The Two *Caenorhabditis elegans* Actin-Depolymerizing Factor/Cofilin Proteins Differently Enhance Actin Filament Severing and Depolymerization†

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ABSTRACT: Actin-depolymerizing factor (ADF)/cofilin enhances the turnover of actin filaments by two separable activities: filament severing and pointed-end depolymerization. Multicellular organisms express multiple ADF/cofilin isoforms in a tissue-specific manner, and the vertebrate proteins are grouped into ADFs and cofilins on the basis of their biochemical activity. A recent comparative study has shown that ADF has greater severing and depolymerizing activities than cofilin [Chen, H., Bernstein, B. W., Sneider, J. M., Boyle, J. A., Minamide, L. S., and Bamburg, J. R. (2004) *Biochemistry 43*, 7127−7142]. Here, we show that the two *Caenorhabditis elegans* ADF/cofilin isoforms exhibit different activities for severing and depolymerizing actin filaments. The ADF-like non-muscle isoform UNC-60A had greater activities to cause net depolymerization and inhibit polymerization than the cofilin-like muscle isoform UNC-60B. Surprisingly, UNC-60B exhibited much stronger severing activity than UNC-60A, which was the opposite of what was observed for vertebrate counterparts. Moreover, UNC-60B induced much faster pointed-end depolymerization of rabbit muscle actin than UNC-60A, while UNC-60A caused slightly faster depolymerization of *C. elegans* actin than UNC-60B. These results suggest that cofilin-like UNC-60B is kinetically more efficient in enhancing actin turnover than ADF-like UNC-60A, while ADF-like UNC-60A is suitable for maintaining higher concentrations of monomeric actin. These functional differences might be specifically adapted for different actin dynamics in muscle and non-muscle cells.

Dynamic rearrangement of the actin cytoskeleton is required for a number of cellular processes. Among a number of actin-binding proteins, actin depolymerizing factor (ADF)[1]/cofilin is one of the essential factors for enhancing actin filament dynamics in vivo (reviewed in refs *1*−*8*). Biochemical studies have shown that ADF/cofilin severs actin filaments and increases the rate of depolymerization from the pointed ends (reviewed in refs *1*−*8*). These two activities can be uncoupled by point mutations (*9*−*12*), suggesting that they are biologically significant in different ways. Enhancement of depolymerization from the pointed ends is critical for enhancing actin turnover, because this process is slow by actin itself and is the rate-limiting step of turnover (*13*, *14*). Filament severing increases the number of free ends where polymerization and depolymerization occur, which is implicated in rapid actin polymerization at the leading edge of motile cells (*15*, *16*). Combination of genetic and biochemical approaches has shown that the activity of ADF/cofilin to disassemble actin filaments is essential for in vivo actin turnover (*10*, *11*, *17*, *18*) and myofibril assembly (*19*). However, little is known about how severing or depolymerizing activity of ADF/cofilin contributes to a specific aspect of actin dynamics in vivo.

Multicellular organisms have multiple ADF/cofilin isoforms (*2*, *5*, *20*). In vertebrates, ADF and non-muscle cofilin (cofilin-1) are widely expressed in a variety of tissues (*21*−*25*), while expression of muscle cofilin (cofilin-2) is limited in muscle and testis (*25*−*27*). Phylogenetic analyses of the ADF/cofilin sequences show that ADF and cofilin diverged within the vertebrate lineage and that nonvertebrate ADF/cofilins are equally distant from ADF and cofilin (*8*, *20*, *28*). ADF (also known as destrin) and cofilin share common actin-regulatory properties. However, comparative biochemical studies have shown that ADF has a stronger activity to induce net actin depolymerization at the steady state than cofilin does (*25*, *29*, *30*) and nonvertebrate ADF/cofilins exhibit either ADF-like or cofilin-like activities (*30*). This is primarily because the ADF−actin complex has a higher critical concentration than the cofilin−actin complex (*29*, *30*). However, inconsistent conclusions about their difference in severing and depolymerizing activities are reported. Assays for severing by fluorescence microscopy (*25*), electron microscopy (*29*), and actin elongation from ADF/cofilin-treated actin seeds (*29*) show that ADF and cofilin have nearly identical activity. In addition, measurement of the rate of actin subunit release from gelsolin-capped filaments indicates that they have little difference with respect to pointed-end depolymerizing activity (*29*). In contrast, a recent

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[1] Abbreviations: ADF, actin depolymerizing factor; AIP1, actin interacting protein 1; Ce-actin, *C. elegans* actin; DTT, dithiothreitol; Lat-A, latrunculin A; r-actin, rabbit muscle actin; SD, standard deviation.

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report by Chen et al. (*30*) demonstrates that ADF is more effective than cofilin in severing and depolymerization using an assay to determine both severing and depolymerizing activities from the depolymerization rate of pyrene-labeled filaments in the presence of various ratios of a barbed-end cap by gelsolin (*11*). At present, the reason for this discrepancy is not clearly understood.

The biochemical difference in ADF/cofilin isoforms might be a key to understanding the difference in their cellular functions. In cells where ADF and cofilin are coexpressed, they have redundant functions in regulating actin turnover (*31*), but their localization and expression levels are differently regulated by changes in the intracellular pH (*32*) and the monomeric actin pool (*33*). In addition, mutations of ADF and cofilin in mice cause distinct phenotypes: the ADF (destrin) mutant mice exhibit corneal disease (*34*), whereas the cofilin-1 knockout mice show embryonic defects in neural tube morphogenesis and migration of neural crest cells (*35*). In both mutants, other ADF/cofilin isoforms are overexpressed (*34*, *35*), but they fail to rescue the phenotypes, suggesting that the ADF/cofilin isoforms play distinct roles in vivo.

