The Role of Gene Rearrangement in Evolution

Saedler, H., Ghosal, D., Nevers, P.

Institut für Biologie III, Schänzlestr. 1. 7800 Freiburg, Federal Republic of Germany

Chromosomes are commonly regarded as conservative structures, in which an exact amount of genetic information is arranged in a definite sequential order. This order is normally preserved when information is exchanged between chromosomes, and guaranteed by a set of recombination enzymes that function only with paired sectors of homologous DNA. But processes such as inversion, deletion, duplication and translocation, often involving recombination between apparently non-homologous chromosomal regions, can alter this sequential order. Chromosomal rearrangements resulting from such events have been observed, in some cases with a disturbingly high frequency, thus inviting speculation about their biological significance. The processes involved have been termed collectively "illegitimate recombination", reflecting our bias for conventional pathways based on sequence homology, but evidence is accumulating which may legitimatize them as important aspects of evolution or even differentiation.

The presence of IS elements in the chromosome of E. coli was originally revealed by the transposition of these DNA elements form their natural positions into indicator systems, resulting in a recognizable mutant phenotype. If, for example, the gal operon is used as an indicator system, mutations can be isolated which are caused by the integration of an IS element into one of the three structural genes of the operon. Not only is the thus interrupted structural gene inactivated, but the expression of the promoter distal genes is also abolished. These mutations therefore are strongly polar. The analysis of the nature of such mutations has been facilitated by the isolation of gal transducing phage and the development of techniques for examining heteroduplex DNA in the electron microscope. With help of these tools it is possible to inspect hybrid DNA molecules consisting of one DNA strand carrying the strongly polar mutation paired with the complementary strand of λ dgal in the electron microscope. The strongly polar mutation is seen as a single stranded DNA loop emerging from a position in the double stranded heteroduplex molecule which corresponds to the map position of the mutation. Analysis of various independently isolated strongly polar mutations with the above technique revealed the existence of different categories of IS elements. The elements were numbered according to the order of their detection. IS1 is about 800 nucleotide pairs long, while IS2, IS3, IS4 and IS5 are each approximately 1400 basepairs long. (For review see Starlinger and Saedler, 1976.) In the following paragraphs we will concentrate on the topics listed below.

A. Chromosomal rearrangements mediated by IS1 (Reif and Saedler, 1975, 1977; Nevers, Reif and Saedler, 1977; Nevers and Saedler, 1978)
B. IS elements found in strategical positions on certain plasmids (Hu et al., 1975)
C. Detection of mini-insertion elements (D. Ghosal and H. Saedler, 1977)

A. Chromosomal Rearrangements Mediated by IS1

IS1 a known to occur in multiple copies in the chromosome of E. coli K12 (Saedler and Heiss, 1973). They also seem to be integral parts of at least some bacterial plasmids like F^+ and R (Hu et al., 1975).

The formation of new chromosomal sequences can result from translocation, duplication, inversion or deletion of genetic material. All these events seem to play a role in the evolution of plasmids as well as chromosomes. IS-elements appear to be responsible for some such chromosomal rearrangements.

Non-adjacent chromosomal regions can be brought together by deletion of the intermittent genetic material, resulting in a new chromosomal order. This reaction has been studied extensively in IS1 induced deletion formation (Reif and Saedler, 1975, 1977). The termini of the integrated IS1 elements are most important in this process. IS1 is retained in the deletion, thus allowing further rounds of rearrangements. IS1 can be considered as a generator for deletions, sometimes fusing the structural genes of the gal operon to other promoters and thus creating a new control circuit (Reif and Saedler, 1977). It is not yet clear, however, which enzymes are involved in this rather unusual type of recombination. Apparently the normal recombination pathways of E. coli are not involved. However, recently mutants were isolated which are deficient in IS1 induced deletion formation. Such mutants may be helpful in the analysis of the enzymes involved in illegitimate recombinational events (Nevers and Saedler, 1977).

B. IS Elements Are Also Found in Strategical Positions on Certain Plasmids

The R-factors of the fi^+ class are composed of two units, each capable of replicating autonomously if dissociated from each other. The RTF unit codes all functions necessary for cell to cell contact, thus allowing the transfer of the plasmid. The r-determinant carries most of the antibiotic resistance genes. An IS1-element separates the RTF unit from the r-determinant at each junction. Both IS1 elements are oriented in the same direction (Hu et al., 1975; Ptashne and Cohen, 1975). This finding suggests a model to explain the formation and dissociation of R-factors as well as the amplification of the antibiotic resistance genes. Rownd and Mickel (1971) showed that R-factors can dissociate into the RTF and the r-determinant in Proteus mirabilis. Dissociation may occur by recombination between the two homologues IS1 substrates of the co-integrate plasmid generating two units, each containing an IS1.

Fusion results from the reverse reaction. Amplification of antibiotic resistance genes could be due to recombination between the homologous IS1 elements of different r-determinant molecules, leading to co-integrate plasmids containing multiple copies of the r-determinant units.

