Kidd *et al.*[3] show genetic interactions between *slit* and *robo* mutants, suggesting that these two molecules act in the same pathway. Brose *et al.*[4] and Li *et al.*[5] show that Slit binds to Robo on cell surfaces and in solution. There are at least three forms of Slit in mammals[14], and at least two of Robo, all of which bind to each other across species boundaries. Moreover, human Slit repels rat motor axons. This is all excellent evidence that Slit is an evolutionarily conserved repulsive ligand for Robo. Interestingly, Slit also binds netrin and laminin *in vitro*, although we don't yet know why.

It is not uncommon for axon-guidance molecules to be used at more than one place in the brain. For example, as well as acting at the midline, netrin is expressed in the vertebrate visual system and in the body-wall muscles of flies. So, the finding of Ba-Charvet *et al.*[6] and Li *et al.*[5] — that the Robo–Slit system is used at other places in the CNS — is not surprising. The first clear case of axons being repelled by a diffusible ligand was in 1993. Adrian Pini[15] showed that cultured axons from the olfactory tract are repelled from a region of the forebrain called the septum. Olfactory-bulb axons, it turns out, express high levels of Robo-2, whereas Slit-2 is highly expressed in the septum. Then there is the hippocampus. Hippocampal axons, which express Robo, do not invade the adjacent entorhinal cortex, which expresses Slit-2. Cell lines expressing Slit-2 can repel both olfactory-tract and hippocampal axons *in vitro*.

Although the new papers show that Slit should join the growing family of evolutionarily conserved, repulsive guidance factors in the CNS, Wang *et al.*[2] report the identification of Slit through a different approach — one that suggests a distinct function for Slit. During development, the axons of pain and temperature receptors enter the spinal cord and travel up and down on the same side for a short distance. They then produce branches along these axon shafts. The branches make synapses with the commissural interneurons that take the message of pain or temperature to the brain. By culturing these pain- and temperature-sensitive neurons in isolation, while exposing them to different CNS fractions, Wang *et al.* discovered that the fraction containing Slit dramatically promoted axonal branching and growth. So, although it has just been identified as a repellent, Slit can also serve as a positive growth- and branch-promoting substance.

The new studies raise further questions. For instance, is the branch-promoting activity of Slit on sensory neurons mediated through Robo? And why does Slit, the repellent, bind netrin, the attractant? Whatever the answers, by uncovering the repellent that keeps some axons from crossing the midline and others from recrossing, these studies shed considerable light on the ancient mysteries of commissure formation. □

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Motor proteins

Another step ahead for myosin

Malcolm Irving and Yale E. Goldman

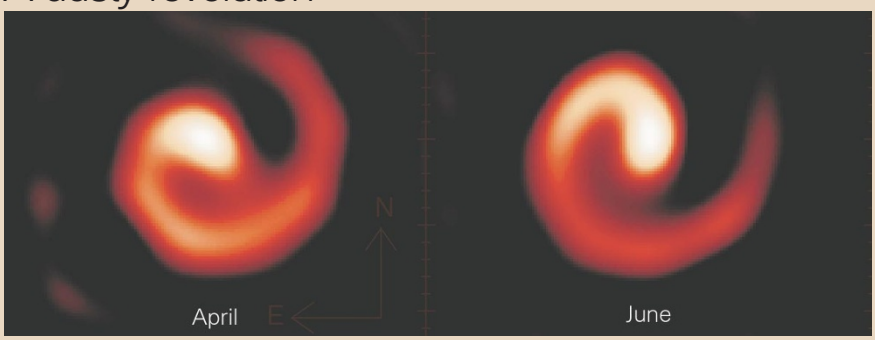
Eukaryotic cells contain many different protein motors, which use metabolic energy to transport cell components along polymer tracks such as actin filaments, microtubules or DNA. A single motor molecule moves along an isolated track in nanometre-scale steps corresponding to hydrolysis of single ATP molecules[1–4]. But two studies on myosin — the motor protein in muscle — by Veigel *et al.*[5] (page 530 of this issue) and Kitamura *et al.*[6], show that each interaction with actin can include two or more sub-steps per ATP hydrolysed.

