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Short Communication

Structure and Properties of the Region of Homology between Plasmids pMB1 and ColE1

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Summary. Physical maps of the two independently isolated *Escherichia coli* plasmids, pMB1 and ColE1, were prepared with 13 restriction endonucleases and compared. A 5.1 kilobase continuous region covering 55% of pMB1 and 75% of ColE1 was found to have similar, but non-identical, restriction maps. The differences in the maps of this region probably arose by localized mutational events rather than by major sequence rearrangements. The F-factor was found to mobilize pMB1 efficiently for conjugal transfer. A region on pMB1 required for its F-mediated transfer was mapped. Results of our study combined with results of other investigators suggest that pMB1 and ColE1 share functional properties such as colicin production, colicin immunity, mode of replication, and mobilization by the F-factor, and that the sequences required to code these functions are contained within the 5.1 kilobase homologous region.

Plasmids with similar properties have been frequently isolated from different bacterial strains and species. The physical relationship between such related plasmids has been mainly studied with one or more of the following techniques-quantitation of hybridization within heteroduplexes by radioisotopic or spectroscopic means, electron microscopic visualization of heteroduplexes, and comparison of restriction endonuclease digestion patterns on electrophoretic gels. Such studies have contributed greatly to plasmid classification and understanding of plasmid evolution (see Broda 1979, for a review).

The two independently isolated *E. coli* plasmids, ColE1 and pMB1 (Bazaral and Helinski 1968; Betlach et al. 1976), present an attractive system for a detailed analysis of plasmid evolution. These plasmids are small (6.8 kb and 9.3 kb, respectively) and share functional properties such as colicin production, conferring immunity to colicin, and the mode of replication. According to Betlach et al. (1976), the major difference between the two plasmids is the existence of an extra segment of DNA on pMB1 which codes for the *Eco*RI restriction endonuclease and the methylase. In order to localize the homologous regions on the two plasmids, we prepared detailed restriction maps of the two plasmids and compared them.

Composite maps of the two plasmids are presented in Fig. 1. Among the 17 enzymes tested, *Bam*HI, *Bgl*I, *Sst*I and *Xho*I did not cut either plasmid. Using the remaining enzymes, a total of 19 sites were mapped on ColE1, while 39 were mapped on pMB1. Although some maps of the two plasmids are available (Bolivar 1978; Staudenbauer 1978), we have greatly extended these maps and also clarified some ambiguities in them.

Sixteen of the 18 distinct restriction sites mapped on ColE1 also appear on pMB1 in the same relative orientations and distances. These are indicated by double-headed vertical arrows in Fig. 1. They span a 5.1 kb region, hereafter referred to as the 'region of homology'. This assignment of homology is based not only on an overall comparison of the two restriction maps, but also on a comparison of the sizes of restriction fragments from the two plasmids on agarose gels.

Figure 2 is representative of such an analysis. Bands indicated by lower-case letters (a–h) identify the fragments from the two plasmids with identical (or nearly so) mobilities on the gel. The positions of these fragments on the restriction maps are indicated at the bottom of Fig. 1. As the sizes of most restriction fragments on agarose gels could be determined only within ± 25–50 bp, these pairs of fragments may not be exactly equal in size. However, based on our data (Fig. 2 and unpublished) we estimate that the two plasmids do not differ by more than 50 base pairs in length within any 1 kb of this region. In other words, no major structural rearrangements such as addition/deletions or inversions are in evidence anywhere within this region.

The restriction maps of the region of homology between the two plasmids are not identical. Seven restriction sites within this segment appear on only one of the two plasmids. These have been indicated by asterisks in Fig. 1. These differences may have arisen by mutating a minimum of seven base pairs out of the 138 base pairs spanned by all the restriction sites mapped in this area. This suggests only about 5% of sequence mismatch between the two plasmids, within a region of overall homology. Thus the major source of evolution for this region may be point mutations.

