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Minimum requirements for the actin-like treadmilling motor system

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Actin is one of the most abundant proteins in eukaryote cells, which forms a double stranded filament. The actin filament is not only a main component of the cytoskeleton, but also acts as a motor protein which moves toward one specific end, the barbed end, driven by polymerization at the barbed end and depolymerization at the other end, the pointed end, without any associated proteins. This motor activity is referred to as "treadmilling" and it represents the simplest motor system known, consisting of only one 42 kDa protein, actin. Here we report the minimum requirements of the actin-like motor system elucidated by computer simulations: (1) Nucleotide binding and ATPase activity in the filament; (2) Polarity in the rates of polymerization and depolymerization between the two ends; and (3) The dependence of the subunit-subunit interactions on the bound nucleotide. These requirements are simple and this knowledge should facilitate the development of artificial molecular motor systems in the future.

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Actin is one of the most abundant proteins in eukaryote cells, which forms a double stranded filament.[1] The actin filament is involved in various kinds of cellular functions, including cell adhesion, cell motility, cell division, cytoskeletal arrangement and muscle contraction. In most cases, the actin filament is dynamic through depolymerization and polymerization at both ends.[2,3] The actin dynamics in lamellipodia and filopodia is typical. Actin filaments move toward one specific end, the barbed end, by polymerization at this end and depolymerization at the other end, the pointed end. This process leads to the outward expansion of the cell membrane.

It is widely known that the actin filament with ATP, in vitro, moves toward the barbed end without any other proteins by polymerization at the barbed end and depolymerization at the pointed end. The polymerization and depolymerization processes occur at the same rate when the steady-state is reached and the movement is referred to as "treadmilling." Therefore, actin acts as a motor system. It is the simplest motor system known, because the actin motor system consists of only the 42 kDa protein actin. The mechanisms of molecular motors are not fully understood, although many motors have been identified, including myosins, kinesins, dyneins and F1-ATPase. Therefore, knowing the minimum requirements of the actin-like motor system may help us to understand not only actin-like motors but also many other motors. We have already reported that a combination of simple parameters reproduce the unidirectional movement of treadmilling.[4] However, this simple set of parameters does not represent the minimum requirements of treadmilling, because the system is still too complex.

The actin-like treadmilling motor system requires three elements. First, ATP hydrolysis occurs within the filament and the exchange of the bound nucleotide in the filament is negligible. Second, polarity in the rates of polymerization and depolymerization exist between the two ends, that is, both rates of polymerization and depolymerization at the fast end (corresponding to the barbed end in the actin filament) are substantially higher than those at the slow end (corresponding to the pointed end in the actin filament). In

the steady-state condition with ATP, ATP hydrolysis and phosphate release tend to occur at the less dynamic slow end before the next monomer is incorporated, whereas ATP-monomer molecules polymerize rapidly at the fast end. Therefore, the fast end is predominantly in the ATP-state, whereas the slow end contains primarily the ADP-state. Here the critical concentration, which is equivalent to the ratio of the depolymerization rate to the polymerization rate, is the same at both ends when the bound nucleotide is the same.[5,6] Third, the critical concentration of the monomer with ATP is lower than that with ADP. This results in a lower critical concentration at the fast end, which consists of the ATP-state, than at the end consisting of the ADP-F-actin (the slow end). Consequently, the motor monomer polymerizes as an ATP-monomer at the fast end and depolymerizes as an ADP-monomer at the slow end (**Fig. 1**).

A computer simulation showed that these three simple requirements were sufficient to reproduce a unidirectional movement (**Fig. 2**). This simulation requires six parameters to represent the three elements described above: k_{ATPase}, C_T, C_D, k_{FT}, k_{FD} and r. k_{ATPase} is the ATPase rate in the filament and corresponds to the first element. C_T and C_D are the critical concentrations of the molecules with ATP and ADP, respectively, where $C_T < C_D$, and these two parameters represent the third element. k_{FT} and k_{FD} are the polymerization rates at the fast end. The depolymerization rates at the fast end with ATP and ADP were calculated as $k_{FT}C_T$ and $k_{FD}C_D$, respectively. Conversely, $k_{ST} = rk_{FT}$ and $k_{SD} = rk_{FD}$, where k_{ST} and k_{SD} are the rate constants for polymerization with ATP and ADP at the slow end, respectively. r is the ratio of the rate constants for the slow and fast ends, where $r < 1$, which represents the second element. The depolymerization rates at the slow end with ATP and ADP were calculated as $rk_{FT}C_T$ and $rk_{FD}C_D$, respectively.