In addition to IS1 other IS-elements are also observed on R-factors, either as mutations or as integral parts of the molecule. For example in R6 of R 100-1 IS2 is found at a position within the transfer genes at which it does not cause a transfer defective mutation but rather contributes to the transfer positive character of the plasmids (Hu et al., 1975). Many of the antibiotic resistance genes can transpose to the various other DNA molecules (Cohen and Kopecko, 1976). At least one of the transposons is flanked by a known IS element (Mac Hattie and Jackowski, 1977).

In the evolution of R-plasmids, IS-elements therefore seem to play an important role.

C. Detection of Mini-Insertion DNA Elements

The detection of IS elements using the heteroduplex technique is limited by the size of the integrated DNA element. If, for example, an IS element is an order of magnitude smaller than IS1 to IS5, it cannot be readily recognized as a single stranded loop using the heteroduplex technique. To analyse very small insertions another technique is more adequate. If suitable restriction fragments are available, one containing an integrated mini-insertion and the same fragment without, they will band at different molecular weight positions when subjected to electrophoresis in agarose or polyacrylamide gels. In this manner two mutants were shown to be due to the integration of a very small piece of additional DNA. Fig. 1 gives the pattern of a Hind II, Hind III double digest of various plasmid DNAs. Slot 3 shows the pattern of the parental plasmid pDG1, which is Gal positive. The pattern of pDG12, in which an IS2 is integrated in the control region of the gal operon, eliminating expression of the gal genes, is presented in slot 4. Note the appearance of the new bands (e and f) and the shift in molecular weight of one band (from a to b), due to the integration of IS2. Slots 1 and 5 show the pattern of two independent Gal positive revertants obtained from plasmid pDG12. Note the increase in molecular weight of only band e in both mutants. This can only be explained by assuming that a small insertion is present in band e. Using appropriate markers (slot 2) as references, the increase in molecular weight can be calculated. Mutation 1 (slot 5) is due to the integration of about a 115 base-pair long piece of DNA, while the other mutation (slot 1) is about a 60 base-pair insertion. The former has been called IS6 and the latter IS7.

Both insertions confer a Gal positive phenotype to the cell carrying the plasmid. Since they seem to have integrated into IS2, they either destroy the polar signal on IS2 or, more likely, each carries its own turn-on signal.

Recently we sequenced both IS6 and IS7 and compared their DNA sequence of IS2 in the region of integration of these mini-insertions.

