Current Biology Vol 16 No 2
R46

Dispatches

AAA+ Molecular Machines: Firing on All Cylinders

AAA+ ATPase protein machines use the power obtained from ATP hydrolysis to remodel macromolecular assemblies in the cell. Recent work in *Escherichia coli* on the ClpX AAA+ protein reveals fundamental mechanical principles that underlie ClpX motor function.

Sarah E. Ades

The AAA+ ATPase motif is found in proteins from all kingdoms of life. The cellular roles of these proteins are diverse; hence the AAA+ designation for ATPase's associated with various cellular activities. Many of these enzymes act as machines, using energy generated from the binding and hydrolysis of ATP to remodel a variety of macromolecular assemblies in the cell [1]. For example, AAA+ subunits of the proteosome in eukaryotes and the Clp protease in prokaryotes denature stably folded proteins [2,3]. Other members of the family disassemble oligomeric protein structures, like NSF, which takes apart membrane fusion complexes [4]. In addition, some AAA+ enzymes, such as the RuvB DNA helicase, act on nucleic acids instead of proteins [5].

Structural and biochemical studies of AAA+ enzymes have shown that they often assemble into oligomeric structures and share a high degree of structural similarity, particularly in the ATP binding domain [6]. Although several models have been proposed for how these molecular machines work, the mechanism is still not well understood. Martin *et al*. [7] have used a novel approach to identify the fundamental, mechanical principles that underlie the bacterial ClpX AAA+ ATPase motor. Their approach can be used with other AAA+ protein machines to determine whether these operating principles are shared by additional members of this diverse protein family. This technique is also applicable to other oligomeric proteins, not just those in the AAA+ family, providing a powerful tool to investigate the function of many protein complexes.

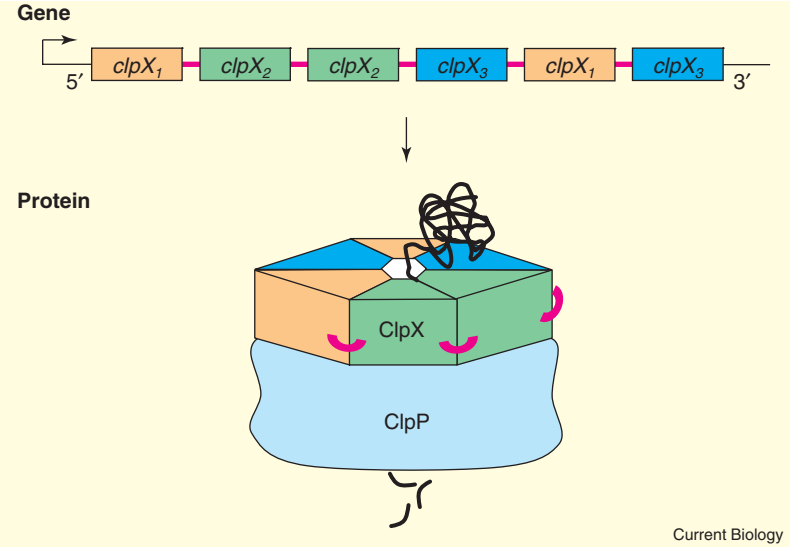

Figure 1. The ClpX protein assembles into a six-membered ring that docks onto the heptameric ClpP protein.

Martin *et al*. [7] constructed a gene that encodes individual ClpX subunits connected by 20 amino acid linkers. Mutations can be introduced into specific subunits in the linked gene (indicated by different colors). The covalently linked ClpX protein is fully functional and can bind, unfold, and translocate substrate to ClpP for degradation.

The ClpX AAA+ protein from *Escherichia coli* forms a barrel-shaped oligomer made of six identical subunits [3]. It can act alone as a chaperone or as a protease when assembled with another barrel-shaped oligomeric protein, ClpP. ClpX binds to folded substrates that have specific recognition sequences [8]. It then uses energy derived from ATP hydrolysis to unfold and translocate substrate proteins through the central pore of the hexamer [9]. Many rounds of ATP hydrolysis are needed to fully unfold and translocate a substrate protein [10]. When assembled with the ClpP protease, ClpX delivers the unfolded substrate protein directly into the central cavity of ClpP for degradation (Figure 1) [3].

