Pulling out the coordination mechanism of myosin-VI

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Efficient transport by single two-headed motor proteins requires coordination of the motor domains. A new single-molecule study sheds light on an important coordination mechanism by demonstrating an asymmetric strain dependence of the weak-to-strong binding transition in myosin-VI heads.

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Many intracellular transport processes are powered by two-headed motor proteins, which literally walk along the cytoskeletal filaments in cells[1]. The walk is powered by ATP-driven conformational changes in the motors' heads (the feet of these motors are for historical reasons called 'heads', while the mode of walking, which is reminiscent of the way humans walk, is referred to as the 'hand-over-hand' model). For a two-headed motor to walk efficiently, the heads must act in a coordinated fashion. The heads of two-headed myosins are separated by a few tens of nanometers a large part of the time, which corresponds to the repeat distance of actin. This indicates that the heads coordinate using intramolecular strain mediated by the lever arms (the myosin equivalent of legs).

In this issue, a single-molecule study by Iwaki *et al.*[2] shows that the strong binding of myosin-IV heads to actin is greatly accelerated by backward strain. This mechanism would allow the diffusing free head to act as a binding ratchet: it increases the probability of forward stepping by favoring strong attachment to forward-facing binding sites.

The myosins are a large superfamily of actin-based motor proteins that perform a wide array of cellular functions. Myosin-VI seems to have both anchoring and trafficking roles *in vivo*[3]. It forms two-headed structures when bound to endocytotic vesicles[4], and dimers *in vitro* are capable of processive unidirectional motion over several microns[3]. In the myosin crowd, it stands out by walking in the opposite direction relative to all other known myosins and by taking what appear to be the largest steps despite not having the longest lever arms[3,5]. To understand how the motor achieves unidirectional and processive walking, several mechanisms must be explained. First, at least one of the heads should be kept tightly bound to actin. Second, the rear head should release first when both heads are bound. This is an example of a gating mechanism: one head is effectively stalled in some state until the other head reaches a certain state (the gate). Third, a free head should bind to a site in front of the other head—a binding reaction that proceeds via an intermediate weakly bound state. This could be achieved by mechanically positioning the head near the forward binding site and/or by biasing the actin binding reaction to favor forward binding in the spirit of the Huxley model for muscle[6]. Myosin-VI has a lot of structural flexibility[7], which might be useful when navigating complex actin networks *in vivo*[8]. This points toward some kind of biased binding[5], but until now, there was no direct evidence to support that assumption.

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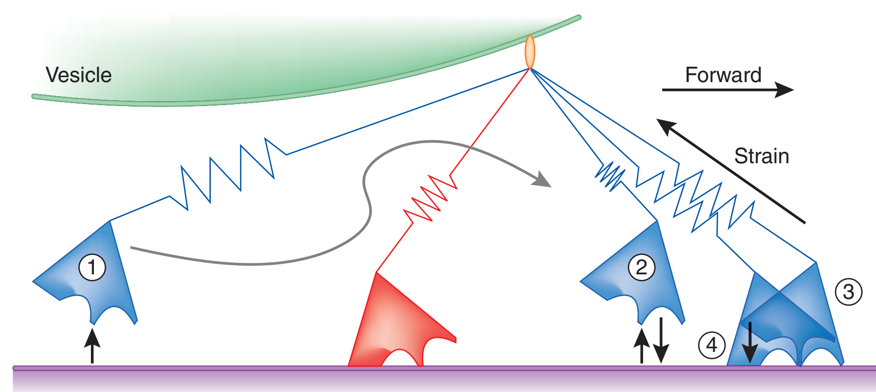

Figure 1 Forward stepping with asymmetric binding rates. The rear head releases (1) and undergoes Brownian motion, occasionally binding and unbinding weakly (2) with the actin filament. Once bound weakly far enough in front of the other head (3), backward strain transmitted through the lever arm accelerates strong binding (4).

In their experiment, Iwaki *et al.*[2] attached single-headed motors to small polystyrene beads. They then used a laser trap to rapidly scan a bead back and forth above an actin filament suspended between microfabricated pillars. During the scans, the motors sometimes interacted with the filament, producing brief attachment events that transiently pulled the bead away from the moving trap. Two kinds of attachment events, with two orders of magnitude difference in average dwell time, could be discerned. The authors interpreted this in terms of weakly and strongly bound states. Supposedly, a free head first becomes weakly bound to actin, and then either detaches directly (short event) or binds strongly before eventually detaching (longer event). The frequencies of long and short events depend on scanning speed and direction, in a way that strongly indicates that the weak-to-strong rate increases with increasing backward strain. This would lead to the biased binding mechanism for forward stepping discussed above, as the free head is strained backward when interacting with a forward binding site (**Fig. 1**). At the highest scanning speed (most strain), the authors could fit their data to a kinetic model and compute the weak-to-strong rate to be about 30 times higher than the (strain-free) value extracted from a bulk assay[9].

