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Kinesin and Myosin ATPases: Variations on a Theme [and Discussion]

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Kinesin and myosin ATPases: variations on a theme

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SUMMARY

The enzymes kinesin and myosin are examples of molecular motors which couple ATP hydrolysis to directed movement of biological structures. Myosin has been extensively studied and its structure and mechanism of coupling are known in detail. Much less is known about kinesin, but many of its major properties are similar to those of myosin. Both enzymes have two catalytic head groups at the end of a long α-helical rod. The head groups contain the sites for ATP hydrolysis and interaction with their respective partners for movement (microtubules or F-actin). In each case the binding and hydrolysis of ATP is rapid and the steady state ATPase rate is limited by a slow step in the region of product release. This slow release of product is accelerated by interaction with actin or microtubules coupled to changes in binding affinity. As there is no evidence for a close evolutionary link between kinesin and myosin, these and other similarities may represent convergence to set of common functional properties which are constrained by the requirements of protein structure and the use of ATP hydrolysis as a source of energy. It will be of particular interest to determine if these common properties are also shared by the large number of divergent proteins which have recently been discovered to possess a domain which is homologous to the head group of kinesin.

1. INTRODUCTION

Myosin is the archetypical ATP-driven molecular motor. It has been the subject of extensive studies by numerous laboratories over a number of years and its structure and mechanism are established, at least in broad outline (Taylor 1979; Hibberd & Trentham 1986). The other well established molecular motor is dynein (Johnson 1985; Vallee & Shpetner 1990). Dynein shares a number of general properties with myosin, but progress on its detailed mechanism has been considerably slower, in part because of its large size and complexity. Recently a number of additional molecular motors have also been discovered and it is of interest to determine whether they operate by a similar mechanism or if unique mechanisms have evolved in each case. These new motors include variations on the basic myosin structure such as single-headed myosin I, as well as the novel families of motors related to kinesin or dynamin (Vale & Goldstein 1990; Shpetner & Vallee 1989). Progress has been made on the analysis of the mechanism of kinesin and it is now possible to begin to evaluate meaningfully how closely its mechanism compares with the myosin paradigm.

2. STRUCTURE

The core of both myosin and kinesin consist of a dimeric structure with three domains as illustrated in figure 1. This view of kinesin is derived from structural predictions based on the amino acid sequence (Yang *et al.* 1989) and on observation by electron microscopy (Amos 1987; Hirokawa *et al.* 1989; Scholey *et al.* 1989). Both enzymes contain two heavy chains which have a globular region (head group) at their N-terminals and an extensive α-helical region through which two chains dimerize into a coiled-coil structure which forms the central domain. The globular head groups are connected to the rest of the molecule by a flexible hinge and can be released by limited proteolysis as soluble fragments (designated S1 in the case of myosin). The isolated head groups contain both the site of ATP hydrolysis and the site of interaction with the filamentous substrate for movement (F-actin or microtubules (MTs)). The C-terminal region consists of a third domain which appears to function in anchoring the motor to the structure which is to be moved (the load). In the case of myosin, the load is other myosin molecules which aggregate into a filament by interaction in this region. In the case of kinesin, the load is a membrane vesicle which will be induced to slide along a MT.

Although this core structure is capable of performing both the ATPase activity and motility activity, there are other smaller subunits present which are likely involved in regulation or modulation of the process. In the case of myosin, each head group also contains two light chains which are required for stability and in some cases for regulation. One light chain binds calcium, can be phosphorylated and is involved in control, particularly in the case of smooth muscle and non-muscle myosin. With kinesin a second pair of subunits (β subunits) are located at the load

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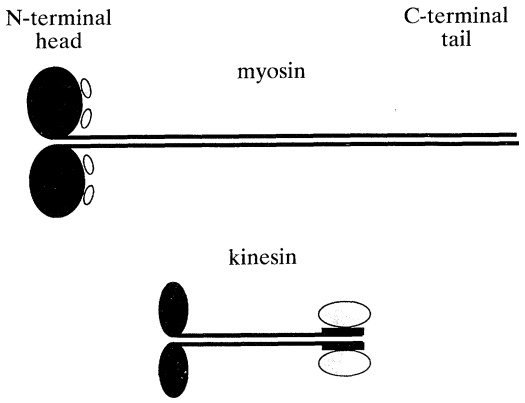

Figure 1. Comparison of myosin and kinesin. The heavy chain of myosin and the α chain of kinesin are indicated with solid filling while the light chains of myosin and the β chains of kinesin are stippled. The region of kinesin at the C-terminal which is not predicted to be α-helical is indicated by a thicker line.

end (Scholey *et al.* 1989; Hirokawa *et al.* 1989) and may be involved in binding to the vesicle surface, although no functional role has been definitely assigned.