The results of the simulation based on realistic parameters determined by direct measurements[7] are presented in **Figure 2A and C**. The filament moves toward the fast end at a constant velocity, indicating that the three elements were sufficient to reproduce the unidirectional movement. The speed of the movement can clearly be changed by altering the parameters. For example, when the critical concentration of the monomers with ADP was increased by nine-fold, the rate also increased by ~7-fold (**Fig. 2B, D and G**), indicating the depolymerization with ADP at the slow end was rate-limiting when the realistic parameters were used (**Fig. 2A and C**).

To elucidate the importance of the three elements, further simulations were performed. When there was no ATPase activity, $k_{ATPase} = 0$, the filament did not

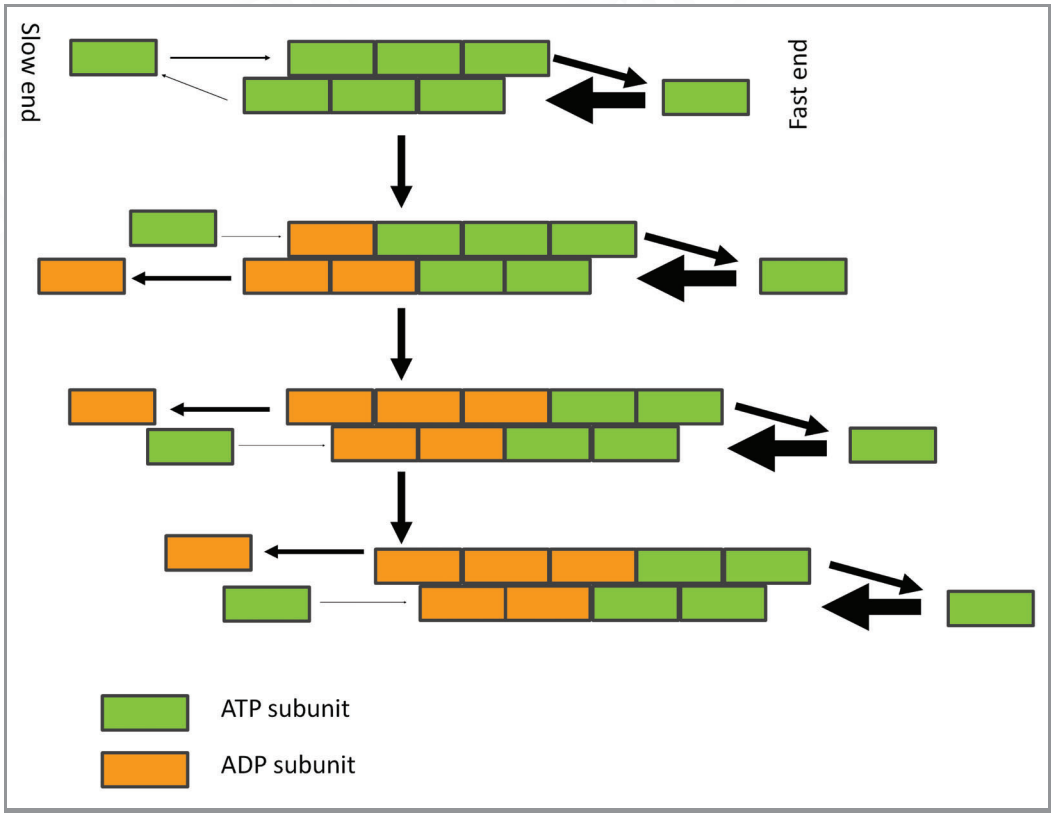

Figure 1. A simplified model for a treadmilling motor system. The thickness of the arrows at each end of the filament is proportional to the polymerization and depolymerization rates which are used in the simulation with the realistic parameters (**Fig. 2A**). In our model, we start with the filament consisting of ATP subunits (the first panel). As time passes, the slow end tends to have ADP subunits because of the ATPase in the filament and the polarity in the dynamics. At the steady-state (the latter two panels), the filament of the actin-like treadmilling motor moves toward the fast end without changing its length by polymerization at the fast end and depolymerization at the slow end.

