SINGLE-MOLECULE PHYSIOLOGY UNDER AN OPTICAL MICROSCOPE: HOW MOLECULAR MACHINES MAY WORK

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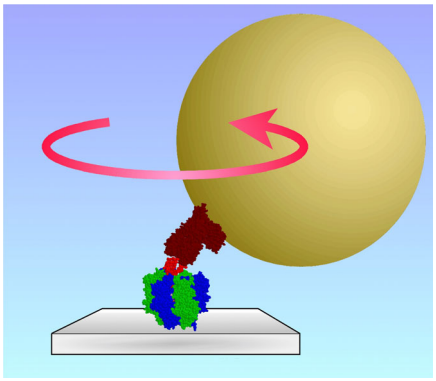

Fig. 1 Imaging rotation of F_1-ATPase through a gold bead attached to the rotor (pink) [2].

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 behaviors 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 [1,2]. RNA polymerase has been shown to be a helical motor that precisely tracks the right-handed double helix of DNA [3], whereas myosin V has been shown to proceed as a left-handed spiral around an actin filament which is a right-handed double helix [4]. Huge tags such as micron-sized plastic beads also allow the manipulation of individual molecules with, *e.g.*, optical or magnetic tweezers [3-6]. I personally believe that molecular machines operate by changing their conformations. Thus, detection of the conformational changes during function is our prime goal.

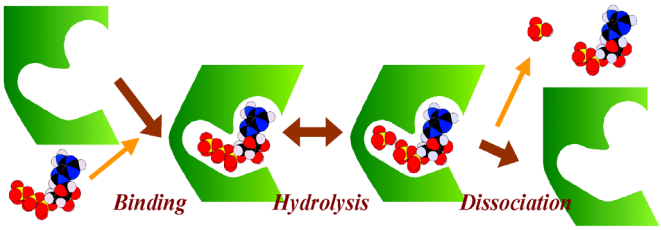

Fig. 2 How an ATP-driven molecular machine (green) may work.

The work on F_1-ATPase is beginning to elucidate how an enzyme can convert the energy derived from chemical reaction (ATP hydrolysis) into mechanical work [2]. High-speed imaging of rotating F_1-ATPase revealed that the rotation consists of pairs of ~90° and ~30° steps. The 90° step is driven by binding of ATP to F_1, and the 30° step by release of a hydrolysis product(s). The hydrolysis reaction *per se*, splitting of ATP into ADP and phosphate, does not do much work. The major source of mechanical energy is a conformational change(s) accompanying ATP binding, and some more energy is liberated upon the release of hydrolysis products (Fig. 2). The role of hydrolysis is to reset the machine for the next round of reaction. This view also accounts for the ATP synthesis by this enzyme when it is forcibly rotated in reverse, and is essentially a consolidation and embodiment of Boyer's binding change model for ATP synthesis. Complete mechanical silence during hydrolysis, however, is not advantageous for high efficiency. I will present a model in which hydrolysis plays a key role in controlling the directionality and efficiency of the overall reaction.

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