Restriction endonucleases: a new role *in vivo?*

from Richard J. Roberts

FEW enzymes have been exploited as thoroughly as the bacterial restriction enzymes. In recent years they have been instrumental in dramatic advances in DNA sequence analysis, genetic engineering, and studies of gene structure and function. Yet surprisingly little is known of their biological role. It has become almost axiomatic to associate them with a defence mechanism by which bacteria insulate themselves against invasion by unwelcome foreign DNA. This view has its origins in the genetic phenomenon of host-controlled restriction and modification. Two enzymes mediate this process: an endodeoxyribonuclease (restriction enzyme) which degrades DNA, and a methylase (modification enzyme) which protects DNA against the action of the restriction enzyme. Because both enzymes coexist in the same cell, the cell's own DNA is protected by the action of the methylase, whereas incoming foreign DNA, which lacks the proper modification, is susceptible to destruction by the restriction enzyme.

Two different types of restriction enzymes have been found and can be distinguished by their mode of cleavage. Although both recognise a specific sequence within a DNA molecule, the Type I enzymes cleave at random sites remote from that sequence and give heterogeneous products, while the Type II enzymes cleave at specific sites within or close to the recognition sequence. It is these latter enzymes that have enjoyed such popularity as the molecular scalpels of the biochemist. Perhaps the best-known example is *Eco*RI from *Escherichia coli* which recognises the sequence, G↓AATTC, and cleaves at the site indicated by the arrow. It produces specific fragments which carry short, single-stranded extensions at each end allowing them to reanneal and become a substrate for a DNA ligase. Thus, any two *Eco*RI fragments can be joined, which has made possible the powerful techniques of genetic engineering.

But paradoxically, the very enzymes which are thought to prevent the exchange of DNA *in vivo* are precisely those which facilitate that exchange *in vitro*. Could it be that the restriction enzymes have a dual role *in vivo* and catalyse both degradation and synthesis just as they do *in vitro?* This possibility has been raised previously but until now it had not received serious attention. It seemed unlikely that bacteria could be as smart as molecular biologists, but apparently they are. For recent experiments by Chang and Cohen (*Proc. natn. Acad. Sci. U.S.A.* **74**, 4811; 1977) show that an *E. coli* strain carrying the *Eco*RI restriction enzyme can indeed mediate genetic engineering *in vivo*. In a series of elegant experiments, they have demonstrated that a variety of site-specific recombination events can be catalysed *in vivo*.

Their experiments centred around the fact that a chloramphenicol (Cm) resistance gene contains an *Eco*RI site located so that cleavage will inactivate the gene. Restoration of gene activity requires precise rejoining at the *Eco*RI site. It was first shown that such precise rejoining could be accomplished *in vivo* by transfecting *E. coli* with two *Eco*RI fragments, each containing a part of the Cm resistance gene. Chloramphenicol-resistant transformants were recovered. Then a plasmid, pSC352, was constructed in which the same Cm gene was inactivated by the insertion of an *Eco*RI fragment. Transfection of pSC352 into an *E. coli* strain (C600, pMB4) which contained the *Eco*RI restriction enzyme resulted in a few transformants (6×10^{-5} of all transformants) which had acquired Cm resistance. *In vitro* analysis showed that such transformants arose from precise excision of the inserted *Eco*RI fragment. In more extensive experiments, they showed that *Eco*RI promoted site-specific recombination could involve multiple and physically separate fragments of plasmid DNA. In each case, the plasmids used for transformation were prepared so as to be unmodified against the action of the *Eco*RI restriction enzyme. What if the sites are already modified? To answer this question, Chang and Cohen propagated pSC352 in *E. coli* C600 (pMB4), where it is modified by the *Eco*RI methylase, and then observed the frequency of excision of the inserted fragment under normal growth conditions. Chloramphenicol-resistant clones developed with a frequency of about 10^{-9}. In this case, therefore, even the presence of the correct modification enzyme within the cell was insufficient to prevent site-specific recombination.

Although the observed frequency of these events *in vivo* is extremely low, their importance should not be underestimated. They raise anew the question of the biological role of restriction enzymes, and have implications for the current heated controversy about *in vitro* recombinant DNA experiments. Because these observations were made in the laboratory, it is difficult to assess their relevance to processes which might occur in the natural environment. Nevertheless, even if they occur at the low frequency observed by Chang and Cohen, the magnitude of the bacterial population on the face of this Earth would still mean that the phenomenon could be of considerable importance to bacterial evolution. Such a possibility has been raised previously (Reanney *Bact. Rev.* **40**, 552; 1976). Clearly, it is necessary that experiments be carried out under conditions resembling those *in vivo* for if indeed bacteria routinely exchange genetic information in this way, such exchange would not be limited to DNA originating from prokaryotes. We would be forced to the conclusion that species barriers exist, not because of a physical barrier to the exchange of genetic information, but rather because a functional barrier prevails. If such is the case, it would surely provide the strongest argument against the strict regulation of recombinant DNA experiments *in vitro*.

This question of frequency has other profound implications. If the frequency can be raised in the laboratory, then it may be possible to construct recombinant DNAs in this way. Shotgun experiments might merely require transformation of the appropriate strain with intact DNA. In this way, both the biochemistry and the present regulations could be circumvented. Of course, ethical questions remain and, presumably, the next round of guidelines for recombinant DNA research will take account of these new observations. It will be of some interest to see how they are phrased. Perhaps all experiments involving recombination, whether *in vivo* or *in vitro*, will fall under their jurisdiction; this could include the whole of classical genetics. Even the most ardent bureaucrats will find it impossible to regulate recombination in the environment. Plants, insects, animals, do it all the time. The line may have to be drawn in the laboratory. There sex will require a Memorandum of Understanding and Agreement, while elsewhere it remains unrestrained. □

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A hundred years ago

THE New York *Tribune* gives an account of a public exhibition in that city of Eddison's Phonograph, which seems to have been very successful. The tones reproduced by the vibrating disk of the machine were so distinct that they could be heard and understood in different portions of the crowded room.

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