Depolymerization of Actin Filaments

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Actin is one of the most abundant and highly conserved proteins in eukaryotic cells. The globular protein assembles into long filaments, which form a variety of different networks within the cytoskeleton. The dynamic reorganization of these networks – which is pivotal for cell motility, cell adhesion, and cell division – is based on cycles of polymerization (assembly) and depolymerization (disassembly) of actin filaments. Actin binds adenosine triphosphate (ATP), and within the filament the actin-bound ATP is hydrolyzed into adenosine diphosphate (ADP) on a time scale of a few minutes.

Because ADP-actin dissociates faster from the filament ends than ATP-actin, it was thought that the filament becomes less stable as it grows older. However, recent depolymerization experiments with single filaments suggested the opposite behavior. Abrupt dynamic changes during filament depolymerization have been observed in buffers containing no free monomers, and indicate that the actin filaments become increasingly stable with time. Several mechanisms for this stabilization have been proposed. The most prominent hypothesis correlates the stabilization with structural transitions of the whole filament helix **[1]**.

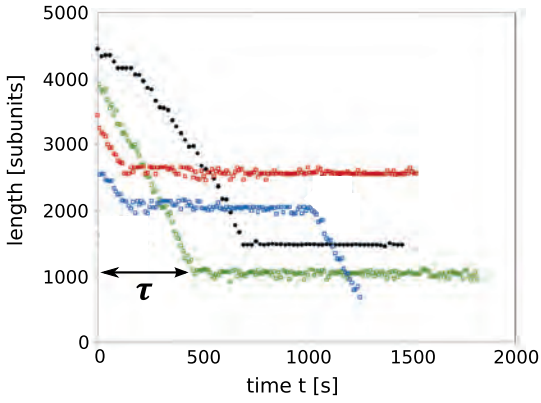

Fig. 1: Length of four actin filaments as a function of time. The filaments depolymerize in buffers containing no free monomers. The shrinkage is suddenly interrupted at a certain interruption time , which differs from filament to filament and represents a stochastic variable.

Interruptions of Depolymerization

In order to study the interruptions of depolymerization, we collaborated with the experimental lab of Marie-France Carlier in Gif-sur-Yvette (France). A combination of single filament microscopy and stochastic modeling allowed us to discover the surprising mechanism of filament stabilization **[2,3]**. In depolymerization experiments on filaments having one end blocked, we confirmed that filaments abruptly cease to shrink and determined the time from the initiation of depolymerization until the occurrence of the first interruption, see **Fig. 1**. This duration time τ differs from filament to filament and represents a stochastic variable.

We considered various hypothetical mechanisms that may cause the observed interruptions. These mechanisms cannot be observed directly, but they lead to distinct distributions of the duration τ and these distributions can be compared with those obtained from single filament experiments. By modeling the underlying stochastic processes – such as the association and dissociation of filament subunits and putative transformations of these subunits – we computed the cumulative distribution functions $P(t) = \text{prob}(\tau \leq t)$ of the stochastic variable τ for all transformation mechanisms in question. For global filament transformations, which were implicitly considered in **[1]**, or transitions that only occur at the depolymerizing filament end, the duration τ is exponentially distributed. Furthermore, many other mechanisms – for instance the copolymerization of actin with already transformed subunits – give rise to an approximately exponential distribution of τ. Successive transformations of subunits starting from a single seed cause the duration τ to have a very narrow Gaussian distribution, and thus result in a sharp rise of P(t) at $t = \langle\tau\rangle$. For the experimentally relevant range of parameters, local transformations of random subunits within the filament lead to a cumulative distribution that is well described by the expression

$$P(t) = 1 - \exp(-\alpha\omega t^2), \qquad (1)$$

where the parameter α is fixed by both the polymerization and depolymerization velocities, and the free parameter ω is the rate of the putative subunit transformations. A comparison of our analytical expressions with the measured distribution, see **Fig. 2**, revealed that the sudden truncation of the shrinkage process does neither arise from blocking of the ends nor from a collective transition of the whole filament. Instead, we have predicted a novel, local transition process occurring at random sites within the filament.

The combination of additional single filament experiments with our theoretical approach – and in particular with a generalization of the distribution in equation (1) – confirmed the notion of a local transition mechanism and identified the postulated transition as the photo-induced formation of an actin dimer within the filaments. Furthermore, we showed that only fluorescently labeled filament subunits may exhibit a transition and that unlabeled actin filaments do not exhibit pauses. This implies that, *in vivo*, older filaments become destabilized by ATP hydrolysis, in contrast to the view expressed in **[1]**.