The head region of myosin, which embodies its motor function, contains a catalytic domain that binds actin and ATP, and an elongated carboxy-terminal domain containing a variable number of calmodulin-like light chains. The light-chain domain is thought to act as a lever arm in the motor mechanism[7,8]. It is often connected to its cargo (which may be a vesicle or filament) through a coiled-coil tail.

Veigel *et al.*[5] exploited the slow kinetics of two single-headed myosins, myr-1 from rat liver and brush-border myosin-I (BBM-I)

Astronomy

A dusty revolution



Collectors of unusual astronomical objects have another to add to their list: the first spiral star ever observed. Elsewhere in this issue (*Nature* 398, 487–489; 1999) Peter Tuthill and colleagues report high-resolution infrared images of a spiral structure in the hot dust around a Wolf–Rayet star (WR104). They use a powerful aperture-masking technique at the 10-m Keck telescope in Hawaii to produce images much better even than those taken by the Hubble Space Telescope.

Wolf–Rayet stars are a phase in the life of exceptionally hot, massive stars, just before they are thought to become supernovae. Some Wolf–Rayet stars are surrounded by shells of dust, but it has been a mystery as to how dust survives the harsh ultraviolet radiation they emit.

Now, not only have Tuthill *et al.* detected a spiral pinwheel in the dust around WR104, but they also watched it rotate every 220 days. The image above shows the dusty spiral as seen in April and June 1998. The authors say the spiral and its rotation are the consequence of a companion star. In their hypothesis, dust is created around the binary star where the stellar winds collide, and is then carried along with the orbital motion.

Whether every dusty star has a binary remains open for debate. But in this case, the images of the spiral are so good that the orbital period, distance and separation of the binary system can be inferred from its effect on the stellar dust, without ever detecting the two central stars. **Sarah Tomlin**

from chicken intestinal epithelium, to reveal the substructure of the interactions between each of these motors and single actin filaments. The authors suspended a filament between two 1-μm plastic beads held by optical tweezers, then brought this into contact with a myosin molecule bound to nitrocellulose. They detected transient interactions between the myosin head and actin by the associated reduction in thermal fluctuations of the beads.

Veigel and colleagues found that the filament is displaced by about 6 nm at the start of each interaction, then, surprisingly, by another 5.5 nm 100–300 ms later. The interval between the two sub-steps did not depend on the concentration of ATP. However, the interval between the second sub-step and the end of the interaction was shorter at higher concentrations of ATP — as expected if each interaction is terminated by the binding of ATP to myosin. With skeletal-muscle myosin heads (termed S1), only one 5.5-nm step was observed, within 5 ms of the start of the interaction.

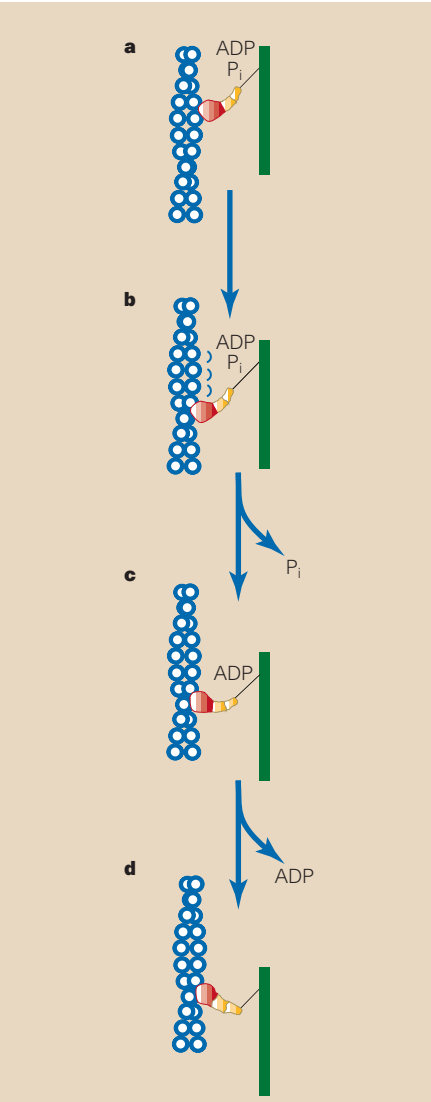


Figure 1 **Different ways in which a myosin S1 head might produce sub-steps as it moves along an actin filament. The catalytic domain is shown in red, with the light chains in yellow. Actin is shown in blue, with the barbed end downwards. The results of Kitamura *et al.*[6] indicate that S1 might jump between actin-binding sites (a → b). Alternatively, or additionally, according to the results of Veigel *et al.*[5], sequential conformational changes in the myosin head might be linked to the release of inorganic phosphate (P_i) and ADP (b → c → d).**

