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VIEWPOINT

The dynamic instability of actin filament barbed ends

Guillaume Romet-Lemonne and Antoine Jégou

The turnover of actin filament networks in cells has long been considered to reflect the treadmilling behavior of pure actin filaments in vitro, where only the pointed ends depolymerize. Newly discovered molecular mechanisms challenge this notion, as they provide evidence of situations in which growing and depolymerizing barbed ends coexist.

Introduction

In cells, actin assembles into filament networks with diverse architectures and lifetimes, playing key roles in functions such as endocytosis, cell motility, and cell division. These filament networks are maintained and renewed by actin turnover, which implies that assembly and disassembly must take place simultaneously and in a controlled manner within the networks. Each actin filament end has the ability to either grow or shrink, depending on the concentration of actin and regulatory proteins, but pure actin treadmills at steady state: ATP-actin is added at the barbed end at a rate matching the departure of ADP-actin from the pointed end, and ATP hydrolysis takes place within the filament. This hallmark feature of actin dynamics has been known for decades (Wegner, 1976) and has been generalized to the cell context, in which it is commonly assumed that actin polymerization takes place at the barbed end, while depolymerization takes place only at the pointed end (whether it be the ends of filaments within the network or the ends of fragments that have detached from it). This notion is reinforced by the fact that the cytoplasm contains high concentrations of monomeric actin (G-actin) in complex with profilin (Funk et al., 2019), which is unable to bind to pointed ends and should drive the elongation of all noncapped barbed ends.

Recently, however, in vitro studies have identified two seemingly independent mechanisms in which, in the presence of profilin-actin, filament barbed ends alternate between phases of growth and depolymerization. This behavior, referred to as "dynamic instability," is widely observed for microtubules but was unexpected for actin filaments. It suggests that cells could use barbed ends for both elongation and disassembly.

Driving the depolymerization of barbed ends with cofilin side-decoration

Proteins of the actin depolymerizing factor (ADF)/cofilin family (henceforth cofilin) are composed of a single ADF-homology (ADF-H) domain and are mostly known for their actin filament–severing activity (De La Cruz, 2009). Cofilin binds cooperatively to the sides of actin filaments, forming clusters where the conformation of the filament is locally altered, leading to its severing at cofilin cluster boundaries. In addition, the barbed ends of cofilin-decorated filaments steadily depolymerize, despite the presence of G-actin and profilin-actin (Fig. 1 A) and even capping protein (CP) in solution (Wioland et al., 2017, 2019). This unexpected result likely originates from the conformational change of actin subunits at the barbed end, induced by cofilin side-binding. As a consequence, filaments exposed to G-actin (with or without profilin), CP, and cofilin alternate between phases of barbed-end elongation and barbed-end depolymerization. In these conditions, actin filament barbed ends thus exhibit a form of dynamic instability.

Driving the depolymerization of barbed ends with twinfilin end-targeting

Twinfilin has two ADF-H domains, but unlike cofilin, it binds poorly to the sides of actin filaments. Rather, twinfilin appears to mainly sequester ADP-actin monomers and target the barbed end to modulate its elongation and capping. Recent in vitro studies have shown that the interaction of twinfilin with actin filament barbed ends could drive their depolymerization, even in the presence of G-actin and profilin-actin (Johnston et al., 2015; Hakala et al., 2021; Shekhar et al., 2021). Very interestingly, the processive barbed-end elongator formin mDia1 is able to protect barbed ends from twinfilin, allowing them to sustain elongation (Shekhar et al., 2021). This leads to a situation in which, as filaments are exposed to profilin-actin and twinfilin, mDia1-bearing barbed ends elongate while bare barbed ends depolymerize (Fig. 1 B). It is safe to assume that, if filaments were continuously exposed to this protein mix including formin in solution, they would alternate between phases of growth and shrinkage over time, as formins come on and fall off the barbed end. This mix of proteins would therefore constitute another situation causing actin filament dynamic instability.

From actin treadmilling to dynamic instability, in cells?

This newly identified versatile behavior of actin filaments is reminiscent of microtubules. While dynamic instability is the

Université de Paris, Centre National de la Recherche Scientifique, Institut Jacques Monod, Paris, France.

Correspondence to Guillaume Romet-Lemonne: romet@ijm.fr.