Functions such as colicin production, colicin immunity, replication functions and mobilizability by the F-factor have been mapped on ColE1 (Dougan et al. 1978). The positions of sequences essential to various ColE1 functions are indicated in Fig. 1. It can be seen that the region of homology between the two plasmids includes all the sequences required for these functions in ColE1. As pMB1 is also known to code for colicin production and immunity and has a mode of replication similar to that of ColE1 (Betlach et al. 1976), it is reasonable to assume that sequences required for these functions on pMB1 lie in the same relative position and orientation as on ColE1. It has, however, not been demonstrated that pMB1 is mobilized by the F-factor or any other conjugative plasmid.

In order to study the mobilization of pMB1 conveniently, we constructed a recombinant of pMB1 (pJL17) containing the

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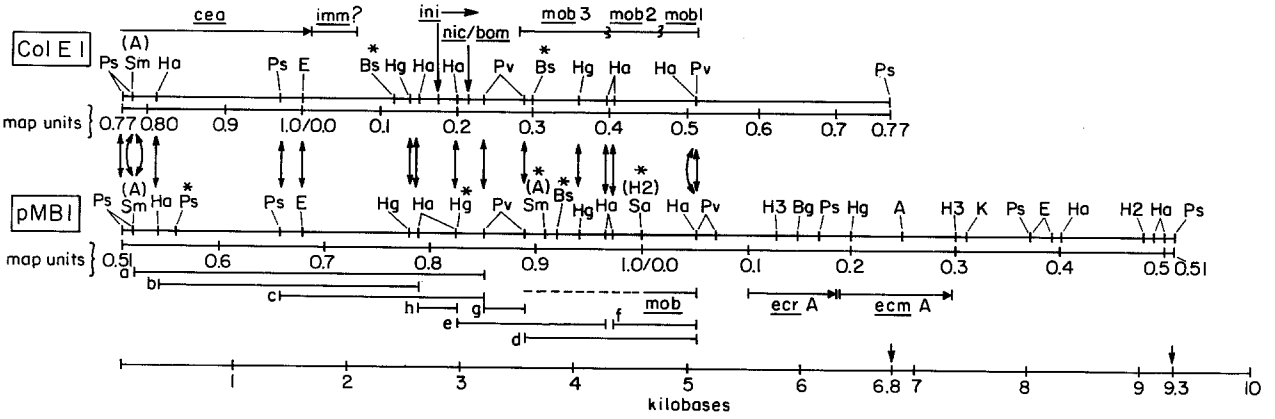

Fig. 1. Restriction maps of pMB1 and ColE1. The plasmids have been linearized for convenient comparison. Positions of most restriction sites have been rounded off to the nearest 1% of the map distance. Map assignment on ColE1 for the colicin production (*cea*) and colicin immunity (*imm*) genes and the initiation of replication (*ini*) are according to Oka et al. (1979). Position of the *nic/bom* site is according to Bastia (1978) and the *mob* gene positions are according to Inselburg and Ware (1979). The *mob* region on pMB1 has been mapped in this study. The positions of *Eco*RI endonuclease (*ecrA*) and the methylase (*ecmA*) were determined by P. Modrich and his colleagues (personal communication). *Hae*II sites at 0.49 and 0.50 on pMB1 are tentative. The region between 0.4 and 0.54 on this plasmid may contain additional *Hae*II sites. Horizontal bars labeled a–h at the bottom left refer to fragments identified in Fig. 2. Key to restriction enzymes: A = *Ava*I, Bg = *Bgl*II, Bs = *Bst*EII, E = *Eco*RI, Ha = *Hae*II, Hg = *Hgi*AI, H2 = *Hind*II, H3 = *Hind*III, K = *Kpn*I, Ps = *Pst*I, Pv = *Pvu*II, Sa = *Sal*I, Sm = *Sma*I

