Complementary Transcription

from our Molecular Genetics Correspondent

It is by now a dogma of molecular biology that only one strand of DNA is transcribed into RNA at any given point. But in a study by RNA–RNA hybridization of sequences transcribed from lambda DNA, Spiegelman *et al.* (*Proc. US Nat. Acad. Sci.*, **69**, 3156; 1972) have found that one gene of the phage is transcribed in opposite directions at different times during infection of a bacterial cell.

The impetus for these experiments came from the observation that different systems of the phage seem to be involved in first establishing and then later maintaining lysogeny, when the lambda DNA is kept in an inactive state within the *Escherichia coli* cell by the action of its repressor protein. This protein is specified by the *cI* gene of the phage, but mutants in three other loci, *cII, cIII* and *cY*, are in general unable to establish lysogeny. But on the rare occasions when they do so, they maintain this condition. Complementation tests show that *cII* and *cIII* mutants make diffusible products, but that *cY* has the *cis*-dominant characteristic of an operator or promoter site, which regulates transcription.

By directly assaying the production of repressor protein in infected cells, Reichardt and Kaiser (*Proc. US Nat. Acad. Sci.*, **68**, 2185; 1971) and Echols and Green (*ibid.*, 2190) found that *cII*, *cIII* and *cY* mutants accumulate greatly reduced amounts of repressor after infection of host cells. In infection by wild type phages, little repressor is made for the first 5 min, after which there is a rapid rate of synthesis for 5 or 10 min, which in turn is succeeded by the low level of synthesis found in lysogenic cells. This low level of synthesis of repressor continues in lysogenic cells where the prophage lambda is mutant in *cII*, *cIII* or *cY*, and this supports the inferences from previous genetic studies.

By assaying the content of repressor protein of cells lysogenic for a temperature sensitive mutant in the *cI* gene, Reichardt and Kaiser found that the presence of active repressor protein seems to be required for its continued (low level) synthesis in lysogens, although it is not required for the establishment of lysogeny in infected cells. Mutants in an operator located immediately adjacent to the *cI* gene cannot make repressor; this operator is usually used to control transcription in the direction away from the *cI* gene (to the right), but may also have the dual function of comprising a site where repressor protein acts to stimulate its own synthesis in the leftward direction.

The model suggested by these studies was that when lysogeny is being established, the *cI* gene is transcribed in the presence of the *cII* and *cIII* functions from a point identified by the *cY* mutation. This means that transcription must proceed through the gene *cro* which lies between *cY* and *cI* and is immediately adjacent to the right side of *cI* on the lambda map. (The order of sites in this region is *cI—cro—cY*.) When lysogeny is established and requires only to be maintained, the *cI* gene is instead transcribed, in the presence of active repressor protein, from a site immediately to its right, that is between *cI* and *cro*. Spiegelman *et al.* now confirm this model biochemically.

Spiegelman *et al.* took advantage of a mutant of lambda which contains only a small part of the phage including the *cro* gene. Labelled RNA from cells lysogenic for this phage should contain the sequences transcribed from *cro* in the usual way; this gene is normally transcribed to the right from a site between *cI* and *cro*. Cells which are in the process of establishing lysogeny, however, should contain molecules of RNA transcribed from the opposite strand of DNA; these will bear a leftward transcript of *cro* at one end and a transcript of *cI* at their other terminus. Cells which are already lysogenic for lambda should lack this long messenger and should have only the short messenger corresponding to *cI* itself. In the first case, the RNA of these cells should anneal with the labelled test preparation; in the second it should not. Using this assay, Spiegelman *et al.* found that the production of the leftward transcripts of *cro* parallels the production of repressor when cells are establishing lysogeny, but is not found at later stages of development.

This means that transcription of the *cI* gene may start from either of two points, separated by the *cro* gene on the phage, depending on whether lysogeny is to be established or maintained. This allows different control systems to act on transcription of the one gene and is presumably necessary to ensure precise timing of synthetic activities in infected cells. Because the startpoint for transcription in the first case is at the far side of a gene (*cro*) usually transcribed in the opposite direction, this gene must be transcribed twice; once (when it must be meaningless) in the wrong direction and then, when the *cro* protein product is needed, in the correct direction. The long messenger with the incorrect transcript of *cro* is presumably translated only in its later part, which implies that the mechanisms for attaching ribosomes to messengers may be changed when phage lambda is establishing lysogeny in *E. coli*. Because the *cro* protein itself has a regulatory function and helps to control repressor synthesis from *cI*, these control circuits make an intricate pattern. Perhaps it is the small size of the lambda genome which is responsible for its making such intensive use of regions of DNA where each strand may be used for a different purpose and control sites may be used in an asymmetrical manner for controlling transcription in either direction.

α-KERATIN

Molecular Fragment Isolated

from a Correspondent

A MOLECULAR fragment has been isolated from α-keratin and shown by a variety of techniques to have the coiled α-helical conformation (Suzuki *et al.*. *J. Mol. Biol.*, **73**, 275; 1973).

α-Keratin has remained an enigmatic protein. Biologically it is a crucial material for terrestrial mammals and historically it has played a central part both in the recognition of the standard molecular conformations adopted by proteins and as a substrate for developing techniques. It is, however, an exceedingly complex substance and has absorbed many man-hours of scientific effort only to reveal the extent of its complexity.

Several structural features of α-keratin have been recognized. Microfibrils of about 70 Å in diameter are visible in electron micrographs of thin transverse sections. These microfibrils are about 100 Å apart on a disordered lattice and are embedded in a matrix. The matrix is identified as the source of the sulphur rich protein fraction which can be isolated from α-keratin. A low sulphur protein fraction can also be isolated and is presumed to originate from the microfibrils. X-ray diffraction patterns from α-keratin show a modulation of the intensity in the high angle region attributable to the coiled α-helix; this modulation indicates that the coiled α-helical regions are packed into the

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