IX-D Single-Molecule Physiology

A single molecule of protein (or RNA) enzyme acts as a machine which carries out a unique function in cellular activities. To elucidate the mechanisms of various molecular machines, we need to observe closely the behavior of individual molecules, because these machines, unlike man-made machines, operate stochastically and thus cannot be synchronized with each other. By attaching a tag that is huge compared to the size of a molecular machine, or a small tag such as a single fluorophore, we have been able to image the individual behaviors in real time under an optical microscope. Stepping rotation of the central subunit in a single molecule of F_1-ATPase has been videotaped, and now we can discuss its detailed mechanism. RNA polymerase has been shown to be a helical motor that rotates DNA during transcription. Myosin V is another helical motor that moves as a left-handed spiral on the right-handed actin helix. Single-molecule physiology is an emerging field of science in which one closely watches individual, 'live' protein/RNA machines at work and examines their responses to external perturbations such as pulling and twisting. I personally believe that molecular machines operate by changing their conformations. Thus, detection of the conformational changes during function is our prime goal. Complementary use of huge and small tags is our major strategy towards this end.
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IX-D-1 Myosin V Is a Left-Handed Spiral Motor on the Right-Handed Actin Helix

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Myosin V is a two-headed, actin-based molecular motor implicated in organelle transport. Previously, a single myosin V molecule has been shown to move processively along an actin filament in discrete ~ 36 nm steps. However, 36 nm is the helical repeat length of actin, and the geometry of the previous experiments may have forced the heads to bind to, or halt at, sites on one side of actin that are separated by 36 nm. To observe unconstrained motion, we suspended an actin filament in solution and attached a single myosin V molecule carrying a bead duplex. The duplex moved as a left-handed spiral around the filament, disregarding the right-handed actin helix. Our results indicate a stepwise walking mechanism in which myosin V positions and orients the unbound head such that the head will land at the 11th or 13th actin subunit on the opposing strand of the actin double helix.

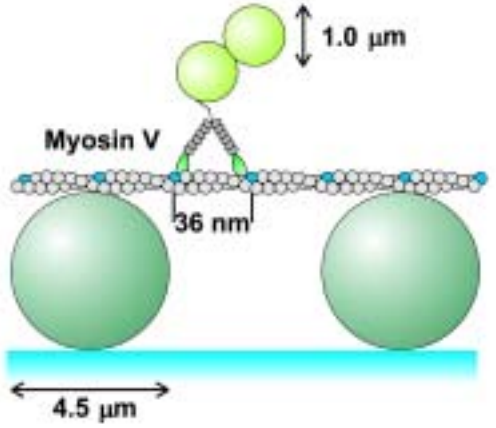

Figure 1. Experimental system for the observation of unconstrained movement of a single molecule of myosin V along an actin filament.

IX-D-2 Pause and Rotation of F_1-ATPase during Catalysis

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F_1-ATPase is a rotary motor enzyme in which a single ATP molecule drives a 120° rotation of the central γ subunit relative to the surrounding $\alpha_3\beta_3$ ring. Here, we show that the rotation of F_1-ATPase spontaneously lapses into long (≈ 30 s) pauses during steady-state catalysis. The effects of ADP-Mg and mutation on the pauses, as well as kinetic comparison with bulk-phase catalysis, strongly indicate that the paused enzyme corresponds to the inactive state of F_1-ATPase previously known as the ADP-Mg inhibited form in which F_1-ATPase fails to release ADP-Mg from catalytic sites. The pausing position of the γ subunit deviates from the ATP-waiting position and is most likely the recently found intermediate 90° position.

IX-D-3 F_1-ATPase Changes its Conformations upon Phosphate Release

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Motor proteins, myosin, and kinesin have γ-phosphate sensors in the switch II loop that play key roles in

conformational changes that support motility. Here we report that a rotary motor, F_1-ATPase, also changes its conformations upon phosphate release. The tryptophan mutation was introduced into Arg-333 in the β subunit of F_1-ATPase from thermophilic *Bacillus* PS3 as a probe of conformational changes. This residue interacts with the switch II loop (residues 308–315) of the β subunit in a nucleotide-bound conformation. The addition of ATP to the mutant F_1 subcomplex $\alpha_3\beta(R333W)_3\gamma$ caused transient increase and subsequent decay of the Trp fluorescence. The increase was caused by conformational changes on ATP binding. The rate of decay agreed well with that of phosphate release monitored by phosphate-binding protein assays. This is the first evidence that the β subunit changes its conformation upon phosphate release, which may share a common mechanism of exerting motility with other motor proteins.