

has been positively identified in the infrared spectrum of the satellite's surface and a strong ultraviolet absorption could also be explained by sulphur; different allotropes of sulphur seem to match the different colours of Io's surface; black spots within its calderas have the same reflectivity as liquid sulphur; and temperatures of the hot spots on Io range up to 600 K, which is consistent with the presence of liquid sulphur. The main reason to doubt that sulphur alone constitutes Io's surface is scepticism that it has the strength to support the observed surface relief. Sulphur is an insulator and is ductile at lower temperatures than are silicates. At volcanic centres, where high conductive-heat flows are likely, temperature gradients in an all-sulphur crust would be steep, and sulphur should flow at relatively shallow depths. Therefore the presence on Io of calderas over 2 km deep suggests that the surface is not predominantly sulphur; the combination of thermal and strength properties required are more consistent with a surface rich in silicates.

Young has recently questioned the suggestion of abundant sulphur on Io on other grounds⁷. Shortly after the Voyager encounters, pictures of Io, richly coloured in tones of red, orange and yellow, were widely distributed. The different colours were generally attributed to different allotropes of sulphur. Precise calibration and examination of the spectral data has since shown that Io is yellowish-green, not orange-red, and that the supposed match between the colours of Io and different allotropes of sulphur is completely spurious. The only stable form of sulphur at Io's surface temperatures is S₈ (all other forms will revert to S₈ in hours) and so the absence of spectral evidence for other allotropes is not, in itself, fatal for the sulphur hypothesis. Young, however, maintains that discrepancies between the reflection spectra of Io and S₈ are so large as to rule out S₈ as a major surface component. He therefore questions the presence of sulphur flows on Io, and cites supporting morphological evidence. Pointing out the pitfalls of comparing spectra of pure elements with those of naturally occurring materials, which usually contain abundant impurities, others maintain that one cannot rule out the presence of sulphur in that way.

The most convincing evidence for sulphur volcanism on Io may be the surface temperatures. Three different kinds of hot spots have been recognized by the Voyager IRIS experiment: stable high-temperature sources of about 600 K; transient high-temperature sources of the same temperature; and stable low temperature sources of less than 400 K. All the sources are dark areas, mostly within calderas. Integrated infrared spectra of Io taken by telescope during eclipses have been successfully modelled with 10⁻⁵-10⁻⁶ of the surface close to 600 K, and none of it at higher temperatures. Even if only 10⁻⁸-10⁻⁹ of the surface is close to 1,000 K, it would be detectable in the spectra. The temperature measurements

are thus consistent with sulphur, which melts at 400 K, but not with silicates, which melt around 1,300 K.

Most investigators now believe that both silicate and sulphur volcanism occurs on Io. Injection of silicates into and on to the silicate/sulphur crust may remobilize the sulphur and cause flows, much in the manner described by Naranjo for Lastarria Volcano², but on a far larger scale. The mobilized sulphur could form pools within calderas where it may be maintained as a liquid by hot silicates beneath. Injection of silicates into the sulphur-rich crust may vaporize sulphur and sulphur dioxide to drive the volcanic plumes. Although studies of sulphur flows at terrestrial volcanoes are

valuable, resolution of the relative roles of sulphur and silicates on Io will have to await acquisition of high-resolution imaging and spectral data. It is planned that this information will be collected by the Galileo spacecraft, which is scheduled to fly by Io on 10 December 1988. □

1. Naranjo, J.A. *Nature* 313, 778 (1985).
2. Greeley, R. *et al. Icarus* 60, 189 (1984).
3. Johnson, T.V. *et al. Science* 226, 134 (1984).
4. Peale, S.J. *et al. Science* 203, 892 (1979).
5. Smith, B.A. *et al. Nature* 280, 738 (1979).
6. Carr, M.H. *et al. Nature* 280, 729 (1979).
7. Young, A.T. *Icarus* 58, 197 (1984).

