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Current Opinion in Cell Biology

# Actin filament dynamics at barbed ends: New structures, new insights

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The dynamic actin cytoskeleton contributes to many critical biological processes by providing the structural support underlying the morphology of most cells, facilitating intracellular transport, and generating forces required for cell motility and division. To execute many of these functions, actin monomers polymerize into polarized filaments that display different structural and biochemical properties at each end. Filament dynamics are regulated by diverse regulatory proteins which collaborate to dictate rates of elongation and disassembly, particularly at the fast-growing barbed (plus) end. This review highlights the biochemical mechanisms of six barbed end regulatory proteins: formin, profilin, capping protein, IQGAP1, cyclase-associated protein, and twinfilin. We discuss how individual proteins influence actin dynamics and how several intriguing complex assemblies influence the polymerization fate of actin filaments. Understanding these mechanisms offers insights into how actin is regulated in essential cell processes and dysregulated in disease.

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**Current Opinion in Cell Biology** 2024, **90**:102419

This review comes from a themed issue on **Cell Dynamics (2024)**

Edited by **Matt Kuyts** and **Robert Grosse**

For a complete overview see the Issue and the Editorial

Available online xxx

**https://doi.org/10.1016/j.ceb.2024.102419**



## Introduction

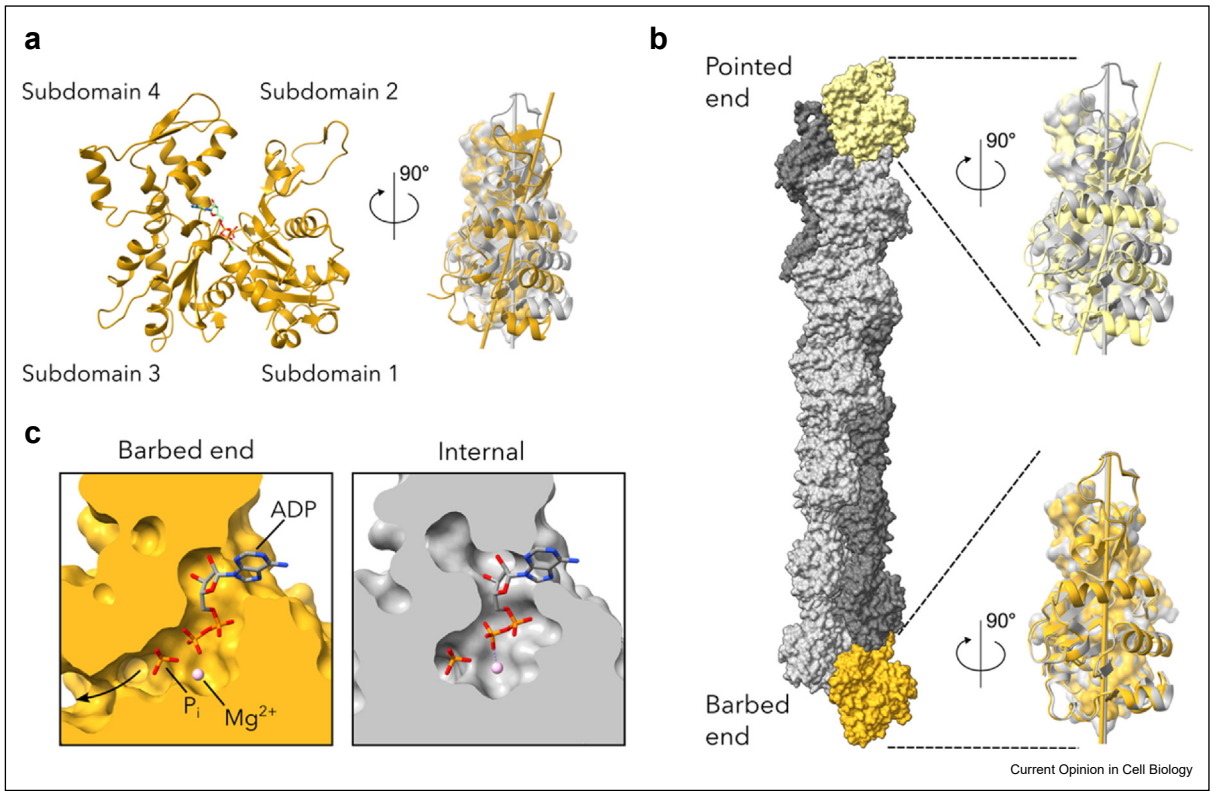
The intricate and dynamic process of polymerizing and recycling actin filaments lies at the heart of fundamental processes that dictate cell shape, establish tracks for intracellular transport, and generate the forces that power motility and division. To execute these functions, actin filament assembly is precisely regulated to produce filaments that polymerize at different speeds, attain specific lengths, and are subject to higher-order interactions. Actin filament dynamics are primarily regulated at the barbed (plus) end by proteins that stimulate polymerization, arrest growth, or promote filament disassembly. We summarize recent biochemical and high-resolution structural studies that examine how actin filaments are regulated at their barbed ends by a diverse ensemble of proteins: formin, profilin, capping protein (CP), IQGAP1, cyclase-associated protein (CAP/Srv2), and twinfilin. These studies further suggest that competitive and collaborative interactions among these proteins coordinate actin dynamics at the barbed end. Unsurprisingly, perturbations to filament assembly or mutations in these regulators contribute to a multitude of pathological conditions including cancer metastasis, neurodegenerative diseases, and immune disorders [1–4].

