Ciliar dyneins

from Roy Burns

THE similarities between the molecular basis of muscle contraction and the beating of cilia flagella have been evident for several years. Both mechanisms involve the sliding of parallel filaments, resulting from the hydrolysis of ATP by enzymes which transiently form crossbridges between the adjacent filaments: the myosin heads in the case of muscle and the dynein-containing side arms of cilia and flagella. Furthermore, the demonstration by Gibbons and Gibbons (*J. Cell Biol.* **63**, 970; 1974) that the crossbridges of sea urchin sperm flagella are frozen when the ATP concentration is suddenly lowered, resulting in rigor, strongly suggests that the dynein-ATPase crossbridge cycle resembles the Lymn-Taylor cycle of muscle (see *News and Views* **235**, 362; 1972). It is however dangerous to extend the analogy too far, and Warner, Mitchell and Perkins (*J. molec. Biol.* **114**, 367; 1977) have recently shown that the outer sidearms of cilia are structurally much more complex than the myosin head crossbridge.

Each of the outer doublets of cilia and flagella bear a pair of sidearms which tangentially approach the neighbouring doublet. These sidearms can in general be removed by dialysis against low ionic strength buffer, and the outer sidearm selectively extracted with 0.5 M KCl (Kincaid, Gibbons & Gibbons *J. supramolec. Struct.* **1**, 461; 1973), and the principal component present in such high salt extracts from sea urchin sperm tail axonemes is the high molecular weight ATPase termed dynein-A. Warner *et al.* have examined such a preparation of dynein-A from cilia from both *Tetrahymena* and the gills of the lamellibranch *Unio* by SDS-gel electrophoresis and by negative staining.

SDS-gel electrophoresis of the *Unio* gill axonemes shows two principal high molecular weight bands, present in a 2:1 ratio, and four additional components with molecular weights greater than 300,000 daltons. Extraction with high salt selectively removes approximately half of the major component (Band-3) which has an estimated molecular weight of 360,000 daltons. When the high salt supernatant from either *Unio* or *Tetrahymena* is examined by negative staining, three principal structures are observed: a 9.3 nm spherical particle with or without a centrally located accumulation

of stain, and aggregates of 2-6 of these 9.3-nm subunits. A fourth unrelated particle is occasionally observed in the *Unio* preparations with dimensions of 9.4×12.7 nm together with some solubilised tubulin and some unidentified 200×3.5 nm filaments. The 9.3-nm particle is consistent with a protein of molecular weight 348,000 daltons, and in view of the extraction conditions is considered to be the monomeric form of dynein-A.

This particle is, however, significantly smaller than the attached sidearm, which in *Tetrahymena* is hook-shaped and measures $20-22 \times 8-10$ nm (Allen *J. cell Biol.* **37**, 825; 1968). Warner *et al.* find that the outer sidearm can be resolved by negative staining into at least three morphological subunits, with a centre-to-centre spacing of 7.5 nm. What then is the relationship between these smaller subunits and the 9.3-nm extracted particles? Three possibilities are suggested; first, that the outer sidearm is composed of one or more non-enzymatic molecules with an identical molecular weight to dynein-A. This possibility should not be discounted as the resolution of SDS-gel electrophoresis is low in the high molecular weight range ($\pm 10,000$ daltons), and the high salt extraction removes only half of the Band-3 component, the remainder presumably being a different protein with the same molecular weight. The second possibility is that the sidearm is a polymer of the 9.3-nm particle. In order to account for the lower centre-to-centre spacing of the subunits of the intact arm it is necessary for a conformational change to occur during extraction, or for the subunits to be superimposed *in situ*, and indeed the sidearms are tilted towards the base at an angle of 28° from the perpendicular. The third possibility is that only the distal portion of the sidearm is extracted, the proximal part remaining attached to the outer doublets but in a form undetected by either negative staining or conventional electron microscopy. However, all of these alternatives indicate that the sidearm is much more complex than the myosin head, and support the view that although the two contractile mechanisms share certain properties, they are unrelated.

Finally, Warner *et al.* have numbered the *Unio* and *Tetrahymena* high molecular weight axonemal proteins 1-6, while Gibbons *et al.* (in *Cell Motility, Cold Spring Harbor Conferences on Cell Proliferation* (eds Goldman, Pollard & Rosenbaum) **3**, 915; 1976) had labelled the sea urchin proteins Band-C, A, D, and B, where Band-A corresponds to dynein-A (also termed dynein-1) and to Warner's

Band-3. At first sight this suggests a proliferation of cumbersome nomenclature. Two points should be made: first, Gibbons has defined the dyneins as high molecular weight (above 300,000 daltons) microtubule-associated ATPases so that certain of the sea urchin high molecular weight proteins, for example Band-B, which lack ATPase activity are not by definition dyneins. Second, it is clear that the same experimental procedure does not necessarily extract the same components from all cilia and flagella; for instance the cilia and flagella of *Aquipten* differ in their extraction properties (Linck *J. Cell Sci.* **12**, 951; 1973) and not all species of sea urchin behave identically. It is probably wise therefore to retain separate nomenclatures until the function and location of each of the high molecular weight components is clear. The differences in the extraction of different cilia and flagella suggest also that the elucidation of the structure and mechanism of movement may be hastened by exploiting obscure organisms rather than confining our attention to a single widely available species. □

Coulson rules OK

from John Walker

THE problem of calculating the electronic energy levels of defects in semiconductors has not been solved, and is currently the subject of much controversy; some new results and re-interpretations of data on point defects in diamond by Gordon Davies (*Nature* **269**, 498; 1977) strongly suggest that the method of calculation proposed by Coulson as far back as 1957 (Coulson & Kearsley *Proc. R. Soc. A* **241**, 433; 1957; see also Coulson & Larkins *J. Phys. Chem. Solids* **32**, 2245; 1971) is the most appropriate one.

Since the 1920s and the development of quantum mechanics it has been possible to calculate with reasonable (and in some cases extreme) accuracy the electronic energy levels of atoms and molecules, and hence their characteristic spectra. The calculations are simplified because an atom is an isolated system; the electrons do not interact with other atoms. Molecules are more difficult, but only a few atoms are involved, at least in the case of simple molecules.

In a solid the analogous problem to the hydrogen atom is an electron bound to a lattice defect such as a vacancy. Here the situation is complex; the surrounding lattice atoms must be taken into account, since they are

Roy Burns is a lecturer in Biophysics in the Department of Physics, Imperial College of Science and Technology, London.