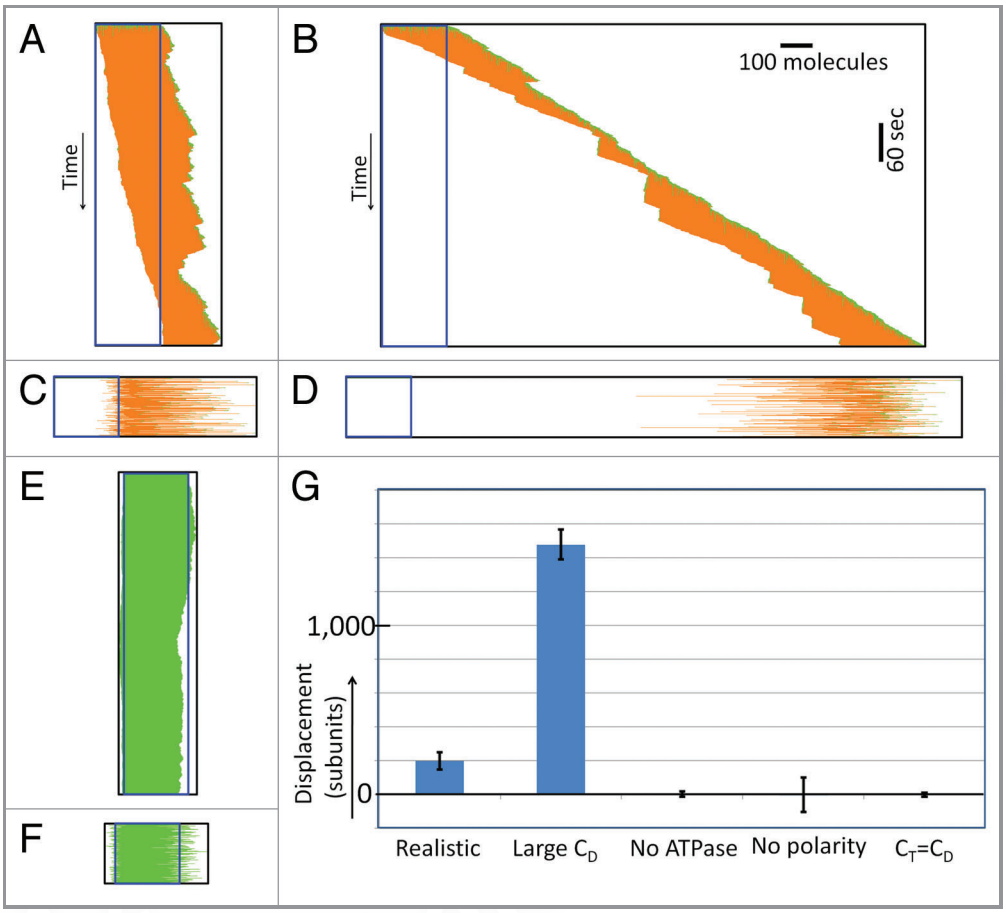

Figure 2. Simulation of the treadmilling motor system with limited parameters. (A–F) The results of the simulations. Each horizontal line represents a single filament of the actin-like treadmilling motor, with the slow end to the left. On the horizontal line, each dot represents a subunit with ATP or ADP in green or orange, respectively. The blue box in each panel indicates the initial position of the filament in the simulation. All panels are presented with the same scale. (A) The result of the simulation with realistic parameters based on a recent communication,[7] k_{ATPase} = 0.3 sec^{-1}, C_T = 0.1 μM, C_D = 2 μM, k_{FT} = 10 $\mu M^{-1}s^{-1}$, k_{FD} = 3 $\mu M^{-1}s^{-1}$ and ***r*** = 0.1. The time course of a single filament is represented with a time interval of 1 sec. The actin concentration was chosen so that the average length of the actin filament remained constant. The time interval of the simulation was 0.005 sec. The filament moves toward the fast end at a constant velocity. (B) The time course of the simulation with the same parameters as (A) except for C_D = 18 μM. The filament moved seven times faster than (A). (C) The status of the filaments after 500 sec with the realistic parameters (A). Each horizontal line represents the result of a single simulation and 180 results were collected. (D) The status of the filaments after 500 sec with the same parameters as (B). (E,F) Results of the simulation with the same parameters as (A) except for k_{ATPase} = 0 sec^{-1}, without the ATPase in the filament. (E) The time course of the simulation. (F) The status of the filaments after 500 sec. The filaments did not show any unidirectional movement. (G) The averaged displacement with the standard deviation after 500 sec for each condition was plotted. Simulations were repeated 180 times for each condition. "Realistic," "Large C_D" and "No ATPase" represent the results with the same parameters as (A), (B) and (E), respectively. "No polarity" represents the result with the same parameters as (A) except for ***r*** = 1, there was no polarity in the dynamics. "C_T = C_D" represents the result with the same parameters as (A) except for C_D = 0.1 μM, the same as C_T. "No ATPase," "No polarity" and "C_T = C_D" showed no significant movement.

move (**Fig. 2E–G**), indicating that the first element was indispensable. When the polymerization and the depolymerization rates were identical at both ends, ***r*** = 1, the filament did not move either (**Fig. 2G**), indicating the second element was essential to the unidirectional movement. The third element was also essential because when the critical concentration was independent of the nucleotide species, C_T = C_D, the filaments did not move. Therefore, the three elements represent the minimum requirements to reproduce the treadmilling movement.

