PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Introduction

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THE ROYAL SOCIETY

Introduction

The Royal Society had not held a Discussion Meeting on muscle since 1972, when the late Edith Bülbring and Dorothy Needham organized a meeting entitled 'Recent developments in vertebrate smooth muscle physiology'. We therefore felt that the time was ripe for another meeting on muscle. At the same time, the great recent progress on other biological structures that cause movement made it appropriate to include some of them.

The emphasis throughout the meeting was on the nature of the physical mechanisms by which movement is actually produced and their coupling to the chemical reactions or physical processes that provide the necessary energy. There was little or nothing on the regulation of the activity of these structures or their application in the life of the organism to which they belong. Many of the contributors are from the physical sciences, reflecting the recent increase of interest in certain aspects of biology on the part of physicists. The intention was to bring together the different approaches: from experimental work, from structure and from theoretical modelling, in the hope that discussion between practitioners of these approaches would bring out fresh interpretations.

There are a great number of motile systems in different organisms and in different parts of the same organism. For example, the different myosins that have so far been recognized are classified into no less than a dozen families. We chose a few systems that are well enough characterized for detailed discussion of their mechanisms to be possible: namely, a few members of the myosin superfamily involved in muscular contraction and cell motility, members of the kinesin family that propel organelles and vesicles along microtubules, and the rotary motor that drives bacterial flagella. These are the now 'standard' molecular motors or motor proteins: myosin and kinesin powered by the hydrolysis of ATP, with a rich literature on their atomic structure, biochemistry, mechanics and theory; the bacterial flagellar motor powered by a hydrogen or sodium ion gradient, a more complicated structure with many subunits, but the major parts identified, and with much research on mechanics, energetics, molecular biology and theory. We have also included the ATP synthase, of which one half, known as F_o, is a rotary motor driven by hydrogen ions entering the mitochondrion. The other half, known as F_1, is normally driven by F_o and synthesizes ATP from ADP and free phosphate, but is able to work backwards as a motor driven by ATP hydrolysis when disconnected from F_o. The biochemistry, structure and mechanics of F_1 are at an advanced stage, so that detailed discussion of its mechanism is therefore possible, whereas that of F_o is much more speculative as its structure is not yet known.

It is natural to look for similarities between the mechanisms of different motors that have similar overall consequences, e.g. myosin molecules propelling a myosin filament past an actin filament, and kinesin molecules propelling an organelle along a microtubule. Any such similarity can be valuable by suggesting a hypothesis to be tested experimentally, but there is a real danger of going a step further and assuming that similar results must be produced by similar mechanisms. Arguments of this kind a hundred years ago had disastrous consequences: it was argued (e.g. by Bernstein 1901) that the striations cannot be of fundamental significance because unstriated (smooth) muscles are able to contract, and this was one of the main reasons for the total loss by 1950 of the excellent knowledge of the striations and their changes that had been gained by light microscopy during the second half of the 19th century. (The other main reason for this loss of structural understanding of muscle was the rise of biochemistry: it was argued that contraction must be a molecular process, molecules cannot be seen with the light microscope, so nothing of importance will be learnt from what can be seen.)

It has recently been found that the atomic structures and amino-acid sequences of the nucleotide binding pocket are closely similar in myosin, kinesin and several of the G-proteins, suggesting a common ancestry for myosin and kinesin (see discussion in paper by R. Vale). But this does not tell us whether the last common ancestor was itself a motor protein or whether the two lineages originated separately from enzymes related to the G-proteins. The lack of sequence resemblance of other parts of the myosin and kinesin molecules seems to make the latter alternative more probable: if so, the resemblance between the nucleotide binding sites is no reason to expect the motor mechanisms to be similar. It could be that there is a common 'trigger' mechanism tied to nucleotide hydrolysis, but that this is coupled in different ways to affect binding at the interface with actin and tubulin, and to produce force. The latter in the case of myosin via a lever-arm mechanism, and in the case of kinesin via a more subtle change affecting the interaction between the two catalytic heads of the molecule. It is noteworthy that kinesin itself and another member of the same family, Ncd, move in opposite directions along microtubules, although they are closely similar in general structure and have 40% identity in their amino-acid sequences.

The first day of the meeting was devoted to the linear motors myosin and kinesin. In an introductory talk, R. M. Simmons identified some of the problems needing to be resolved in the actomyosin system as (i) whether the conformational change suggested by X-ray structures does occur in the normal cycle of operation; (ii) if so, whether this is the 'working stroke' by which force or movement is produced; (iii) whether myosin can make multiple interactions with actin for the use of one ATP molecule; and (iv) whether the mechanism can usefully be described as a 'Brownian ratchet'.

