Supplemental Information

Inventory of Supplemental Information:

Supplemental data:
- Supplemental Figures: S1 and S2 related to Figure 2; S3 related to Figure 5.
- Supplemental Movies: S1 related to Figure 1; S2 and S3 related to Figure 2; S4 related to Figure 3; S5 related to Figure 4; S6 related to Figure 5.

Supplemental Experimental Procedures:
- Experimental methods required to perform the experiments described in this study.

Supplemental Text
- ADF/cofilin binding is controlled by the chemical state of actin-bound nucleotide throughout elongating networks
- Description of the mathematical model and the numerical simulation methods used to account for the spatiotemporal distribution of ADF/cofilin throughout actin filament networks.

Supporting References
**Figure S1. ADF/cofilin binding is controlled by the chemical state of actin-bound nucleotide throughout elongating networks**

GST-pWA coated beads were added to a motility medium containing 4 μM Alexa568-actin monomers, 12 μM profilin, 150 nM Arp2/3 complex, and 0.3 μM Alexa488-ADF/cofilin. (A) is a montage of time-lapse images showing the formation of a star-shaped actin network elongating from the nucleating beads; the corresponding graphs represent the evolution of fluorescence intensities (taken along the dashed line) for actin and ADF/cofilin within the actin tail at different times during polymerization. (B) is a representative image taken after one hour of actin assembly of star-like network and the cartoon in the rightmost part of the image highlights the positioning of the different zones of ADF/cofilin recruitment. Blue chevrons indicate the localization of the polymerizing zone close to nucleating beads, whereas blue arrowheads point to the filament elongation sites at bundles tips. (C) and (D) are zooms of bead surroundings in (A) and (B) respectively. The scale bars represent 5 μm. (See also Movie S3).

**Figure S2. Distinct modes of spatiotemporal incorporation for CP and ADF/cofilin during actin based motility.**

GST-pWA coated beads were added to a motility medium containing 4 μM actin monomers, 12 μM profilin, 150 nM Arp2/3 complex, 0.3 μM Alexa568-ADF/cofilin, 45 nM Alexa488-CP. Images were taken at the indicated times during polymerization. Alexa488 is colorized in green and Alexa568 in red. Linescans of the fluorescence intensities taken along the actin gel (using Metamorph), as represented by the dashed curves, are associated under each image of the time-lapse it is linked to. The scale bar represents 5 μm.
Figure S3. Effect of the Intensity and duration of laser photobleaching during FRAP experiences with CP.

(A-D) Time-lapse images of photobleaching experiments of Alexa488-CP on the propulsive actin network nucleated from GST-pWA coated beads in the absence of ADF/cofilin. In these examples, the intensity of the laser and the duration of the illumination were modified for each experiment and the values for CP fluorescence intensity in the bleached zone are indicated in (E). GST-pWA coated beads were added to a motility medium containing 4 μM actin monomers, 12 μM profilin, 150 nM Arp2/3 complex, 45 nM Alexa488-CP. Images were taken at the indicated times during polymerization.

Time-lapsed images were acquired by fluorescent microscopy. The photobleached zone, surrounded by a dashed rectangle on the images, was obtained by a local intense laser illumination. For a better visualization images are also shown in pseudocolor. The scale bar represents 5 μm. (E) Graph representing the recovery over time of the fluorescence intensity within the bleached zones of the different experiments presented in this figure. Analysis was achieved using the threshold function of Metamorph. For an easier comparison data were normalized by the fluorescence intensity pre-bleaching. The different curves are superimposed on the right, showing that the photobleaching intensity has an effect on the depth of the first rapid phase of recovery (linked to the capping of ends resulting from fragmentation subsequent to laser photodamage) but not on the slopes neither on the second slower phase of recovery (linked to the replacement of bleached proteins).
Supporting Movies

**Movie S1: Spatiotemporal incorporation of CP (green) throughout elongating actin networks (red) during reconstituted bead motility.**
The movie corresponds to the time-lapse series in Figure 1B. Total elapsed time is 43 min 58 s and compressed to a 1-second AVI movie.

**Movie S2: Spatiotemporal incorporation of ADF/cofilin (green) throughout elongating actin networks (red) during reconstituted bead motility.**
The movie corresponds to the time-lapse series in Figure 2. Total elapsed time is (left) 1 hour 30 min, or (right) 1 hour 14 min, and is compressed to a 4-second AVI movie.