3. BASAL ATPase REACTION IN ABSENCE OF MTs

Initial experiments to determine the mechanism of hydrolysis of ATP by kinesin were complicated by the fact that kinesin binds ADP so strongly that the enzyme as isolated contains an ADP at its active site (Hackney 1988). This occurs even though the final stages of purification are conducted in the absence of ADP or ATP. The release rate of this bound ADP was found to be strongly dependent on the concentration of free magnesium and chromatography in the presence of excess EDTA results in enzyme which does not contain a nucleotide at the active site. This nucleotide-free kinesin (Hackney *et al.* 1989) was used successfully in single turnover measurements to establish the scheme indicated in figure 2. The initial binding and hydrolysis of ATP are fast, but product release is slow. The rate of ADP release following hydrolysis can be determined by cold chase experiments using pyruvate kinase and phospho(*enol*) pyruvate as a trap for free ADP. The active site of kinesin is first loaded with radioactive ADP in presence of pyruvate kinase and then chased out by addition of a large excess of unlabelled ATP. Under these conditions, any free ADP is rapidly converted to ATP by pyruvate kinase and any ADP which is detected following an acid quench must be inaccessible to pyruvate kinase by virtue of being bound at the active site of kinesin. The data of figure 2 with an ADP release rate of 0.008 s^{-1} were obtained in a buffer containing a free magnesium concentration of 0.4 mM. The buffer for most of our more recent work contains 1.9 mM free magnesium and consequently has a slower ADP release rate of 0.0015 s^{-1} (Hackney *et al.* 1991).

The rate of P_i release appears to be considerably faster than the rate of ADP release, based on rapid gel filtration and oxygen exchange measurements (Hackney 1988). When kinesin is incubated with radioactive ATP and rapidly passed down a centrifuge gel filtration column to remove free nucleotide and P_i different results are obtained depending on the location of the label. With ^{14}C-labelling of the adenine ring, much of the label is retained with the protein, consistent with tight binding and slow release of the ADP. With [γ-^{32}P]ATP labelling, essentially none of the label is retained by kinesin during passage down the column indicating that P_i is released rapidly after hydrolysis. Because the time required for separation on the centrifuge gel filtration column is not precisely defined, the rate of release of P_i is only approximated by this method, but release of P_i is clearly much faster than release of ADP. These initial experiments could be improved by use of nucleotide-free kinesin so that equilibration of radioactive label into the pool of bound kinesin product complexes would be more rapid and by use of techniques which allow a differentiation between free and bound P_i (Webb & Hunter 1991).

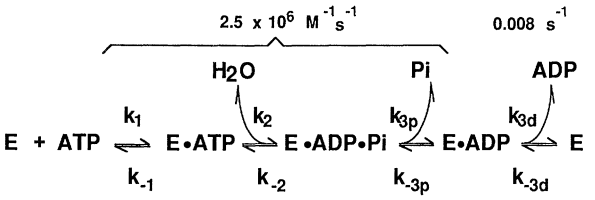

Figure 2. Scheme for ATP hydrolysis by kinesin in absence of MTs.

Oxygen exchanged experiments are also consistent with a rapid release of P_i. With myosin and other ATPases that have a slow release of P_i following hydrolysis, the reverse reaction to reform ATP occurs at a rate comparable to the rate of release of products. This reversal of the hydrolysis step results in the incorporation of multiple water-derived oxygens into the P_i which is finally released and the extent of incorporation of water-derived oxygens provides an estimate of the relative rate of product release versus reformation of bound ATP (see Sleep *et al.* 1980). With kinesin there is no extra incorporation of water-derived oxygens during ATP hydrolysis (Hackney 1988) which indicates that release of products is fast with respect to reformation of bound ATP. This could be due either to a slow rate of reformation of bound ATP or to a fast release of products. The later case would be consistent with the fast release of P_i indicated above. Once P_i has left the active site, no further incorporation of water-derived oxygens can occur even though ADP remains bound and its rate of release is slow. These experiments have not yet been performed in the total absence of MTs due to the very slow rate of steady state ATPase which is exhibited under those conditions, but no extra incorporation of water-derived oxygens was detected over a wide range of MT concentrations from levels which are only weakly stimulating to levels which are close to saturating. This is in contrast to the results with myosin in which the actin concentration modulated the extent of incorporation of water-derived oxygens by virtue of its stimulation of P_i release.

