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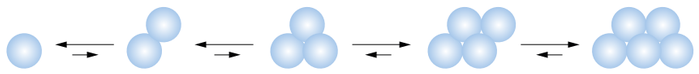
THE PRINCIPALS OF ACTIN POLYMERIZATION

BOX 1: ACTIN NUCLEATION

Helical polymer are stabilized by multiple interaction sites between adjacent subunits. Actin filaments are formed from monomeric actin (G-actin) as a consequence of nucleation. The nucleation is initiated, when two actin monomers bind to each other and form a dimer. This complex is weak and can easily dissociate, unless a third monomer adds to form a more stable trimer. Trimers are also referered to as nuclei, because they initiate the elongation process of filaments by fast addition of actin monomers. In the scheme below, the lenghts and direction of the arrows indicate the tendency for addition or dissociation of monomers at each step of the actin polymerization.



After the trimer has formed, further actin monomers are added at high speed to the complex.

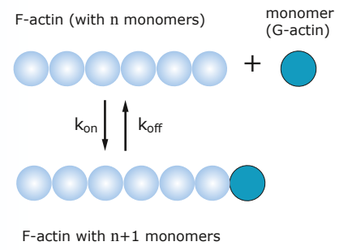
The assembly of nuclei is a relatively slow and reversible process, reflected by the lag phase of actin polymerization. By addition of pre-formed nuclei, e.g. gelsolin-actin nuclei or short actin filaments, the lag phase can be completely eliminated, as shown in figure 2 of box 2 below.

The physiologically relevant form of actin is Mg^{++}-actin (Mg-actin). In comparison to Ca-actin, Mg-actin has a higher propensity to nucleate. Thus a larger number of filaments forms and the overall polymerization is faster.

ON RATES AND OFF RATES

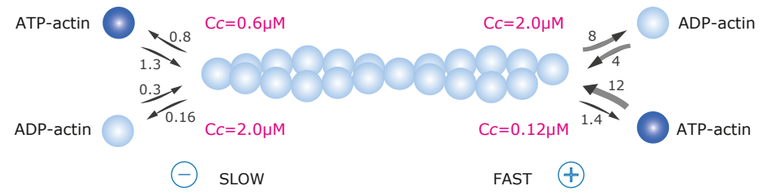
Actin filaments (F-actin) are polar, double helical, linear polymers of monomeric actin molecules. G-actin assembles (polymerizes) by the addition of other G-actin molecules and it disassembles (depolymerizes) by the loss of G-actin at both ends of the actin filament.

The polymerization rate (monomer addition) is given by the rate constant k_{on} (unit: $\mu M^{-1}s^{-1}$) , while the depolymerization rate (loss of monomers) is given by k_{off} (unit: s^{-1}) .



BOX 3: THE PLUS AND MINUS ENDS OF ACTIN FILAMENTS

Both ends of the actin filament polymerize at different rates. The fast-growing end is termed the plus end and the slow-growing end is termed the minus end. The rapid growth of the plus end is based on the ~10 times higher association rate of Mg-ATP actin compared to the relatively low association rate of Mg-ATP actin at the minus end. In contrast, the dissociation rates are very similar for both ends.



The ratio between the rate constants for association (unit: $\mu M^{-1}s^{-1}$) and dissociation (unit: s^{-1}) defines the critical concentration for Mg-ATP actin and Mg-ADP actin. The association rate at the plus end of ~12 $\mu M^{-1}s^{-1}$ and ~ 1.3 s^{-1} at the minus end means, that 1 μM of free ATP-actin leads to the addition of on average 12 monomers per second to the plus end of already existing filaments. Thus, the rate of association depends on the concentration of free ATP-actin. Since the dissociation rates of ATP-actin from both ends of the filament are almost equal, the dissociation is defined only by the loss of monomers from the filament ends, and does not depend on the concentration of free ATP-actin.

SYNONYMS

⊕
plus end
barbed end
fast polymerizing end

⊖
minus end
pointed end
slow polymerizing end

BOX 2: ACTIN POLYMERIZATION

The typical time course of the salt induced actin polymerization, as e.g. obtained in Fluorometry with Pyrene Actin, is characterized by three different phases as shown in the figure 1:

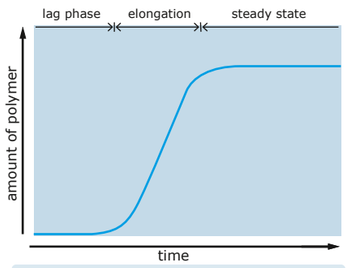


Fig. 1: Salt-induced actin polymerization, e.g. by PolyMix.

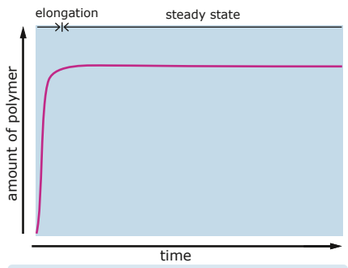


Fig. 2: Course of actin polymerization with pre-made nuclei, e.g. gelsolin-actin complexes.

