Molecular Motors Take Tension in Stride

James A. Spudich[1,*]
[1]Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305, USA
*Contact: jspudich@stanford.edu
DOI 10.1016/j.cell.2006.07.009

Mechanical tension controls the function of a wide variety of eukaryotic motor proteins. Single-molecule analyses have revealed how some of these proteins sense and respond to tension. The single motor studies on dynein by Reck-Peterson et al. (2006) described in this issue pave the way to understand molecular mechanisms used by this unique machine.

Molecular motors are a class of enzymes that exquisitely couple ATPase activity and precise changes in protein conformation. Because these mechanical changes are linked to the binding of the motor to its protein track (Figure 1, top left), the net result is directed motion. There are nearly one hundred different molecular motors in a eukaryotic cell, and the diversity of mechanisms by which the various motors work is remarkable, each being designed to carry out its particular physiological function. Studies of molecular motors reveal many principles about how proteins behave, such as the importance of long-range communication between different regions of a protein and the special roles of large unstructured regions. As only a handful of motors have been characterized in detail, other concepts fundamental to protein structure and function will certainly emerge as more are analyzed. In an extensive study reported in this issue of *Cell*, Ronald Vale and colleagues (Reck-Peterson et al., 2006) use single-molecule techniques to describe the processive motion and stepping behavior of cytoplasmic dynein.

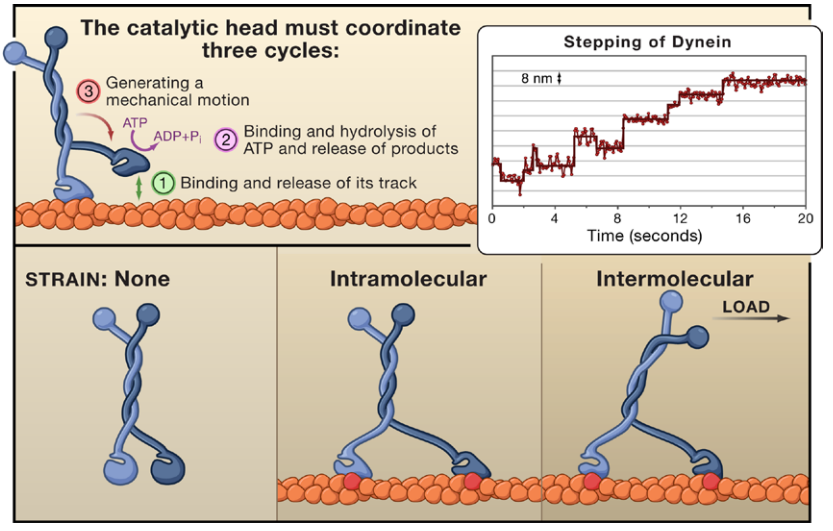

Figure 1. Coordinating the Movement of Motor Proteins
(Top left) The schematic diagram indicates the three cycles that molecular motors must coordinate, as depicted for a myosin motor. (Top right) Single-molecule analysis (Reck-Peterson et al., 2006) indicates that dynein primarily takes steps of 8 nm along microtubules. (Bottom) A homodimeric motor (left) with its two heads in identical configurations represents the motor in the absence of tension. (Middle) Walking along a polarized track with no external load, the homodimeric motor experiences intramolecular tension that causes the conformation of the active site to be distorted in different ways for each head. (Right) An external load changes the enzyme kinetics of a motor protein. These changes may be different from those that result from intramolecular tension. Note that there are preferred binding sites of processive motors along there tracks, here indicated in red. Myosins V and VI, for example, both prefer to step along the helical pseudo-repeat of an actin filament (~36 nm) rather than undergo the distortions necessary to reach off the longitudinal axis to bind to other actin monomers.

Cytoplasmic dynein is a 1.2 megadalton microtubule motor that is important for spindle formation, chromosome segregation, and transport of numerous cargoes. Dynein is found on a divergent evolutionary branch of the AAA+ family of ATPases and is therefore very different from both myosins and kinesins, which share many mechanistic similarities (Vale and Milligan, 2000). Reck-Peterson et al. (2006) used the budding yeast *S. cerevisiae* to produce a functional recombinant dimeric dynein and developed single-molecule fluorescence assays to directly observe its processive motion. They show that cytoplasmic dynein motors must be dimeric for processivity. This conclusion is supported by the creation of a dynein that can be converted between monomer and dimer states using a small molecule. For this recombinant dynein, processive motion was only observed in the presence of the small molecule that induced dimer formation. Remarkably, the processivity of dynein does not require any of the known dynein-associated subunits in the yeast genome.

