news and views

Self-control by bacteriophage lambda

from a Correspondent

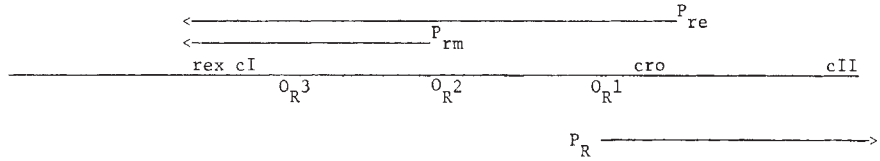
It has been known for some time that the control of repressor synthesis in bacteriophage lambda is not a simple phenomenon. Such are the intricacies of the regulatory circuits involved in fact, that the subject was largely confined to European laboratories. Now, much of the lambda DNA sequence containing the *cis*-regulatory elements of repressor synthesis has been deciphered (Waltz *et al.*, *Nature* **262,** 665; 1976; Ptashne *et al.*, *Science* **194,** 156; 1976), and we can all take a crack at explaining the mechanism.

Shortly after infection of a sensitive *E. coli* by lambda, large quantities of repressor protein are synthesised. This repressor is translated from message initiating at a promoter, P_{re}, located several hundred nucleotide pairs to the right of the repressor structural gene, *cI*. Transcription from P_{re} depends on two phage products, *cII* and *cIII* which are not synthesised in an established lysogen. The maintenance level of repressor, about 200 repressor monomers per cell, is much lower than the infection level and depends on the activity of a different promoter, P_{rm}, located close to *cI*.

Transcription from P_{rm}, like transcription from P_{re}, is not constitutive. Repressor can be inactivated and its rate of reappearance measured over a long period. What is found is that, once a cell has lost repressor, it is very slow to resynthesise it. Since a prophage makes but two phage products, repressor and Rex, it would appear that one of these is responsible for maintaining the activity of P_{rm}. The experiments of Waltz *et al.* and Ptashne *et al.* demonstrate that repressor is its own inducer.

By the use of restriction endonuclease DNA fragments containing the *cI* control elements, the control of repressor expression can be shown *in vitro* at the level of mRNA synthesis. If the fragment is incubated with RNA polymerase, no transcription of *cI* is observed. Instead, all the transcripts initiate at the P_R promoter—the rightward promoter which is active in lytic phage development but repressed in a lysogen. Addition of a small amount of repressor to the incubation mixture turns off P_R transcription and activates *cI* transcription from P_{rm}. If large quantities of repressor are added, all transcription from the fragment is eliminated, including *cI*.

A simplified map of the control region is given below.



To the right of *cI* are three operators, or repressor binding sites, O_R1, O_R2 and O_R3. These sites are 17 base pairs long and are separated by spacers 3 to 7 base pairs long. They differ slightly in sequence and function from each other; O_R1 binds repressor more strongly than O_R2 or O_R3. Sequences recognised by RNA polymerase for rightward transcription, P_R, are located within O_R1 and O_R2. Repressor, by binding to these operators, prevents RNA polymerase from binding to P_R. O_R3 is not required to repress P_R; its role is to regulate the synthesis of repressor in lysogens. The control region displays a symmetry centred around the middle of O_R2, such that O_R3 contains a sequence similar to P_R, but with the opposite orientation. This is P_{rm}.

The sequence of the control regions from mutant phage have been obtained and support these assignments. Mutations in O_R1 or O_R2 destroy their capacity to bind repressor. A mutation located between O_R1 and O_R2 inactivates P_R, and a base change in the spacer between O_R3 and O_R2 destroys P_{rm}.

Speculating on the control of repressor synthesis on the basis of the DNA sequence we can see that the sequences of P_{rm} and P_R are not quite identical. This could explain why an additional factor, repressor for example, is required for P_{rm} activation. Moreover, the P_{rm}-promoted mRNA begins with AUG, and the codon corresponding to the amino terminus of repressor is found immediately adjacent to this triplet. Thus the P_{rm} message contains no leader sequence, hitherto thought to be essential for ribosome binding. This is not the case for the mRNA initiated at P_{re}, which carries the sequence GGTGAT. Since, under optimal conditions, P_{rm} and P_{re} are both very efficient promoters, the low level of repressor in a lysogen is due to inefficient translation of the P_{rm} mRNA, presumably because it is leaderless.

Unfortunately, it is still not obvious how repressor stimulates *cI* transcription. Perhaps P_{rm} competes with P_R for RNA polymerase, and the role of repressor is to block the binding of RNA polymerase at P_R. If this were the case, we would expect that mutations which reduce the efficiency of P_R as a promoter would relieve the repressor requirement of P_{rm}. Mutations isolated so far in P_R do not have this character. Alternatively, repressor bound at O_R1 may interact with RNA polymerase to stimulate directly its binding at P_{rm}. Ptashne notes that the distance between O_R1 and the initiation of *cI* transcription is the same as the distance between the start point of *lacZ* transcription and the site in the *lac* operon where the postive-control cyclic AMP binding protein interacts.

With either model, repressor synthesis in a lysogen will be negatively regulated at O_R3, where repressor protein competes with RNA polymerase for binding to DNA. The interplay of these positive and negative control circuits assures that the maintenance level of repressor will be confined to narrow limits.

The ability to isolate and sequence mutations in regulatory regions is certain to be tested again at other interesting lambda loci; notably at P_{re} and at the sites of action and recognition of the lambda control functions *N*, *Q*, *cII*, and *cIII*. □