In the nematode *Caenorhabditis elegans*, two ADF/cofilin family proteins, UNC-60A and UNC-60B, are expressed from the *unc-60* gene by alternative splicing (*36*). Our previous studies suggested that UNC-60A and UNC-60B are functionally differentiated ADF/cofilin isoforms. UNC-60A is expressed in various tissues and required for early embryogenesis, whereas UNC-60B is specifically expressed in body wall muscle and essential for myofibril assembly (*19*, *37*). In vitro, these two isoforms have different activities. In a steady-state assay, UNC-60A causes much greater actin depolymerization than UNC-60B, while UNC-60B binds to filaments more strongly than UNC-60A (*38*). In addition, UNC-60B is a preferential isoform for UNC-78/actin-interacting protein 1 (AIP1) for disassembly of actin filaments (*39*, *40*). However, we have recently observed that misregulation of alternative splicing of the *unc-60* gene can increase the level of expression of UNC-60A in body wall muscle and can suppress the cytoskeletal defects in *unc-60B* mutants (*41*). This observation suggests that UNC-60A may be able to compensate for the function of UNC-60B in muscle cells and led us to investigate further details of their biochemical activities.

In this study, we specifically compared the activities of UNC-60A and UNC-60B to sever filaments and to enhance pointed-end depolymerization using actins from rabbit muscle and *C. elegans*. Our results indicate that UNC-60B has much stronger severing activity than UNC-60A, whereas UNC-60A enhances pointed-end depolymerization of *C. elegans* actin slightly faster than UNC-60B does. These findings suggest that the two ADF/cofilin isoforms have distinct roles in regulating actin turnover. UNC-60B is a kinetically more efficient enhancer of actin turnover than UNC-60A, while UNC-60A is capable of maintaining higher concentrations of monomeric actin.

EXPERIMENTAL PROCEDURES

Proteins. Rabbit skeletal muscle actin was purified as described previously (*42*). *C. elegans* actin was purified from a wild-type N2 strain as described previously (*43*). Bacterially expressed recombinant UNC-60A and UNC-60B were purified as described previously (*38*). Gelsolin was purified from bovine newborn calf serum (Invitrogen) as described previously (*44*).

Assay for F-Actin Depolymerization by Sedimentation. The sedimentation assay of rabbit muscle actin or *C. elegans* actin with UNC-60 proteins was performed as described previously (*45*) in F-buffer [0.1 M KCl, 2 mM $MgCl_2$, and 1 mM dithiothreitol (DTT)] containing 20 mM MES-KOH (pH 6.5), 20 mM Hepes-NaOH (pH 7.5), or 20 mM Tris-HCl (pH 8.5). Ultracentrifugation was performed in a Beckman TLA100 rotor at 80 000 rpm for 20 min.

Quantification of Steady-State F-Actin Levels. Rabbit muscle G-actin or *C. elegans* G-actin in G-buffer [2 mM Tris, 0.2 mM $CaCl_2$, 0.2 mM ATP, and 0.2 mM DTT (pH 8.0)] was polymerized at 2−16 μM in F-buffer (pH 7.5) for 15 h in the absence or presence of UNC-60A or UNC-60B at a 1:2.5 molar ratio. The light scattering signals of the samples were measured at an angle of 90° and at a wavelength of 500 nm with a Perkin-Elmer LS50B fluorescence spectrophotometer. The data were analyzed by linear regression using SigmaPlot 2000 (Systat Software Inc.).

Assay for Exchange of Actin-Bound Nucleotides. Effects of UNC-60A or UNC-60B on the exchange rate of actin-bound nucleotides were examined as described by Hawkins et al. (*46*). Briefly, G-actin was labeled with etheno-ATP (ϵ-ATP) as described previously (*46*), and 5 μM labeled actin was incubated with 0−10 μM UNC-60A or UNC-60B in G-buffer containing 20 μM ϵ-ATP for 10 min at room temperature, and then 1 mM ATP was added to displace actin-bound ϵ-ATP. Dissociation of ϵ-ATP was monitored by the loss of fluorescence (excitation at 360 nm and emission at 410 nm), and the exponential rate (k_{obs}) was calculated with SigmaPlot 2000 (Systat Software Inc.). The dissociation constants were calculated by A. Weeds (Medical Research Council Laboratory of Molecular Biology, Cambridge, U.K.) using the equation described by Hawkins et al. (*46*).

Assay for Barbed-End Elongation from F-Actin Seeds. A spectroscopic assay for examining actin elongation from filament ends was performed as described previously (*19*). Briefly, 10 μM F-actin from rabbit muscle or *C. elegans* was mixed for 30 s with 0−10 μM UNC-60A or UNC-60B in F-buffer at various pHs and used as seeds for polymerization of pyrene-labeled rabbit muscle G-actin. The kinetics of actin polymerization was measured, and the initial rate in the presence of UNC-60 proteins was compared with that of actin alone.

Direct Observation of Actin Filament Severing. Observation of actin filament severing by fluorescence microscopy was performed as described previously (*47*, *48*) with slight modifications. Briefly, unlabeled actin (1.4 μM), Alexa488-labeled actin (1.5 labels/molecule on amines, Molecular Probes) (0.4 μM), and biotin-labeled actin (1.0 label/molecule on amines, Cytoskeleton Inc.) (0.2 μM) were copolymerized at room temperature for 2 h in ISAP buffer [50 mM KCl, 5 mM EGTA, 2 mM $MgCl_2$, 1 mM ATP, 1 mM DTT, and 20 mM Hepes-NaOH (pH 7.5)]. Alexa488 and biotin-labeled actin was attached to a perfusion chamber using anti-biotin antibody (Molecular Probes) as described previously (*48*). To label barbed ends of filaments, 0.4 μM rhodamine-labeled G-actin (1.0 label/molecule on amines, Cytoskeleton Inc.)

in wash buffer (ISAP buffer containing 0.5 mg/mL bovine serum albumin) was perfused and incubated for 2 min. After being washed twice with antibleaching buffer (ISAP buffer with 5 mg/mL bovine serum albumin, 0.036 mg/mL catalase, 0.2 mg/mL glucose oxidase, 6 mg/mL glucose, and 100 mM DTT) containing 0.2 μM cytochalasin D, the chamber was mounted onto the stage of a Nikon Eclipse TE2000 inverted microscope. UNC-60A or UNC-60B was diluted in antibleaching buffer, perfused into the chamber, and incubated for 3 or 10 min. When the assays were performed at different pHs, 20 mM MES-KOH (pH 6.5) or 20 mM Tris-HCl (pH 8.5) was added in ISAP buffer instead of 20 mM Hepes-NaOH.

