## 8. Biological Science

### 8-1. Crystal Structure of the Electron-Transfer Complex between Ferredoxin and Ferredoxin-NADP$^+$ Reductase [1]

Plants use light energy for the reductive assimilation of a inorganic compounds, carbon dioxide, nitrate and sulfate in the chloroplast. The reductive power for assimilation originates from water through a series of photochemical reactions in the thylakoid membranes. An electron-transfer protein ferredoxin (Fd) is the ultimate destination in this electron transport, and Fd reduced by photosystem I donates an electron to several Fd-dependent enzymes (ferredoxin-NADP$^+$ reductase (FNR), ferredoxin-thioredoxin reductase (FTR) et al.). Although these Fd-dependent enzymes vary in molecular size, primary structure and the prosthetic group composition, they all have the ability to form a productive electron-transfer complex with Fd. The crystal structures of single Fd [2], FNR [3] and FTR [4] have been reported. Still, the structures of Fd:Fd-dependent enzyme complexes remain undetermined, and are essential for a clear understanding of the photosynthetic electron-transfer process. We optimized the conditions for the cocrystallization of maize leaf Fd and FNR and determined the complete structure at 2.59 Å resolution [1].

In our crystal structure, the complex consists of one molecule each of FNR and Fd (Fig. 1), whose redox centers are in close proximity. These distances are sufficiently close for direct electron transfer through space between the two prosthetic groups. The accessible surface area buried in the interface is about 800 Å$^2$ for each partner, which constitutes 5% and 15% of the total surface areas of FNR and Fd, respectively; the proportion of polar atoms in this interface is about 50%. There are five Fd residues and four FNR residues at the intermolecular contact region, producing a hydrophobic environment near the two prosthetic groups. On both sides of this hydrophobic region, there are twenty-one charged residues in total, five pairs of which form intermolecular salt bridges (Fig. 2). This electrostatic interaction apparently has a major role in determining the orientation of the two proteins and in stabilizing the complex.

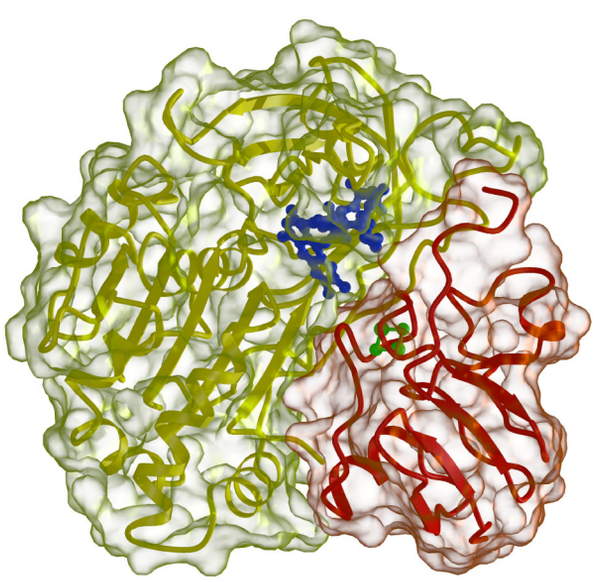

Figure 1.
Ribbon model with a transparent molecular surface of the complex between ferredoxin-NADP$^+$ reductase and ferredoxin from maize leaf.

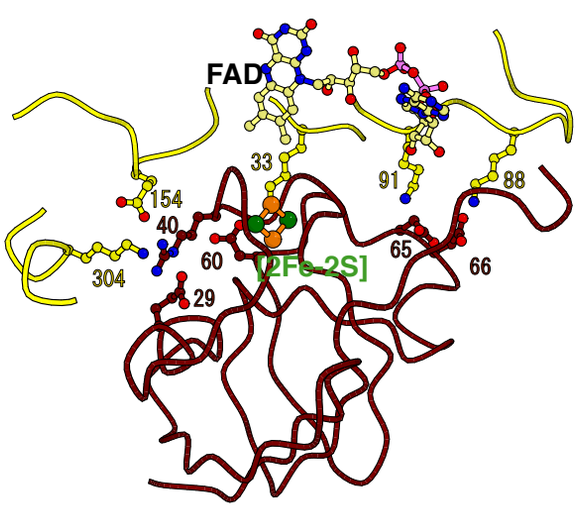


Figure 2.
Structure of the electrostatic interaction sites at the interface. Amino acid residues forming intermolecular salt bridges are drawn as ball-and-stick models.

