genes offers a beautiful system in which to test such theories; so few experiments can be performed on protein evolution that the possibilities offered by this system can scarcely be disregarded.

BACTERIOPHAGE

A *cis*-Acting Protein

from our Cell Biology Correspondent

COMPLEMENTATION tests, involving simultaneous infection of a bacterium with two mutant bacteriophages, have become one of the standard tools for defining the number of genes in the chromosome of a bacteriophage and ascertaining whether independently isolated mutants of a particular phage carry mutations in the same or different genes. Such tests implicitly depend on the assumption that the gene products specified by the phage genes are free to diffuse about the infected cell, and the successful use of complementation tests with a wide variety of coliphages proves that this condition is usually fulfilled. Lindahl (*Virology*, **42**, 522; 1970) has, however, found an interesting exception which proves the rule. He has isolated two temperature sensitive mutants and two amber mutants of the lysogenic coliphage P2 which carry a mutation in the so-called gene *A*. These four mutants conspicuously fail to complement with any of the other P2 mutants so far tested, which carry mutations not in gene *A* but in other genes essential for the replication of the phage; because the two temperature sensitive mutants of gene *A* fail to complement, this result cannot be caused by any polar effect. Furthermore, the four gene *A* mutants have an unusual physiological property; they are unable to replicate their DNA when they infect bacteria in non-permissive conditions even if another P2 genome is replicating in the same cell.

Lindahl concludes from all this that a functional A protein is required for the replication of the P2 genome, and because a phage specifying a functional A protein cannot complement a mutant specifying a defective A protein, the A protein appears to act only on the chromosome from which it was transcribed. Finally an active A protein must be an essential prerequisite for the expression of other P2 genes. Because the A protein is *cis*-acting it cannot freely diffuse in an active form about the entire infected cell, but, beyond that, its nature and function remain, not surprisingly, a matter for speculation. One of the more attractive suggestions is that the A protein is involved in the attachment of the P2 chromosome to some "essential site", perhaps on the bacterial membrane, without which neither replication nor transcription can occur. There are precedents for such a mechanism. Sinsheimer and his colleagues, for example, have reported that replication of the ϕX174 chromosome depends on attachment to such an "essential site". And there are further parallels between P2, ϕX174 and another coliphage, S13. Mutants of these latter two phages have been isolated which fail to replicate their DNA, and coinfection with a replicating phage fails to provide the defective function of the mutant. Lindahl suggests, therefore, that some *cis*-acting protein may be involved in the replication of the chromosomes of S13 and ϕX174 as well as P2, and to bolster his case further he points out that both P2 and ϕX174 fail to replicate in the REP strain of *E. coli*.

The moral of this story is clear enough; proteins which act only on the chromosomes from which they were transcribed may be more widespread and more generally important than is currently appreciated. Their occurrence should certainly not be overlooked by anyone using complementation tests to define bacteriophage mutants particularly if a class of mutants which fail to complement has been detected.

HAEMOGLOBIN

The Truth about Haemoglobin

from our Molecular Biology Correspondent

WHEN nearly ten years ago Perutz and Kendrew caught up with the Holy Grail, it brought into focus the question which the pursuit of this object must always have raised in the minds of all but the most myopic, of what, having found it, one would do with it: absorbing and pleasurable as it might be to contemplate, it was not clear that its form would provide any kind of explanation of its attributes.

The similarity of the haemoglobin and myoglobin chains in a sense compounded the disappointment, for not only was the nature of the Bohr effect not vouchsafed, but the accepted explanation in terms of protonation of the haem-linked histidines was undermined. Perutz, however, did not flag, and only now after many man-years of toil is he garnering the ultimate rewards that were promised by the initial success. In last week's *Nature* (**228**, 726; 1970) he described the results of comparisons of the oxygenated and reduced states of haemoglobin, which have at last become possible. In the first place inspection of models shows that, depending on its binding state, the iron atom can be in the plane of the haem, as is the case in low-spin complexes, such as oxyhaemoglobin, or the greater part of an Å out of plane in other derivatives, including deoxyhaemoglobin. This effect is sufficient to trigger off a series of conformational consequences. What happens within the subunit when oxygen is bound was deduced from the structure of a derivative in which the more obvious change involving relative displacement of the four subunits is inhibited by a chemical cross-link. In the α-chains the movement of the iron carries with it its bound histidine and therefore displaces the segment of α-helix of which this residue is a member. This squeezes the haem pocket, and pushes out the tyrosine side chain next to the C-terminal arginine, which is pulled away with it, so breaking ion-pair bonds with two groups in the other α-chain. In the β-chain the haem pocket is constricted, and has to open if it is to admit a ligand, but in other respects a closely similar chain of events can be traced.

Then, supposing the α-chains oxygenate first, by reason of their more accessible iron atom, the disruption of four ion-pairs allows the transition to a new inter-subunit geometry—that of oxyhaemoglobin—to take place. Oxygenation of the β-chains, with ejection of the tyrosines from the pockets and displacement of the C-termini, now no longer involves the separation of ion-pairs, since these have already been broken by the inter-subunit displacement. Thus the oxygen affinity is increased by the energy-equivalent of two coulombic interactions. The high-affinity intermediate is then forthwith oxygenated. The energetically important subunit interactions are confined to two of the interfaces ($\alpha_1\beta_2$, $\alpha_2\beta_1$ and not $\alpha_1\beta_1$, $\alpha_2\beta_2$) with local displacements

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