Monitoring Actin Depolymerization from Pointed Ends. G-Actin (5 μM) from rabbit muscle or *C. elegans* in G-buffer was incubated with 5, 15, or 50 nM gelsolin for 5 min, and then polymerization was initiated by adding 2 mM $MgCl_2$ and 100 mM KCl. After polymerization for 3 h at room temperature, the gelsolin-capped actin filaments were diluted to 0.5 μM with F-buffer at various pH values in the absence or presence of 1 μM latrunculin A (Biomol), UNC-60A, or UNC-60B to induce depolymerization. Changes in light scattering at an angle of 90° and at a wavelength of 400 nm were monitored with a Perkin-Elmer LS50B fluorescence spectrophotometer.

RESULTS

UNC-60A Causes More Net Actin Depolymerization Than UNC-60B with Weak pH Sensitivity. Our previous studies have shown that two ADF/cofilin isoforms in *C. elegans*, UNC-60A and UNC-60B, interact differently with F-actin from rabbit muscle: UNC-60A depolymerizes but does not remain bound to F-actin, whereas UNC-60B binds to but does not depolymerize it (*38*). First, we re-examined this difference using actins from rabbit muscle or *C. elegans* at different pH values. Using sedimentation assays, UNC-60A consistently showed stronger activity to increase the amount of unpelletable actin than UNC-60B on either rabbit muscle actin (r-actin) (panels a and b of Figure 1A,B) or *C. elegans* actin (Ce-actin) (panels c and d of Figure 1A,B). As demonstrated previously, UNC-60B depolymerized Ce-actin more strongly than r-actin (*43*) (compare panels b and d of Figure 1B), while UNC-60A had similar depolymerizing activity on both actins (panels a and c of Figure 1B). In the range of pH values between 6.5 and 8.5, both UNC-60A and UNC-60B exhibited slightly stronger activity at higher pHs on both actins (Figure 1B). However, the observed pH sensitivity is much weaker than that for other ADF/cofilin proteins, especially vertebrate ADF, that shows much greater actin depolymerizing activity at high pHs than at low pHs (*14*, *30*, *46*, *49*, *50*). Both UNC-60A and UNC-60B cosedimented with F-actin (Figure 1A). Quantification of the sedimented actin and UNC-60A or UNC-60B indicates that the molar ratios of actin and UNC-60A or UNC-60B did not exceed 1:1 in a manner independent of pH values (Figure 1B, insets), suggesting that binding of ADF/cofilin to actin at a 2:1 ratio as demonstrated by electron microscopy (*51*) is not likely to occur under these conditions.

The difference in the effects of UNC-60A and UNC-60B on the actin critical concentration explains their different activities in the sedimentation assays (Figure 2). In the presence of excess UNC-60B (1:2.5 actin:UNC-60B molar ratio), there was only a minor effect on the steady-state levels of polymerized actin [Figure 2a,b (■)]. In contrast, excess UNC-60A strongly inhibited actin polymerization [Figure 2a,b (▽)]. In the presence of UNC-60A, a significant increase in the level of light scattering due to actin polymerization was observed only when the actin concentration was 30 μM (Figure 2). An increase in the level of light scattering of Ce-actin in the presence of 30 μM UNC-60A was relatively small compared to that of r-actin, and we cannot exclude the possibility that it may still be within the extension of the baseline. Assuming that UNC-60A-bound F-actin scatters light to an extent similar to that of UNC-60B-bound F-actin given that UNC-60A and UNC-60B have similar molecular masses, lines with the same slopes as UNC-60B-bound actin were drawn at the 30 μM datum point, which yielded the estimated critical concentration of the UNC-60A−actin complex (27−29 μM). The effects of UNC-60A or UNC-60B were nearly identical on either r-actin or Ce-actin (compare panels a and b of Figure 2). Thus, UNC-60A is a much more effective inhibitor of actin polymerization than UNC-60B.

To determine if UNC-60A and UNC-60B have different affinity with G-actin, we examined their effects on the exchange rate of actin-bound nucleotides (Figure 3). ADF/cofilins bind to G-actin and inhibit exchange of actin-bound nucleotides (*46*, *50*, *52*) with the exception of *Plasmodium* ADF that stimulates nucleotide exchange (*53*). In the absence of UNC-60A or UNC-60B, Ce-actin exchanged nucleotide >2-fold faster than r-actin (Figure 3). Both UNC-60A and UNC-60B inhibited nucleotide exchange in a dose-dependent manner (Figure 3). Estimation of the dissociation constants indicates that UNC-60A and UNC-60B bind to G-actin with a relatively small difference in the affinity, while both proteins bind to r-actin with much higher affinity than to Ce-actin (Figure 3). These results show that the difference in the two proteins in the actin depolymerizing and sequestering activities is not simply due to the difference in their affinity with G-actin.

UNC-60B Has Stronger F-Actin Severing Activity Than UNC-60A. The actin filament severing activity of UNC-60A and UNC-60B was examined by two different methods, and surprisingly, cofilin-like UNC-60B exhibited much stronger severing activity than ADF-like UNC-60A (Figures 4 and 5). In the actin-seed elongation assay (Figure 4), severing of seed filaments increases the number of free barbed ends and enhances the initial rate of polymerization from the seeds (*14*, *46*, *54*, *55*). UNC-60B enhanced the elongation rate of r-actin ~2.5-fold (Figure 4b), while UNC-60A did not alter the rate at a 1:1 molar ratio (Figure 4a). There was no significant difference in their activities in the pH range of 6.5−8.5 (Figure 4a,b). They exhibited similarly different severing activities on Ce-actin (Figure 4c,d), although the elongation rate was only enhanced 1.2−1.4-fold by UNC-60B (Figure 4d), suggesting that Ce-actin is less frequently fragmented than r-actin. UNC-60B did not enhance the elongation rate of Ce-actin at pH 6.5 in three independent experiments [Figure 4d (●)]. However, due to large deviations in the data as compared to the relatively small increase in the elongation rate, we were not able to further confirm whether this is a meaningful pH-sensitive effect.