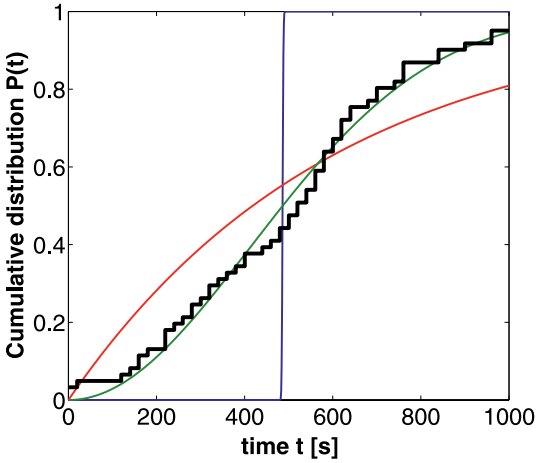

Fig. 2: The cumulative distribution P(t) = prob ($\tau \leq t$) of the duration τ, i.e., the probability that the interruption occurs at any time prior to time t, provides a fingerprint of the mechanism that causes the interruption. The exponential distribution (shown in red) is implied by many possible mechanisms such a transformations that may occur only at the shrinking filament end. The step-like function (shown in blue) is caused by sequential transformations of the filament subunits. Local transformations of random subunits lead to a cumulative distribution given by equation (1) and displayed in green. Experimental data are shown in black. The red, blue, and green curves corresponding to theoretical distributions were obtained by least-square-fitting of the respective distributions to the experimental distribution. Each fitting procedure involves only one fit parameter provided by the transition rate ω. Since the data can only be described by the green curve, we conclude that the interruptions arise from local transitions of random subunits within the filament. As soon as such a transformed subunit arrives at the shrinking end, it causes the interruption of depolymerization.

Mechanism of ATP Hydrolysis

The filament destabilization by ATP hydrolysis becomes apparent as an acceleration of the depolymerization prior to the interruption: In **Fig. 1**, the black data, corresponding to a filament grown from ATP-actin, exhibit an increase of the depolymerization velocity, whereas the red, green and blue data points, obtained for three filaments grown from ADP-actin, exhibit shrinkage with constant velocity. The mechanism of ATP hydrolysis has remained elusive for many years: Both the so called "random model", where ATP is hydrolyzed at each subunit with the same rate, as well as the "vectorial model", where ATP hydrolysis exclusively takes place at a subunit neighboring an ADP-actin subunit, have been proposed in the literature. The measurement of the time-dependent depolymerization velocity using fluorescence microscopy in conjunction with a theoretical description of the depolymerization process and a careful data analysis reveals that the rate of ATP hydrolysis is constant within the filament, corresponding to a random hydrolysis mechanism **[3,4]**. This method also provided novel insight into the function of profilin, a protein that accelerates actin depolymerization in cells, thus demonstrating the method's potential in the functional analysis of actin regulators.

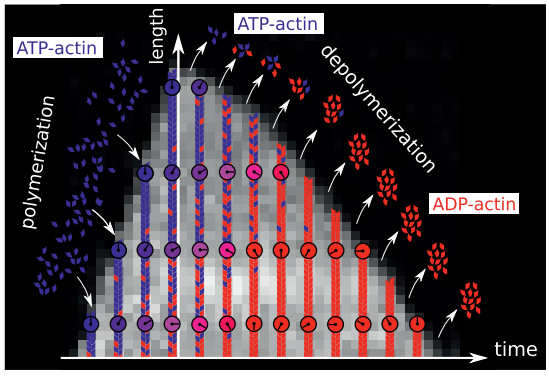

Fig. 3: The time-dependent extension of an actin filament is shown in light gray. Left of length axis: During polymerization, ATP-actin is incorporated into the filament. The subsequent hydrolysis of the bound ATP gives rise to ADP-actin. Right of length axis: The velocity of depolymerization increases over time, since ADP-actin dissociates more rapidly from the filament end than ATP-actin. The local composition of the filament (i.e. the fraction of ATP-actin) can be inferred from the time-dependence of the depolymerization velocity and is indicated by the coloring of the small clocks. These clocks measure the `age' of the subunit at the respective position within the filament, that is the time that has elapsed since the incorporation of the respective filament segment. The correspondence between the color of the clocks and the local time indicates that the rate of ATP hydrolysis is constant along the filament.

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