## New, detailed looks at the barbed end

ATP-bound actin monomers polymerize into filaments, which elongate more rapidly at their barbed end than at their pointed end [5,6]. Structural studies of actin filaments have been limited to fiber diffraction and electron microscopy, which have traditionally yielded relatively low-resolution structures. However, technological advancements enabling direct detection of electrons have recently expanded the limits of cryo-EM studies by significantly improving the resolution of reconstructions [7,8]. In the past two years, several studies have reported structures of actin filaments at resolutions below 2.5 Å [9,10]. These exquisitely resolved structures have elucidated conformational differences at the barbed and pointed ends that underlie the mechanisms of polarized filament elongation and aging.

Newly polymerized actin subunits undergo conformational "flattening", which involves a ~20° rotation of the outer subdomains relative to the inner subdomains (Figures 1a and 1b) [11]. The two terminal subunits at the barbed end adopt the classical flat conformation that is typical of internal subunits [12]. This creates a surface that is favorable for binding an incoming monomer. In contrast, at the pointed end, the final two subunits of

**Figure 1**




**Structures of the barbed and pointed ends of actin filaments.** (**a**) Canonical front- and side-facing views of an ATP-actin monomer (PDB ID: 1ATN). Due to their location when incorporated into filaments, subdomains 1 and 2 are referred to as the outer subdomains, and subdomains 3 and 4 are referred to as the inner subdomains. The side view of the monomer is superimposed with the structure of an internal, filamentous actin subunit (gray; PDB ID: 8F8P) via superposition of the inner subdomains (surface representation) to highlight differences in the relative orientations of the outer subdomains (ribbon representation). Gray and orange axes extend through the major axis of the outer subdomains. (**b**) *Left*, A structural model of an actin filament shown as a surface representation. *Right*, Side-facing views of the terminal subunits at the pointed (PDB ID: 8F8S) and barbed (PDB ID: 8F8R) ends (top and bottom) are superimposed with the structure of an internal, filamentous subunit, as in (**a**). This figure was adapted from Ref. [12] and made using ChimeraX [89]. (**c**) Slices through surface representations of the terminal, barbed end subunit (left) and an internal subunit (right) of an actin filament near the nucleotide-binding site depict the backdoor pathway for phosphate release. The actin subunits are positioned in a side view, with their front-facing surface oriented to the right. ADP and $Mg^{2+}$ are bound in the nucleotide binding cleft located on the front-facing surface. A $P_i$ moiety from the structure of an actin filament in the $Mg^{2+}$-ADP-$P_i$ state (PDB ID: 8A2S) was fit into the binding pocket and included to highlight the location of the $P_i$-binding site. The arrow depicts the path of $P_i$ release through the backdoor. This figure was adapted from Ref. [15] and made using ChimeraX [89].

the filament are present in a twisted, monomer-like conformation [12,13]. Thus, the diverging structures at the barbed and pointed ends provide a structural framework for polarized actin filament elongation. Future studies will undoubtedly illuminate how this structural asymmetry gives rise to the distinct polymerization kinetics at each end [14].

In addition to providing a binding site that supports polymerization, conformational flattening promotes nucleotide hydrolysis, which is followed by phosphate release as the filament ages [10,11]. Structures of filamentous actin bound to ADP-Pi and ADP have now resolved the pathway for phosphate release, which occurs through a "backdoor" that opens via disruption of a hydrogen bonding network involving residues R177 and N111 (Figure 1c) [15]. In internal subunits, the backdoor is predominantly closed and opens only transiently [10]. In contrast, this exit route is found largely open at the barbed end, enabling rapid phosphate release. Thus, in conditions favoring depolymerization, phosphate can dissociate from the barbed end subunit without requiring structural rearrangements. This explains why phosphate is released from the terminal subunit at rates that are orders of magnitude faster than from internal filament subunits [14,16].