**Movie S3: Spatiotemporal incorporation of ADF/cofilin (green) throughout reconstituted star-shaped actin networks (red), in the absence of CP.**
The movie corresponds to the time-lapse series in Figure S1. Total elapsed time is 58 min 56 s and compressed to a 2-second AVI movie.

**Movie S4: In silico reconstitution of the chemical state of actin-bound nucleotide of elongating comet tails controlling ADF/cofilin incorporation.**
Left, in the absence of ADF/cofilin; right, in the presence of 1 µM of ADF/cofilin. The movie corresponds to the time-lapse series in Figure 3.

**Movie S5: Evidence for ADF/cofilin fragmentation activity within dense actin networks.**
Fluorescence recovery after photobleaching experiments on Alexa-Capping protein incorporated in the comet tail during bead motility, in conditions similar to that used in Figure 4. Three zones are successively bleached using local intense laser illumination. Total elapsed time is 49 min and compressed to a 7-second AVI movie.

**Movie S6: Actin networks turnover through the macroscopic release of their aged portions due to ADF/cofilin stochastic severing activity.**
The movie corresponds to the time-lapse series in Figure 5. In the first movie Alexa568-actin is colorized in red, and Alexa488-ADF/cofilin in green. Total elapsed
time is 1 hour 14 min and compressed to a 3-second AVI movie. In the second movie, representing a zoom of the fragmenting rear of the comet tail, only actin-fluorescence is showed. The dark blue arrowhead points to the motile bead, the light blue arrowhead follows the thick actin shell assembled prior to symmetry breaking and its macroscopic release (red arrowhead) into small meshwork portions (blue arrowheads).
Supplemental Experimental Procedures:

Quantification of the laser photodamage during FRAP experiments

A well-defined zone of the actin comet tail away from the bead center was bleached using a local intense laser illumination (Figures 4 and S3). The variation of the fluorescence recovery over time of these zones followed a double exponential (Figure 4D) that reads:

\[ y = m_1 + m_2 \cdot \exp(-k_{\text{obs}1} \cdot x) + m_4 \cdot \exp(-k_{\text{obs}2} \cdot x) \]

with

\[ k_{\text{obs}1} = k_{\text{th, CP}} \cdot (\text{CP}) - k_{\cdot \text{CP}} \]
\[ k_{\text{obs}2} = k_{\cdot \text{CP}} \]

and where \( m_1 \) corresponds to the intercept, \( m_2 \) and \( m_4 \) to the amplitudes of the fluorescence recovery, \( k_{\text{obs}1} \) to the fast rate of recovery, and \( k_{\text{obs}2} \) to the slow rate of recovery. To determine the reactions that contributed to the kinetics of the fluorescence recovery after photobleaching, we first tested the effect of a variation of the intensity of the laser illumination during the FRAP on the fluorescence recovery (Figures 4A and S3). We found that the intensity of illumination had an impact only on the amplitude of the rapid phase of the fluorescence recovery signal (Figure S3). Indeed, the amplitude of the rapid phase of recovery increased with the laser intensity (Figure S3). We conclude that the FRAP illumination fragments the actin filaments; thereby creating free barbed ends available for the rapid recruitment of unbleached CP (Figure 4A and S3). However, as the amplitude of the photodamage of actin networks was related to the laser intensity, it was comparable between different sets of FRAP experiments performed at equal laser intensity. This effect was quantified in the absence of fragmentation by ADF/cofilin and factored into the
equation of double exponential curve fits used to analyze the FRAP experiments in the presence of ADF/cofilin. As a consequence, $k_{obs1}$ accounted for the rapid incorporation of unbleached CP, which is limited by both the concentration of free CP present in the motility medium and the on-rate constant ($3 \mu M^{-1}.sec^{-1}$, (Schafer et al., 1996)), and corresponds to the phase of the rapid fluorescence recovery. The $k_{obs2}$ corresponds then to the slow replacement of bleached CPs, which is limited by their slow off-rate constant from actin filaments barbed ends ($4 \times 10^{-4} sec^{-1}$ in (Schafer et al., 1996)) and is related to the phase of slow fluorescence recovery. Importantly, the rapid phase of recovery involves the generation of free barbed ends within the defined area of photobleaching assay. For experiments achieved in the absence of ADF/cofilin, we determined the rate constant for slow fluorescence recovery corresponding to the dissociation rate constant of CP from the barbed ends of actin filaments, $0.0015 \pm 0.0002 sec^{-1}$, a value 3 to 5 times faster than that already published data (Schafer et al., 1996; Kuhn and Pollard, 2007). Moreover, the occurrence of laser photodamage would probably induce loss of actin filaments that drift away, which may explain that lack of recovery of the initial signal of photobleached fluorescent CP (Figures 4D and S3).
**Supplemental Text**