In addition, the crystal structure of maize leaf FNR in the free state was determined at 2.2 Å resolution. There are significant alterations in the structures of the Fd-bound and free FNRs. The loop structure from Gly 82 to Lys 91 in FNR moves notably, enabling Lys 88 and Lys 91 to serve as salt bridge residues with Fd. A remarkable alteration is found at Glu 312, which is one of the conserved residues surrounding the isoalloxazine ring of FAD in the active site of FNR. It has been reported, based on a series of mutations for Glu 312, that the charge, size and polarity of this residue are crucial for proper binding of the nicotinamide ring of NADP(H), and have a definite effect on the electron transfer between FNR and Fd [5]. Recently, the side chain of Glu 312 has been shown to hydrogen bond with the nitrogen atom of the carboxamide group of NADP(H) based on a crystallographic study [6]. A comparison of our crystal structures of free and Fd-bound FNRs shows that the OE1 atom of Glu 312 moves an additional 2 Å toward the active site, bringing it into the hydrogen bonding distance. This side-chain movement seems to be caused by contact of the Glu 312 OE1 atom with CO of Ser 38 of Fd. This is the first evidence, to our knowledge, for an induced-fit like structural change in the FNR active site upon Fd-binding.

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## 8-2. Crystal Structures of the HslVU Complex and its ATP-Dependent Proteolysis Mechanism

ATP-dependent proteases are responsible for degradation of the major proteins of the cell. The bacterial ATP-dependent protease HslVU is a homolog of the eukaryotic 26S proteasome [1]. HslVU is composed of two distinct polypeptides, the heat shock locus HslV peptidase and HslU ATPase. HslV forms a two-stacked dodecamer. HslU forms a hexameric ring and binds to HslV. Crystallographic studies of HslVU should provide an understanding of the ATP-dependent protein unfolding, translocation, and proteolysis by this and other ATP-dependent proteases.

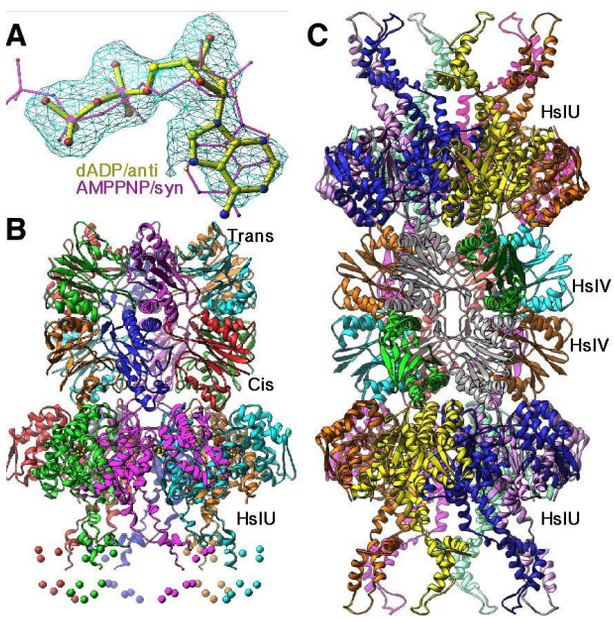


Figure 3.
The structures of HslVU. (A).A composite-omit electron density map at 3.0 Å resolution reveals that the bound dADP is in an anticonformation. This map was generated before dADP was built into the model. (B).The HslVU complex in the asymmetric $U_6V_6V_6$ configuration. Parts of HslU domain I could not be built into the final electron density and are indicated by spheres for their approximate locations. (C).The HslVU structure in the symmetric $U_6V_6V_6U_6$ configuration. Theorientation of the complexes in (1b) and (1c) differs by 30°.

*Overall Structure*

We have determined two crystal structures (Fig. 3) of the HslVU protease: an asymmetric complex at 3.0 Å resolution and a symmetric complex at 7.0 Å resolution. In the asymmetric complex, there is one HslU hexamer bound to the end of the HslV dodecamer in a $U_6V_6V_6$ configuration (Fig. 3B). In the symmetric complex, there are two HslU hexamers bound to both HslV ends in a $U_6V_6V_6U_6$ configuration (Fig. 3C). The structures were determined by molecular replacements with HslU and HslV structures of Bochtler et al. [2].