## Formin and profilin

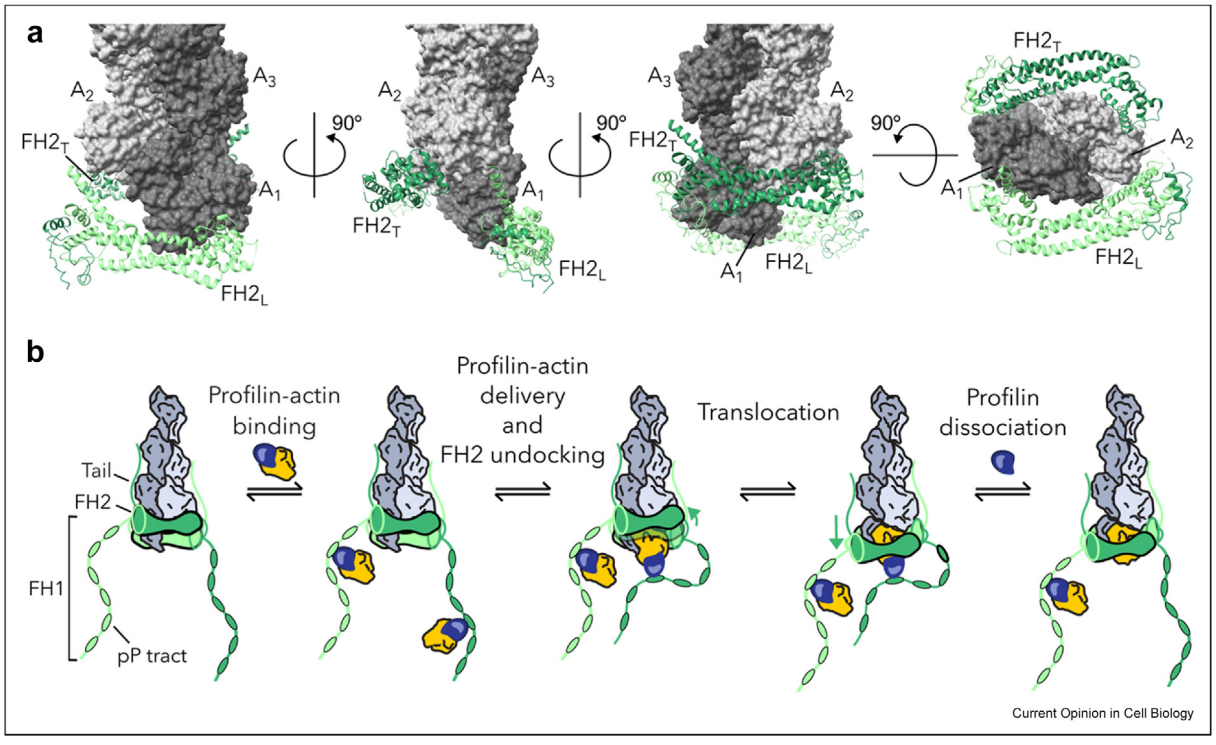
Although actin monomers can assemble spontaneously into filaments, cells express diverse families of "nucleators" to overcome the kinetic barriers to filament formation [6]. Among these proteins, formins are unique in their ability to remain processively associated at barbed ends following nucleation, where they regulate filament

elongation [17]. Formins encircle the terminal subunits of barbed ends with their dimeric formin homology 2 (FH2) domains [18–20], which "step" forward and bind incoming actin monomers at rates that are specific to each formin [17].

High-resolution cryo-EM structures of actin filaments bound by the mammalian formins mDia1 and INF2 [21,22], and the fission yeast formin Cdc12 [21], have provided new molecular insights into the FH2-barbed end interaction. One study revealed that all three formins adopt a conserved asymmetric conformation in which three of the four actin-binding sites in the FH2 dimer engage the terminal actin subunits at the barbed end (Figure 2a) [21]. No major changes in actin subunit flattening or helical twist were observed at the barbed ends of formin-bound filaments. However, the trailing FH2 subunit was found to sterically occlude the binding site for an incoming actin monomer, thus requiring repositioning to allow actin binding [21]. This repositioning may occur through an "undocking" event, which enables translocation of the FH2 subunit in the barbed end direction and subsequent binding of the new actin monomer. The mDia1 dimer was shown to adopt a wider conformation, which may better alleviate the steric clash than the Cdc12 and INF2 dimers do, consistent with the faster polymerization kinetics measured for mDia1 [21,23,24]. A second study reported that mDia1 binds the barbed end via a smaller contact surface than does INF2, which may also contribute to the differing elongation activities of these formins [22]. Binding of an incoming actin monomer was observed to be coupled with an inward rotation and displacement of the leading FH2 domain toward the filament. Collectively, these studies support a model in which the extent and nature of each formin's interactions with the barbed end contribute to the rate of filament elongation.