The structural basis of the second element has been characterized. We determined the three-dimensional structure of the actin pointed end by cryo-electron microscopy and single-particle analysis[8] and the resultant structure showed that a specific loop to loop interaction at the pointed end is the basis of the slower dynamics at the pointed end than at the barbed end.[4] The requirements for the polarity in the dynamics is reviewed by Narita, 2011.[6] There are two simple requirements. The double (or more) stranded form of the filament and one specific interaction at the slow end were essential.

Unfortunately, the structural basis of the first and the third elements remain unresolved. Three models of the actin filament determined by X-ray fiber diffraction[1] and electron microscopy[9,10] have been published, the first was with Ca^{2+} and

ADP,[1] the second with Mg^{2+} and ADP[9] and the third with Mg^{2+} and ADPPi.[10] However, the mechanisms of the ATPase in the filament and the affinity change between the subunits according to the bound nucleotide species are not well understood. This is because of a lack of resolution in each structure, and due to the absence of a structure when ATP is bound. To understand the ATPase mechanism and the small affinity change (~20 fold) within the structure requires a clear understanding of the side-chain conformations, which could not be seen in any of the three models. For a full understanding of the actin motor system, the actin filament structures at higher resolution with ADP, ADPPi and ATP are required.

Nonetheless, the computer simulation has shown that the three elements represent the minimum requirements to reproduce the treadmilling one-way movement (**Fig. 2**). The requirements are simple and relatively easy to achieve using an artificial motor system. This knowledge may facilitate the development of artificial molecular motor systems in the future.

References

1. Oda T, Iwasa M, Aihara T, Maeda Y, Narita A. The nature of the globular- to fibrous-actin transition. Nature 2009; 457:441-5; PMID:19158791; http://dx.doi.org/10.1038/nature07685
2. Pollard TD, Borisy GG. Cellular motility driven by assembly and disassembly of actin filaments. Cell 2003; 112:453-65; PMID:12600310; http://dx.doi.org/10.1016/S0092-8674(03)00120-X
3. Pantaloni D, Le Clainche C, Carlier MF. Mechanism of actin-based motility. Science 2001; 292:1502-6; PMID:11379633; http://dx.doi.org/10.1126/science.1059975
4. Narita A, Oda T, Maeda Y. Structural basis for the slow dynamics of the actin filament pointed end. EMBO J 2011; 30:1230-7; PMID:21378753; http://dx.doi.org/10.1038/emboj.2011.48
5. Ward JH. Hierarchical grouping to optimize an objective function. J Am Stat Assoc 1963; 58:236-44; http://dx.doi.org/10.2307/2282967
6. Narita A. Merits of the double-stranded form of the actin filament revealed by structures of the filament ends. Commun Integr Biol 2011; 4:692-5; http://dx.doi.org/10.4161/cib.4.6.17137
7. Fujiwara I, Vavylonis D, Pollard TD. Polymerization kinetics of ADP- and ADP-Pi-actin determined by fluorescence microscopy. Proc Natl Acad Sci USA 2007; 104:8827-32; PMID:17517656; http://dx.doi.org/10.1073/pnas.0702510104
8. Narita A, Maeda Y. Molecular determination by electron microscopy of the actin filament end structure. J Mol Biol 2007; 365:480-501; PMID:17059832; http://dx.doi.org/10.1016/j.jmb.2006.06.056
9. Fujii T, Iwane AH, Yanagida T, Namba K. Direct visualization of secondary structures of F-actin by electron cryomicroscopy. Nature 2010; 467:724-8; PMID:20844487; http://dx.doi.org/10.1038/nature09372
10. Murakami K, Yasunaga T, Noguchi TQ, Gomibuchi Y, Ngo KX, Uyeda TQ, et al. Structural basis for actin assembly, activation of ATP hydrolysis, and delayed phosphate release. Cell 2010; 143:275-87; PMID:20946985; http://dx.doi.org/10.1016/j.cell.2010.09.034