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THE ENGINES OF BIOMOLECULAR MOTORS

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INTRODUCTION

The majority of biomolecular motors are powered by nucleoside triphosphate (NTP), especially adenosine triphosphate (ATP). These motors consist of a β-sheet with highly conserved motifs and the nucleotide binding domain around it. The highly conserved protein folds are the *engines* of these motors, which convert the energy of NTP hydrolysis cycle to mechanical work. Although functions of molecular motors are widely diverse, (including cargo movement, DNA unwinding, protein degradation, ion pumping, etc), the nucleotide binding domains are very similar. In the binding site, NTP undergoes a hydrolysis cycle

$$E + NTP \rightleftarrows E \cdot NTP \rightleftarrows E{\bullet}NTP \rightleftarrows E{\bullet}NDP{\bullet}P_i \rightleftarrows E{\bullet}NDP + P_i \rightleftarrows E + NDP + P_i$$

where E is the enzyme (motor protein), the small dot represents the docking of NTP, and the large dot represents the tightly-bound states. The hydrogen bond network formed in the NTP binding step, as shown in Figure 1 [1], deforms the β-sheet and adjacent structures. The local deformation propagates to conformational changes of functional residues to do mechanical work or to change the affinity to the substrate [2]. For multimeric motor proteins, we must also consider the stress paths among subunits which control the sequence and the activity of the protein. Stress trajectories emanating from a binding site either passes through a circumferential stress loop or a stress loop through the substrate.

Here we describe the mechanisms of T7 hexameric helicase, F_1 ATP synthase, and ϕ29 portal protein to illustrate the similarity and the difference of the mechanochemical couplings.

T7 HELICASE

Bacteriophage T7 helicase is a hexameric motor protein that couples energy from dTTP (deoxythymidine triphosphate) hydrolysis cycle to unwind double-stranded DNA by translocating along one DNA strand [3]. Based on the crystal structure [4] and the biochemical measurements [3, 5], we propose a 6-state, 6-subunit, sequential kinetic-network model.

The catalytic sites communicate via mechanical strain. The central player in this inter-site communication is the β-sheet that lies close to each catalytic site. The closing of the catalytic site around the nucleotide deforms the ß-sheet, and this deformation moves the contacting DNA. The elastic energy stored in this sheet as it deformed will power the recovery stroke that resets the power loop after it detaches from the DNA. At the same time, a third ß-sheet loop transfers strain to the P-loop of the next catalytic site. The data for the transient kinetics and the simulation results based on the mechanical consideration are shown in Figure 2A.

F_1 ATP SYNTHASE

F_1 ATP synthase is a rotary motor protein which can either manufacture ATP or hydrolyze ATP to pump ions [1, 2]. This motor is composed of a hexamer and a central rotating γ-subunit. The hexamer contains 3 α-subunits and 3 β-subunits. Only 3 β-subunits are catalytic. The rotation of the γ-subunit was observed in the hydrolysis direction by attaching an actin filament to this subunit [6]. We developed a mechanochemical model to explain the experimental results [2, 7].

In the catalytic site, the sequential hydrogen bond formation during ATP binding is converted to elastic strain energy of ~24 kT. This strain energy bends the β-subunit, which is an elastic element to store some of the energy for later use [2, 7, 8]. The bending stress is propagated to the γ-subunit as a rotary torque. Each of the subunits cycle through its conformational range in sequence, and all 3 β-subunits provide a continuous rotational torque to rotate the γ-subunit by 360º. Stresses propagate through at least two switch points on γ-subunit that interact with specific sites of the hexamer, and also communicate to other catalytic sites through circumferential contacts. We thus build the potential energy term by term by including elastic energy of the subunits and electrostatic energy of interactions. In order to reach the observed high efficiency, each subunit must exert two power strokes in two different steps: ATP binding and Pi release. The energy of the second step comes from the stored energy when ATP binds. This model quantitatively explains major features of the F_1 motor.