*Nucleotide Dependent Translocation Pore*

The current and previous structures [2] provide a nucleotide-dependent translocation pore mechanism. There are three HslU conformational states: (A) Closed state (Fig. 4A) occurs in the current HslU structure with 6dADP per hexamer. In this state, the pore is closed and Tyr91 of the GYVG pore motif points toward HslV. (B) The open state (Fig. 4B) has has been shown to occur in a previously determined HslU with 3 nucleotides. In this state, the pore is hydrophobic and opened, and Tyr91 points inside HslU. (C) The filled state (Fig. 4C) has also been shown to occur in a HslU structure with 4 nucleotides. In this state, two of Tyr91 residues occupy the pore. These states suggest that Tyr91 can move from inside HslU toward HslV through the pore. With these motions, the substrate should bind to Tyr91, the translocator residue, and can be translocated into the catalytic chamber of HslV (Fig. 4D).

An analysis of nucleotide binding-induced conformational changes in the current and previous HslU structures suggests a protein unfolding-coupled translocation mechanism. In this mechanism, unfolded polypeptides are threaded through the aligned pores of the ATPase and peptidase and translocated into the peptidase central chamber.

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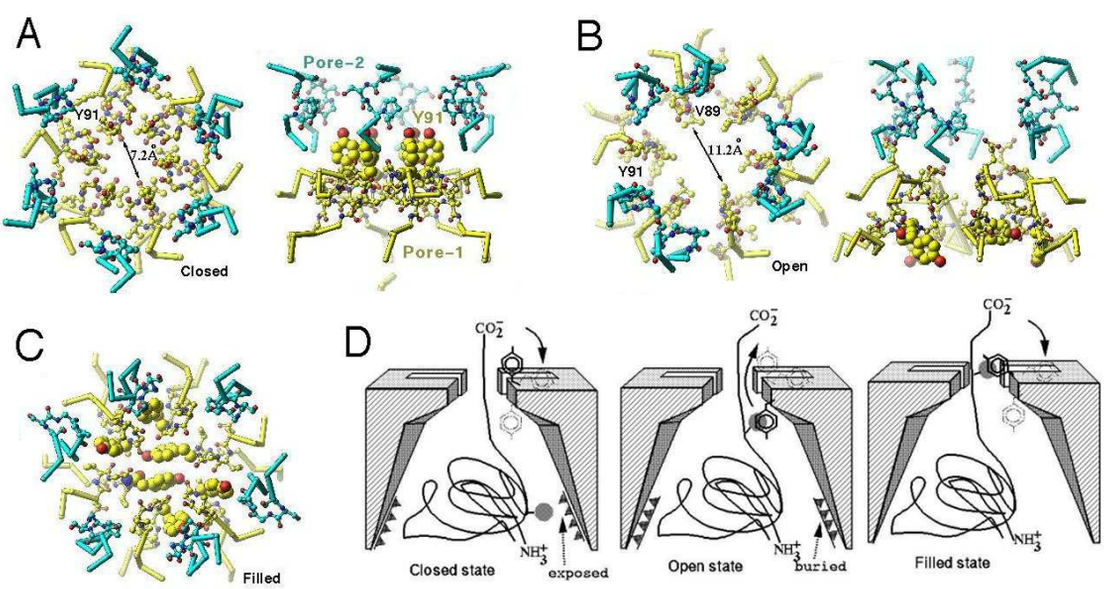


Figure 4.
Nucleotide-dependent motions of the HslU pore.

## 8-3. Structural Basis for the ADP-Specificity of a Novel Glucokinase from a Hyperthermophilic Archaeon

ATP plays universal roles in the storage of free energy in biological systems and as a phosphoryl donor in kinase reactions. However, certain hyperthermophilic archaea, such as *Thermococcus litoralis* and *Pyrococcus furiosus*, utilize unusual ADP-dependent glucokinases and phosphofructokinases in their glycolytic pathways. These ADP-dependent kinases are similar to each other, but show no sequence similarity to any of the hitherto known ATP-dependent enzymes [1]. Because no other kinases that use ADP are known to date, this novel kinase group is a very rare example that can shed light on the evolutionary origin of kinases.