Formins modulate filament elongation through interactions with the abundant cytoplasmic protein profilin. In cells, a substantial pool of actin monomers are bound by profilin at their barbed end surface [25,26]. This interaction inhibits filament nucleation and the elongation of free pointed ends [27–29]. However,

**Figure 2**




**Formin regulates subunit addition at the barbed end.** (**a**) Structural views of the dimeric FH2 domain of the formin mDia1 bound to the barbed end of an actin filament. The actin filament (dark gray and light gray) is shown as a surface and the formin (green and light green) is depicted using a ribbon representation (PDB ID: 8RU2). The actin subunits are labeled $A_1$, $A_2$, etc. The leading and trailing FH2 domains are labeled as $FH2_L$ and $FH2_T$. (**b**) Schematic of filament elongation mediated by formin and profilin. Profilin-actin complexes (blue and yellow) bind polyproline tracts (pP tracts) encoded in the formin FH1 domain and are delivered to the barbed end. Incorporation of the incoming actin monomer at the barbed end requires undocking and translocation of the trailing FH2 domain (dark green). This is followed by dissociation of profilin from the actin and the polyproline tract. This figure was adapted from Ref. [21].

profilin-actin complexes readily associate with barbed ends [29,30], enabling filament elongation. Profilin binding at the terminal subunit is thought to sterically preclude the addition of an actin monomer to the penultimate subunit [31]. Thus, profilin's lifetime at the barbed end limits the rate of elongation under physiologically relevant conditions [32]. Profilin bound to a barbed end also increases the dissociation rate of the terminal subunit, thereby promoting depolymerization in conditions where actin is limited [16,31].

Binding of profilin-actin complexes to polyproline tracts encoded in the flexible FH1 domain enables direct delivery of actin to the barbed end, thus accelerating elongation beyond the diffusion-limited rate observed for spontaneous polymerization (Figure 2b) [33,34]. FH2 domains and profilin bind overlapping surfaces on actin, suggesting that formin stepping may promote profilin detachment following the delivery step [21]. The number of polyproline tracts encoded by FH1 domains ranges from two to fourteen, giving rise to a wide range of formin polymerization activities [23,35]. The ensemble of tracts establishes a gradient of affinity for profilin-actin complexes which promotes efficient delivery to the growing barbed end from each position in the FH1 domain [36]. Competition for delivery from multiple profilin-actin-bound polyproline tracts to the single FH2-bound barbed end further limits the polymerization rate for each formin [37].

In addition to the FH1 and FH2 domains, the highly variable C-terminal "tail" regions of formins play important roles in polymerization [38]. Formin tails have been shown to influence formin processivity through electrostatic interactions with the sides of elongating filaments [38]. An investigation of the impacts of alternative splicing encompassing the tail region of the *Drosophila* formin Fhod further refined this model by revealing sequence-dependent tail functions [39]. In contrast to previous measurements using the formin Capu [38], longer Fhod tails were observed to inhibit processive filament elongation by promoting dissociation of Fhod from the barbed end. This effect originates from a stretch of nine residues, demonstrating that tails can modulate formin activity in a highly sequence-specific manner. The tail domain of the formin INF2 also plays a role in actin filament severing [40]. Cryo-EM structures revealed that the WH2 motif encoded in INF2's tail region binds a site adjacent to the hydrophobic cleft on subdomain 1 of the actin subunits neighboring the FH2-bound barbed end [22]. Thermal motions may enable repositioning of both the WH2 motif and FH2 dimer, ultimately weakening inter-subunit contacts along the filament and promoting filament severing. The binding of diverse effector proteins to tail regions can also enhance the polymerization properties of formins via synergistic filament nucleation [41–44]. Expansion of existing structural models to incorporate the flexible FH1 and C-terminal regions will further enrich the understanding of formin functions.