Later in the meeting, after the contribution from R. D. Astumian, the distinction between Brownian ratchets and other types of mechanism was vigorously discussed. In his paper, Astumian distinguishes between a Brownian ratchet, defined as a conformational change which 'requires thermal activation, with a Poisson-distributed stochastic completion time' on the one hand and on the other a 'working stroke', defined as a 'process more like a viscoelastic relaxation with a deterministic completion time'. It seems to us that this distinction is real but that the restriction of the phrase 'working stroke' to the second type of mechanism does not correspond with current use of the words. We certainly regard the mechanisms proposed by Huxley (1957) and by Huxley & Simmons (1971) as producing a 'working stroke', but both of them fall squarely within Astumian's definition of a Brownian ratchet. Two issues are confounded: one is whether there really is a conformational change, and the second is, whatever the mechanism, whether force and movement are generated by random fluctuations.

The seven full papers on the first day of the meeting were reviews of biochemical, structural and mechanical aspects of linear motors, myosin and kinesin. There are striking differences between these two systems both in biochemical respects and in their overall performance. In myosin, the detached state has ATP bound as an equilibrium mixture of ATP itself and ATP with the terminal phosphate bond broken but with both the ADP and the inorganic phosphate still bound, while in kinesin the detached state has ADP bound. As regards overall behaviour, kinesin moves 'processively' along a microtubule, i.e. a single kinesin molecule can move for a distance corresponding to many monomers in the microtubule without detaching, whereas a myosin usually detaches from actin after a single step. The paper of T. Yanagida, however, presents recent results, in which a myosin molecule moving along actin may make up to five steps, each closely equal to the spacing of monomers along each strand of an actin filament. This behaviour has so far been seen only in a situation where there was very little resistance to the motion. It is perhaps worth pointing out that this is not the first time that a processive movement of myosin along actin has been suggested: 'slippage' mechanisms based on indirect mechanical evidence enjoyed a vogue some years ago (reviewed by Simmons 1992; Burton 1992), but have not been widely pursued, lacking direct confirmation. On the other hand, A. F. Huxley gave reasons for considering the possibility that in myosin there are two distinct mechanisms for generating force, one the 'lever-arm' conformational change suggested from recent structural studies and the other a change in attachment of the catalytic domain to actin, reminiscent of earlier models of force production (Huxley 1969).

The second day of the meeting started with three experimental papers on rotary motors and ended with five theoretical papers, three on rotary motors and two on linear motors. Though there are clear differences between linear and rotary motors, it was nonetheless intriguing and instructive to hear them discussed alongside each other in the same meeting, and to see whether there are at least conceptual similarities. Do all the proteins have separate catalytic, binding and actuator domains? In the case of the ATPases, is the basic chemical to mechanical conversion similar? Do they all work as Brownian ratchets? Can rotary motors be regarded in a general sense as linear motors which have been 'circularized', that is as one or more linear motor proteins facing a circularized track, or for that matter can myosin and kinesin be viewed as linearized rotary motors?

In the case of the ATP synthase, while the motor can be regarded as a single molecule, there are three identical subunits which, as Boyer showed many years ago, possess considerable cooperativity in the relative phasing of their ATP hydrolysis cycles. Cooperativity is also a feature in the two-headed binding of kinesin in its 'hand-over-hand' processive movement, but there the similarity ends. The ATP synthase 'track' is not a polymeric array but a relatively simple asymmetrical (cam-like) stator. Direct observation of the rotation of the rotor as it interacts with each subunit in succession was provided by K. Kinosita Jr, with convincing dwell periods separated by 120° at low ATP concentration, reminiscent of experiments on kinesin which established for the first time its stepwise processivity.

The other two rotary motors, the bacterial flagellar motor and F_o, are both powered by a hydrogen ion gradient rather than by ATP hydrolysis. Though obviously in a different class from the ATPases, they are somewhat more closely related to linear motors in having a number of identical motor proteins on the rotor that are apposed to one or more interacting units on the stator. There is less information about their kinetics because, essentially as ion channels, intermediate states are difficult to detect compared with an ATPase. Indeed, one class of possible mechanism (the turbine model; see paper by R. Berry) for these motors puts them in a category for which a kinetic approach might prove futile.

In conclusion, it cannot be said that any of the various motor mechanisms is completely understood. While great strides have been taken in recent years towards understanding the structural mechanism of the ATPase in myosin, kinesin and F_1, there is little information about how this is transmitted into the interaction with the other molecules involved (actin, etc.). The biochemistry is also well characterized for myosin, kinesin and F_1, and though the final word on the size and nature of the 'working stroke' has still to be said, the advent in the last decade of single-molecule methods has brought about a sea-change in the mechanics field. The situation is less well advanced for the more complex systems of the bacterial flagellar motor and F_o, where biochemistry is clearly problematic, and the size of the motors militates against simple structural solutions. Theoretical studies, if not yet definitive, are in a healthy state of flux. In future years, more motors will be added to the list: the dynein family, RNA polymerase (which has already made its mark), and there are many more DNA processing enzymes to come, and one hopes that new systems will be discovered.

While it is possible to define what would constitute a 'solution' in our current state of knowledge, two members of the audience at the Meeting raised the question of whether quantum effects are involved. One negative answer is that biological macromolecules lie on the border between macroscopic 'classical' structures and quantum objects, and effects and observations of quantization are likely to be smeared out across the large number of bonds. Another is that there is no evidence that the energy of ATP hydrolysis is stored directly in some localized structure. A new generation may think differently.

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