BACTERIOPHAGES

Differentiation of λ

from a Correspondent

DIFFERENTIATION is a stable change in the phenotype of a cell without an alteration of its genotype. It has long been thought of as an attribute of higher organisms, but in 1968 two groups of workers showed independently that in certain conditions, *Escherichia coli* λ lysogens can exist stably in one of two states, immune or non-immune.

E. coli lysogenic for wild-type bacteriophage λ are immune to superinfection with other λ phage. This immunity is caused by λ repressor, which binds to λ DNA and prevents transcription of all but a small portion of the genome. Normally if λ prophages are induced, for example, by heating a lysogen which contains a thermo-labile repressor, cell death rapidly ensues. But when mutations are present in viral genes whose functions are lethal to the cell, immunity can be lifted without killing the cell. Once immunity is lost, it is not regained when the temperature is lowered.

The lysogen has responded in a permanent way to a transient environmental change. The cell has not lost the prophage and, in fact, no mutations can be demonstrated in the structural gene for repressor, the C_I gene. Because the prophage in the non-immune cell is genetically identical to the prophage in the immune cell—the lysogen is genetically capable of producing repressor but does not—the cell has differentiated. And, in further analogy to higher systems, occasional rare cells dedifferentiate spontaneously, forming colonies immune to superinfection. These, in turn, can be heated to destroy repressor, and colonies form which are, once again, stably non-immune.

Early last year, it seemed as though the mechanism of this curious phenomenon could easily be explained when Eisen *et al.* showed (*Proc. US Nat. Acad. Sci.*, **66**, 855; 1970) that the non-immune cells contain a phage gene product which prevents the re-establishment of immunity. Mutants in this gene, named *cro* (for amusing but irrelevant reasons), do not differentiate. Heated cultures of *cro* lysogens do not remain non-immune, but regain their immunity over a relatively short period of time after cooling; in about 20 min the level of repression reaches about half that of fully repressed levels. It seemed that a simple two-element regulatory circuit could account for everything. Repressor prevents the expression of the *cro* gene; thus immunity is a stable feature of lysogens. Expression of the *cro* gene, on the other hand, prevents the expression of immunity; repressor, once lost from the cells, never again reappears.

But Kourilsky *et al.* (*Cold Spring Harb. Symp. Quant. Biol.*, **35**, in the press) and Heinemann and Spiegelman (*Proc. US Nat. Acad. Sci.*, **67**, 1122; 1970) have now presented data describing the transcription of the C_1 gene which are not in accord with the above model. They have found that immediately after induction of a lysogen, transcription of this gene ceases. Furthermore, the presence or absence of the *cro* gene product is of no importance; C_I transcription stops in any case. *Cro* therefore acts to keep C_1 transcription off once it is turned off, but something other than *cro* is responsible for turning it off in the first place.

The behaviour of other mutants ruled out another theory, the crossed promoter hypothesis. This theory had been popular in Paris before the discovery of *cro*. Stated in its original form, it conjectured that the promoter for rightward transcription, which is located in the *x* region of the lambda genome, lies upstream and on the opposite strand from the promoter for C_I. Messenger RNA chains starting from one promoter grow over the other, preventing it from binding RNA polymerase and initiating transcription. Brought up to date, this theory assigns a secondary role to *cro*, that of assuring that transcription of C_I, once shut off, will not resume, even if counter-current transcription should momentarily falter.

This hypothesis has been directly tested by Kourilsky *et al.* and by Heinemann and Spiegelman. They have found that in an *x* promoter mutant, where rightward transcription does not occur, the leftward transcription of C_I is nevertheless turned off a few minutes after induction. This demolishes the crossed promoter theory, at least in its present form.

Passing to the problem of what keeps C_I on after induction, it seems that even highly defective mutants which produce no known functions after induction turn off immunity transcription. As there are only two phage genes known to be expressed in an immune lysogen, the choice is very narrow. One is the *rex* gene, whose product makes lysogens immune to infection by T4rII. Because *rex* λ-immune lysogens exist, *rex* function cannot be implicated in the maintenance of C_I transcription. The other λ gene expressed in a lysogen is the C_I gene whose product is the λ repressor. Repressor therefore must be responsible for the transcription of the C_I gene, that is, repressor is its own inducer.

Some lambdologists argue, however, that only one thermo-labile repressor, C_I857, has been examined, and that this mutant might be unique. Finally, there does not seem to be unanimous agreement over the data; one school of *messagistes* in fact denies that C_1 transcription turns off after induction of an *x* mutant.

CHROMOSOMES

Fluorescent Markers

from our Cytogenetics Correspondent

FLUORESCENCE microscopy of chromosomes stained with quinacrine dihydrochloride is clearly going to change the face of human cytogenetics. This emerged at the session on human autosomes on the second day of the meeting of the Genetical Society in London on December 18 and 19. Dr P. L. Pearson (Oxford) described the fluorescent patterns of human chromosomes. There are bands of different intensity of fluorescence along the chromosomes that are specific for each pair of homologues, thus allowing each chromosome to be identified with a degree of certainty not previously possible (Caspersson *et al.*, *Exp. Cell Res.*, **62**, 490; 1970).

In addition to these constant and reproducible bands there are certain intensely fluorescent regions on some autosomes, notably the centromere of chromosome 3, the satellites of *D* and *G* group chromosomes, that are more variable in occurrence. Pearson presented data on the frequency with which these autosomes displayed the intensely fluorescent landmarks in a sample of nearly 200 individuals. The fluorescent marker chromosomes are inherited and it may thus be possible to identify chromosomes contributed by each parent.

The ability to identify each chromosome now makes it possible to describe translocations more accurately. For example, translocations involving the hitherto intractable *C* group were among a number of translocations identified by fluorescence by Dr Pearson and by Dr Karin E. Buckton and Professor H. J. Evans (Edinburgh). Translocation chromosomes were found to be present in a number of males with a low sperm count; the origin and meiotic behaviour were described by Dr C. Chandley (Edinburgh). She has also discovered a small supernumerary chromosome in two of these sub-fertile individuals.

Identification of the origin of these chromosomes, as well as the marker chromosomes found in many cancer cells, may now be possible with fluorescence microscopy. Already the so-called Philadelphia chromosome, found in cases of chronic myeloid leukaemia, has been identified by Evans (and recently published by Caspersson *et al.*, *Exp. Cell Res.*, **63**, 238; 1970) as being chromosome number 22 and can be distinguished from chromosome 21, which is of similar morphology and is found in triplicate in cells from mongoloid idiots. One point that emerges from these fluorescent studies on human chromosomes is that some standardization of chromosome identification is now necessary to bring up to date the Denver nomenclature of 1960.