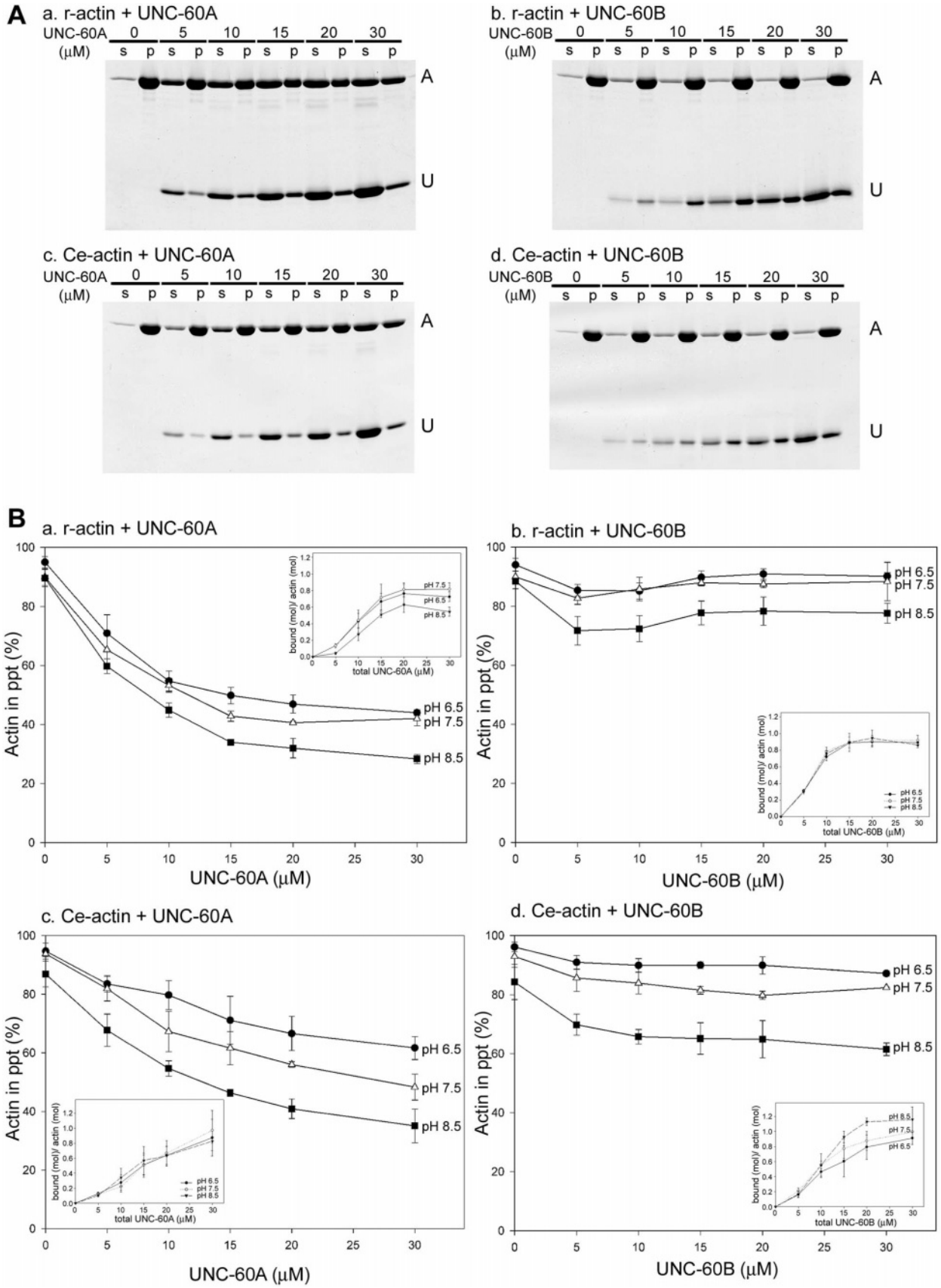

FIGURE 1: Effects of UNC-60A and UNC-60B on actin filament examined by a sedimentation assay at various pH values. F-Actin (10 μM) from rabbit muscle (a and b) or *C. elegans* (c and d) was incubated for 30 min with varied concentrations of UNC-60A (a and c) or UNC-60B (b and d) and ultracentrifuged to sediment F-actin. (A) Representative gels of the assays at pH 7.5. Supernatants (s) and pellets (p) were analyzed by SDS−PAGE and Coomassie blue staining. Positions of actin (A) and UNC-60A or UNC-60B (U) are indicated at the right. (B) Quantitative analysis of the sedimentation assay. Percentages of actin in the pellets were plotted as a function of total concentrations of UNC-60A or UNC-60B. Insets show quantification of molar ratios of actin and UNC-60A or UNC-60B that cosedimented. Data shown are the means ± SD of three experiments.

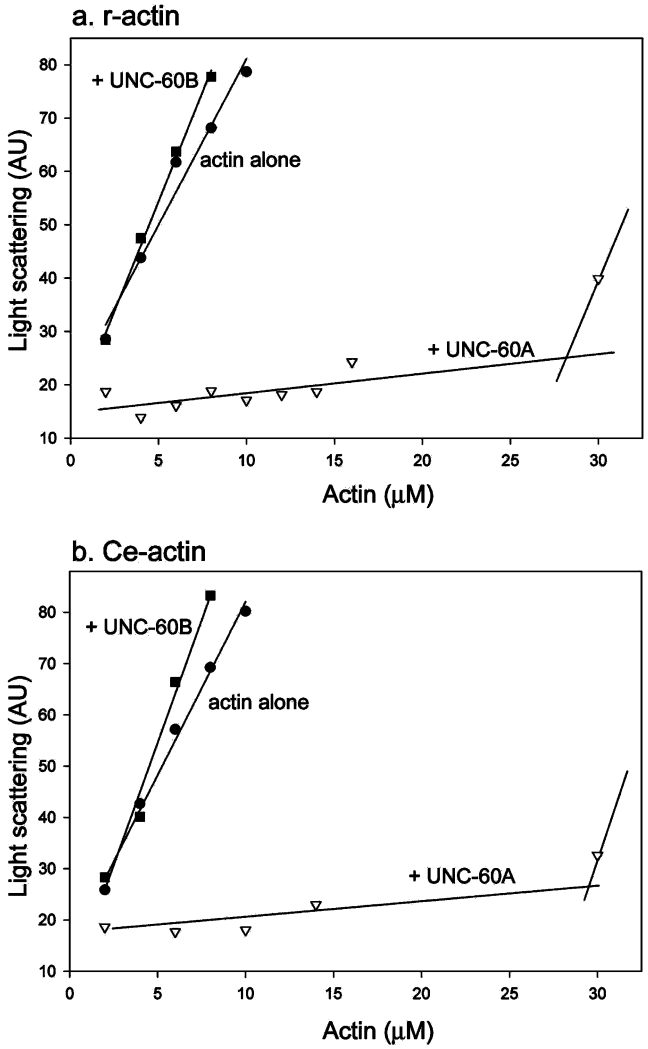

FIGURE 2: Effects of UNC-60A and UNC-60B on steady-state actin polymerization. Various concentrations of actin from rabbit muscle (a) or *C. elegans* (b) were polymerized in F-buffer (pH 7.5) either in the absence or in the presence of UNC-60A or UNC-60B at a 1:2.5 molar ratio. The light scattering signals (arbitrary units) of the samples were plotted as a function of actin concentration.