## Capping proteins

The precise regulation of barbed ends by capping proteins dictates filament length [45], enables the assembly of actin arrays with precise architectures [46], and maintains the integrity of barbed ends by preventing subunit loss [47,48]. Barbed-end capping proteins are diverse and include the calcium-sensitive gelsolin family [49], the barbed-end-membrane linking adducin family [50], and the highly abundant capping factor, heterodimeric capping protein (CP) which is comprised of α and β subunits [46,48]. Crystal and cryo-EM structures have elucidated the molecular basis for CP's direct interactions at the barbed end [12,46,51,52]. Notably, CP's residence at the barbed end does not appreciably change the structure of the actin filament but instead induces major conformational changes to the CP itself [12]. CP contains two C-terminal extensions, the α-tentacle and β-tentacle [12,46,51,52]. CP's α-tentacle blocks the site of new monomer addition, effectively arresting filament growth [12,46–48]. Whereas binding of the β-tentacle to the filament contributes to the overall affinity of CP for the barbed end, it is not essential for directly arresting polymerization [12,46,48]. Instead, the β-tenacle plays a significant role in inhibiting interactions between the barbed end and additional regulatory proteins, like formin, twinfilin, and diverse Class I nucleation promoting factors [46,53–55]. *In vitro*, CP's interactions with barbed ends have been shown to be high affinity ($k_D$ = 0.1 nM), rivaling those of assembly-promoting formin proteins [48,54–56]. Further adding to its potency *in vitro*, CP dissociates from barbed ends slowly ($t_{1/2}$ = 25 min) [48,56].

Based on CP's biochemical properties and the presence of additional capping factors, some have speculated that all barbed end growth could be effectively arrested in cells [56,57]. However, while CP's off-rate is very slow *in vitro* [48,56], the turnover rate of CP in lamellipodia is much faster (~2 s in fibroblasts [58]), indicating that mechanisms regulating filament uncapping occur. Post-translational modifications of residues in CP's β-tentacle promote filament uncapping [59]. CP is also regulated by diverse proteins that contain an ~30 amino acid residue 'capping protein interaction' (CPI) motif that expedites a direct interaction with CP. Binding of CPI-containing proteins to CP prevents its barbed end association by sequestering free CP molecules and further facilitates CP's dissociation from the barbed end [48,51,60–62]. CPI-motif proteins display a large range of uncapping potency and also inhibit interactions with additional regulators of CP including V-1/myotrophin [48,60,63]. Uncapping events also occur through

competitive interactions for barbed end binding sites (explored below).

### IQGAP1, cyclase-associated protein (CAP/Srv2), and twinfilin

IQGAP1, CAP/Srv2, and twinfilin each attenuate barbed-end growth, albeit by different mechanisms. IQGAP1 is a large (189 kDa) homodimeric protein that influences actin filaments in two ways: (1) filament bundling via side-binding interactions mediated by a calponin homology domain and (2) short ($\sim$20 s) elongation-pausing events at the barbed end [64–66]. Distinguishing IQGAP1's two activities remains challenging due to the absence of full structural data and IQGAP1's tight affinity and slow off-rate (0.006 $s^{-1}$) from filament sides [65]. However, truncation and amino acid substitution analysis of purified IQGAP1 has identified two residues in the IQ-motif region as essential for pausing barbed end elongation [57]. Substitution of these residues with alanine does not affect the protein's dimerization or bundling activities [57]. In NIH-3T3 cells, these substitutions fail to fully restore changes in actin filament arrays, cell shape, or motility, produced by deletion of the protein, while having no effect on microtubules [57]. This suggests that these residues are specific and essential for IQGAP1's barbed end activities.