Structural and biochemical studies of AAA+ ATPase proteins have generated two basic models for how these motors function [1]. In some structures, nucleotides are bound to all subunits, suggesting that the motor functions via a concerted mechanism in which all subunits hydrolyze ATP simultaneously [11,12] (Figure 2). In contrast, other structures have nucleotides bound to only a few of the subunits [12,13]. This asymmetry in nucleotide binding has led to models proposing a sequential mechanism for motor function. According to these models, the enzyme functions like a rotary engine: individual subunits hydrolyze ATP asynchronously and ATP hydrolysis proceeds in a predetermined, sequential order around the AAA+ ATPase ring (Figure 2).

A key experiment to test whether AAA+ motors use either a concerted or sequential mechanism would be to form mixed oligomers of active and inactive subunits, varying both their number and placement in

the oligomer. Since the concerted mechanism requires that all subunits (or a defined subset of subunits) act simultaneously, any oligomer with fewer than the required number of active subunits should not be active. For a sequential mechanism to be valid, motor function should be constrained by the placement of active subunits in the hexamer. Because oligomers form stochastically, however, it is extremely difficult to control their assembly in order to obtain a pure population of proteins with the desired number and arrangement of active subunits. Martin *et al*. [7] solved this problem by constructing a gene that encodes a tandem array of individual ClpX subunits connected by 20 amino acid linkers (Figure 1). The resulting covalently linked ClpX enzyme assembled with ClpP and degraded substrates as well as the unlinked protein. Variants with defined arrangements of active and inactive subunits could then be made by introducing mutations at specific locations in the tandem gene array (Figure 1).

Two different mutations that block ATP hydrolysis were used in these studies [7]. ClpX variants with the E185Q mutation in the Walker B motif can bind ATP and substrates with near wild-type affinity. However, they cannot unfold and translocate substrates, because they cannot hydrolyze ATP [14]. As a result, the E185Q subunits are binding-competent, but catalytically inactive. ClpX variants with the R370K mutation in the sensor II domain cannot hydrolyze ATP or bind substrate and are in a resting, inactive state [15]. These mutations were placed in individual subunits of the tandem gene array to generate a series of covalently linked ClpX proteins with defined orders of catalytically active, binding-competent but catalytically inactive, and resting subunits. With these proteins, Martin *et al*. [7] addressed a series of fundamental questions about the biochemical and geometric constraints on ClpX motor function.

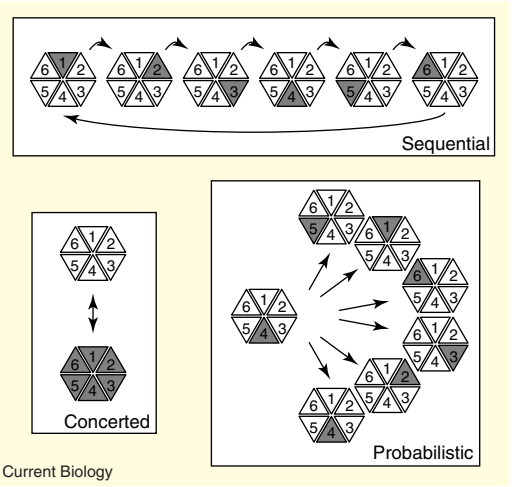

Figure 2. Models proposed for AAA+ motor function.

The subunit of the hexamer actively hydrolyzing ATP is shaded. For ease of illustration, ATP hydrolysis in every subunit is depicted for the concerted model and in a single subunit for the sequential and probabilistic models. For motors that use a sequential mechanism, each round of ATP hydrolysis proceeds from one subunit to the next in a fixed order around the hexameric ring. For motors that use a concerted mechanism, all subunits hydrolyze ATP simultaneously, and the enzyme cycles between active and inactive states. For motors that use the probabilistic mechanism, individual subunits hydrolyze ATP asynchronously, and the sequence of ATP hydrolysis from round to round is not restricted to a defined order.