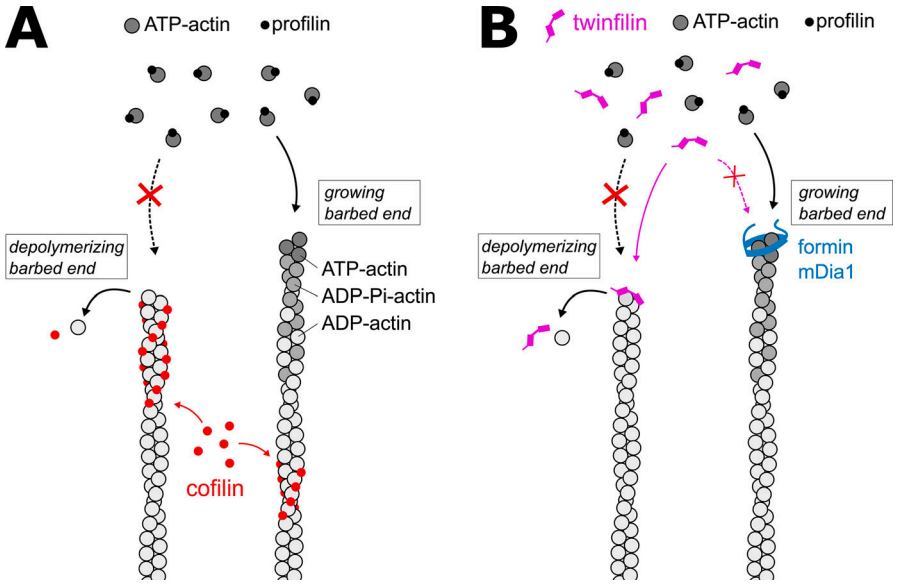

Figure 1. **Two mechanisms that give rise to barbed-end depolymerization in elongation-promoting conditions. (A)** When a cofilin side-decorated region reaches the barbed end, adding a new actin or profilin-actin becomes very difficult, and the barbed end depolymerizes. Not represented: Capping by CP can lead to depolymerization, as it allows the cofilin cluster to reach the barbed end, which then has a much weaker affinity for CP and steadily depolymerizes. Also, severing events occur at cofilin cluster boundaries, creating new barbed ends, either bare or cofilin-decorated. **(B)** Twinfilin binds to the barbed end, preventing its elongation and causing its depolymerization. Whether twinfilin remains processively attached to the depolymerizing barbed end or departs with the actin subunits is still unknown. Twinfilin has no impact on the elongation of mDia1-bearing barbed ends.

hallmark behavior of microtubules, they can also be made to treadmill steadily by adding 4 microtubule-associated proteins (Arpağ et al., 2020). In cells, both microtubule dynamic instability and treadmilling have been clearly observed (Wittmann et al., 2003). In contrast, the disassembly of single actin filaments, either embedded in a network or severed from it, has not yet been directly observed in cells. Despite insights from techniques such as single-molecule speckle microscopy, it is still unclear from which end actin filaments depolymerize, even in networks that appear to globally treadmill, such as the lamellipodium. Pointed end depolymerization alone cannot account for what is observed in cells (Miyoshi et al., 2006) and alternative mechanisms have been proposed, including brutal filament-to-monomer transitions occurring in bursts, driven by cofilin, coronin, and Aip1 (Brieher, 2013; Tang et al., 2020).

In cells, the high amounts of available G-actin (tens of micromolars; Funk et al., 2019) should limit barbed-end depolymerization. Based on the reported on-rate for ATP–G-actin at the barbed ends of cofilin-decorated filaments (Wioland et al., 2017, 2019), we can estimate that these barbed ends, under such conditions, would depolymerize for tens of seconds before being "rescued," which is enough to remove tens of subunits from each filament. In contrast, twinfilin concentrations similar to those of G-actin appear necessary to drive barbed-end depolymerization (Hakala et al., 2021; Shekhar et al., 2021). As proteomics studies in HeLa cells report that twinfilin is 50-fold less abundant than actin, this may be difficult to achieve in cells (Bekker-Jensen et al., 2017). However, future studies may uncover proteins, or posttranslational modifications of actin, that enhance the ability of twinfilin to drive barbed-end depolymerization in the presence of high concentrations of profilin-actin.

Molecular insights and possible synergies

While cofilin and twinfilin both interact with actin via ADF-H domains, they appear to drive barbed-end depolymerization through different mechanisms: twinfilin by directly targeting the barbed end, and cofilin by decorating the filament sides, thereby changing the conformation of the filament and putting its barbed end in a depolymerization-prone state.

The two mechanisms, nonetheless, share clear similarities. For instance, cofilin side-binding and twinfilin end-targeting both slow down ADP-actin barbed-end depolymerization, compared with bare ADP-actin filaments (Wioland et al., 2017; Hakala et al., 2021; Shekhar et al., 2021). Strikingly, a crystal structure of the actin/twinfilin/CP complex indicates that the actin conformational change induced by twinfilin binding at the barbed end is similar to that induced by cofilin decorating the sides (Mwangangi et al., 2021). It is thus possible that the dynamic instability of actin filament barbed ends reflects the same conformation changes, triggered either by cofilin side-decoration or twinfilin end-targeting.