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4. REACTION IN THE PRESENCE OF MTs

Actin stimulates the low steady state ATPase rate of myosin by binding to the myosin-products complex and increasing the rate of product release. In an analogous manner, MTs also accelerate the rate limiting release of ADP by kinesin (Hackney 1988). This conclusion is based on cold chase experiments in which MTs are also included in the chase and the rate of release of ADP is observed to be markedly increased. There are two major problems with these results, however, which were not compatible with this simple model. The first is that the kinetics of ADP release in the presence of MTs did not obey first order kinetics and were biphasic or triphasic. The second problem is that the ADP release rate of most of the kinesin active sites was much too slow to account for the rapid steady state hydrolysis rate which was observed in parallel reactions with the same level of MTs. In a related issue, various preparations of kinesin have been reported to have a wide range in their maximum rate of ATP hydrolysis in the presence of MTs. Some of these problems were resolved by the discovery that limited proteolysis of kinesin can stimulate the rate of MT-stimulated ATP hydrolysis (Kuznetsov *et al.* 1989). In particular we have shown that kinesin preparations often contain a small variable amount of enzyme which has lost it β subunits due to partial proteolysis during isolation (Hackney 1991; Hackney *et al.* 1991). These α_2 dimers are not readily separated from the $\alpha_2\beta_2$ tetramers during most purification steps, but can be resolved by velocity sedimentation in a sucrose density gradient where the $\alpha_2\beta_2$ tetramer migrates with an apparent $s_{20,w}$ of 9.5 *S* compared to an apparent $s_{20,w}$ of 6.7 *S* for the α_2 dimer. The dimer has a 5-fold elevated ATPase rate and has a correspondingly more rapid rate of ADP release during a cold chase in the presence of MTs. This rapid release of ADP by the dimer likely accounts for part of the variability in steady state ATPase rates between different preparations and for the small variable component with a very rapid phase of ADP release which is observed in some kinesin preparations. The presence of the dimer does not, however, remove all of the problems since the isolated 9.5 *S* tetramer still has biphasic kinetics of ADP release and releases ADP at a rate which is too slow to account for the rate of steady state ATP hydrolysis. The most direct interpretation of these results is that the steady state ATPase reaction is produced by a small fraction of the enzymes which turn over extremely rapidly while the bulk of the active sites turn over slowly at the rate observed in the cold chase experiments. Definitive resolution of these discrepancies will require additional work, but the observation of a major conformatinal change in kinesin provides a possible mechanism for the inhibition of the bulk of the sites as discussed below. Interestingly, when the tetramer or the dimer are adsorbed to glass surfaces, they both drive the sliding of MT bundles at the same velocity (Hackney *et al.* 1991) in spite of a 5-fold difference in MT-stimulated ATPase rates as measured in solution.

5. CONFORMATIONAL TRANSITION OF KINESIN

Electron microscopic observation of kinesin indicated the presence of a flexible hinge in the stalk and the tendency for the molecule to fold into a more compact conformation at low ionic strength, *I* (see Hisanaga *et al.* 1989). This conformational transition has also been observed by direct hydrodynamic observation (Hackney *et al.* 1992) in which the $s_{20,w}$ value of kinesin shifts from 9.5 *S* at low *I* to 6.5 *S* at high *I* (adjusted with NaCl or KCl). The midpoint for this transition is at an *I* value of 0.25 at pH 6.9 and it is thus expected that the enzyme will be in the folded conformation under both physiological conditions and the usual conditions for measurement of ATPase kinetics and *in vitro* motility. The α_2 dimer undergoes a similar conformational transition indicating that the β subunits are not required for formation of the folded conformation, although the midpoint *I* value for the transition is shifted.