The authors also labeled dynein in specific locations with fluorescent dyes or quantum dots and tracked single molecules to reveal dynein's

stepping behavior. In contrast to a previous report of very large steps (24–32 nm) of cytoplasmic dynein under low external force (Mallik et al., 2004), Reck-Peterson et al. and others (Toba et al., 2006) find that dynein, like Kinesin-1, moves primarily in 8 nm increments through alternating movements of its two motor domains (Figure 1, top right). Reck-Peterson and colleagues observe considerable variability in step size and direction, suggesting that the dynein step has a large diffusional component, which differs from Kinesin-1 and is more like myosin VI.

How do homodimeric processive motors such as dynein coordinate the alternating stepping of their two motor domains in a unidirectional manner? An important concept to emerge from single-molecule analyses of molecular motors is the importance of tension sensing to coordinate stepping. Coordination of motor activity via mechanisms of tension sensing has long been suspected by biologists studying muscle. For example, the motor domains of muscle myosin II influence one another during contraction. Some heads undergo their mechanical motion (referred to as a stroke), while others are still bound in their post-stroke states. But the characteristics of muscle myosin II, specifically its small step size, its nonprocessive movement as a single molecule, and the complicated multimolecular interactions occurring in the sarcomere, make it difficult to assess how tension or load affects its nucleotide biochemistry.

Motors that are processive are easier to probe with regard to tension sensing compared to nonprocessive motors such as muscle myosin II. This is because processive motors have long runs of stepping as single molecules (Figure 1, top right). By analyzing the distribution of dwell times (the time between steps), one can determine the kinetic rates that describe transitions in the stepping cycle. It is in the analysis of dwell times that the power of the single-molecule approach is realized. Applying a load to a motor specifically perturbs mechanical transitions in the stepping cycle, resulting in a force-dependent shift in the dwell time distribution. By observing changes in the dwell distribution resulting from both varying load and nucleotide concentration, one can identify mechanical transitions that alter the conformation of the active site and thus affect nucleotide binding, ATP hydrolysis, and product release.

Using this approach, several groups have reported changes in the rates of nucleotide binding or release to either the leading head or trailing head of myosin V as a result of intramolecular strain. In myosin V, this strain develops in the stretch of protein between the two heads when they are both bound to an actin filament (Baker et al., 2004; Purcell et al., 2005; Veigel et al., 2005). Similarly, the two-headed kinesin motor walks processively along a microtubule, taking 8 nm steps, alternately stepping with each of its catalytic heads in a hand-over-hand fashion. Here, too, the intramolecular strain that results when the two heads are bound to the microtubule changes the kinetics of stepping (Guydosh and Block, 2006). These changes presumably result from strain-induced modifications of the active site (Figure 1, bottom). The two heads are identical when not bound to their track but are both altered in different ways when in their bound state.

In addition to intramolecular tension sensing, intermolecular tension sensing can also regulate motor activity. This type of regulation was demonstrated for the myosin VI motor, which acts both as a transporter (for example, in the movement of endocytic vesicles) and as a structural linker (to maintain the spatial organization of the Golgi complex). The motor is able to switch between these two functions by sensing external forces. A biochemical mechanism has been worked out for this mode of behavior using single-molecule laser trap techniques where known forces can be applied to the myosin VI molecule (Altman et al., 2004). ATP binding is required to weaken the affinity of myosin VI for actin. Thus, if ATP binding is inhibited, the translocation cycle will be halted and the motor will clamp onto the actin filament with both heads strongly bound. Remarkably, single-molecule studies show that this is achieved by a dramatic increase in the rate of ADP binding when the motor experiences load against its stepping ($\sim$100-fold increase in the rate of ADP binding for $\sim$2 pN of load). Thus, ADP favorably competes with ATP for binding to the active site and prevents the ATP-induced dissociation of the actin-myosin VI complex.

Will cytoplasmic dynein reveal unique forms of tension sensing? The work of Reck-Peterson et al. (2006) sets the stage for answering this question. Dynein is likely to use different mechanisms compared with myosin and kinesin for sensing intramolecular tension to coordinate its two motor domains during processive motion. Unlike myosin and kinesin whose motor domains are physically separated while walking on their tracks, Reck-Peterson et al. suggest that dynein's two motor domains may overlap and potentially interact, which could result in intramolecular strain leading to changes in the kinetic cycles of the two heads. In addition, angular displacements of dynein's microtubule binding domain, which is located at the tip of a 10 nm long stalk, could also result in strain that alters nucleotide affinity or enzymatic steps in any of the four ATP binding sites in the catalytic motor domain. Finally, although the dynein-associated proteins are not essential for processivity in vitro, they could play a role in tension sensing.

