MOLECULAR BIOLOGY

Isolation of Genetic Repressors

In such a rapidly expanding and fiercely competitive field as molecular biology, comparisons are bound to be invidious, but few would deny that the outstanding achievements in the past 12 months have been the isolation of two repressors and the consequent confirmation of Jacob and Monod's model of genetic regulation.

A brilliant series of deductions led Jacob and Monod from their kinetic data on enzyme induction in *E. coli* to the operon concept and to a model of genetic regulation in which operon-specific repressor molecules prevent the expression of sets of genetic information—the operons—until these are required by the cell. The classic example is the negative control of the lactose operon which envisages a repressor molecule preventing the expression of three genes involved in lactose metabolism, until the inducer, normally lactose, is presented to the cell, whereupon the genes are derepressed, enzymes are made and the lactose metabolized. The simplest hypothesis to account for the action of inducer and repressor proposes that the repressor actually binds to the DNA, preventing transcription, and that the inducer by binding to the repressor somehow modifies it, perhaps by allosteric changes, so that it can no longer bind to the DNA.

Confirmation of this hypothesis, which has become central to much of molecular biology, awaited the isolation of a repressor molecule and there was understandably great excitement when Gilbert and Müller-Hill announced (*Proc. US Nat. Acad. Sci.*, **56**, 1891; 1966) that this had been done for the lactose operon repressor. The approach was elegant and direct, based on the minimal assumption that repressor and inducer interact. Gilbert and Müller-Hill reasoned that because an uninduced *E. coli* must contain some lactose repressor molecules, it should be possible to isolate them simply by fractionating the total cell protein. That done, the repressor fraction could be identified by its ability to bind inducer specifically and reversibly. The isolation of suppressible amber mutants of repressor genes had already shown that repressors are proteins, not nucleic acids. Gilbert and Müller-Hill made cell extracts of a mutant strain of *E. coli* with a lactose repressor which appeared to bind inducer more tightly than wild-type repressor, and after a crude fractionation they dialysed the fractions against a radioactive inducer, not lactose but an analogue. With minds sharpened by many months of negative results, they immediately grasped the significance of one fraction which bound inducer very slightly more than all the others above the background level. Further fractionation of this material yielded the repressor.

Two other mutant strains provided negative controls. First, Gilbert and Müller-Hill could not detect binding between their inducer and fractions from a strain of *E. coli* which always makes the lactose enzymes even in the absence of lactose. These cells evidently lack the repressor. Second, all the fractions from a mutant strain which has a modified repressor molecule and is not induced *in vivo* by the inducer they were using failed to bind the same inducer.

The lactose repressor has a sedimentation coefficient of 7 to 8*S*, and an estimated molecular weight of 150,000 to 200,000. It is a rather large protein. Gilbert and Müller-Hill also estimated that the repressor corresponds to only about one part in 10^4 of the total cellular protein. This gives an indication of the magnitude of their achievement in isolating it.

Closely following this success, Ptashne (*Proc. US Nat. Acad. Sci.*, **57**, 306; 1967), from the same Harvard laboratory, isolated the λ phage repressor which is responsible for completely repressing the entire phage genome, allowing the establishment of lysogeny, and for the immunity of lysogenic bacteria to superinfection. By taking advantage of a strain of *E. coli* which can, after massive ultra-violet irradiation, maintain phage protein synthesis even though its own protein synthesis is greatly reduced, and by making use of super-infection immunity, Ptashne isolated the λ repressor using column chromatography and a double-labelling technique. He identified the repressor fraction as such by showing that it is not made by λ phage with an amber mutation in the repressor gene and that phage with a temperature sensitive mutation in this gene make a modified form. The λ repressor is an acidic protein and, with a sedimentation coefficient of 2·8*S* and an estimated molecular weight of 30,000, it is much smaller than the lactose repressor.

Ptashne has gone on to show that the λ repressor does indeed bind to DNA (*Nature*, **214**, 232; 1967). A phage known as λ imm^{434} lacks a small region of the wild-type genome including both the repressor gene itself and the site which determines the sensitivity of the phage to λ repressor. In other words, λ imm^{434} neither makes λ repressor nor is repressed by it. The protein Ptashne had isolated and identified as the λ repressor binds to λ DNA but not to λ imm^{434} DNA. This simple experiment not only proves that the isolated protein is in fact the specific repressor but also strongly suggests that the repressor acts by binding directly to DNA and thus preventing transcription. Further support for this conclusion comes from Gilbert and Müller-Hill's demonstration that the lactose repressor binds to lactose operon DNA and moreover can be removed from the DNA by an inducer. Neither of these observations, of course, proves that the binding of repressor to DNA prevents transcription, but Gilbert and Müller-Hill are now in a position to show this unambiguously with *in vitro* experiments.

There is no doubt that this important work has opened the way to a complete elucidation of the molecular events that occur during genetic repression and induction.