**ADF/cofilin binding is controlled by the chemical state of actin-bound nucleotide throughout elongating networks**

Based on its ability to bind the aged part of actin filament networks with a 40-fold higher affinity than to the newly-polymerized zone, at low concentration (0.3 µM) ADF/cofilin may serve as a “late” marker of actin networks; We investigated the macroscopic organization of dynamic actin networks in the absence of CP. We initiated the assembly of Arp2/3 complex branched-actin networks on the surface of NPF-coated beads in the absence of CP and in presence of 0.3 µM of ADF/cofilin. As predicted, removing CP from the motility medium led to the formation of a star-like actin structure and a spherical scattered actin gel initiated around the bead (Vignjevic et al., 2003; Achard et al., 2010) (Figure S1). Moreover, in agreement with previously published results (Loisel et al., 1999; Achard et al., 2010), no symmetry breaking of the actin network was observed in absence of CP (Figure S1). To further describe the dynamic organization of this actin network, we quantified the spatial and temporal incorporation of fluorescently-labeled ADF/cofilin with linescans of fluorescence intensity taken along both the diameter of the nucleating bead and the center of an actin bundle from the star-shaped network (Figure S1). We found that at the initial times of actin polymerization, up to 4 minutes, ADF/cofilin localized around nucleating beads and thereafter at the distal part of the actin bundles assembled around the particle. During actin polymerization, ADF/cofilin accumulated along the bundles of the star-shaped actin network (up to 38 minutes); it was enriched in the central part of the bundles, but less abundant in the vicinity of the bead and excluded from the distal tips of bundles (Figure S1, linescans, arrowheads and chevrons for
exclusion). In addition, although the spatio-temporal enrichment of ADF/cofilin in the central part of the bundles was associated with a stable amount of actin along the dashed lines (Figure S1A, linescans), its was closely related to the increase in bundle thickness over time (Figure S1A, timelapse images). The spatial and temporal incorporation of ADF/cofilin is in agreement with the orientation of actin filaments with their barbed ends growing away from the nucleating particle as recently observed at single filament resolution (Achard et al., 2010; Reymann et al., 2010), and consistent with the progressive gathering of actin filaments into large bundles to form the star-like structure (Achard et al., 2010).

Furthermore, in the absence of CP, we observed that ADF/cofilin accumulated in a thin layer of actin network immediately surrounding the bead (Figure S1, B green rings, C and D), whereas it was simultaneously excluded from the distal actin network (Figure S1A, red shell). This ADF/cofilin distribution highlighted that the preexisting actin filaments that host the autocatalytic formation of the Arp2/3-branched actin networks were ADP-loaded (Achard et al., 2010).

**Kinetic model for the spatiotemporal incorporation of ADF/cofilin within growing actin filament networks**

**A. Dynamics of actin network growth from nucleating beads.**

We modelled the growth of actin network after the breaking of the initial shell and the establishment of the polar expansion of the actin comet tail. The velocity of actin gel growth is then controlled by the elongation or nucleation of new actin filaments at the bead surface only. Therefore, we considered the actin comet tail as a continuum
medium generated on the bead surface (half a bead) and growing at a constant velocity, V. Furthermore, the newly created actin filaments were transported with the same velocity V to form a comet tail with a constant section and a length proportional to the polymerization duration. The definition and value of V is given in the table S1.