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Protein structures

Two for the price of one

from Stephen C. Harrison

USING the techniques of molecular biology, large quantities of proteins can now be produced to order. One consequence is that it is becoming possible to determine the three-dimensional structures of new classes of proteins that were not previously available in sufficient quantities to crystallize. For example, on page 762 of this issue Ollis *et al.* report a crystallographic analysis of the large ('Klenow') fragment of Pol I, the DNA polymerase of *Escherichia coli* and the first polymerase structure to be solved¹. An accompanying paper on page 811, documents the similarity between the protein sequences of Pol I and the DNA polymerase of bacteriophage T7, and suggests an interesting three-dimensional relationship². This comparison neatly illustrates how knowledge of the three-dimensional structure of one protein can give useful information about the structure of its homologues and reminds us that recognizing similarity may be the only reliable way to predict structure from sequence.

The studies of Pol I by Arthur Kornberg and colleagues have a central role in the history of nucleic-acid enzymology, and it is satisfying to see initial three-dimensional correlates of this classic work. The Klenow fragment of Pol I is the carboxy-terminal two-thirds of the protein, which contains two of the three enzymatic activities (the polymerase and the editing exonuclease). As seen at 3.3 Å, it comprises two distinct domains — one of about 200 amino-acid residues, the other of about 400. The smaller domain, which roughly resembles the nucleotide-binding domain of kinases and dehydrogenases, binds deoxynucleotides; the position of the deoxynucleotide dTMP in the crystal probably marks the location of the editing exonuclease since deoxynucleotides inhibit this activity but not the polymerase.

The larger domain has a very deep cleft, about 20-24 Å wide and 30 Å deep. A six-

stranded β -sheet structure forms its base and several α -helical structures form its walls. The top of the cleft could be closed off by the folding down of a spatially-disordered subdomain (about 50 residues in the middle of the sequence, joining two α -helices that form one side of the cleft). Ollis *et al.* suggest that double-stranded DNA binds in this cleft, with the primer 3' terminus near the end that faces the smaller domain. In support, they cite the concentration of positively-charged amino-acid side chains in the cleft as well as the location of amino-acid changes in certain Pol I mutants.

The notion that the disordered subdomain might close down 'on top' of DNA bound in the cleft is an attractive way of explaining the ability of the enzyme to move along the DNA chain, and is reminiscent of proteins that bind to nucleic acids. Tyrosyl tRNA synthetase has a loosely attached domain, disordered in crystals, that might fold down on the tRNA³; the subunits of viruses such as tomato bushy stunt virus⁴ and southern bean mosaic virus⁵ have flexibility-tethered R domains to draw RNA into assembling shells; the tobacco mosaic virus subunit has a loop disordered in the protein disk that clamps around RNA in the virus⁶; the repressor of bacteriophage λ embraces DNA with N-terminal arms⁷; and the bacterial histone-like protein is thought to enfold DNA with a β -loop⁸.

Regarding the similarities between sequences of T7 DNA polymerase and the Klenow fragment of Pol I there are several striking points. First, the similarities occur in local segments of varying length (9-45 residues), eight of them in the larger domain. Second, these eight segments constitute most of the structures that form the cleft described above, including the flexibly-linked subdomain. Between these segments, the polypeptide chain in Pol I makes excursions to the surface of the do-

mains, and Ollis *et al.* believe that the insertions or deletions necessary to accommodate the T7 DNA polymerase could readily be formed².

Comparisons of the Pol I amino-acid sequence with those of various eukaryotic DNA polymerases do not reveal any significant alignments. As Ollis *et al.* point out, similar structures need not have obviously similar sequences (evolutionarily distant globins, for example), but the converse does generally seem to be true. Indeed, using a known structure to deduce the structure of a homologue is an approach that is as old as the first protein structures — haemoglobin structure was deduced from myoglobin, and trypsin from chymotrypsin. But now that nucleotide sequencing is leading to a rapid expansion in the library of amino-acid sequences, the detection of similarity takes on new importance.