We solved the crystal structure at 2.3 Å resolution of an ADP-dependent glucokinase from *Thermococcus litoralis* (tlGK) complexed with ADP [2]. The overall structure can be divided into large and small $\alpha/\beta$-domains, and the ADP molecule is buried in a shallow pocket in the large domain (Fig. 5). The approximate shape, consisting of large and small domains with the active site between them, is similar to that of conventional ATP-dependent hexokinases, but the tertiary structure is completely different.

A structure database search revealed that tlGK has a similar fold to two 'ATP-dependent' kinases: *Escherichia coli* ribokinase (RK) and human adenosine kinase (AK). A comparison based on the three-dimensional structure revealed that several motifs important both in the structure and the function are conserved, and that the recognition of the $\alpha$- and $\beta$-phosphate of the ADP in the tlGK is almost identical with the recognition of the $\beta$- and $\gamma$-phosphate of ATP in these ATP-dependent kinases (Fig. 6). In other words, its rare nucleotide specificity is caused by a shift in the nucleotide binding position by one phosphate unit. The strict conservation of the binding site for the terminal and adjacent phosphate moieties suggests a common ancestral origin of both the ATP- and ADP-dependent kinases.

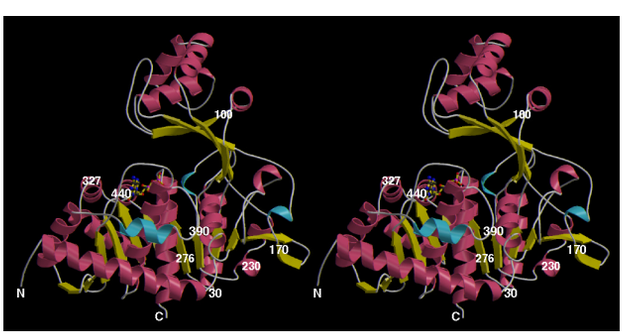


Figure 5.
Stereographic ribbon diagram of tlGK including an ADP molecule. Helices/$3_{10}$-helices/strands are shown in pink/cyan/yellow, respectively.

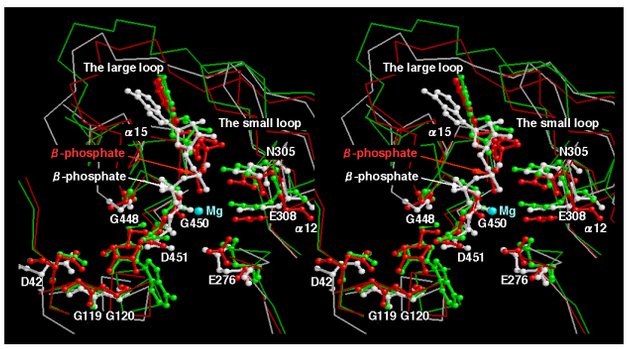


Figure 6.
Stereographic multiple superpositioning of conserved tlGK/RK/AK active sites. Each model is shown in white/red/green, respectively. ADP of tlGK/RK, ribose of RK and two adenosines of AK are presented as ball-and-stick models in the corresponding colors. Highly conserved and catalytically important residues are shown as a ball-and-stick model and labeled. The Mg atom of AK is shown in cyan. The spatially conserved secondary structure related with nucleotide recognition is labeled with white letters.

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## 8-4. Crystal Structure of a *cis*-Prenyl Chain Elongating Enzyme, Undecaprenyl Diphosphate Synthase [1-3]

Isoprenoid compounds, which are constructed by $C_5$ (5 carbons) units, are the most structurally diversed in natural products. Over 23,000 isoprenoids have been found and characterized. They serve as hormones, vitamins, pigments, electron carriers, constituents of membranes, photoreceptors and so on. All of them are derived from linear prenyldiphosphates, which are synthesized by a family of prenyltransferases. These enzymes can be classified into two major groups according to the *cis-trans* isomerism of the products. Undecaprenyl diphosphate synthase (UPS), which belongs to the *cis*-type prenyltransferase family, catalyzes the *cis*-prenyl chain elongation onto farnesyl diphosphate (15 carbons; FPP) as a primer to yield undecaprenyl diphosphate (55 carbons; UPP) by 8-times condensation with isopentenyl diphosphate (5 carbons; IPP) (Fig. 7). This enzyme is essential for cell-wall biosynthesis in many bacteria. Although many enzymes for the *trans*-type prenyl chain elongation have been cloned and characterized, little is known about *cis*-enzymes. The crystal structure of UPS from *Micrococcus luteus* B-P 26 has been determined using BL-6A and BL-6B. This is the first structure among *cis*-prenyltransferases.

