self-assembly would offer the possibility of both left- and right-helical hands. Better comparisons can be made with the fibre patterns in plant cell walls; for instance, helical windings in cotton fibre cells follow both left-handed or right-handed paths and often abruptly change hands[13]. Neville[14] has suggested that there is an interplay between molecular self-assembly into helicoidal structures and mechanical reorientation due to growth forces. And it is worth noting that Lloyd[15] has suggested that the central role in the formation of these helical patterns is played by microtubules.

In the past few years there has been decided progress in uncovering how genes control patterns of early development (the segmented pattern of insect embryos for example), and how the genetic information is converted into a sort of relief map of morphogenic gradient[16]. But there is more than one way to skin a cat and more than one way to control shape. Handedness is a fundamental quality of biological form, as D'Arcy Thompson appreciated, and Mendelson's group has found an interesting experimental system in which to look at it. □

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MEMBRANE BIOLOGY

The shadow and the substance

Walter Gratzer

THE injunction that one should waste no pure thoughts on impure proteins, often imputed to Kornberg, was (according to the man himself) actually voiced by Racker. The aphorism retained its pith until molecular genetics finally revealed how protein chemistry might be done without any protein. No one who has laboured to purify refractory and uncooperative proteins from membranes will fail to be stirred by the amount of information that Kopito *et al.*, writing in *Cell* (**59**, 927–937; 1989), have garnered about the nature, structure and properties of the neuronal membrane anion-exchange protein without even reaching for a bottle of detergent.

It has been known for some years that the well-characterized anion exchanger of the human red cell — the preponderant membrane protein, band 3, or in the new terminology, AE1 — has immunologically detectable counterparts in other tissues, including brain, but almost nothing has until now been discovered about their properties. Band 3 itself has been sequenced by way of the gene. First the chicken and mouse sequences were established, and in 1988 Tanner and his colleagues (*Biochem. J.* **256**, 703–712; 1988) finally got the human sequence out. It has now been independently determined by Lux *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **86**, 9089–9093; 1989), and the two DNA sequences agree — remarkably, for it is a sizeable protein — down to a single base in the coding sequence. The two separate domains, recognized by earlier chemical studies, are apparent: there is a largely membrane-associated N-terminal half and a cytoplasmic piece. The evidently α-helical membrane-spanning segments can be discerned, and it seems that the chain makes 14 passes through the lipid bilayer, with small loops of varying length on the outer cell surface. The intracellular domain has binding sites for various red-cell proteins, most importantly ankyrin, the main link between the membrane skeletal network and the membrane. At the extracellular surface there is a binding site, containing at least one lysine, for a family of stilbene disulphonate anion-transport inhibitors.

From Passow's laboratory in Frankfurt came the news that messenger RNA from mouse spleen, or indeed band 3 complementary RNA, when injected into *Xenopus* oocytes leads to the appearance of band 3 in the membrane. It is functional and subject to inhibition by the stilbene disulphonate inhibitor, DIDS (Bartel *et al. EMBO J.* **8**, 3601–3609; 1989). Garcia and Lodish (*J. biol. Chem.* **264**, 19607–19713; 1989) obtained a similar result with the messenger for the human protein. Both groups set out to identify the lysine(s) at the inhibitor binding site. It had been confidently predicted that a sequence-conserved lysine (558 in the mouse, equivalent to 539 in man), or possibly another lysine, three residues along in the sequence (missing, however, in the chicken), would be the spot. Garcia and Lodish mutated lysine 539, but to no effect; it must then, they inferred, be the other lysine, if earlier data in the literature were correct. Bartel *et al.*, however, went a step further with their mouse clone, and replaced both. The DIDS still bound and inhibited, although covalent attachment, which requires a reactive lysine side-chain, was expunged. Clearly the site is near; but, as so often, nature is more complex than the biochemist desires.

Back then to Kopito and the neurons: a red-cell cDNA probe picked out a mouse genomic restriction fragment, which was revealed as representing a new band-3-like protein (AE3). The whole sequence was found in two overlapping cDNA clones and after that all was plain sailing. First the sequence, when determined, turned out to be quite closely related to that of red-cell band 3 (AE1) and a corresponding kidney protein sequence (AE2), obtained by a similar procedure in Lodish's laboratory a year ago. In the C-terminal, anion-transporting part of the molecule all three sequences are closely related: the similarity between AE3 and AE1 amounts to some 80 per cent of the amino acids, with 70 per cent identity. Hydrophobicity plots show superimposable systems of peaks and valleys; the pattern of bilayer-traversing segments stands out.

Next, Kopito and his colleagues demonstrated that transfected cultured (COS) cells expressed the AE3 with considerable efficiency. The cells that showed expression were also positive by immunofluorescence, and their cytoplasmic pH was measured with a fluorescent indicator. Anions were transported through the membrane, and when the external medium contained carbonate–bicarbonate, the cytoplasm became acidified. The inhibitor, DIDS, did its thing. Moreover deletion of the entire N-terminal half of the sequence (645 amino acids) from the DNA still led to a functional protein, although the acidification that it brought about was a little less. A survey by immunofluorescence indicated that, in nature, AE3 is probably confined to neurons.

So more or less without seeing the protein, Kopito *et al.* have discovered that neurons possess an acidifying anion exchanger, the transport properties of which they have characterized; that it will home in on and insert itself into the membrane without benefit of a leader sequence; that the C-terminal half of the molecule suffices to express its function; and that the activity is very probably regulated by the interaction of the business end with the pendant cytoplasmic domain. And more than that one scarcely needs to know. □

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