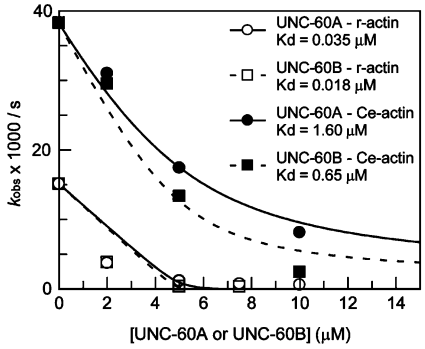

FIGURE 3: Effects of UNC-60A and UNC-60B on nucleotide exchange of G-actin. The rate of exchange of ϵ-ATP (k_{obs}) that had been bound to 5 μM r-actin (empty symbols) or Ce-actin (filled symbols) was measured in the presence of various concentrations of UNC-60A (circles) or UNC-60B (squares). Data are averages of three independent experiments. Standard deviations are less than $4.9k_{obs} \times 1000\ s^{-1}$.

Filament severing activity of UNC-60 proteins was further examined by direct observation of fluorescently labeled actin filaments (Figure 5). Alexa488- and biotin-labeled actin filaments were attached to a glass coverslip in a perfusion chamber, treated with UNC-60A or UNC-60B, and observed before and after the treatments. Our previous study demonstrated that UNC-60B had strong severing activity in this assay, and a significant number of filaments are dissociated after the treatment (*48*). Therefore, to better distinguish severing from shortening from the pointed ends, pre-existing barbed ends were labeled with rhodamine-labeled actin. If the filaments are frequently severed, the population of filaments with the rhodamine label will become minor because ends newly created by severing will not have the label and severing near the barbed end will liberate the label. In contrast, if the filaments are only shortened by depolymerization from the pointed ends, the rhodamine label will be retained in most of the filaments. In the control treatment with buffer alone, the filaments were stable and largely intact for 3 or 10 min (Figure 5a−d), and the percentage of rhodamine-labeled filaments was only slightly decreased after the treatment (Figure 5k). Treatment with UNC-60A for 3 min caused only occasional filament severing (Figure 5e,f, arrowheads) with minor alterations in the morphology of the filaments. After 10 min, UNC-60A induced more frequent severing (Figure 5g,h, arrowheads), and the proportion of rhodamine-labeled filaments decreased (Figure 5k). In contrast, UNC-60B, at a lower concentration than UNC-60A, strongly fragmented the filaments (Figure 5i,j) and caused the loss of the rhodamine label at the ends (Figure 5k) after treatment for 3 min. In this assay, UNC-60B had a slightly higher severing activity as the pH increases, while UNC-60A did not exhibit significant pH sensitivity (data not shown). These data provide strong evidence that UNC-60B has much stronger filament severing activity than UNC-60A.

UNC-60A and UNC-60B Differently Enhance Pointed-End Depolymerization. Next, we examined the effects of the ADF/cofilin isoforms on the rate of depolymerization from the pointed end of the actin filament. Actin (5 μM) was polymerized in the presence of gelsolin at variable gelsolin: actin molar ratios (1:1000, 1:333, and 1:100) to alter the number of pointed ends. Depolymerization was induced by dilution to 0.5 μM actin (slightly below the critical concentration at the pointed end) in the presence or absence of UNC-60 proteins at 1 μM. Since UNC-60B strongly quenches fluorescence of a pyrene label on Cys-374 of actin (our unpublished data), the process of depolymerization was monitored by light scattering at 400 nm. Filament binding by ADF/cofilin increases the level of light scattering at a maximum of 30% (*13*) and contributes to the initial increase in the magnitude of the signal (see Figure 6d,h). In the absence of ADF/cofilin, both r-actin (Figure 6a) and Ce-actin (Figure 6e) slowly depolymerized under these conditions. Latrunculin a (Lat-A), an actin monomer-sequestering agent (*56*), did not enhance depolymerization of r-actin (Figure 6b), confirming that the conditions favor depolymerization from the pointed ends. However, Lat-A slightly enhanced depolymerization of Ce-actin (Figure 6f), suggesting that residual polymerization might occur under these conditions, which could be inhibited by Lat-A. By a comparison of the depolymerization curves induced by Lat-A, UNC-60A slightly enhanced the depolymerization of r-actin (Figure 6c), whereas UNC-60B induced depolymerization more rapidly (Figure 6g). In both cases, the de-