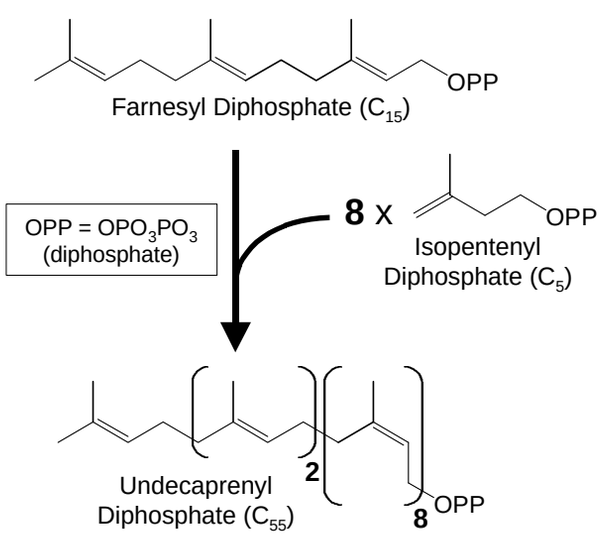


Figure 7.
Enzymatic reaction catalyzed by undecaprenyl diphosphate synthase (UPS).

The crystal structure (Fig. 8) is completely dissimilar to those of the other isoprenoids biosynthesis-relating enzymes. Although it had been believed that all of such enzymes have a common structural motif, called “isoprenoids synthases fold”, composed of 10-12 antiparallel $\alpha$-helices, it was elucidated that UPS has only $\beta$-sheets in the core. In addition, the UPS structure belongs to a new protein folding family.

UPS has a large cleft on its molecular surface composed of highly conserved amino acid residues among *cis*-prenyltransferases. The interior of this cleft mainly consists of hydrophobic residues, and positively charged arginine residues are located at the entrance. A common structural motif for phosphate recognition, called “structural P-loop”, was

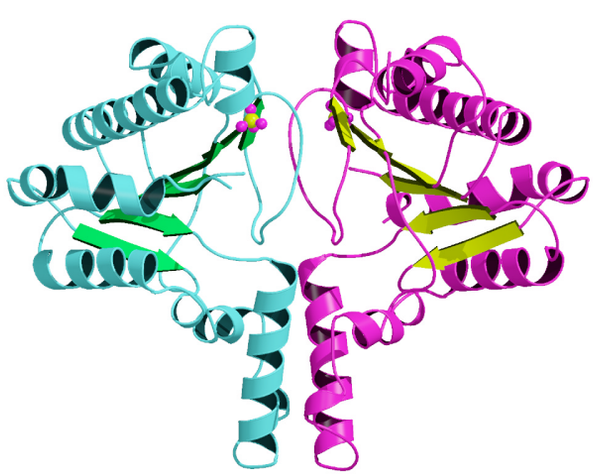

Figure 8.
Overall structure of undecaprenyl diphosphate synthase (UPS). UPS acts as a homodimer; one subunit is shown in cyan and green, and the other by magenta and yellow. Small molecules drawn by yellow and magenta spheres indicate sulfate ions, which are suggested to locate the substrate-binding position.

found at the entrance of the cleft. This motif is composed of completely conserved residues among cis-prenyltransferases. A sulfate ion, which was used for crystallization, was bound to this motif. This position is assumed to be the substrates-binding site. The “structural P-loop” may recognize the allylic substrate like FPP, and the size of the cleft may control the final product chain length.

