

SUPPLEMENTAL DATA

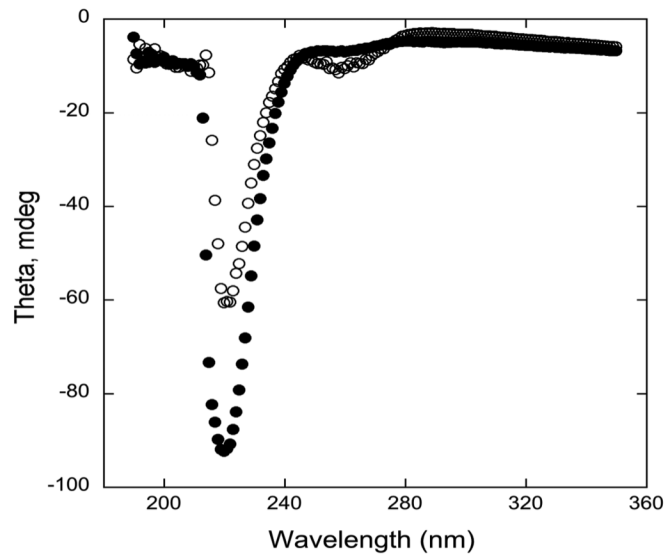


FIGURE S1. Circular dichroism spectra of (○) *S. pombe* actin, and (●) chicken skeletal muscle actin at 4 °C. Conditions: 2.5 mL 2 μM actin in 2 mM Tris, 0.1 mM CaCl₂, 1 mM NaN₃, 0.05 mM ATP, 0.125 mM DTT, pH 8.0. The data points are the average of three measurements.

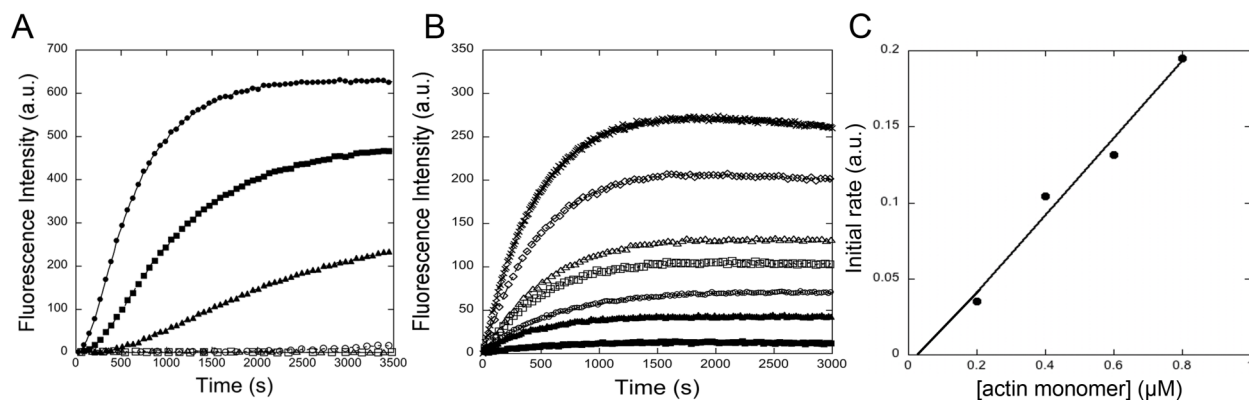


FIGURE S2. Kinetics of chicken muscle actin polymerization measured by the fluorescence of pyrene-labeled actin. Conditions: KMEI buffer containing 50 mM KCl, 1 mM MgCl₂, 1 mM EGTA, 10 mM imidazole, 0.1 mM ATP, and 1 mM DTT, pH 7.0. (A) Time course of spontaneous polymerization of a range of concentrations of 5% pyrene-labeled muscle actin monomers: (●) 4 μM, (■) 3 μM, (▲) 2 μM, (○) 1 μM, (□) 0.5 μM and (△) 0.3 μM. (B) Time course of elongation of 0.2 μM muscle actin filament seeds with a range of concentrations of 5% pyrene-labeled muscle actin monomers: (×) 2 μM, (◇) 1.5 μM, (△) 1 μM, (□) 0.8 μM, (○) 0.6 μM, (▲) 0.4 μM, (■) 0.2 μM, and (●) 0 μM. (C) Dependence of the initial elongation rate on the concentration of actin monomers. A line with a slope of 0.25 fit the data with $R^2 = 0.97$ and an X-intercept equal to the critical concentration of 0.04 μM.