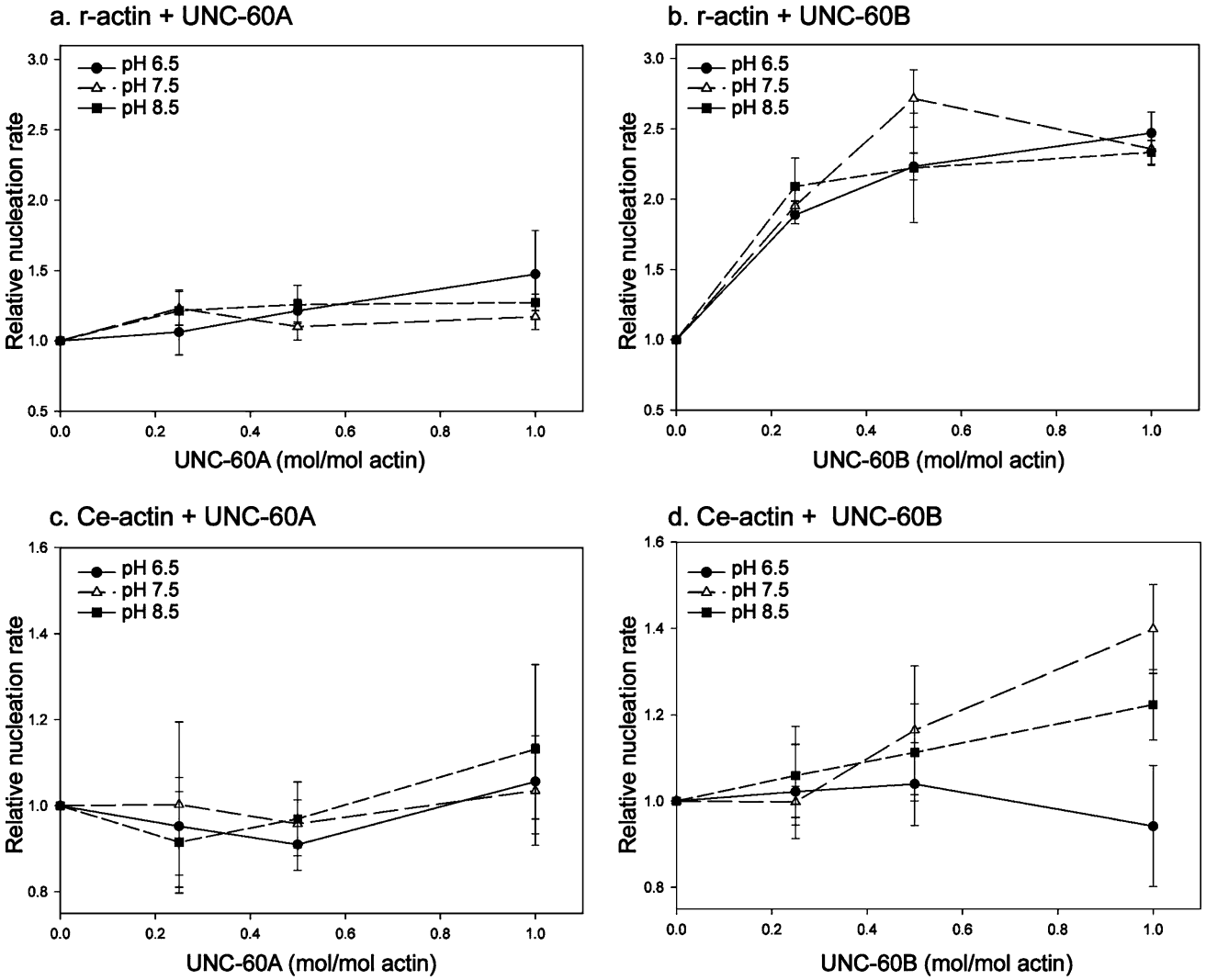

FIGURE 4: Effects of UNC-60A and UNC-60B on the nucleating activity of F-actin seeds. F-Actin (10 μM) from rabbit muscle (a and b) or *C. elegans* (c and d) was mixed with UNC-60A (a and c) or UNC-60B (b and d) at various molar ratios and used as nuclei at 1.25 μM to induce polymerization of pyrene-labeled G-actin. Nucleation rates were determined as the initial rates of the increase in the pyrene fluorescence, which correlates linearly with the number of free barbed ends. The data are expressed as relative nucleation rates to that of F-actin alone. Data shown are the means ± SD of three experiments.

polymerization was faster as the number of pointed ends increased, indicating that the data reflect their difference in the pointed-end depolymerizing activity. There was no significant pH-dependent difference in their activities at pH 6.5−8.5 (data not shown). Interestingly, both UNC-60A and UNC-60B caused relatively rapid depolymerization of Ce-actin at pH 6.5 and 7.5 (data not shown). Under these conditions, rapid depolymerization proceeded during manual setting of the samples in the spectrophotometer before starting the measurement (average 15 s), and reliable comparison in their activities was difficult. At pH 8.5, depolymerization was slower than at other pHs tested, and interestingly, UNC-60A caused faster depolymerization than UNC-60B (compare panels g and h of Figure 6). Again, the depolymerization rate was dependent on the number of pointed ends, indicating that this is due to their difference in the pointed-end depolymerizing activity. These results demonstrate that UNC-60A has stronger depolymerizing activity on *C. elegans* actin, but not on rabbit muscle actin, than UNC-60B, and that matching the source of actin and ADF/cofilins for in vitro experiments is important in studying biologically relevant activities.

DISCUSSION

In this study, comparison of the effects of UNC-60A and UNC-60B on actin filaments demonstrates that they have different filament severing and pointed-end depolymerizing activities in addition to the difference in their activity in increasing the level of net actin depolymerization. In particular, direct microscopic observation of the effects on actin filaments revealed a striking difference in their severing activity. UNC-60B has stronger severing activity than UNC-60A, while UNC-60A induces faster depolymerization from the pointed end of Ce-actin than UNC-60B. However, the difference in the pointed-end depolymerizing activity was relatively small, and UNC-60B has a greater impact on actin dynamics than UNC-60A. In contrast, UNC-60A maintains much higher concentrations of monomeric actin than UNC-60B does.

We detected a difference in the severing activity of two ADF/cofilin isoforms by two different methods. UNC-60B exhibited significant severing in both assays, whereas UNC-60A caused modest severing in only the direct microscopic observation. The seeded actin elongation assay might be less