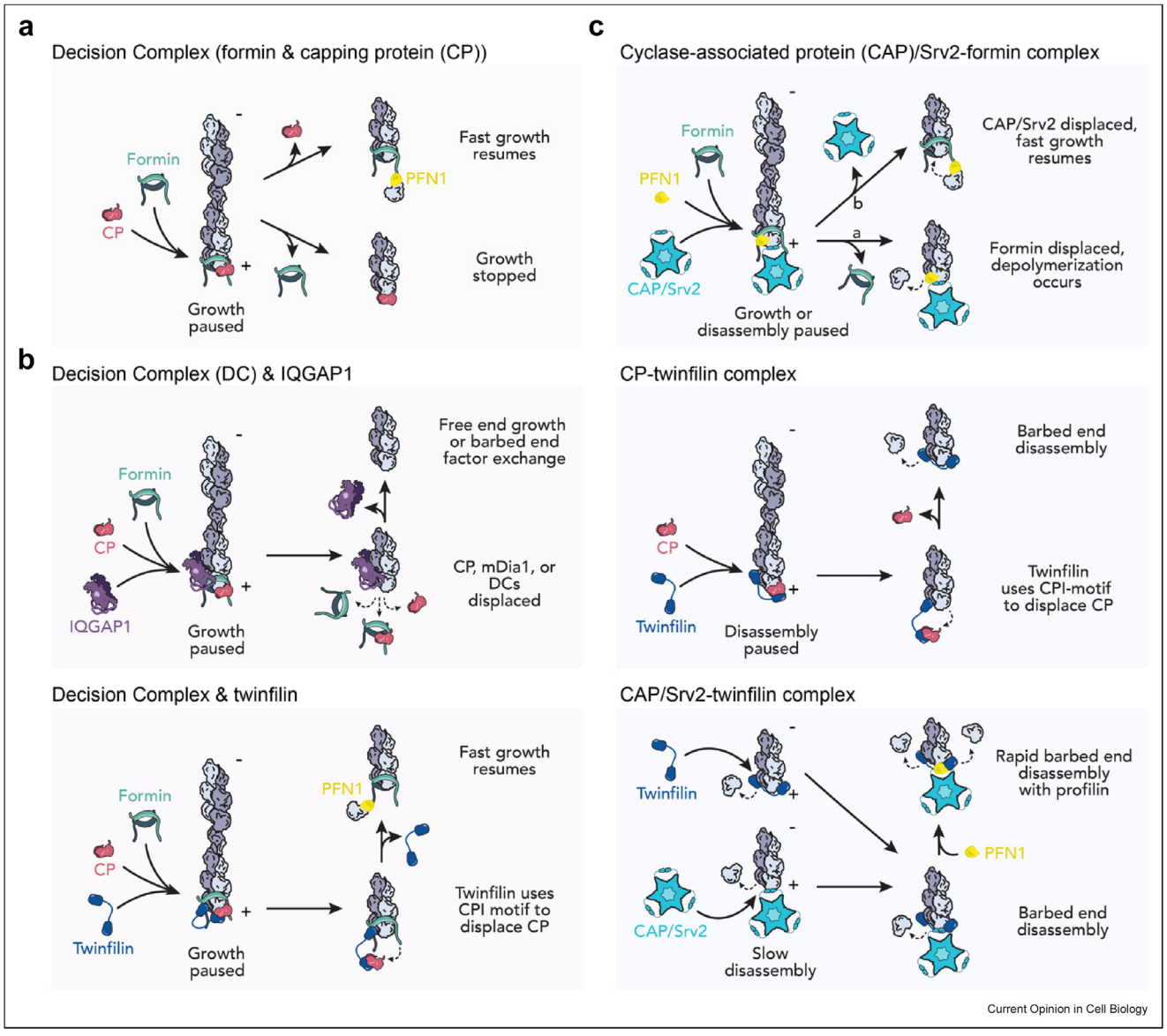
CAP/Srv2 and twinfilin are both historically classified as individual actin turnover factors. Notably, the ability of either protein to disassemble actin filaments is greatly enhanced by synergy with cofilin, albeit at much different potencies [67–71]. Ortholog-specific variations add further complexity to the disassembly effects produced by CAP/Srv2 and twinfilin. For example, yeast Srv2 and yeast twinfilin each display stronger pointed end disassembly activities compared to related mammalian proteins [67,72]. In a recent paradigm shift, several studies have revealed that CAP/Srv2 and twinfilin can also be strategically positioned to restrict growth and facilitate filament disassembly from the *barbed* end [53,67,69,73,74]. Single-molecule microscopy assays performed with purified proteins suggest that complexes form at or near the barbed end to promote actin filament disassembly [67,75]. However, available structures of CP and twinfilin do not provide enough detail to infer the structural implications of a CAP-twinfilin complex on a filament barbed end [76]. One model postulates that mammalian CAP promotes barbed end disassembly by binding and stimulating the removal of terminal actin subunits with its WH2 motif and its actin monomer-binding CARP domains [74,77]. In contrast, twinfilin binding to the barbed end is hypothesized to alter the conformation of the terminal subunits in a manner that disrupts the CP–barbed-end interaction [69,76]. The resulting loss of CP would destabilize the barbed end, promoting twinfilin-mediated filament disassembly [69,76]. Until higher resolution structures of these proteins and complexes can be determined, the current biochemical data reinforce the concept that competition for specific binding sites at the barbed end drives important filament dynamics. For additional details on mechanisms of filament disassembly, we point readers to this recent review [70].