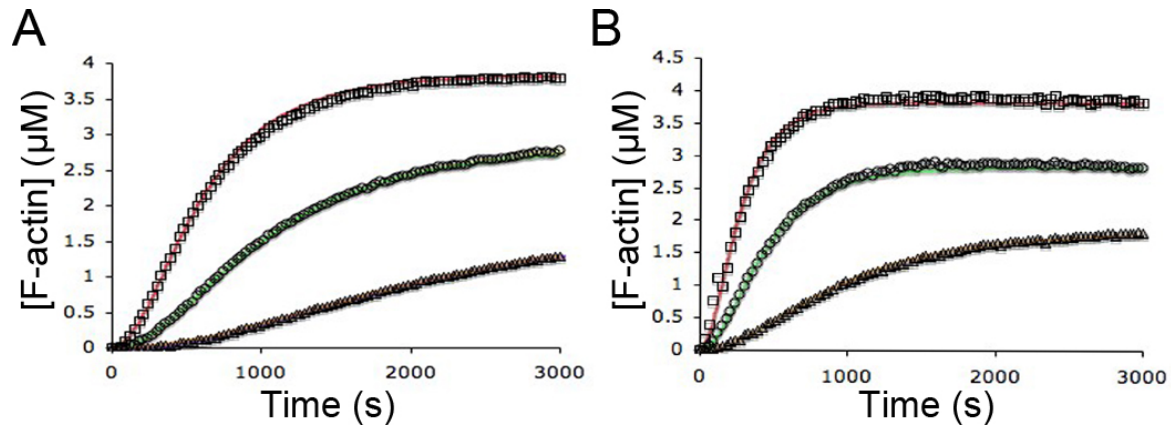


FIGURE S3. Comparison of simulated time courses of actin polymerization (colored smooth curves) with the fluorescence of pyrene-actin (open symbols). *A*, Time course with (\square) 4 μM , (\circ) 3 μM and (\triangle) 2 μM muscle actin, and a good fit with trimer nuclei forming with an apparent rate constant of $0.035 \mu\text{M}^{-1}\text{s}^{-1}$, $0.0275 \mu\text{M}^{-1}\text{s}^{-1}$, and $0.02 \mu\text{M}^{-1}\text{s}^{-1}$ for the three concentrations. *B*, Time course with (\square) 4 μM , (\circ) 3 μM and (\triangle) 2 μM *S. pombe* actin, and a good fit with trimer nuclei forming with apparent rate constants of $0.175 \mu\text{M}^{-1}\text{s}^{-1}$, $0.15 \mu\text{M}^{-1}\text{s}^{-1}$ and $0.1125 \mu\text{M}^{-1}\text{s}^{-1}$ for the three concentrations.

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Pombe      —EEEIAALVIDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHHGIMVGMGQKDSYVGDEAQ 58
Chicken    CDEDETTALVCDNGSGLVKAGFAGDDAPRAVFPSIVGRPRHQGMVGMGQKDSYVGDEAQ 60
Cerevisiae —DSEVAALVIDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGMVGMGQKDSYVGDEAQ 58
          *   **   *****   *****   *****   *   *****   *****
Pombe      SKRGILTLKYPIEHGIVNNWDDMEKIWHHTFYNELRVAPEEHPCLLTEAPLNPKSNREKM 118
Chicken    SKRGILTLKYPIEHGIITNWDDMEKIWHHTFYNELRVAPEEHPTEAPLNPKANREKM 120
Cerevisiae SKRGILTLRYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPMNPKNREKM 118
          *****   *****   *****   *****   *****   *   **   *****
Pombe      TQIIFETFNAPAFYVAIQAVLSLYASGRRTGIVLDSGDGVTHVPIYEGYALPHAIMRLD 178
Chicken    TQIMFETFNVPAMYVAIQAVLSLYASGRRTGIVLDSGDGVTHNVPYIYEGYALPHAIMRLD 180
Cerevisiae TQIMFETFNVPAFYVSIQAVLSLYSSGRRTGIVLDSGDGVTHVPIYAGFSLPHAILRID 178
          ***   *****   **   **   *****   *****   *****   *   *****   *
Pombe      LAGRDLTDYLMKILMERGYTFSTTAEREIVRDIKEKLCYVALDFEQELQTAQSSSLEKS 238
Chicken    LAGRDLTDYLMKILTERGYSFVTTAEREIVRDIKEKLCYVALDFENEMATAASSSLEKS 240
Cerevisiae LAGRDLTDYLMKILSERGYSFSTTAEREIVRDIKEKLCYVALDFEQEMQTAQSSSIEKS 238
          *****   *****   *****   *   *****   *****   *   **   *****
Pombe      YELPDGQVITIGNERFRAPEALFQPSALGLENAGIHEATYNSIMKCDVDIRKDLYGNVVM 298
Chicken    YELPDGQVITIGNERFRCPETLFQPSFIGMESAGIHETTYNSIMKCDIDIRKDLYANNVM 300
Cerevisiae YELPDGQVITIGNERFRAPEALFHPSVLGLESAGIDQTTYNSIMKCDVVRKELYGNIVM 298
          *****   *****   **   *   *   *   *   *   *   *   *   *   *
Pombe      SGGTTMYPGIADRMQKEIQALAPSSMKVKIVAPPERKYSVWIGGSILASLSTFQQMWISK 358
Chicken    SGGTTMYPGIADRMQKEITAPLAPSTMKIKIIAPPERKYSVWIGGSILASLSTFQQMWITK 360
Cerevisiae SGGTTMFPGIAERMQKEITAPLAPSSMKVKIIAPPERKYSVWIGGSILASLSTFQQMWISK 358
          *****   *****   *****   *****   **   **   *****   *****   *
Pombe      QEYDESGPGIVYRKCF 374
Chicken    QEYDEAGPSIVHRKCF 376
Cerevisiae QEYDESGPSIVHHKCF 374
          *****   **   **   ***

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FIGURE S4. Comparison of the amino acid sequences of actins from fission yeast *S. pombe*, chicken skeletal muscle and *S. cerevisiae*. The asterisks show the residues conserved among the three species. The alignment was done by using Kalign (2.0) with 0.45 terminal gap penalty, 11.0 gap open penalty, 0.85 gap extension penalty, and the alignment is in ClustalW format (<http://www.ebi.ac.uk/Tools/services/web/toolform.ebi?tool=kalign>).