This result fills an important gap in our qualitative understanding of myosin-IV. It also points toward a mechanism that could be at work in other motor proteins as well and adds to the list of strain-dependent gating mechanisms. Previous data show that backward strain favors binding of ADP over ATP in myosin-VI (ref. 10). Because ATP binding induces head detachment, this hints at a gating mechanism where intramolecular strain hinders the leading head from detaching before the rear head[3]. The theoretical framework for understanding motor

proteins is often called mechanochemistry, referring to the fact that reactions that produce movement must depend on applied load. However, putting together a coherent picture of flexible two-headed motors requires a characterization not only of how different transitions depend on strain but also of how the strain is distributed among the various parts and joints. It seems this purely mechanical aspect is becoming more important the more we learn about these systems.

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S1P1 signaling just keeps going and going and going…

Stuart Cahalan & Hugh Rosen

Synthetic agonists of sphingosine-1-phosphate receptor subtype 1 are able to generate long-term signaling that persists for hours after ligand-induced receptor internalization. These signals are independent of agonist potency, can be reversed after receptor internalization by specific antagonists, and show a distinct acyl-chain-length structure-activity relationship.

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Sphingosine-1-phosphate (S1P) is a pleiotropic signaling lipid that activates a family of G protein–coupled receptors (S1P1–S1P5). The S1P receptor prodrug agonist FTY720 (fingolimod) is currently in phase 3 clinical trials for relapsing-remitting multiple sclerosis. After phosphorylation by sphingosine kinase 2, the active agonist FTY720-phosphate (FTY720-P) elicits some of its therapeutic effects by causing systemic retention of lymphocytes in lymph nodes, thus preventing autoreactive T cells from causing pathology. In this issue, Mullershausen *et al.*[1] provide new chemical insights into the propensity of FTY720 and its analogs to induce and maintain sustained, and perhaps tonic, signaling of the S1P1 receptor despite its complete internalization, and they also highlight the importance of differing hydrophobic interactions in these processes (**Fig. 1**).

The *in vivo* mechanism leading to the phenotypes of S1P1 agonist treatment has been long debated. Initially, FTY720-P was demonstrated to be a potent agonist of S1P1, S1P3, S1P4 and S1P5 (ref. 2). It was later shown that FTY720-P could lead to rapid, sustained internalization and degradation of the S1P1 receptor[3]. Genetic studies established that S1P1 on thymocytes was required for their exit from the thymus into blood, and when thymocytes lacking S1P1 were transferred intravenously into recipient mice, they were sequestered from the blood and the lymph[4]. These findings led to a proposed mechanism by which FTY720-P causes a loss of surface S1P1 expression on the lymphocytes, rendering them functionally null and unable to respond to an S1P gradient to leave the secondary lymphoid organs. However, it has also been shown that synthetic S1P1-specific agonists, some of which cause similar receptor internalization and recycling as S1P itself, can also cause lymphocyte sequestration[5]. Additionally, S1P1-specific antagonists do not induce sequestration, and can in fact rapidly reverse agonist-induced

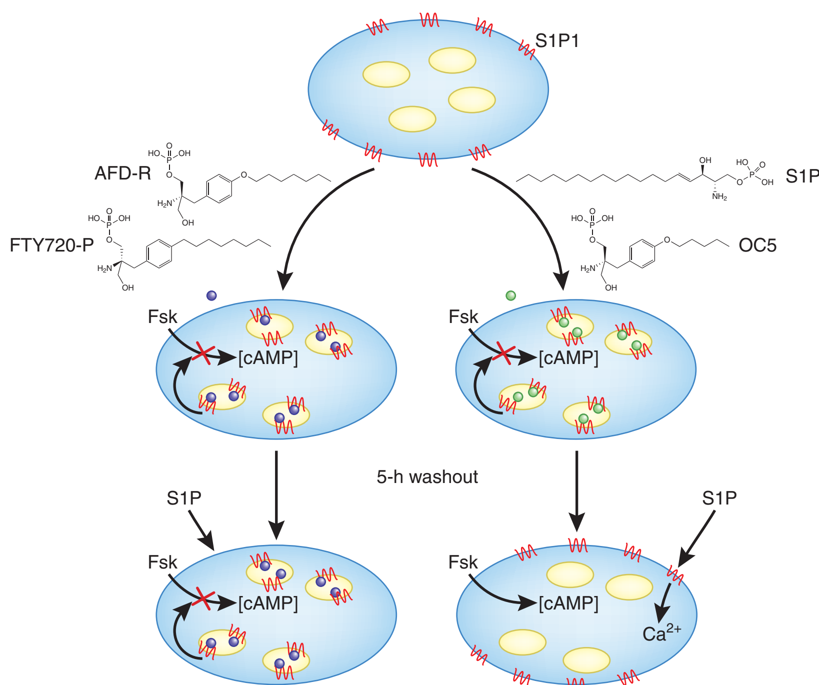

Figure 1 Effect of S1P1 signaling and trafficking in response to different agonists. Upon agonist treatment, S1P1 induces G_i-mediated inhibition of cAMP production by forskolin (Fsk) and internalization of the receptor. 5 h after removal of agonist, cells treated with FTY720-P and AFD-R still inhibit forskolin-dependent cAMP production through persistent signaling of internalized receptors, whereas cells treated with S1P and OC5 respond normally to forskolin. After removal of agonist, cells treated with FTY720-P and AFD-R are unable to release Ca^{2+} in response to S1P treatment due to receptor internalization, whereas cells treated with S1P and OC5, which have membrane-associated S1P1, release Ca^{2+} in response to S1P treatment.

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