### Competitive, cooperative, and higher-order complex mechanisms coordinate barbed end dynamics

At first glance, the notion that multiple large proteins might occupy a single barbed end seems dubious, especially given the often-opposing roles of many end binding proteins. However, several examples have recently emerged demonstrating how barbed end proteins work together and compete to regulate actin dynamics. A key mechanism involves CP and the formin mDia1, which bind barbed ends with similar high affinities but perform opposing roles [54,55]. CP and mDia1 do not directly interact but both can reside on a single barbed end while interacting with different actin surfaces [54,55]. When both proteins co-occupy a barbed end, polymerization is stalled [54,55]. If formin dissociates first, CP continues to pause growth. Conversely, if CP dissociates first, filament growth resumes. As such, "decision complexes" comprised of the formin, CP, and the barbed end, play a critical role in regulating actin assembly (Figure 3a). Although a full structure of the decision complex at the barbed end is not yet available, high resolution cryo-EM structures of individual CP or mDia1 molecules bound to barbed ends provide some insights into the possible mechanisms of these interactions [12,21,78]. When CP joins a filament, it undergoes major structural changes that do not substantially alter the shape of the actin filament or barbed end [12]. As mDia1 steps forward to facilitate the addition of new monomers to the barbed end [21], CP might either bind to a previously unoccupied end or leverage its existing end-bound, "flat" conformation to stabilize itself through its α- and β-tentacles at the barbed end. CP and mDia1 mutually destabilize each other at barbed ends through several steric clashes related to the body and tentacles of CP and the FH2 domains of the formin [12,21,54,55,78]. Some formins have also been shown to diffuse away from the barbed end and along the lengths of actin filaments [24,55,78]. Such translocation events may further increase the availability of the barbed end for binding by CP. Whether the ability to assemble into decision complexes is a feature that extends to all formins is not yet known.

Additional regulators further refine 'decision complex' dynamics to promote actin assembly (Figure 3b). For example, IQGAP1 can directly bind, activate [66,79], and displace formin from barbed ends [57]. Time-resolved, four-color microscopy assays of these proteins revealed that IQGAP1 also promotes the dissociation of

**Figure 3**




**Complexes directly affecting barbed end dynamics**. Cartoon depiction of dynamics associated with highlighted barbed end complexes. (**a**) Dynamics of the formin-capping protein (CP) decision complex (DC). The DC pauses filament growth until either CP or the formin dissociates from the barbed end. When CP dissociates, fast formin-mediated growth with profilin is promoted, whereas when the formin dissociates via steric competition with the α and β-tentacles of CP, CP prevents additional filament growth. (**b**) DC modulating proteins that promote actin filament growth. IQGAP1 and twinfilin further regulate DCs. IQGAP1 promotes barbed end displacement of CP, mDia1, or DCs. In contrast, twinfilin directly interacts with CP through CPI-motifs to displace CP and promote fast formin-mediated actin filament polymerization. (**c**) Complexes with traditional disassembly factors present at the barbed end. The top panel explores two proposed mechanisms concerning formin-cyclase-associated protein (CAP/Srv2) interactions in the presence of profilin (PFN1). In the first (depicted by the a), CAP/Srv2 displaces formin, while remaining associated with the barbed end. This promotes the depolymerization of the filament in the presence of PFN1 [74]. In contrast, the second mechanism (depicted by the b), postulates the presence of the formin promotes the dissociation of CAP/Srv2, stimulating fast filament growth [77]. The middle panel depicts twinfilin removing CP from the barbed end via its CPI-motif and then its continued association promotes the removal of additional actin subunits from the barbed end. The bottom panel shows how twinfilin and CAP/Srv2 synergize in the presence of PFN1 to rapidly depolymerize filaments from the barbed end.

decision complexes [57]. Thus, IQGAP1 "refreshes" barbed ends by decreasing the residence time of several barbed end factors (i.e., mDia1, CP, and decision complexes) [57]. The large, unstructured nature of IQGAP1 suggests that it might sterically hinder or directly compete with formin or CP for access to barbed end binding sites. In a parallel mechanism, twinfilin, which does not interact with mDia1, is able to displace CP via

its CPI motif to reinitiate formin-based assembly [75]. Each of these studies was performed with constitutively active, truncated versions of mDia1, thus the conservation of these activities with the full-length protein or other formins remains unclear. Twinfilin and IQGAP1 are also predicted to interact [80], raising exciting questions about how these factors collectively influence the barbed end. These observations further underscore the need for high-resolution structural studies and time-resolved biochemical assays to understand these complex mechanisms.

Several examples of cooperative barbed-end-binding complexes build upon established mechanisms of filament depolymerization or uncapping events (Figure 3c). Multiple efforts have demonstrated that the combinations of the barbed end disassembly factors CAP/Srv2 or twinfilin along with profilin synergistically promote filament depolymerization [67,68,71,73,74,77]. Two proposed mechanisms further explore how CAP/Srv2 influences formin-capped barbed ends [67,74]. In the first, CAP/Srv2 promotes the displacement of formin (mDia1) and stimulates subsequent filament depolymerization from the barbed end due to the presence of profilin [67,74]. Coarse grain modeling of dimers of CAP/Srv2's CARP domain suggests that it promotes formin dissociation through steric clashes that disrupt filament elongation [74]. Notably these predicted clashes still occur with a variant of CAP/Srv2 that is unable to depolymerize barbed ends [74]. The second mechanism differs in that the presence of CAP/Srv2 increases the lifetime of mDia1 at the barbed end and promotes the barbed-end disassociation of CP [77]. While the outcomes of the two proposed mechanisms are opposite, both may be possible depending on the context, particularly in cells where these factors coexist. Additional biochemical and high-resolution studies will be needed to distinguish these details and assess whether the displacement mechanism is a universal feature of formins or unique to mDia1.