This conformation transition is strikingly similar to the 10 *S* to 6 *S* transition which smooth muscle myosin undergoes (Suzuki *et al.* 1982; Trybus *et al.* 1982). In this case the myosin is in a folded inhibited conformation at physiological *I* and phosphorylation shifts the equilibrium towards the active unfolded species (see Cross *et al.* 1988). In the case of kinesin it is not known what if anything regulates the conformational transition, but it is interesting to speculate that the folded form of kinesin represents an inhibited soluble pool of the enzyme which can be activated into an unfolded conformation. The high rate of steady state ATP hydrolysis in the presence of MTs, which appears to be due to the rapid turnover of a minor fraction of the kinesin molecules, could be due to a limited number of activated molecules whereas the bulk of the kinesins are in the inhibited conformation. This model also provides a way to account for the pronounced influence of the β subunits on the steady state ATPase rate. In the unfolded conformation, the β subunits are far removed from the head groups and it was unexpected that they should have such a pronounced effect on ATP hydrolysis, but their presence in close proximity to the head groups in the folded conformation provides a reasonable mechanism for their influence. The isolated head group has been reported to have a MT-stimulated ATPase rate which is even higher than that of the α_2 dimer (Kuznetsov *et al.* 1989). This may indicate a progressively weaker interaction of tail domains with the head groups on going from the $\alpha_2\beta_2$ tetramer to the α_2 dimer to isolated head groups. This, in turn, may be associated with a progressive increase in MT-stimulated ATPase rates.

6. COMPARISON TO MYOSIN

The mechanisms of myosin and kinesin show a number of overall similarities. Both enzymes bind and hydrolyze ATP rapidly and have rate-limiting product release in the absence of actin or MTs. This low

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basal rate is accelerated by interaction of the products complex with actin or MTs and this acceleration is specifically due to stimulation of product release. The major difference in the schemes relates to the relative release rates of P_i and ADP and even that difference is one of degree rather than a qualitative difference. Kinesin appears to release P_i much more rapidly than it releases ADP whereas myosin releases both ADP and P_i at a rate which is more rapid than that of a preceding conformational change which can be accelerated by interaction with actin. But even in the case of myosin, P_i release is more rapid than ADP release and selective ADP release can become rate limiting at low temperature. These differences may be important since the region between P_i release and ADP release has been postulated to be a critical one for functioning of myosin as a motor (Hibberd & Trentham 1986). This model has the power stroke for actomyosin occurring in the region between P_i release and ADP release which is precisely the region of greatest difference in the mechanisms of myosin and kinesin. Thus although the mechanisms are generally similar, important differences occur in the entry and exit rates to states which may contain the power stroke and these differences could have significant consequences for the physiological properties of the two motors.

7. GENERAL CONSIDERATION ON THE PROPERTIES REQUIRED OF BIOLOGICAL MOTORS

What are some of the general properties expected for the mechanism of coupling of ATP hydrolysis to long range movement of macromolecular structures relative to each other (as opposed to repetitive movements such as simple conformational changes which do not produce cumulative displacement)? Simple arguments based on the amount of energy available per ATP indicate that multiple cycles of ATP hydrolysis will be required for movement over the distances which are commonly observed. This in turn requires that the relative movement occurs along a directional filamentous (or at least polymeric) framework so that similar cycles of ATP hydrolysis and coupled interactions with the framework subunits can occur at progressive greater distances. In particular experimental arrangements, it may be either the motor or the framework which physically moves with respect to external reference, but the distinction can still be made between one component which undergoes repetitive cyclic conformational changes and a polymeric framework whose components undergo sequential interactions with the cycling component and whose principal function is to provide the road along which movement occurs.

In general the physiological role of the motor is not to move the motor itself along the road, but rather to move some load which is attached to the motor. Thus the motors as the cycling component will need to have at least two functional domains, one for interaction with the road and a second for interaction with the load and this road–load polarity is likely to be an intrinsic property of all motors. Both myosin and kinesin as well as dynein possess an extended flexible linker region between the road and the load domains. These linkers are useful in that they provide a way for the two other domains to be sufficiently separated so that the load and the road do not interfere during the cycling of the motor. Such an extended linker is not required, however, and some motors such as myosin I can accomplish movement with a shorter separation of the two domains. These general considerations apply not only to myosin and kinesin, but also to motors which operate on other filamentous roads including such motors as helicases which move along a polymeric road of DNA and motors such as that of bacterial flagella which move along circular roads using proton translocation as the driving force.