In addition to decorating the filament sides, cofilin targets ADP-actin barbed ends. Unlike twinfilin, the direct interaction of cofilin with the barbed end cannot cause its depolymerization in the presence of ATP-actin monomers. Indeed, cofilin end-targeting accelerates the depolymerization of ADP-actin barbed ends in the absence of G-actin, but cofilin does not appear to interact with growing ATP-actin barbed ends (Wioland et al., 2017). This is in stark contrast with twinfilin end-targeting, which slows down ADP-actin depolymerization and accelerates ADP–Pi-actin depolymerization (Shekhar et al., 2021). These different behaviors regarding the nucleotide state of actin are intriguing and should be investigated further.

Cofilin thus needs to decorate the filament sides in order to have an impact on barbed-end dynamics in elongation-promoting conditions. However, it is unknown whether cofilin side-decoration extends all the way to the terminal subunits and occupies sites that twinfilin would target. Thus, it is unclear whether cofilin and twinfilin would compete or synergize to drive barbed-end depolymerization.

Synergies with other proteins are also worth further investigation, CP being an interesting candidate. Cofilin side-decoration drastically decreases the barbed-end affinity for CP, and capped filaments are thereby an efficient intermediate to turn growing barbed ends into depolymerizing barbed ends (Wioland et al., 2017). Twinfilin interacts with CP and the barbed end to enhance uncapping (Hakala et al., 2021; Mwangangi et al., 2021). Since CP can bind mDia1-bearing barbed ends and displace mDia1 (Bombardier et al., 2015; Shekhar et al., 2015), perhaps CP can also

contribute to turn growing, mDia1-bearing barbed ends into depolymerizing barbed ends, by removing mDia1 from barbed ends and subsequently getting displaced from the barbed end by twinfilin.

Finally, it is worth noting that profilin, which does not contain an ADF-H domain, also interacts with the barbed face of G-actin and with the barbed end of the filament. When profilin is in sufficient excess, it is able to promote barbed-end depolymerization in the presence of ATP–G-actin (Pernier et al., 2016). Unlike twinfilin, its depolymerization-promoting activity is not prevented by formin mDia1, and it thus does not lead to dynamic instability (bare and mDia1-bearing barbed ends all either grow or depolymerize). The coexistence of growing, mDia1-bearing barbed ends and depolymerizing, twinfilin-targeted barbed ends (Fig. 1 B) was observed in the presence of profilin (Shekhar et al., 2021), but profilin actually may not be required. Future studies should determine the exact role of profilin in this mechanism.

Conclusion

The extent to which barbed-end dynamic instability contributes to actin turnover in cells is not known, but possible molecular mechanisms have now been identified. They should change the way we envision actin network dynamics, as we must now consider the possibility that cells also exploit the barbed end for disassembly. More work is needed to further document these mechanisms, but the idea of a "generalized treadmilling" has now been contradicted at its source: in vitro experiments.

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References

Arpağ, G., et al. 2020. *Proc. Natl. Acad. Sci. USA*. https://doi.org/10.1073/pnas.2003191117

Bekker-Jensen, D.B., et al. 2017. *Cell Syst*. https://doi.org/10.1016/j.cels.2017.05.009

Bombardier, J.P., et al. 2015. *Nat. Commun*. https://doi.org/10.1038/ncomms9707

Brieher, W. 2013. *Mol. Biol. Cell*. https://doi.org/10.1091/mbc.e12-09-0694

De La Cruz, E.M. 2009. *Biophys. Rev*. https://doi.org/10.1007/s12551-009-0008-5

Funk, J., et al. 2019. *eLife*. https://doi.org/10.7554/eLife.50963

Hakala, M., et al. 2021. *Nat. Cell Biol*. https://doi.org/10.1038/s41556-020-00629-y

Johnston, A.B., et al. 2015. *Nat. Cell Biol*. https://doi.org/10.1038/ncb3252

Miyoshi, T., et al. 2006. *J. Cell Biol*. https://doi.org/10.1083/jcb.200604176

Mwangangi, D.M., et al. 2021. *Sci. Adv*. https://doi.org/10.1126/sciadv.abd5271

Pernier, J., et al. 2016. *Dev. Cell*. https://doi.org/10.1016/j.devcel.2015.12.024

Shekhar, S., et al. 2015. *Nat. Commun*. https://doi.org/10.1038/ncomms9730

Shekhar, S., et al. 2021. *J. Cell Biol*. https://doi.org/10.1083/jcb.202006022

Tang, V.W., et al. 2020. *J. Biol. Chem*. https://doi.org/10.1074/jbc.RA120.015018

Wegner, A. 1976. *J. Mol. Biol*. https://doi.org/10.1016/S0022-2836(76)80100-3

Wioland, H., et al. 2017. *Curr. Biol*. https://doi.org/10.1016/j.cub.2017.05.048

Wioland, H., et al. 2019. *Biochemistry*. https://doi.org/10.1021/acs.biochem.8b01001

Wittmann, T., et al. 2003. *J. Cell Biol*. https://doi.org/10.1083/jcb.200303082