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## 8-5. Three Conformations of the MutS Protein Regulated by Adenine Nucleotides

In living organisms, DNA is continuously being damaged as a result of errors introduced by DNA replication, genetic recombination or other processes [1]. These DNA lesions may lead to mutations, genetic diseases and tumors. To remove such DNA lesions, all organisms have developed various DNA repair systems, including the DNA mismatch repair (MMR) system. It has been shown that MutS protein (about 90 kDa) recognizes and binds specifically to mismatched base pairs in DNA, constituting the first step of MMR [1, 2]. Then, MutL and other proteins cooperate in subsequent steps to complete the MMR pathway. The pathogenic genes of human hereditary non-polyposis colorectral cancer (HNPCC) appear to share a high degree of homology with bacterial MutS and MutL. Moreover, MutS homologues have also been isolated from plants. These observations suggest that the MMR system is essential for all living organisms from bacteria to eukaryotes. MutS has a weak ATP hydrolysis activity for which no role has yet been identified. However, recent reports underscore the importance of ATP for the function of these proteins, since ATP and its poorly hydrolyzable analogue, ATPγS [adenosine-5’-O-(3-thiotriphosphate)], can affect the DNA binding activity of MutS.

Small-angle X-ray scattering (SAXS) is a powerful technique for measuring the size and general shape of molecules in solution [3]. SAXS measurements of MutS were performed at BL-10C both in the presence and in the absence of adenine nucleotides. The results demonstrate the existence of three different conformations of MutS in solution: ATP-bound, ADP-bound and nucleotide-free forms. The ATP-bound form has the most compact conformation with a maximum particle dimension ($d_{max}$) of 110 Å and with a radius of gyration ($R_g$) of 37 Å. The ADP-bound form has the most stretched conformation with a $d_{max}$ of 130 Å and an $R_g$ of 44 Å.

The nucleotide-free form shows values intermediate between the two forms (120 Å for $d_{max}$ and 41 Å for $R_g$). These conformational changes induced by adenine nucleotides have been confirmed by an analysis of the distance distribution function ($P(r)$).

Recently, methods for reconstructing three-dimensional density maps of macromolecules in solution have been developed [4]. By using these techniques, the shapes of MutS in solution under various conditions were calculated. In the structure models, one axis of the molecule is longer than the other, and a hole is present in the lower part (Fig. 9). The rendering of the three-dimensional density maps shows three different conformations for MutS bound to different nucleotides. The shapes of the model structures differ from each other, especially in the upper region. When bound to ADP, the molecule appears to have two holes (Fig. 9b). This is similar to the crystallographic structure which clearly displays two holes (Fig. 9d). In the latter, the upper hole is occupied by bound DNA [5]. An open/close movement of the upper region may occur by binding of adenine nucleotides, consequently modulating the DNA binding activity of MutS. We have also demonstrated that a MutS mutant, in which a lysine belonging to the nucleotide binding motif is replaced by an alanine, can not undergo conformational changes. Based on these findings, we conclude that the DNA-binding activity of MutS may depend on conformational changes triggered by both the binding and hydrolysis of ATP [6].

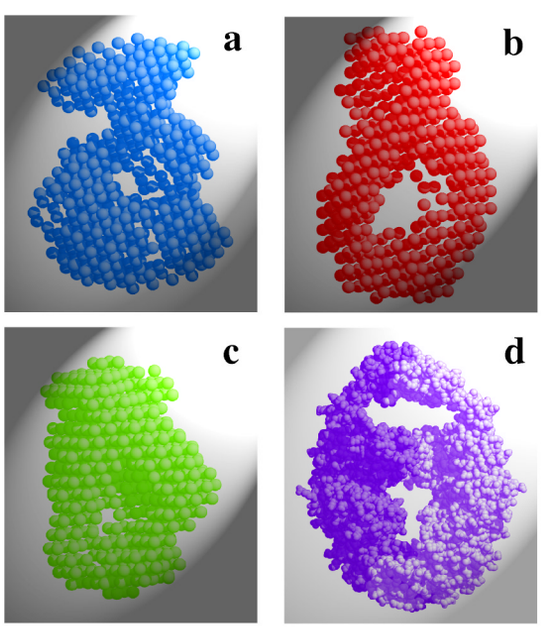


Figure 9.
Simulated models of the three-dimensional structure for MutS in solution. The shapes of nucleotide free (**a**), ADP bound (**b**) and ATP bound (**c**) forms were calculated from the $P(r)$ functions. (**d**) shows the space filling model of *E. coli* MutS dimer, in which the bound DNA was removed from the MutS-DNA complex of the crystal structure (PDB accession number 1E3M).