8. SLIPPAGE DURING COUPLED ATP HYDROLYSIS

As developed by Jencks (1980), coupling requires that the process of ATP hydrolysis be halted at a designated step until some particular part of the coupled process (the conformational changes) occurs. In the case of myosin, ATP hydrolysis proceeds rapidly in the absence of actin until product release is required. At this point the process is blocked because product release is slow in the absence of actin. Binding of actin to the product complex induces conformational changes which result in an accelerated release of products to complete the ATPase cycle. These conformational changes also represent the completion of the conformational cycle which produces the movement and coupling in this way ensures that both processes are intimately intertwined and that one cannot proceed without the other. Release of P_i and ADP from the myosin products complex in the absence of actin thus represents slippage through the ATPase cycle without coupling to the conformational cycle. The rate of this slippage reaction relative to the maximal rate of the fully coupled process is a measure of the tightness of coupling. In the case of S1 from rabbit skeletal myosin, typical rates for ATP hydrolysis at low ionic strength are $0.06\ s^{-1}$ in the absence of actin and $15\ s^{-1}$ in the presence of saturating actin levels. This represents a coupling ratio of 250:1 indicative of tight coupling of the two processes. Coupling by kinesin is even tighter with corresponding rates of 0.0015 and $7\ s^{-1}$ for turnover of the α_2 dimer at low I in the absence and presence of saturating levels of MTs. This yields a coupling ratio of 4700:1 and the true ratio may be even higher as there is reason to believe that the folded form of the α_2 dimer may still be partially inhibited in its ability to be stimulated by MTs.

With enzymes which do not show tight coupling, it remains unclear whether this represents true loose coupling which would be of relevance to the physiological situation or if this represents partial denaturation or other complications which result in abnormal behaviour for the isolated components. The pathway for uncoupled slippage has a high-energy barrier, but a second low-energy pathway must also be available which can be utilized when the coupling requirements

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have been satisfied. In effect the kinesin–ADP complex in the absence of MTS is poised and ready to release ADP, but release is blocked by the conformational constraints of the enzyme. Partial denaturation or partial proteolysis may relieve some of these constraints and permit the enzyme to slip through this step in the absence of the coupling process, perhaps by following the same pathway as used for coupling, but without coupling actually taking place. Myosin from smooth muscle provides a good example of such a situation (Cross *et al.* 1988). In this case the proteolytic fragment heavy meromyosin has lost the constraint required for formation of the trapped product complex.

The desirability of reducing slippage may play a role in how biological motors have evolved. For example, there is no absolute reason why the framework components could not be the sites of ATP hydrolysis, but in all known cases, it is the cycling component which hydrolyses ATP. This is reasonable, however, when considered from the view point of the coupling reactions. If the cycling component is the site of ATP hydrolysis, then comparatively little time is spent in the state awaiting coupling since each individual cycling component turns over during each cycle. If the framework units, such as individual tubulin monomers, are the site of ATP hydrolysis, then each one would have to remain halted at the stage susceptible to slippage for an extensive length of time before a cycling component interacted with that particular framework unit. Slippage could occur through this whole extended period and this would result in wasteful hydrolysis of ATP without production of useful work.

REFERENCES

Amos, L.A. 1987 Kinesin from pig brain studied by electron microscopy. *J. Cell Sci.* **87**, 105–111.

Cross, R.A., Jackson, A.P., Citi, S., Kendrick-Jones, J. & Bagshaw, C.R. 1988 Active site trapping of nucleotide by smooth non-muscle myosins. *J. molec. Biol.* **203**, 173–181.

Hackney, D.D. 1988 Kinesin ATPase: rate-limiting ADP release. *Proc. natn. Acad. Sci. U.S.A.* **85**, 6314–6318.

Hackney, D.D. 1991 Isolation of kinesin using initial batch ion exchange. *Methods Enzymol.* **196**, 175–181.

Hackney, D.D., Levitt, J.D. & Wagner, D.D. 1991 Characterization of $\alpha_2\beta_2$ and α_2 forms of kinesin. *Biochem. biophys. Res. Comm.* **174**, 810–815.

Hackney, D.D., Levitt, J.D. & Suhan, J. 1992 Kinesin undergoes a 9 to 6 S conformational transition. *J. biol. Chem.* (In the press.)

Hackney, D.D., Malik, A. & Wright, K.W. 1989 Nucleotide-free kinesin hydrolyzes ATP with burst kinetics. *J. biol. Chem.* **264**, 15943–15948.

Hibberd, M.G. & Trentham, D.R. 1986 Relationship between chemical and mechanical events during muscular contraction. *A. Rev. Biophys. biophys. Chem.* **15**, 119–161.