It is clear that simple pair by pair comparisons of amino-acid sequences, while often revealing, have several limitations. Different criteria can lead to different alignments, especially in marginal cases. Even with the serine proteases, sequence alignment and three-dimensional alignment get out of register in the less conserved regions⁹. A more powerful approach is the detection of homology groups. A dramatic example is the analysis of sequences from bacterial repressor and activator proteins^{10,11}, where a conserved helix-turn-helix motif of three-dimensional structure can be predicted and a group alignment established, despite the fact that many individual pairs of sequences cannot be related unambiguously. Indeed, the alignment has permitted Wharton *et al.*¹² to predict the location of this motif, and hence the effects of certain *in vitro* mutagenesis experiments on two repressors whose structures have not yet been solved crystallographically. It should be noted *en passant* that a strictly comparable helix-turn-helix is not present in the large fragment of Pol I, although the J and K helices of the larger domain are reminiscent of the motif.

In other words, in predicting aspects of protein structure from sequence, what we need to recognize are entire domains or significant subdomains — not just elements of secondary structure. The poor correlation between local sequence and secondary structure has been aptly demonstrated by Kabsch and Sander¹³, who show that the same pentapeptide in different proteins has fundamentally different backbone configurations in each; before anyone runs a secondary-structure prediction program on a new sequence again they should carefully study this paper.

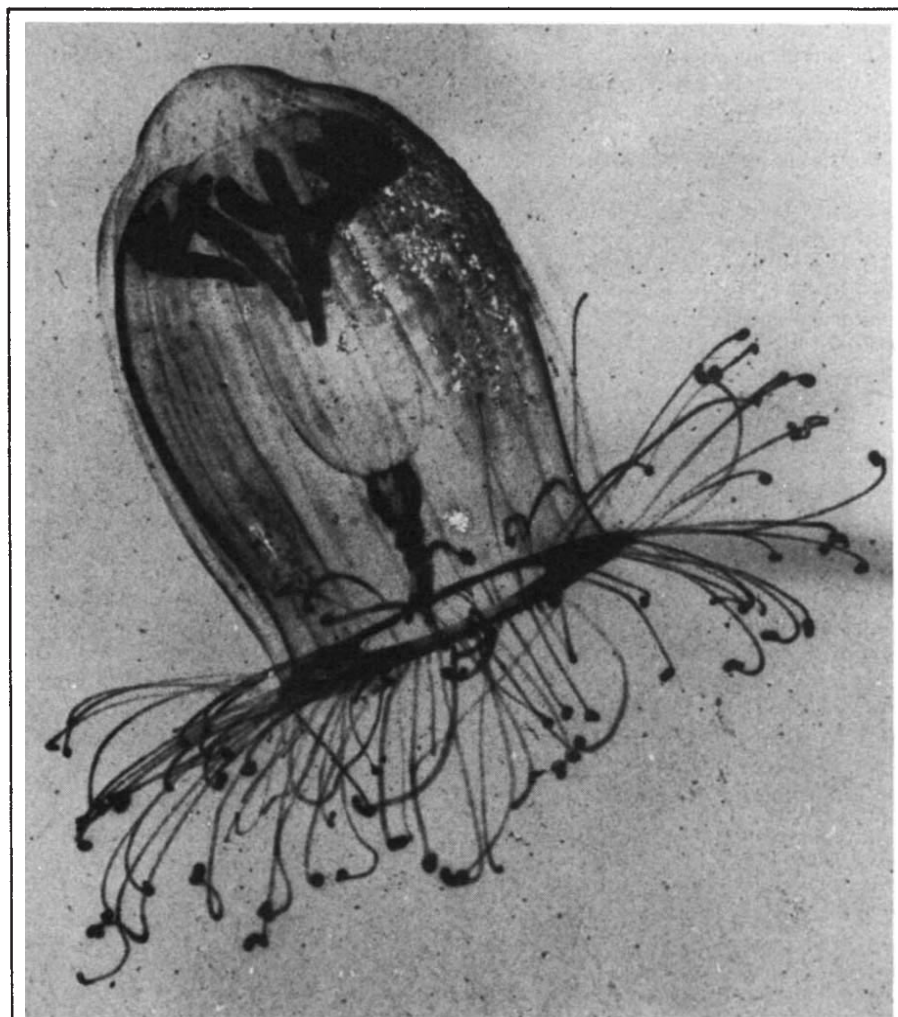
Thus, sequence similarity is currently the only reliable method for detecting structural similarity; several suggestions immediately follow. First, there is an important need for better ways to recognize similar sequences and to align them correctly. Will comparisons in more than two