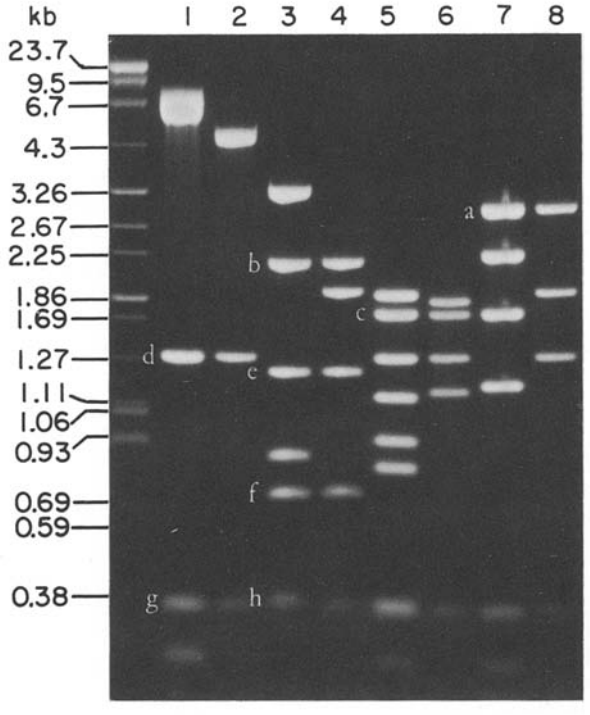

Fig. 2. Comparison of pMB1 and ColE1 restriction fragments. Products of single or multiple digestions of pMB1 and ColE1 were electrophoresed on a 1.2% agarose gel. Channels 1, 3, 5 and 7 contain pMB1 DNA, while the rest contain ColE1 DNA. The enzymes used in the digests were: Channels 1 and 2. *Pvu*II; 3 and 4, *Hae*II; 5 and 6, *Pst*I + *Pvu*II; 7 and 8, *Ava*I + *Pvu*II. The molecular weight markers were derived from λ DNA or pBR322 DNA. Bands marked a–h are pMB1 and ColE1 fragments of identical mobilities. Their positions on the restriction maps are indicated in Fig. 1

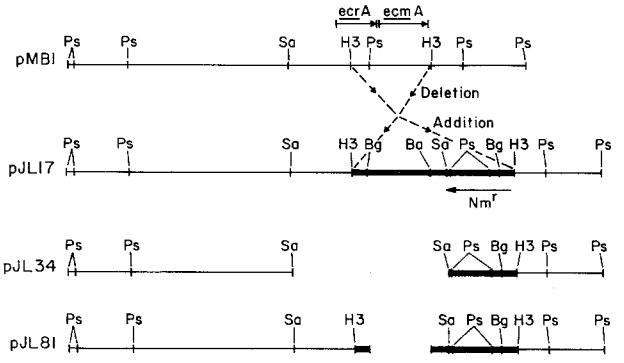

Fig. 3. Derivatives of pMB1. The source of the neomycin resistance gene (Nm^r) was a *Hind*III fragment from pRZ102 (ColE1::Tn5) (Jorgensen et al. [1979]). The recombinants were prepared by standard methods. The Tn5-derived sequences within the plasmids are indicated by heavy lines. The extents of deletions within pJL34 and pJL81 compared to their parent, pJL17, are indicated by gaps within their maps. Key to enzymes: Ba = *Bam*HI, Bg = *Bgl*II, H3 = *Hind*III, Ps = *Pst*I, Sa = *Sal*I

neomycin-resistance gene from Tn5 (Fig. 3). pJL17 was found to be mobilized efficiently by an F′ *lac pro* (Table 1). In order to locate the sequences on pJL17 necessary for its mobilization, two deletion mutants (pJL34 and pJL81) were constructed (Fig. 3). Both plasmids confer neomycin resistance on their hosts. pJL81 is mobilized efficiently by the F′ *lac pro*, while mobilization of pJL34 is reduced by more than 100-fold (Table 1). These data suggest that the mobilization properties of pJL17 are not due to the Tn5-derived sequences but are due to the pMB1 sequences and that the sequences of pMB1 required for the mobilization are in a region homologous to the *mob* region of ColE1. Within the *mob* region of ColE1, the two plasmids differ in four out of 10 restriction sites. Apparently, these mutations have not resulted in different phenotypes for the plasmids.