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## 8-6. Actin as the Generator Element of Force in Muscle Contraction

Muscle contraction takes place when actin in thin filaments and myosin heads protruding from thick filaments interact with each other, powered by the hydrolysis of ATP. In the current model of muscle contraction, the force might be generated by a tilting or rotating motion of the myosin heads on the actin filament, which is assumed to be very stiff. Such motion stretches the elastic elements residing somewhere around or within the myosin heads, pro-

ducing a force that displaces thin filaments. However, the assumption that the actin filaments are effectively inextensible has not been verified.

Recently, we have provided direct evidence of the extensibility of the actin filaments through precise spacing measurements of an actin-based meridional reflection at ~1/2.7 $nm^{-1}$ corresponding to the intersubunit distance between actin monomers by synchrotron X-ray diffraction at PF [1-3]. It was found that the actin filaments became shortened at the onset of activation of muscle, and then extended as the force developed. The results show that the extensibility of actin filaments is purely elastic, and that a fairly large part of the sarcomere elasticity (~60%) of an active muscle originates by the elasticity of the actin filament (Fig. 10). There is also evidence for some extensibility in the myosin filament. Extensibility changes proceed in parallel to force changes, implying that elastic energy is stored in the actin filaments and serves as the contractile force. Differential spacing changes of the reflections coming from the pitches of the left- and right-handed basic helices in the actin filaments and from the intersubunit distance indicate that the actin filament twists when it shortens during an initial activation and untwists when it elongates during the development of force. Twisting motions result in the change of the helical symmetry of the actin filament, providing an appropriate geometry for the active interaction between actin and myosin heads. Thus, the actin filament has a highly elastic property and plays a much more active role than a simple rigid-force transmitter in the force-generation mechanism. High compliance in the actin filaments (and myosin filaments too) challenges the current ideas about how muscle works [4] (Fig. 11). Synchrotron X-ray studies reveal that the actomyosin motor system (muscle) has a property of a rotary motor.

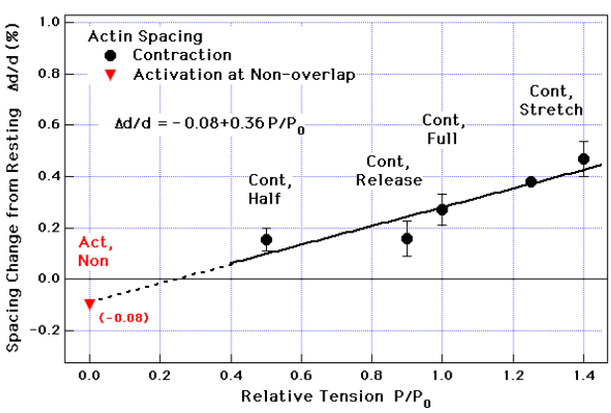


Figure 10.
Spacing changes of the 2.7 nm actin meridional reflection against tension relative isometric tension ($P_0$) of active muscle at full-filament overlap. The data of 'Act, Non' are from the overstretched muscle upon activation.

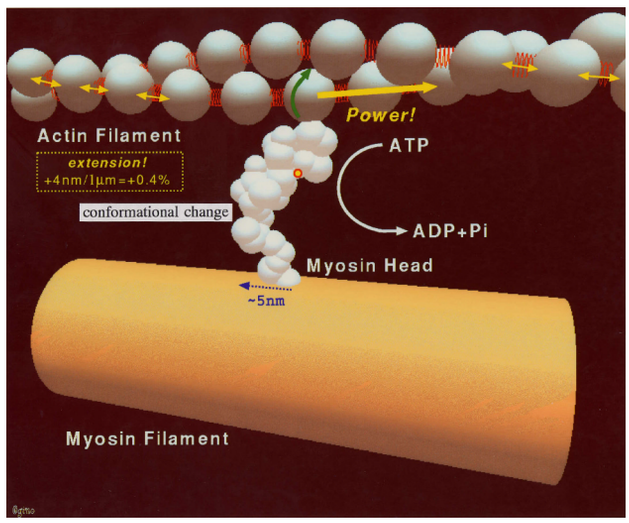


Figure 11.
Schematic representaion of the role of a highly elastic actin filament in the force generation mechanism of muscle.

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