The authors suggest that the sub-steps seen with myr-1 or BBM-I are coupled to two intermediate steps in the ATP hydrolysis cycle. These steps could be the release of inorganic phosphate (P_i) from the active site (Fig. 1; b → c), followed by the release of ADP (Fig. 1; c → d). This idea fits with image reconstructions of BBM-I heads bound to actin filaments[9]. When ADP dissociates, the light-chain domain of BBM-I tilts by about 6 nm, similar to the sub-steps seen by Veigel *et al.*[5] for this motor. Moreover, S1 from skeletal muscle does not tilt in response to ADP (ref. 10; R. Diaz and R. A. Milligan, personal communication), supporting the identification, by Veigel *et al.*, of BBM-I's second sub-step with ADP release.

However, Kitamura *et al.*[6] have reported multiple sub-steps for skeletal muscle S1. These authors captured S1 on the tip of a scanning-probe microscope, then moved it close to a bundle of actin filaments in the presence of ATP. Interactions between the myosin and actin could again be detected by decreased thermal fluctuations. A series of up to five sub-steps of about 5.3 nm each, usually all in the same direction, was observed at the start of each interaction. Although the duration of a single interaction varied inversely with ATP concentration, the interval between the sub-steps, only about 4 ms, did not depend on the concentration of ATP. This is strong evidence that an interaction containing several sub-steps requires only a single molecule of ATP.

Although the two papers[5,6] report sub-steps of the same amplitude, the multiple sub-steps observed for S1 by Kitamura *et al.*, but not by Veigel and colleagues, produce a large discrepancy in the total displacement per interaction. This discrepancy is the latest episode in a long-running controversy. The divergent results probably reflect differences in the techniques used by the two groups: the methods of attaching actin and myosin to the transducers; the geometrical relationships between the two proteins; and the mechanical compliance and dynamic response of the experimental systems. For example, actin filaments slide over an S1-coated surface at least three times faster with the biotin/avidin attachment method used by Kitamura *et al.* than with the nitrocellulose method used by Veigel *et al.*, and the faster velocity is close to that seen with the native protein[11]. Another possibility is that the high stiffness perpendicular to the filament of the scanning probe used by Kitamura *et al.* suppresses the dissociation of myosin from actin.

What can the new results tell us about the mechanism of the myosin motor? The isoform differences probably reflect functional specializations — BBM-I and myr-1 may control vesicle transport and the viscoelasticity of the cell cortex, whereas skeletal-muscle S1 must work with hundreds of partners to produce rapid filament sliding. These characteristics may require differences in the step size, the fraction of time spent attached to actin, or the relationship between ADP release and filament sliding[12].

Because the S1 lever arm is about 9 nm long, and the BBM-I and myr-1 arms are even longer, the presence of one or two 5–6-nm sub-steps is consistent with the tilting lever-arm model (Fig. 1; b → d). But this model cannot readily explain the total displacements of almost 30 nm sometimes seen by Kitamura *et al.* for S1, or the uniform 5.3-nm sub-steps that they report. These sub-steps are roughly equal to the 5.5-nm separation of actin monomers along each strand of the actin filament, suggesting that myosin heads can jump repeatedly between actin-binding sites during a single ATPase cycle (Fig. 1; a → b). A series of as many as five such jumps in the same direction, as observed by Kitamura *et al.*, would seem to require a mechanism with novel structural and physicochemical concepts.

Whatever the final interpretation of these new studies, they show that single-molecule techniques can reveal intermediate transitions in protein–protein interactions, and give us a first glimpse of mechanical substructure within single actin–myosin interactions. This is a big step towards understanding the mechanism of energy transduction by motor proteins. □

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