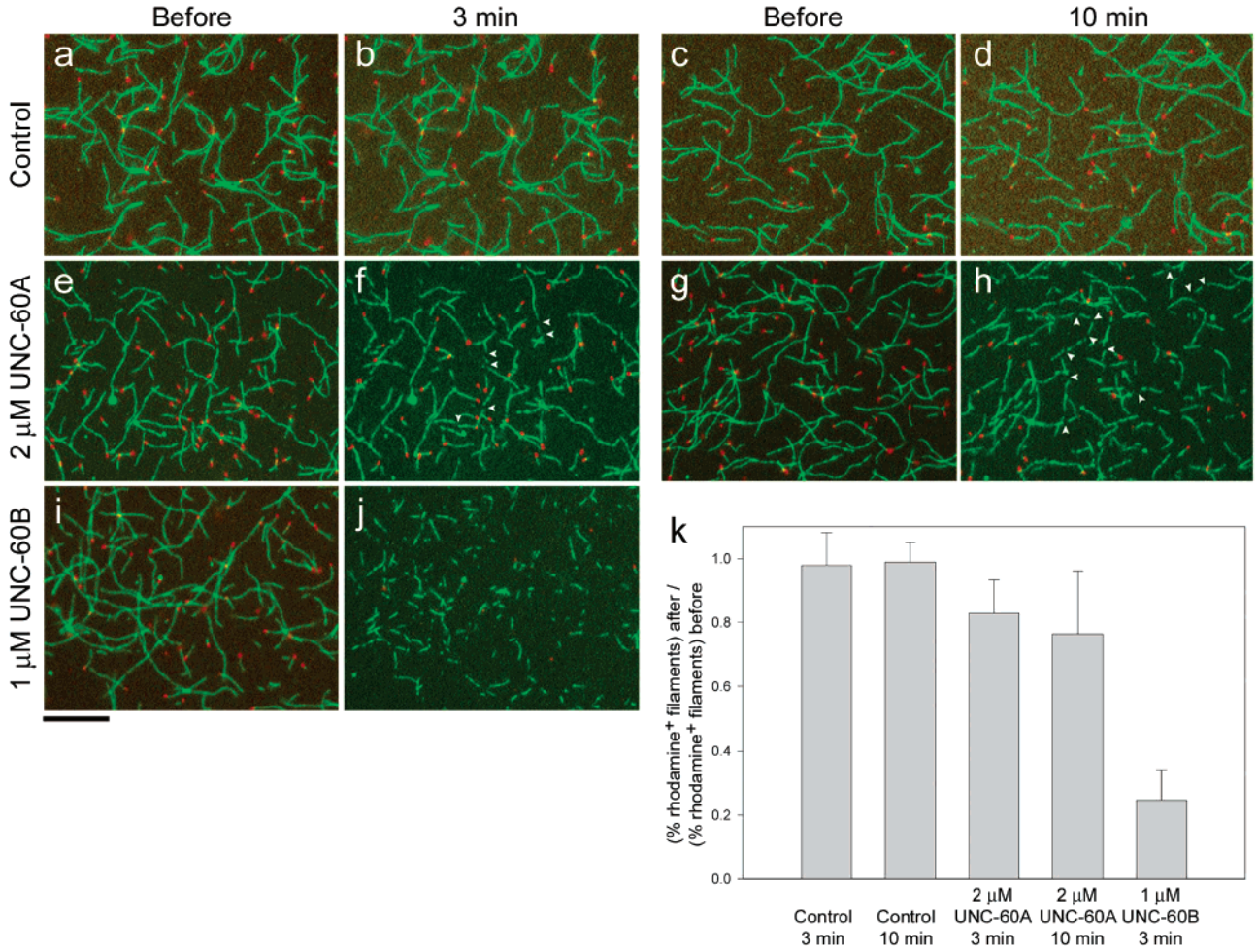

FIGURE 5: Direct observation of F-actin severing by UNC-60A and UNC-60B. (a−j) Alexa488- and biotin-labeled actin filaments with rhodamine labels at the pre-existing barbed ends were treated with buffer alone (a−d), 2 μM UNC-60A (e−h), or 1 μM UNC-60B (i and j). The filaments were observed before (a, c, e, g, and i) or 3 min (b, f, and j) or 10 min (d and h) after the incubation by fluorescence microscopy, and the same fields are shown for each experiment. Arrowheads in panels f and h indicate severing events induced by UNC-60A. These micrographs show the results at pH 7.5. The bar is 10 μm. (k) Changes in the proportion of rhodamine-labeled (rhodamine$^+$) filaments. Fields in micrographs containing 100−200 filaments each were compared before and after the treatment, and the percentages of rhodamine-labeled filaments after the treatment were divided by those before the treatment to calculate the ratios. Filament severing will generate filaments with no label and is expected to decrease the ratio. The data are from four separate fields from two independent experiments and are means ± SD.

sensitive for detecting severing than the microscopic assay, because quenching of the pyrene fluorescence by ADF/cofilin, re-annealing of filaments, depolymerization, and repolymerization can affect the results. Indeed, the seed activity declines after prolonged incubation of actin with ADF (*14*). In contrast, under the conditions used for the microscopic assay, re-annealing and re-polymerization are expected to be very infrequent. In addition, tethered filaments are physically restricted and more susceptible to severing by ADF/cofilin than free filaments (D. Pavlov and E. Reisler, personal communication). Therefore, although the seeded elongation assay was previously used to demonstrate little difference in the severing activity of human ADF and cofilin (*29*), the direct microscopic assay might reveal possible difference in their activity.

The comparative study on a number of ADF/cofilin proteins classified UNC-60A into the ADF subgroup and UNC-60B into the cofilin subgroup (*30*). This study showed that ADF is more effective than cofilin in both severing and depolymerization and more pH-sensitive. Our results agree with their classification in the sedimentation assay and the measurement of critical concentration. However, our data revealed several unique features of UNC-60A and UNC-60B. First, both proteins have only weak pH sensitivity. Second, cofilin-like UNC-60B has much stronger severing activity than ADF-like UNC-60A, which is the opposite of the characteristics of the vertebrate counterparts. Third, UNC-60A induced pointed-end depolymerization only weakly on rabbit muscle actin, but slightly more strongly on *C. elegans* actin, than UNC-60B. As previously reported for UNC-60B (*43*), UNC-60A also exhibited somewhat different activity on Ce-actin, and the results using Ce-actin are likely to be more relevant to their cellular functions. These unique activities are probably evolved to effectively regulate actin dynamics in *C. elegans*. Analysis of a number of ADF/cofilin sequences shows that UNC-60A and UNC-60B are equally distant from vertebrate ADFs and cofilins in a phylogenetic tree (*8*, *20*, *28*), suggesting that they are uniquely evolved members of the ADF/cofilin family.