B. Kinetics of actin modification in the comet.

The hydrolysis of the bound nucleotide and the phosphate release are important processes that control ADF/cofilin binding to ADP-actin filaments (or F-ADP) before filament severing and network fragmentation. Since the state of the nucleotide bound to actin subunits depends only on the age of the subunit in the actin network, we subdivided the comet into slices of constant thickness. We assumed that the nucleotide state was identical for all subunits in a given slice and depended only on the age at which the slice was generated at the bead surface. More specifically, we assume that each slice can be in one of the following states

\[
\{ F - \text{ATP}, \ F - \text{ADP} - \text{Pi}, \ F - \text{ADP}, \ F - \text{ADP} - \text{ADF}, \ F - \text{ADP} - (\text{ADF})_2 \},
\]

with the probability

\[
\{ S_1(t), S_2(t), S_3(t), S_4(t), S_5(t) \}.
\]

The changes of nucleotide state or ADF/cofilin binding is governed by a simple set of linear differential equations

\[
\begin{align*}
\frac{dS_1}{dt} &= r_1 - (r_1 + r_2)S_2 - r_3S_3 - r_4S_4 - r_5S_5, \\
\frac{dS_2}{dt} &= r_2S_1 - r_3S_2, \\
\frac{dS_3}{dt} &= r_3S_2 - r_4S_3, \\
\frac{dS_4}{dt} &= r_4S_3 - r_5S_4, \\
\frac{dS_5}{dt} &= r_5S_4 - r_5S_5.
\end{align*}
\]

\[\tag{1}\]
Where we used the fact that

\[ S_1(t) + S_2(t) + S_3(t) + S_4(t) + S_5(t) = 1. \quad (2) \]

The reaction rates in eq. (1) represent ATP hydrolysis \( r_1 \), phosphate release \( r_2 \), the binding of ADF/cofilin to ADP-actin subunits \( r_3 \), and the cooperative binding of a second ADF/cofilin molecule with the rate \( r_4 \). All rates definition and numerical values are listed in the table S1. Finally, severing of filaments and comet tail fragmentation occurs for slices in the final state \( S_5 \) at rate \( r_5 \) (Blanchoin and Pollard, 1999). When constraining the \( r_5 \) constant to the value measured previously by Blanchoin et al (Blanchoin and Pollard, 1999), the model accounted for a comet tail length obtained experimentally.

C. Fitting the ATP/ADP-Pi cap length.

The ATP/ADP-Pi cap length is controlled by (i) the overall growth of the actin comet, (ii) the release of Pi following ATP hydrolysis and (iii) the depletion of actin monomers in the course of time. We adopted a very simple model, assuming that the concentration of monomeric actin is governed by

\[
\frac{d[G]}{dt} = k_{on} [B] \times ([G]_c - [G])
\]

where \([B]\) is the concentration of barbed ends (assumed constant), \(k_{on}\) is the rate of binding of actin monomers at the barbed end of actin filaments, \([G]_c\) is the critical concentration of actin monomers. The corresponding velocity for barbed ends is classically given by

\[
Vel = \delta k_{on} [B]([G] - [G]_c)
\]

where \(\delta\) is the length increase of an actin filament upon polymerization of a single actin monomer. The total filament length at time \( t \) is now:
\[ L(t) = \delta k_{\text{orn}} [B] \int_0^t \left( [G](s) - [G] \right) ds \]

The proportion of monomers bound to ATP or ADP-Pi is then given by

\[ L_{\text{cap}}(t) = \int_0^t L(t-s) \exp(-k_p s) ds \quad (3) \]

where \( k_p \) is the rate constant of Pi release (here, we neglected the ATP hydrolysis since this process is extremely fast compared to Pi release). This last equation expresses that the fraction of F-actin filament created at time \( t-s \), with a length \( L(t-s) \) has a proportion of \( \exp(-k_p s) \) to be in the ATP/ADP-Pi state. The ATP/ADP-Pi cap length is the integral of this last quantity.