Hirokawa, N., Pfister, K.K., Yorifuji, H., Wagner, M.C., Brady, S.T. & Bloom, G.S. 1989 Submolecular domains of bovine brain kinesin identified by electron microscopy and monoclonal antibody decoration. *Cell* **56**, 867–878.

Hisanaga, S., Murofushi, H., Okuhara, K., Sato, R., Masuda, Y., Sakai, H. & Hirokawa, N. 1989 The molecular structure of adrenal medulla kinesin. *Cell Motil. Cytoskeleton* **12**, 264–272.

Jencks, W.P. 1980 The utilization of binding energy in coupled vectorial processes. *Adv. Enzymol.* **51**, 75–106.

Johnson, K.A. 1985 Pathway of the microtubule-dynein ATPase and the structure of dynein: a comparison with actomyosin. *A. Rev. Biophys. biophys. Chem.* **14**, 161–188.

Kuznetsov, S.A., Vaisberg, E.A., Rothwell, S.W., Murphy, D.B. & Gelfand, V.I. 1989 Isolation of a 45-kDa fragment from the kinesin heavy chain with enhanced ATPase and microtubule-binding activities. *J. biol. Chem.* **264**, 589–595.

Scholey, J.M., Heuser, J., Yang, J.T. & Goldstein, L.S.B. 1989 Identification of globular mechanochemical heads of kinesin. *Nature, Lond.* **338**, 355–357.

Shpetner, H.S. & Vallee, R.B. 1989 Identification of dynamin, a novel mechanochemical enzyme that mediates interactions between microtubules. *Cell* **59**, 421–432.

Sleep, J.A., Hackney, D.D. & Boyer, P.D. 1980 The equivalence of phosphate oxygens for exchange and the hydrolysis characteristics revealed by distribution of $[^{18}O]P_i$ species formed by myosin and actomyosin ATPase. *J. biol. Chem.* **255**, 4097–4099.

Suzuki, H., Kamata, T., Ohnishi, H. & Watanabe, S. 1982 Adenosine triphosphate-induced reversible change in the conformation of chicken gizzard myosin and heavy meromyosin. *J. Biochem.* **91**, 1699–1705.

Taylor, E.W. 1979 Mechanism of actomyosin ATPase and the problem of muscle contraction. *CRC Crit. Rev. Biochem.* **6**, 103–164.

Trybus, D.M., Huiatt, T.W. & Lowey, S. 1982 A bent monomeric conformation of myosin from smooth muscle. Proc. natn. Acad. Sci. U.S.A. **79**, 6151–6155.

Vale, R.D. & Goldstein, L.S.B. 1990 One motor, many tails: an expanding repertoire of force- generating enzymes. *Cell* **60**, 883–885.

Vallee, R.B. & Shpetner, H.S. 1990 Motor proteins of cytoplasmic microtubules. *A. Rev. Biochem.* **59**, 909–932.

Webb, M.R. & Hunter, J.L. 1991 Continuous assay for phosphate release based on UV absorbance measurements and its use to measure GAP-activated hydrolysis of GTP bound to p21ras. *Biophys. J.* **59**, 348a.

Yang, J.T., Laymon, R.A. & Goldstein, L.S.B. 1989 A three-domain structure of kinesin heavy chain revealed by DNA sequence and microtubule binding analyses. *Cell* **56**, 879–889.

Discussion

K. A. JOHNSON (*Department of Biochemistry, The Pennsylvania State University, U.S.A.*). First, I wanted to amend slightly one of the comments Dr Hackney made in respect of work on dynein. I would say that in spite of the size and complexity of dynein, we know quite a bit about its mechanism in terms of rates and equilibrium constants of most steps in the pathway. One of the features that shares and follows some of the work that Dr Hackney had done on kinesin is that ADP release is rate limiting in the overall pathway. I am puzzled by this. He suggested that this may not be important, but it may be important because there is a free energy change associated with the release of phosphate and potentially there could be some steps in the coupling mechanism involving a change in conformation prior to reassociation of the kinesin-products or dynein-

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products complex with the microtubule. Does he have any comments on that?