The result that UNC-60B is kinetically more effective than UNC-60A in enhancing actin dynamics is surprising to us. On the basis of the previous studies, we hypothesized that

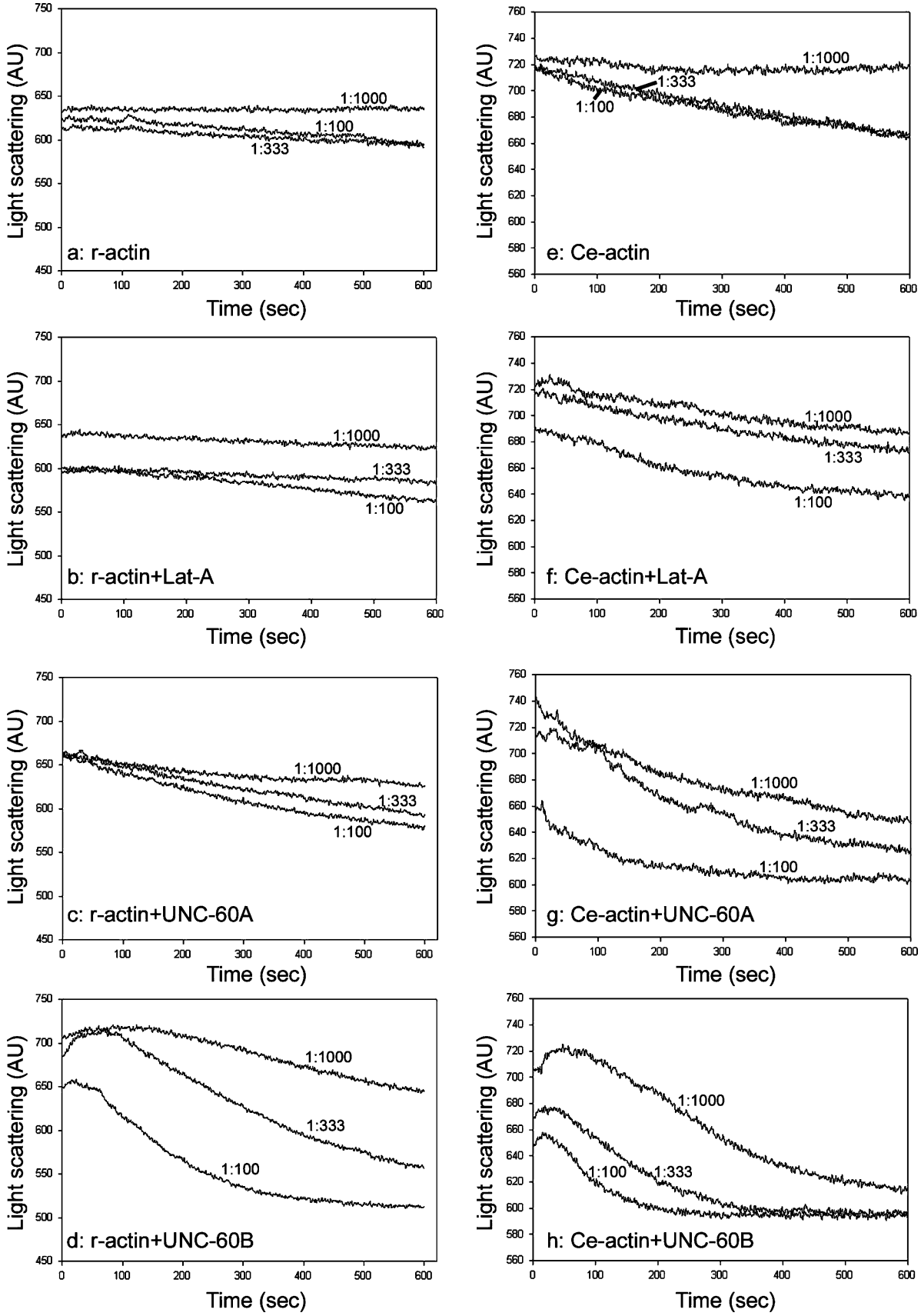

FIGURE 6: Effects of UNC-60A and UNC-60B on depolymerization from pointed ends of gelsolin-capped actin filaments. Depolymerization of gelsolin-capped F-actin (0.5 μM) from rabbit muscle (a−d) or *C. elegans* (e−h) with the indicated gelsolin:actin molar ratios in the absence (a and e) or presence of 1 μM latrunculin A (Lat-A) (b and f), 1 μM UNC-60A (c and g), or UNC-60B (d and h) was monitored by light scattering (arbitrary units) at 400 nm. Experiments whose results are depicted in panels a−d were performed at pH 7.5, and those in panels e−h at pH 8.5. Although relatively large deviations in the baseline signal (±35 arbitrary units) were observed among experiments, the results were qualitatively consistent in three independent experiments, and the representative data are shown.

UNC-60A has stronger activity than UNC-60B to depolymerize actin filaments and functions in cellular processes that require very rapid actin dynamics such as cytokinesis and embryogenesis (*37*), and that UNC-60B is required for less dynamic events in muscle cells (*19*, *37*). However, our prediction that the actin filaments in muscle cells are less dynamic than those in non-muscle cells might not be true. An early study demonstrated that exchange of actin subunits is minimal in adult skeletal muscle (*57*). However, a more recent study using cultured muscle cells showed that actin subunits within the myofibrils undergo turnover (*58*). In addition, during assembly of myofibrils, drastic reorganization of the actin cytoskeleton should involve very rapid actin turnover. Therefore, an ADF/cofilin isoform with a stronger activity might be appropriate for muscle cells. Relatively weak activity of UNC-60A on filament severing was unexpected, but it is suitable for maintaining high concentrations of monomeric actin. To support rapid actin dynamics in non-muscle cells, UNC-60A will need to cooperate with other actin-regulatory proteins, such as AIP1 for filament disassembly (*5*), and profilin (*59*, *60*) and cyclase-associated protein (*61*−*64*) for recycling actin monomers for polymerization. Although UNC-60A does not cooperate with UNC-78/AIP1 for filament disassembly (*40*), *C. elegans* has a second AIP1 gene, and this gene product may preferentially interact with UNC-60A. Also interestingly, *C. elegans* has three profilin isoforms (*65*) and two cyclase-associated protein isoforms. Therefore, future analysis of these proteins may reveal novel functional interactions among the actin-regulatory proteins in isoform-specific manners, which may explain how the actin cytoskeleton is structurally and functionally differentiated in cell type-specific manners in multicellular organisms.

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