During the fitting of figure 3, C and D, we adjusted the parameter \( k_p \) to 0.0012 s\(^{-1}\) (compared to 0.0019 s\(^{-1}\) measured in bulk solution (Blanchin and Pollard, 1999)) to ensure matching between experimentally measured ADF/cofilin exclusion zone length and the ATP/ADP-Pi cap length predicted by equation (3).
| Parameter         | Definition                                                                 | Numerical value or Expression |
|-------------------|-----------------------------------------------------------------------------|-------------------------------|
| $k_{\text{on, Barbed end}}$ | Association rate constant at the barbed end (Pollard, 1986) | $11.6 \, \mu M^{-1}.s^{-1}$ |
| $k_{\text{Pi-release}}$ | Rate constant of the release of the inorganic phosphate without ADF/cofilin (Melki et al., 1996) | $0.0012 \, s^{-1}$ |
| $k_{\text{Pi-release-ADF}}$ | Rate constant of the release of the inorganic phosphate in the presence of ADF/cofilin (Blanchoin and Pollard, 1999) | $0.035 \, s^{-1}$ |
| $k_{\text{ATP-hydrolysis}}$ | Hydrolysis rate constant of ATP-nucleotide bound to subunits (Pollard and Borisy, 2003) | $0.35 \, s^{-1}$ |
| $k_{\text{on, ADF}}$ | Binding rate constant of ADF/cofilin on ADP-actin subunits (Blanchoin and Pollard, 1999) | $0.0085 \, \mu M^{-1}.s^{-1}$ |
| $k_{\text{off, ADF}}$ | Dissociation rate constant of ADF/cofilin (Blanchoin and Pollard, 1999) | $0.005 \, s^{-1}$ |
| $k_{\text{coop, ADF}}$ | Rate constant of cooperative binding/unbinding of ADF/cofilin to FADP subunits (Blanchoin and Pollard, 1999) | $0.075 \, \mu M^{-1}.s^{-1}$ |
| $k_{\text{severing}}$ | Severing rate constant (Blanchoin and Pollard, 1999) | $0.012 \, s^{-1}$ |
| $V$ | Elongation rate | $k_{\text{on, Barbed end}}[\text{Actin}] - k_{\text{off, Barbed end}}$ |
| $r_1$ | ATP hydrolysis rate | $k_{\text{ATP-hydrolysis}}$ |
| $r_2$ | Phosphate release rate | $k_{\text{Pi-release}}$ |
| $r_3$ | Rate of binding/unbinding of ADF/cofilin to F-ADP subunits | $k_{\text{on, ADF}}[\text{ADF/cofilin}] - k_{\text{off, ADF}}$ |
| $r_4$ | Rate of cooperative binding/unbinding of ADF/cofilin to FADP subunits | $k_{\text{coop, ADF}} \cdot [\text{ADF/cofilin}] - k_{\text{off, ADF}}$ |
| $r_5$ | Rate of severing | $k_{\text{severing}}$ |

Table S1: Kinetic rate constants and reaction rates.
Supplemental References

Achard, V., Martiel, J.L., Michelot, A., Guerin, C., Reymann, A.C., Blanchoin, L., and Boujemaa-Paterski, R. (2010). A "primer"-based mechanism underlies branched actin filament network formation and motility. Curr. Biol. 20, 423-428.

Blanchoin, L., and Pollard, T.D. (1999). Mechanism of interaction of Acanthamoeba actophorin (ADF/cofilin) with actin filaments. J. Biol. Chem. 274, 15538-15546.

Kuhn, J.R., and Pollard, T.D. (2007). Single molecule kinetic analysis of actin filament capping. Polyphosphoinositides do not dissociate capping proteins. J. Biol. Chem. 282, 28014-28024.

Loisel, T.P., Boujemaa, R., Pantaloni, D., and Carlier, M.F. (1999). Reconstitution of actin-based motility of Listeria and Shigella using pure proteins. Nature 401, 613-616.

Melki, R., Fievez, S., and Carlier, M.-F. (1996). Continuous Monitoring of Pi release Following Nucleotide Hydrolysis in Actin or Tubulin Assembly Using 2-Amino-6mercapto-7-methylpurine Ribonucleoside and Purine-Nucleoside Phosphorylase as an Enzyme-linked Assay. Biochemistry 35, 12038-12045.

Pollard, T.D. (1986). Rate constants for the reactions of ATP- and ADP-actin with the ends of actin filaments. J. Cell Biol. 103, 2747-2754.

Pollard, T.D., and Borisy, G.G. (2003). Cellular motility driven by assembly and disassembly of actin filaments. Cell 112, 453-465.

Reymann, A.-C., Martiel, J.-L., Cambier, T., Blanchoin, L., Boujemaa-Paterski, R., and Théry, M. (2010). Nucleation geometry governs ordered actin networks structures. Nat. Mat. 9, 827-832.

Schafer, D.A., Jennings, P.B., and Cooper, J.A. (1996). Dynamics of capping protein and actin assembly in vitro: uncapping barbed ends by polyphosphoinositides. J. Cell Biol. 135, 169-179.

Vignjevic, D., Yarar, D., Welch, M.D., Peloquin, J., Svitkina, T., and Borisy, G.G. (2003). Formation of filopodia-like bundles in vitro from a dendritic network. J. Cell Biol. 160, 951-962.
Figure S3