1. Lag phase: this phase corresponds to the time the actin monomers need for nucleation.
2. Elongation phase: during this growth phase, new monomers add to the ends of the growing filament, which leads to the elongation of actin filaments.
3. Steady-state: the equilibrium phase is reached, when the growth of the actin filament by monomer addition balances the loss of monomers from the filament. Monomers that disassemble become part of the free G-actin pool again.

THE CRITICAL CONCENTRATION

The number of monomers adding to the actin filament is proportional to the concentration of free monomers ($k_{on}C$), while monomers leave the filaments end at a constant rate (k_{off}), that does not depend on C.

As the actin filament grows, the number of free actin monomers decreases. However, the concentration drops until it reaches a constant value, called the critical concentration (Cc). At this concentration the rate of monomer addition equals the rate of monomer loss.

This equilibrium is described: $k_{on}C = k_{off}$

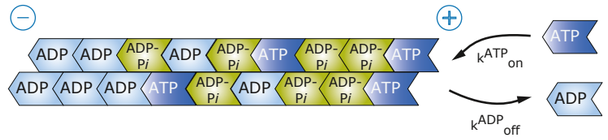
Which means: $Cc = \frac{k_{off}}{k_{on}} = \frac{1}{K^*}$

* K is the equilibrium constant for monomer addition.

BOX 4: ACTIN ATPase - THE ATP HYDROLYSIS

The actin molecule has a high affinity ATP binding pocket. Upon polymerization ATP is first hydrolyzed to ADP-*Pi*. ADP and the free phosphate bind for several minutes to the ATP pocket, before the P*i* dissociates and actin turns into ADP-actin. The ability of actin to hydrolyze ATP classifies actin as an ATPase and the activity of the actin-ATPase is e.g. influenced by the interaction of a actin by actin-binding proteins. The ATP hydrolysis to ADP-actin reduces the affinity for adjacent monomers, and thus eases its dissociation of from the filaments end.

Usually ATP-actin associates and ADP-actin dissociates from the plus end of the actin filament:

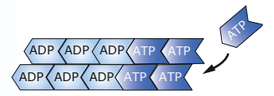


The actin filament grows until the actin concentration adjusts somewhere between the critical concentrations of both ends. However, ATP-actin also dissociates from the plus end and ADP-actin associates (for k^{ADP}_{on} and k^{ATP}_{off} see box 5 for further details). They are usually very small, so that the growth of the filaments reaches steady-state when:

$$Cc = \frac{k^{ADP}_{off}}{k^{ATP}_{on}}$$

The ATP that is hydrolyzed to ADP during polymerization, is exchanged by ATP in the free G-actin molecule. Thus ADP-actin is recharged to become ATP-actin. In *in vitro* assays it is thus essential to maintain a sufficient quantity of ATP in the actin solution.

THE ATP CAP



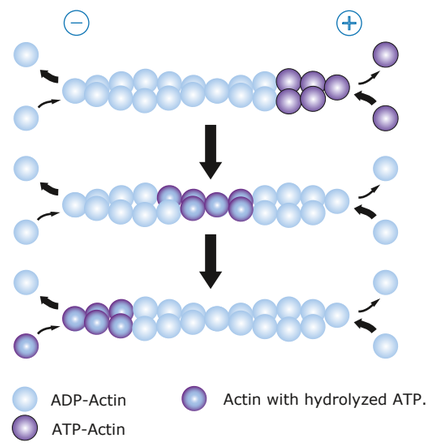
The rate of monomer addition during polymerization of actin filament can be faster than the rate at which ATP is hydrolyzed. In this case, the end is termed to have a "cap" . The cap defines the G-actin carrying a nucleoside triphosphate, resp. the actin filament ATP cap.

BOX 5: THE TREADMILLING MECHANISM OF F-ACTIN

If the plus end of the actin filament is freely available, the polymerization proceeds until the concentration of free ATP-actin is above the Cc for the plus end, but below the Cc for the minus end. Thus the lenghts of polymerizing actin filaments increase, while the concentration of free ATP-actin drops. This is the steady state, where monomers show a balance between net assembly at the plus end and net disassembly at the minus end at an identical rate. The actin filament maintains a constant length, despite of the net flux of monomers through the filament.

At the minus end the Cc is 0.6 μM, thus below this value dissociation occurs. The plus end however, has a Cc of 0.12 μM and grows above this value, obviously at concentrations where the minus end already shrinks. Because the Cc for both ends are different, the concentration of free ATP-actin is between the Cc of both ends. The plus end grows while the minus end shrinks and actin monomers progress from the plus to the minus end. This phenomenon is therefore called treadmilling.

The ATP-actin which has initially associated to the plus end leaves the minus end as ADP-actin and enters the pool of free G-actin. This free ADP-actin is subsequently reloaded with ATP and can participate in the process of actin polymerization.



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BUFFERS & COMPONENTS

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MonoMix (G-actin buffer, 50x) 2x1.0ml	Cat. #: 5100-01
ME-Buffer (Mg-Exchange Buffer, 10x) 2x1.0ml	Cat. #: 5111-01
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ACTIN & ACTIN BINDING PROTEINS

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