What are the basic steps that the ClpX motor drives, and what is the minimal power-generating unit of the motor? To deliver a substrate to ClpP for degradation, ClpX must perform three basic steps. It must specifically bind the substrate, unfold it, and translocate the unfolded polypeptide into the proteolytic cavity of ClpP. Previous work with the E185Q mutation suggested that the binding step is separable from the catalytic step, and therefore independent of motor function [14]. This result was confirmed and extended with data obtained from experiments with different covalently linked ClpX variants [7]. The authors found that the requirements for substrate binding and motor function are different. Linked ClpX enzymes must contain three or more active or binding-competent subunits to interact with ClpP and substrate. However, only one of these subunits needs to be catalytically active to unfold and translocate the substrate. Therefore, the power stroke of the ClpX motor provides the energy for protein unfolding and translocation, and can be driven by multiple rounds of ATP hydrolysis in a single subunit. Like a car running on one cylinder, a ClpX variant with only one active subunit is underpowered and degrades substrates at a much slower rate than variants with more active subunits. Nevertheless, it can function.

Does the ClpX motor use a concerted or sequential mechanism? One of the surprising outcomes of this work was that many unrelated combinations and permutations of active and inactive ClpX subunits yielded enzymes whose activities, as measured by both the rate of ATP hydrolysis and the rate of substrate degradation, were proportional to the number of active subunits [7]. This result is inconsistent with both strictly sequential and concerted models for motor function. For example, the three covalently linked ClpX variants WRWRWR, WWWRRR, and RWERWE (the W subunit is active, R is resting, and E is binding-competent, but inactive) have very different subunit arrangements, yet the overall activity of each is proportional to the number of active subunits [7]. A few variants, particularly those with fewer than three active subunits, did yield proteins with activities lower than expected based on the number of active subunits, indicating that some interactions between neighboring subunits contribute to overall enzyme activity.

Martin *et al*. [7] suggest a compelling new model for the mechanism used by ClpX which posits that the order of ATP hydrolysis by individual subunits

in the hexamer is probabilistic, and not held to a particular firing order as prescribed by concerted or sequential models (Figure 2). As the authors point out, this mechanism is also well suited to the biological properties of the system. When an unfolded polypeptide chain is translocated through the hexameric ClpX ring, each segment of the substrate is conformationally and chemically unique and may be located anywhere in the ring. At any given time, one ClpX subunit may be better positioned than another to interact with the substrate. As the enzyme need not follow a specified firing order, the subunit best positioned to interact with the substrate can hydrolyze ATP, driving that particular round of unfolding and translocation.

Is the probabilistic model for AAA+ ATPase motor function used by other proteins? Many AAA+ machines act on heterogeneous substrates, suggesting that this mechanism would be advantageous for other members of the family. In addition, mechanisms that invoke a specified firing order for motor function require specific, ordered interactions between subunits. The probabilistic mechanism is not bound by such constraints and can function similarly with the wide array of quaternary structures adopted by diverse AAA+ enzymes. When appropriate linkers can be designed, comparable experiments with other AAA+ ATPase machines will determine whether the probabilistic model is indeed a general mechanism and will provide new insights into how these remarkable machines function.