It is quite obvious that both IS6 and IS7 can be derived from IS2 sequences

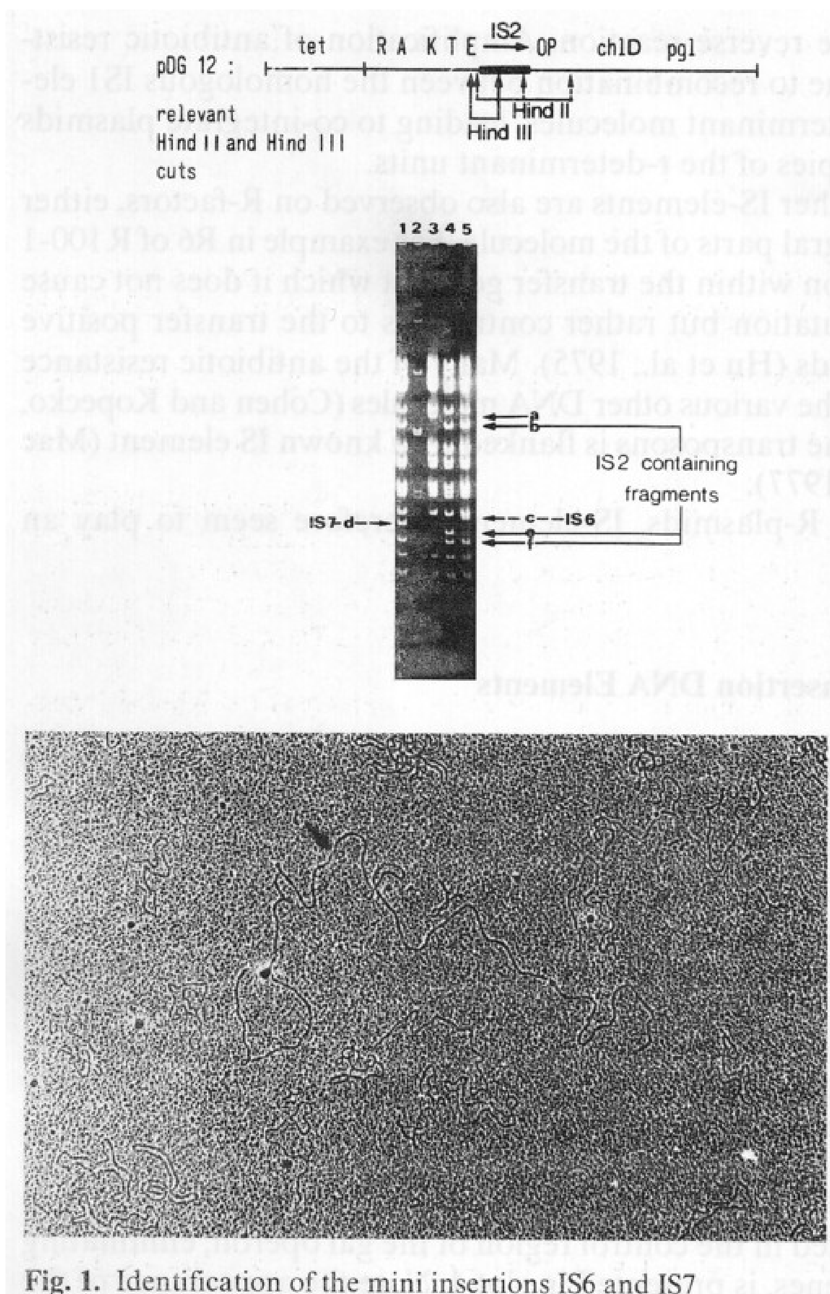

Fig. 1. Identification of the mini insertions IS6 and IS7

in a complicated manner. That is, genetic information from both DNA strands of IS2 seems to have multiplied and re-integrated in a rearranged form, resulting in the formation of a turn-on signal. (For detailed discussion see Ghosal and Saedler, 1978.)

Conclusions

IS elements are natural components of the E. coli chromosome. They can translocate from one position in the chromosome to another. Besides stimulating a number of illegitimate recombinational events, like deletion and transposition of other genes, which is thought to be of evolutionary importance, they also carry signals necessary for gene expression (Saedler et al.,

1974; Ghosal and Saedler, 1977, 1978). Similar events are also known to occur in higher organism (Nevers and Saedler, 1977).

References

Cohen, S.N., Kopecko, D.J.: Structural evolution of bacterial plasmids: Role of translocating genetic elements and DNA sequence insertions. Federation Proceedings **35**, 2031–2036 (1976)

Ghosal, D., Saedler, H.: Isolation of the mini insertions IS6 and IS7 of E. coli. Molec. Gen. Genet. **158**, 123–128 (1977)

Ghosal, D., Saedler, H.: DNA sequence of the mini-insertion JS2–6 and its relation to the sequence of IS2. Nature **275**, 5681 (1978)

Hu, S., Ohtsubo, E., Davidson, N., Saedler, H.: Electron microscope heteroduplex studies of sequence relations among bacterial plasmids. XII. Identification and mapping of the insertion sequences IS1 and IS2 in F and R plasmids. J. Bacteriol. **122**, 764–775 (1975b)

MacHattie, L.A., Jackowski, J.B.: Physical structure and deletion effects of the chloramphenicol resistance element Tn9 in phage λ. DNA Insertion Elements, Plasmids and Episomes, p. 219–228. Cold Spring Harbor Lab. 1977

Nevers, P., Reif, H.J., Saedler, H.: A mutant of E. coli defective in IS1-mediated deletion formation. DNA Insertion Elements, Plasmids and Episomes, p. 125–128. Cold Spring Harbor Lab. 1977

Nevers, P., Saedler, H.: Transposable genetic elements as agents of gene instability and chromosomal rearrangements. Nature **268**, 109–115 (1977)

Nevers, P., Saedler, H.: Mapping and characterization of an E. coli mutant defective in IS1-mediated deletion formation. Molec. Gen. Genet. **160**, 209–214 (1978)

Ptashne, K., Cohen, S.N.: Occurrence of insertion sequence (IS) region on plasmid deoxyribonucleic acid as direct and inverted nucleotide sequence duplications. J. Bacteriol. **122**, 776–781 (1975)

Reif, H.J., Saedler, H.: IS1 is involved in deletion formation in the gal region of E. coli K12. Mol. Gen. Genet. **137**, 17–28 (1975)

Reif, H.J., Saedler, H.: Chromosomal rearrangements in the gal region of E. coli K12 after integration of IS1. DNA Insertion Elements, Plasmids, and Episomes, p. 81–91. Cold Spring Harbor Lab. 1977

Rownd, R., Mickels, S.: Dissociation and reassociation of RTF and R-determinants of the R factor NR1 in proteus mirabilis. Nature Newbiol. **234**, 40–43 (1971)

Saedler, H., Heiß, B.: Multiple copies of the insertion DNA sequences IS1 and IS2 in the chromosome of E. coli K12. Molec. Gen. Genet. **122**, 267 (1973)

Saedler, H., Reif, H.J., Hu, S., Davidson, N.: IS2, a genetic element for turn-off and turn-on of gene activity in E. coli. Molec. Gen. Genet. **132**, 265–290 (1974)

Starlinger, P., Saedler, H.: IS elements in microorganisms. Current Topics in Microbiology and Immunology. Vol. 75, p. 111–152. Berlin, Heidelberg, New York: Springer 1976