D. D. Hackney. We know what the rate constants are but not how they fit into a model for the full cycle. Another problem is the stimulation of the ATPase rate can be saturated by adding microtubules without saturating the physical binding of the enzyme to the microtubule, very much analogous to older work done with the refractory state in the actomyosin ATPase scheme. It is even worse here, but we think that the reason for this is that most of the enzyme remains unbound. It is some smaller subpopulation which binds to the microtubule and turns over ATP rapidly so producing most of the flux. So all of those things will make it very hard to work out the exact mechanism until there is a form of the enzyme that we know is a single population and that works in a truly active way. We have not reached that stage yet.

K. A. Johnson. There have been some suggestions of phosphorylation of the beta chains regulating kinesin. Does Dr Hackney have any evidence for phosphorylation in his preparations?

D. D. Hackney. We have no evidence at all because we do not isolate kinesin from sources where it is feasible to do radioactive labelling. We have not got into that aspect of the work.

K. A. Johnson. We have some recent results on the cloned head fragment of kinesin from *Drosophila* that does not show this apparently higher steady state ATPase activity in the absence of microtubules seen with the proteolytic fragments. The steady-state turnover rate of the isolated head is on the order of 0.005 s^{-1} in the absence of microtubules and around 5 s^{-1} in the presence. So it correlates quite closely with the numbers that Dr Hackney obtained, surprisingly considering it is from *Drosophila* rather than cow.

D. D. Hackney. Yes, it may well turn out that it is a somewhat slower enzyme when isolated in that way. That is always a thing to watch for when dealing with a subfragment of an enzyme.

K. C. Holmes (*Max Planck Institut für medizinische Forschung, Heidelberg, Germany*). Does kinesin show the glycine-rich loop similar to myosin, the 'P loop'?

D. D. Hackney. It does have the loops that are normally thought to play a role in the binding of a nucleotide.

M.-F. Carlier (*Laboratoire d'Enzymologie, CNRS, Gif-sur-Yvette, France*). Does kinesin 'walk' along protofilaments in microtubules and has Dr Hackney been able to make it 'walk' in circles around rings of tubulin that are just curled protofilaments with enhanced ATPase activity?

D. D. Hackney. If Dr Carlier means 'walk' as a single molecule in a precessive mode, as a single enzyme moves down a microtubule, then that is not work that we have done, but others have tried to show that a single kinesin molecule attached to glass can drive the sliding of a microtubule. In the normal actomyosin scheme, of course, myosin dissociates from the actin filament during each cycle, and kinesin probably does the same so that the microtubule could diffuse away. However, in that situation when one protein is attached to a glass surface, it is probable that the large microtubule cannot diffuse away rapidly and the kinesin binds again. In that way it can walk along the microtubule. Some laboratories like the model that has one head attached and the next head reaching over ready to bind, but that is in the range of speculation.

G. F. Elliott (*Open University Research Unit, Boars Hill, Oxford, U.K.*). I would like to make the suggestion that the unfolding effect could be a very simple electrostatic one, if one makes the assumption that kinesin α-helical rod binds anions as we have shown in the case of myosin.

K. Burton (*King's College, London, U.K.*). Is it possible to do these ATPase rate or ADP release measurements with the kinesin bound to small lipid vesicles?

D. D. Hackney. It may be, but one would need to know that the binding was meaningful physiologically and I do not believe anyone has yet shown that. Many laboratories have tried to investigate the nature of the site on the surface of vesicles to which kinesin binds but this has been very slow. If one could get a really good, uniform population in which all the molecules were bound in a physiologically relevant way, then one might get very useful results.

D. R. Trentham (*National Institute for Medical Research, Mill Hill, London, U.K.*). I would like to make a comment relating to an experiment by Clive Bagshaw which he recently did in Standford on myosin filaments (Bagshaw *et al.* 1992). He was using fluorescent nucleotides and because of the difference of the local concentration of the nucleotide when it was bound to the protein versus that in the background medium, he was at least able to look at some elementary steps of the ATPase.

Reference

Bagshaw, C.R., Matuska, M. & Spudich, J.A. 1992 Measurement of ATP turnover by single immobilised myosin filaments. *J. Mus. Res. Cell. Motil.* **13,** 266.

C. Cremo (*Department of Biochemistry, Washington State University, Pullman, U.S.A.*). Does Dr Hackney know that he has not removed part of the kinesin tail when the beta subunits come off?

D. D. Hackney. No, we do not. We know from a high resolution run on an SDS gel that it is the same size, but it could have lost a few residues either end. That is the main reason why we wanted to find salts which remove the beta subunits in a mild way. We do know that it is possible to nick the alpha subunit, and that causes a big rise in the rate.

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