the possibility for a role for relaxation complex in ColE1 mobilisation. Consistent with this is the observation that the site of the nick introduced by relaxation complex is some 300 nucleotides anticlockwise of the origin of DNA replication (Tomizowa et al., 1977): the former site could be a distinct transfer origin, as found in a number of conjugative plasmids. ColE1 derivatives containing deletions extending into the mobility region (e.g. pMB8 and pVH51 and their derivatives) are also Mob^-, complementable in trans, and have little or no relaxation complex (Warren and Sherratt, 1977; Hershfield et al., 1976).

Insertions that map between 0.58 and 0.76 produce no detectable alterations in phenotype. Either this is a non-essential region or it encodes for an unknown function. In contrast we have so far been unable to isolate an insertion of any transposon, (and know of no other transposon insertions), between 0.92 and 0.26. This region is known to carry the origin of replication as well as gene(s) for immunity to colicin E1. Insertions into this region may be 'lethal' to the plasmid and/or the cell. However DNA can easily be inserted at the *Eco*RI site (1.0) (Hershfield et al., 1974): perhaps relevant to this is our preliminary observation that transposon insertions are more highly polar than many insertions at the *Eco*RI site obtained after in vitro genetic manipulation.

As yet we are able to say little about control of ColE1 replication and control of colicin synthesis. All ColE1::Tn plasmids tested maintain the plasmid at a normal level (fraction of total DNA as plasmid) though some show a reduced ability to replicate in chloramphenicol (Dougan and Sherratt, 1977a). Similarly all ColE1::Tn plasmids that have an intact colicin gene are able to synthesize colicin normally both spontaneously or after mitomycin C treatment. As

yet we do not know if expression of plasmids with insertions in the colicin gene is derepressed after treatment of cells with mitomycin C. Selker et al. (1977) have shown that functional expression of the *S. typhimurium trp*A gene inserted at the *Eco*RI site of ColE1 can be derepressed after mitomycin C treatment. We propose that such expression originates at a promoter adjacent to the colicin gene as it has been shown that mitomycin C treatment of cells containing ColE1 results only in the derepression of colicin E1 (and possibly immunity protein) and not other ColE1-specified proteins (Tyler and Sherratt, 1975; Sherratt unpublished).

In conclusion it is clear that ColE1 possesses definable functional regions. We are at present studying the expression of these plasmids, as well as other ColE1 derivatives, in minicells in the hope that we will be able to identify and characterise the proteins produced from these regions.

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Note Added in Proof

Only 10 of the 14 *Hae*III sites are shown in Figure 1. There are three additional sites between 0.25–0.37, and one between 0.54–0.70 (Tomizowa et al. (1977)).