References

1. Ogura, T., and Wilkinson, A.J. (2001). AAA+ superfamily ATPases: common structure–diverse function. Genes Cells *6*, 575–597.
2. Pickart, C.M., and Cohen, R.E. (2004). Proteasomes and their kin: proteases in the machine age. Nat. Rev. Mol. Cell Biol. *5*, 177–187.
3. Sauer, R.T., Bolon, D.N., Burton, B.M., Burton, R.E., Flynn, J.M., Grant, R.A., Hersch, G.L., Joshi, S.A., Kenniston, J.A., Levchenko, I., *et al.* (2004). Sculpting the proteome with AAA(+) proteases and disassembly machines. Cell *119*, 9–18.
4. May, A.P., Whiteheart, S.W., and Weis, W.I. (2001). Unraveling the mechanism of the vesicle transport ATPase NSF, the N-ethylmaleimide-sensitive factor. J. Biol. Chem. *276*, 21991–21994.
5. Caruthers, J.M., and McKay, D.B. (2002). Helicase structure and mechanism. Curr. Opin. Struct. Biol. *12*, 123–133.
6. Hanson, P.I., and Whiteheart, S.W. (2005). AAA+ proteins: have engine, will work. Nat. Rev. Mol. Cell Biol. *6*, 519–529.
7. Martin, A., Baker, T.A., and Sauer, R.T. (2005). Rebuilt AAA + motors reveal operating principles for ATP-fuelled machines. Nature *437*, 1115–1120.
8. Flynn, J.M., Neher, S.B., Kim, Y.I., Sauer, R.T., and Baker, T.A. (2003). Proteomic discovery of cellular substrates of the ClpXP protease reveals five classes of ClpX-recognition signals. Mol. Cell *11*, 671–683.
9. Singh, S.K., Grimaud, R., Hoskins, J.R., Wickner, S., and Maurizi, M.R. (2000). Unfolding and internalization of proteins by the ATP-dependent proteases ClpXP and ClpAP. Proc. Natl. Acad. Sci. USA *97*, 8898–8903.
10. Kenniston, J.A., Baker, T.A., Fernandez, J.M., and Sauer, R.T. (2003). Linkage between ATP consumption and mechanical unfolding during the protein processing reactions of an AAA+ degradation machine. Cell *114*, 511–520.
11. Gai, D., Zhao, R., Li, D., Finkielstein, C.V., and Chen, X.S. (2004). Mechanisms of conformational change for a replicative hexameric helicase of SV40 large tumor antigen. Cell *119*, 47–60.
12. Wang, J., Song, J.J., Seong, I.S., Franklin, M.C., Kamtekar, S., Eom, S.H., and Chung, C.H. (2001). Nucleotide-dependent conformational changes in a protease-associated ATPase HslU. Structure *9*, 1107–1116.
13. Singleton, M.R., Sawaya, M.R., Ellenberger, T., and Wigley, D.B. (2000). Crystal structure of T7 gene 4 ring helicase indicates a mechanism for sequential hydrolysis of nucleotides. Cell *101*, 589–600.
14. Hersch, G.L., Burton, R.E., Bolon, D.N., Baker, T.A., and Sauer, R.T. (2005). Asymmetric interactions of ATP with the AAA+ ClpX6 unfoldase: allosteric control of a protein machine. Cell *121*, 1017–1027.
15. Joshi, S.A., Hersch, G.L., Baker, T.A., and Sauer, R.T. (2004). Communication between ClpX and ClpP during substrate processing and degradation. Nat. Struct. Mol. Biol. *11*, 404–411.

303 S. Frear, Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania 16802, USA.

DOI: 10.1016/j.cub.2006.01.006

Insect Navigation: No Map at the End of the Trail?

Although the hunt for cognitive maps in insects may not have reached the end, the search itself has been fruitful in sharpening our understanding of the ways that insects navigate through familiar surroundings.

Matthew Collett[1] and Thomas S. Collett[2]

Almost every year new discoveries increase one's appreciation of the behavioural sophistication of ants and bees, making one wonder how the cognitive capacities of these small-brained animals measure up to those of much larger-brained mammals. Studying cognitive capacities is particularly informative within a behavioural domain, such as navigation, where different species do roughly similar things. A paper by Wehner and colleagues [1], published recently in *Current Biology*, introduces an interesting new method for asking how flexibly ants use landmark memories when navigating within familiar terrain.

Habits often mask behavioural flexibility. On our habitual route to work. we tend to perform, as if in a trance, a sequence of stereotyped actions that are often cued by landmarks along the route. Should we be stopped for directions mid-route, then we may wake up and, as we formulate a reply, become aware of the many types of spatial memories that we have at our disposal, but which are normally masked while we follow our route. By pointing in the direction of the requested location, we can communicate its position relative to where we are. Or we can give a sequence of instructions that describe a route to the location, possibly choosing between several routes. Such route instructions, moreover, are