Thus, the 5.1 kb homologous region is most likely derived from the same ancestral sequence and codes on both plasmids for colicin production, colicin immunity, replication functions, and mobilization by the F-factor. Among these functions, one is essential (replication), while the other three are not essential (colicin production, colicin immunity, and mobilization). While the colicin production and immunity functions confer a survival

Table 1. Mobilization frequencies of pMB1 derivatives

Plasmid	% mobilization
pJL17	61
pJL34	≦0.4
pJL81	59

Mobilization frequencies were determined by a protocol similar to that of Warren et al. (1979). The donor strain was CSH41 (F′*lac pro*, Strep^s) with the appropriate plasmid, and the recipient was RR1 (Strep^r, Pro$^-$). Percent mobilization = 100 × (No. of Pro$^+$ Strep^r Nm^r cells)/No. of Pro$^+$ Strep^r cells

advantage upon their host-cell species, the mobilization functions allow for a spread of the plasmid through bacterial population. It is, therefore, not surprising that all these functions have been conserved during the separate evolutions of the two plasmids.

Outside the region of homology, the restriction maps of the two plasmids are completely different. Thus, no basis for the alignment of the plasmids outside the 5.1 kb region of homology exists. If a random alignment of the plasmid regions is made, then 5 out of 5 to 8 out of 8 restriction site differences are found. It seems clear that the two plasmid sequences are widely different here.

Smith et al. (1976) have reported isolation of a plasmid (NTP13) with a size (9.5 kb) and properties similar to those of pMB1. It codes for colicin production and immunity, has a mode of replication similar to ColE1, and carries the *Eco*RI endonuclease and methylase genes. These authors performed DNA-DNA reassociation experiments to determine the extent of homology between ColE1 and NTP13. They found that about 75% of ColE1 was homologous to about 61% of NTP13 (Smith et al. 1976). Our estimate that 75% of ColE1 is homologous to about 55% of pMB1 is consistent with these results, if NTP13 and pMB1 are one and the same plasmid. This is probably true, not only because of the size and functional similarities of the two plasmids, but also because they originated in the same *E. coli* strain (Betlach et al. 1976; Smith et al. 1976).

Betlach et al. (1976) created heteroduplexes between *Eco*RI-digested ColE1 and the large *Hind*III fragment of pMB1. Electron microscopic examination of these heteroduplexes suggested that all of ColE1 was homologous to pMB1. This is in conflict with our observation that about 25% of ColE1 (1.7 kb) has no sites common with pMB1. If NTP13 is pMB1, then the results reported by Betlach et al. (1976) are also in conflict with the results of Smith et al. (1976).

Finally, two more comments concerning the mobilization properties of pMB1 seem appropriate. (1) Comparison of restriction maps suggests that the *nic/bom* region of pMB1 should be on pBR322. Thus pBR322 should be *bom*$^+$ *mob*$^-$: indeed this has been shown to be the case by Twigg and Sherratt (1980) (2) As one of the domains required for R751 or R388-mediated mobilization of ColE1 lies outside the region of homology between pMB1 and ColE1 (Warren et al. 1979), pMB1 may not be mobilized by these R-factors.

Nomenclature of Genes of Type II Restriction Endonucleases and Methylases

We have used the symbols *ecrA* and *ecm*A for the *Eco*RI restriction and modification genes, respectively. These three-letter designations have been adapted from the suggestion of Smith and Nathans (1973) on the nomenclature of restriction enzymes. They indicate the source of the plasmid (*E. coli*), distinguish between restriction and modification genes (*r* or *m*), and allow ordering of the genes (*A, B, C,* etc.) as more genes are found in the same bacterium. We feel that they are likely to be more informative and less ambiguous than the designations *hsr* and *hsm* suggested by Novick et al. (1976).

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