ϕ29 PORTAL PROTEIN

Bacteriophage ϕ29 portal protein is a hexameric or a pentameric ATPase which pushes DNA into the phage's capsid

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against high pressure [9]. The sequence alignment against other NTPases shows that it is likely that the β-sheet and the ATP contacting residues are conserved. We thus assume the coupling between the hydrogen bond network and the conformational changes is similar to other NTPases we have studied. We develop a mechanochemical model to explain the observation in the pulling experiments [9].

According to the experimental results, we propose two possible DNA translocation mechanisms for the portal protein. The mechanical step can be either coupled to the ATP binding step or the ADP release step. In the first model, the ATP-bound state has high affinity to DNA, so that the hydrogen bond network formed in the ATP binding step propagates stress to move the DNA contacting residues. The energy of hydrogen bond formation is directly transduced to the mechanical work of DNA movement. In the other model that ADP release is coupled to the mechanical step, the ADP-bound state has high affinity to DNA, so that DNA can be moved only in the ADP-bound state. The energy of hydrogen bond formation during ATP binding is stored as elastic energy, and the energy is released when the site is recoiled to its original conformation during the ADP release process. A possible experiment to distinguish these two models is to conduct a bulk experiment to test the DNA affinity to the enzyme in both ATP abundant and ADP abundant environments. Figure 2B shows the simulation results for different external pulling forces under various [ATP] based on the ATP binding model. The simulation is in good agreement with the experimental data.

CONCLUSIONS

In all three biomolecular motors, NTP binding forms the hydrogen bond network to deform the structures around the conserved β-sheet. The stresses initiated at the nucleotide binding domain are propagated either through the circumferential paths of subunit-subunit interfaces, or through the substrates such as DNA (portal protein and helicase) and γ-subunit (F_1 motor). The cooperativity among subunits is accomplished by the change of affinities to substrates or ligands coupled to the chemical reactions. Thus, the conserved motifs around the β-sheet, or the *engine* parts, play the same mechanical role in all of these biomolecular motors.

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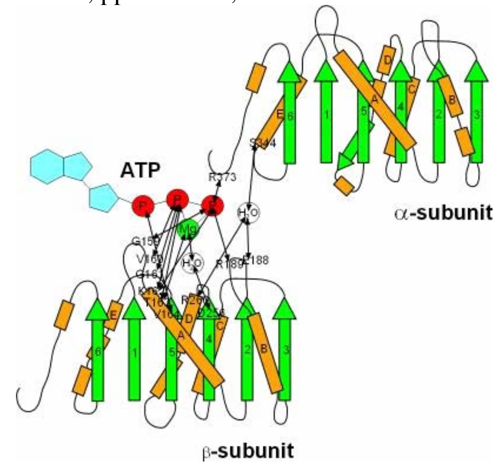

Fig. 1 Plot of the secondary structures of two adjacent F_1 subunits and an Mg-ATP molecule. The hydrogen bonds between subunits and Mg-ATP are shown.

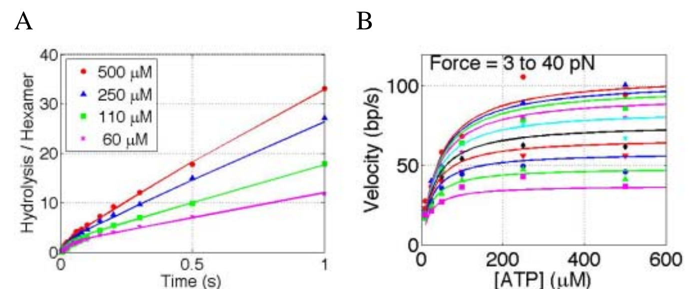

Fig. 2 The agreements of simulations and experiments for two motor proteins. (A) The transient kinetics of T7 helicase hydrolysis. Each curve corresponds to a different initial [dTTP]. (B) The steady-state translocation velocities of ϕ29 portal protein under various [ATP]. Each curve corresponds to a different external pulling force.

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