dimensions (three or more sequences at once) be useful? Second, there is a real possibility that many interesting structures — or parts of structures — will be solved by 'catalogue look up', as used by Ollis *et al.* to suggest a structure for parts of T7 DNA polymerase.

Sequence data bases grow vaster each day. New crystal structures also appear more quickly (with the aid of two-dimensional position-sensitive detectors, as used by Ollis *et al.* and described in these columns last May¹⁴). If even some of the new structures tells us something about a whole family of homologues, progress will be rapid indeed. A solution to 'the protein folding problem' — given a primary sequence, divine the tertiary fold — has been regarded as the pot of gold at the end of the protein-crystallographer's rainbow. Perhaps a portion of the gold already lies at our feet. □

- Ollis, D.L., Brick, P., Hamlin, R., Xuang, N.G. & Steitz, T.A. *Nature* 313, 762 (1985).
- Ollis D.L., Kline, C. & Steitz, T.A. *Nature* 313, 811 (1985).
- Bhat, T.N., Blow D.M., Brick, P. & Nyborg, J. *J. mol. Biol.* 158, 699 (1982).
- Harrison, S.C., Olson, A.J., Schutt, C.E., Winkler, F.K. & Bricogne, G. *Nature* 276, 368 (1978).
- Abad-Zapatero, C., Abdel-Meguid, S.S., Johnson, J.E., Leslie, A.G.W., Rayment, I., Rossmann, M.G., Suck, D., & Tsukihara, T. *Nature* 286, 33 (1980).
- Klug, A. *Harvey Lectures* 74, 141 (1979).
- Pabo, C.O., Krovatin, W., Jeffrey, A. & Sauer, R.T. *Nature* 298, 441 (1982).
- Tanaka, I., Appelt, K., Dijk, J., White, S.W. & Wilson, K.S. *Nature* 310, 376 (1984).
- Greer, J. *Proc. natn. Acad. Sci. U.S.A.* 77, 3393 (1980).
- Sauer, R.T., Yocum, R.R., Doolittle, R.F., Lewis, M. & Pabo, C.O. *Nature* 298, 447 (1982).
- Anderson, W.D., Takeda, Y., Ohlendorf, D.H. & Matthews, B.W. *J. mol. Biol.* 159, 745 (1982).
- Wharton, R., Brown, E.L. & Ptashne, M. *Cell* 38, 361 (1984).
- Kabsch, W. & Sander, C. *Proc. natn. Acad. Sci. U.S.A.* 81, 1075 (1984).
- Harrison, S.C., *Nature News and Views* 309, 408 (1984).

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Aglantha digitalis is a jellyfish that swims by contracting the wall of its bell-shaped body to expel a jet of water from its base. Swimming can be either slow, when it is fishing, or fast, when escaping from predators. One contraction during fast swimming takes the animal five times as far as in slow swimming, when it moves about 15 cm, one body length. In a paper on page 791 of this issue, G.O. Mackie and R.W. Meech show that both swimming behaviours are produced by the same muscle sheet, innervated by the same giant motor axons. Fast swimming is evoked by rapidly-conducted, sodium-dependent action potentials, whereas slow swimming results from a low-amplitude calcium potential in the same axon. This is the first time that two types of impulse propagation have been reported in a single nerve fibre.