Protein structure

More geese . . .

from Roger Pain

THE story of Mendel's gardener pinching out his peas in order to satisfy his intuitive sense of reality may have its origin in the countryman's legendary closeness to nature, but it is not entirely foreign to the day-to-day activity of the scientist. This week's issue of *Nature* (**303**, 828; 1983) brings a further essay in the art of observation in the form of a new chapter in the lysozyme story. Most lysozymes, like peas, come in convenient prepackaged containers from which they are easily extracted, and have a good shelf life making them suitable for structural investigation.

To date, lysozymes from a wide range of species have shown reasonable homology and structural similarities, with the exception of phage lysozyme. Despite complete lack of sequence homology, however, the central portion of the molecule supporting the catalytic and binding sites was found to bear a strong conformational homology to the equivalent region in hen egg-white lysozyme — once people had learnt how to look at the structure. This was in keeping with the growing experience that in globular proteins conformation can prove a clearer marker of family relationship than amino acid sequence.

Grütter, Weaver and Matthews, in solving the structure of goose lysozyme (p.828), have challenged the molecular evolutionist with yet more intriguing data. The goose group, which includes the black swan, has longer polypeptide chains than either hen or phage lysozymes and bears no similarity in sequence to either. Again, the central region bearing the active site carboxyls and the substrate-binding cleft is homologous by conformational criteria to the analogous regions in hen and phage. In the sequence to the N-terminal side of this region there is a stretch of 10–20 residues whose α-carbons are positioned in conformational homology with hen, whereas on the C-terminal side, a similar number of residues have their α-carbons positioned in conformational homology with phage. These relationships are summarized in the figure (compare with Grütter *et al.*'s Fig. 1).

The authors argue that the conformational similarities in this trio of enzymes support divergence from a common ancestor rather than convergent evolution towards a common function. They also point out, again very reasonably, that the goose enzyme, which has all the features of both hen and phage, could have evolved from — or into — either; but that hen and phage enzymes could not have evolved directly into one another (see the figure).

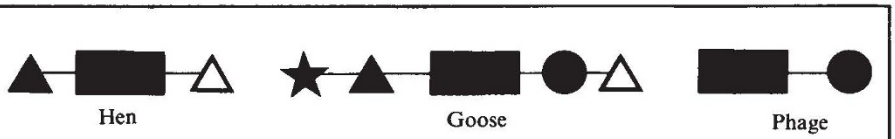
The difficulty in this field is that one is faced with competing probabilities in a process which is itself highly improbable, and gene manipulation techniques are not yet sufficiently developed to allow easy multiple experiments that could allow the effects of 'mutations' at potentially interesting loci to be investigated. Problems suggested by, but not exclusive to, these fascinating lysozyme structures include the following. First, the chance coincidence of two sequences of α-carbons, each arranged in aperiodic three-dimensional conformations, is highly improbable. The probability is very much increased however if the α-carbons in each case form a rigid secondary structure such as an α-helix, and more so still if those helices have to depend for stabilization on each being packed against similar conformationally homologous folding units or domains. How does one tell whether the homology in such cases reflects divergent evolution from a common genetic origin, or convergent evolution under selective pressure from conformational constraints?

Second, in describing residues outside the central catalytic region as catalytically 'non-essential', Grütter and colleagues are being careful not to rule out the possibility of functions for these various groups of residues. These functions, which may involve interaction with substrate structures or just stabilization of the catalytic core structure, are at present unknown as also is the selection pressure to retain these groups of residues in their conformation. Fossilization of a region of conformation may occur if further mutation would lead to a different conformation which would decrease the stability of the molecule as a whole, even though its original function is no longer required.

Finally, a familiar problem for the classical morphologist is that, in comparing structures, we readily 'see' similarities and therefore sense continuity. What we cannot see in the molecular fossil record is the possible process of interconversion between dissimilar conformations, sequence clues having already disappeared. These two conformations are therefore regarded as unrelated in a genetic sense. However, it is not difficult to envisage a critical mutation in a stabilizing region of the molecule which triggers a change in its conformation to one which is still able to interact with and stabilize say the catalytic region.

Given the tools and the insight to make the appropriate mutations, it should be possible at least to visualize some of the possibilities for change in the evolutionary process. In the present state of the art however, Grütter, Weaver and Matthews have taken the useful intuitive course through these problems, as latter-day mendelian gardeners. The only other way leads to frustration and despair:

Farewell all joys, O death come close mine eyes,
More Geese than Swans now live, more fools than wise.



Diagrammatic comparison of the structures of hen, goose and phage lysozymes.

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Oncogenic intelligence

One cancer gene: two mutations

from Peter Newmark

SINCE the discovery, about a year ago, that there was a difference of only one nucleotide (and one amino acid) between the transforming gene of a human bladder cancer cell line and its nontransforming version in normal cells, there has been an unsuccessful hunt for other examples of the same mutation (see, most recently, Feinberg *et al. Science* **220**, 1175; 1983). Now, instead, a different mutation in the same gene has been found by Yuasa *et al.* in a human lung cancer cell line (see page 775 of this issue of *Nature*).

The gene is that known as c-Ha-*ras* (or c-*bas/has*), the cellular gene from which the oncogene of the Harvey murine sarcoma virus is presumed to have been derived. Its product is a 21,000-molecular-weight protein, p21. In the human bladder cancer cell line T24/EJ, a point mutation has changed the twelfth amino acid of p21 from glycine to valine. By contrast the transforming c-Ha-*ras* gene isolated and cloned by Yuasa *et al.* from the Hs242 human lung carcinoma cell line has glycine at amino acid 12, as normal, but a substitution of leucine for glutamine at amino acid 61.

Since amino acid 61 does not form part of the predicted site whose binding of nucleotides is conceivably affected by a change in amino acid 12 (see *Nature* **301**, 262 and **302**, 842; 1983), one is, unfortunately, left without even a working hypothesis for why two different mutations should both confer upon p21 the apparent ability to transform NIH3T3 cells.

Are there other point mutations that have the same effect? A series of experimentally engineered mutations throughout the c-Ha-*ras* gene might provide the answer.

Peter Newmark is Deputy Editor of Nature.

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