Beyond formin, interactions between CAP/Srv2, ADP-bound actin filaments, and additional disassembly factors like cofilin may amplify the rate of filament disassembly [68,69,71,77]. Some WH2 domain-containing proteins like CAP/Srv2 and VopF mediate removal of CP from the barbed end to initiate or amplify filament disassembly mechanisms [77,81]. Further, CPI-mediated interactions between CP and twinfilin have consequences for barbed end dynamics. For example, twinfilin, which promotes filament disassembly on its own, and can synergize with CAP/Srv2 to drive enhanced depolymerization, can also remove CP [53,69,73]. These concepts are supported by observations from mammalian cells, where loss of twinfilin disrupts actin filament arrays and reduces the turnover rate of filaments present in lamellipodia [73]. The indirect roles of additional proteins that modulate the activity of *bona fide* barbed end regulators further increase the complexity of these mechanisms. For example, while both CARMIL and twinfilin contain capping protein interaction (CPI) motifs that allow them to bind to and remove CP from the barbed end, CARMIL is 30-fold more effective [53,73,82]. In sum, these findings reinforce the idea that the barbed end is a molecular hub that is regulated through intricate mechanisms in which multiple proteins compete and collaborate to assemble filament arrays with precise dynamics.

### Regulation of cellular actin dynamics by higher-order mechanisms

Actin filament barbed ends exist in diverse subcellular environments, including the tips of filopodia, the dense actin meshworks present in lamellipodia, and the ends of stress fibers [83]. Cleverly designed live-cell studies have begun to probe areas of active actin assembly. Measurements of formin-mediated filament elongation obtained by tracking fluorescently labeled mDia1 in HT1080 cells closely matched maximal elongation rates observed *in vitro*, thus supporting current biochemical models for formin-mediated polymerization [32]. Reverse genetics and live imaging have elucidated the interactions between CP and Arp2/3 activating factors in lamellipodia [46], as well as CP's essential role in filopodia formation in combination with Ena/VASP [84]. Genetic manipulation and super-resolution imaging have confirmed the competitive dynamics of CP-formin decision complexes in live yeast [85]. These studies have further revealed decision complex dynamics to be slower in yeast than in mammalian cells and *in vitro* assays [58,85]. Finally, sophisticated studies measuring actin filament turnover in live-cells with genetically depleted twinfilin levels revealed a more stable association of CP with barbed ends and a reduced rate of actin filament turnover, consistent with measurements from *in vitro* protein assays [73]. Collectively, these studies demonstrate that many of the general principles gleaned from biochemical assays are indeed applicable to the complex cellular interior. Continued exploration will broaden our understanding of these mechanisms by revealing the range of activities exhibited by protein orthologs and isoforms, probing the significance of binding affinities, rates, and stoichiometries, and identifying the unique and unifying features of diverse cellular systems.

## Conclusions

Recent studies have provided valuable insights into the network of molecular interactions that precisely modulates actin filament assembly, stability, and turnover at barbed ends. They also raise exciting research questions, including: How do additional binding partners further modulate dynamics at the barbed end? How do actin isoforms, which deviate structurally at their N-termini

and interact differentially with many barbed-end regulators, increase the complexity of these interaction networks [86–88]? While biochemical and structural studies have yielded many important mechanistic details, exactly how these mechanisms are translated and executed in the cellular context is currently underexplored. A complete understanding of the regulation of actin dynamics will require insights gleaned from additional high-resolution and time-resolved studies performed both *in vitro* and in cells.

## Author contributions

Conceptualization: N.C., J.L.H–R. Writing – original draft: N.C., J.L.H–R. Writing – review and editing: N.C., J.L.H–R. Visualization: N.C., J.L.H–R.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The only data included are publically available PDB files. Each structure used is identified.

## Acknowledgements

We would like to acknowledge the many excellent studies that enriched our understanding on this topic, but we were unable to include due to space constraints. We are grateful to Mark Zweifel, Marc Ridilla, and Brian Haarer for their helpful comments on this manuscript. Additional thanks to Katherine M. Swinton for being a model of creativity and authenticity. This work was supported by the National Institutes of Health [R01GM122787 to N.C. and R35GM133485 to J.L.H–R.].

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