Although tension sensing as a means of regulating function is usually associated with molecular motors, I believe that the ability of mechanical strain to dramatically alter the function of proteins will be found to be a widespread form of regulation of enzyme activity in cells. The dense cytoskeletal net-

work with its large cadre of molecular motors is associated with many if not most other cell constituents and enzymes. Thus, a wide variety of enzyme systems are likely to feel transient loads that could have dramatic effects on their biochemical behaviors. As in the past, a detailed understanding of molecular motors provides us with insights into how proteins in general are able to achieve their remarkable and diverse activities.

REFERENCES

Altman, D., Sweeney, H.L., and Spudich, J.A. (2004). Cell *116*, 737–749.

Baker, J.E., Krementsova, E.B., Kennedy, G.G., Armstrong, A., Trybus, K.M., and Warshaw, D.M. (2004). Proc. Natl. Acad. Sci. USA *101*, 5542–5546.

Guydosh, N.R., and Block, S.M. (2006). Proc. Natl. Acad. Sci. USA *103*, 8054–8059.

Mallik, R., Carter, B.C., Lex, S.A., King, S.J., and Gross, S.P. (2004). Nature *427*, 649–652.

Purcell, T.J., Sweeney, H.L., and Spudich, J.A. (2005). Proc. Natl. Acad. Sci. USA *102*, 13873–13878.

Reck-Peterson, S.L., Yildiz, A., Carter, A.P., Gennerich, A., Zhang, N., and Vale, R.D. (2006). Cell, this issue.

Toba, S., Watanabe, T.M., Yamaguchi-Okimoto, L., Toyoshima, Y.Y., and Higuchi, H. (2006). Proc. Natl. Acad. Sci. USA *103*, 5741–5745.

Vale, R.D., and Milligan, R.A. (2000). Science *288*, 88–95.

Veigel, C., Schmitz, S., Wang, F., and Sellers, J.R. (2005). Nat. Cell Biol. *7*, 861–869.

CK2 and PML: Regulating the Regulator

Valérie Lallemand-Breitenbach[1] and Hugues de Thé[1,*]
[1]Université de Paris 7, CNRS, UMR 7151, Hôpital St. Louis, 75475 Paris Cedex 10, France
*Contact: dethe@paris7.jussieu.fr

DOI 10.1016/j.cell.2006.07.004

The PML protein induces senescence, and, upon oncogenic stress, its absence promotes cellular transformation. In this issue of *Cell*, Scaglioni et al. (2006) show that phosphorylation of PML by CK2, a kinase frequently activated in human cancers, promotes PML degradation. Therefore, pharmacological inhibition of CK2-induced PML loss could be used to offset tumor establishment.

Analysis of the chromosomal translocations in tumor cells from acute promyelocytic leukemia patients led to the discovery of the *PML* gene. Fusion between the retinoic acid receptor α gene (*RARα*) and several other genes, most frequently *PML*, results in the production of chimeric proteins that drive the uncontrolled cellular proliferation and block in differentiation that leads to leukemia. Interestingly, the PML protein accumulates in subnuclear domains termed PML nuclear bodies, the function of which has remained a puzzle to cell biologists. Despite the fact that *Pml*-deficient mice exhibit only subtle defects, a variety of biological functions have been assigned to PML, ranging from senescence and apoptosis to combating viral infection. The many biochemical processes (such as transcription, DNA repair, and proteolysis) proposed to be under PML control add to the mystery surrounding this protein.

In this issue of *Cell*, Scaglioni et al. (2006) demonstrate that casein kinase 2 (CK2), a kinase associated with cancer promotion, phosphorylates PML and targets it for degradation by the proteasome. Loss of the critical CK2 phosphorylation site in PML results in stabilization of this protein, enhancement of PML-induced apoptosis and senescence, and abrogation of sensitivity to CK2 inhibitors. Moreover, in human non-small cell lung cancers, there is an inverse relationship between PML expression and CK2 activity. PML degradation upon CK2 activation could account for the frequent loss of PML expression observed in multiple human tumors. This loss of PML could promote tumor formation because the enforced expression of activated Ras (which leads to cancer) induced more aggressive lesions in the lungs of *Pml*-deficient mice compared to wild-type animals (Scaglioni et al., 2006). PML is upregulated during senescence and is also required for the induction of senescence caused by Ras activation or PTEN loss ex vivo and in vivo (Scaglioni et al., 2006; Trotman et al., 2006). Similarly, PML overexpression in primary fibroblasts is sufficient to trigger senescence (Bischof et al., 2002). CK2 activation could thus inhibit oncogene-induced senescence by mediating PML loss. Hence, *PML* transcriptional induction by growth-suppressive pathways (such as the interferon, p53, or TGF-β signaling pathways) may be balanced by