An antiparallel actin dimer has been proposed to be an intermediate species during actin filament nucleation. We now show that latrunculin A, a marine natural product that inhibits actin polymerization, arrests polylysine-induced nucleation at the level of an antiparallel dimer, resulting in its accumulation. These dimers, when composed of pyrene-labeled actin subunits, give rise to a fluorescent excimer, permitting detection during polymerization in vitro. We report the crystallographic structure of the polylysine-actin-latrunculin A complex at 3.5-Å resolution. The non-crystallographic contact is consistent with a dimeric structure and confirms the antiparallel orientation of its subunits. The crystallographic contacts reveal that the mobile DNase I binding loop of one subunit of a symmetry-related antiparallel actin dimer is partially stabilized in the interface between the two subunits of a second antiparallel dimer. These results provide a potential explanation for the paradoxical nucleation of actin filaments that have exclusively parallel subunits by a dimer containing antiparallel subunits.

Actin filament nucleation occurs very slowly de novo, but it occurs rapidly as a necessary step in actin-based motility (1). The formation of a dimer from monomeric subunits is the most thermodynamically unfavorable nucleation step with an estimated equilibrium dissociation constant of 4.6 M (in contrast to the formation of a dimer from monomeric subunits, which occurs rapidly as a necessary step in actin-based motility (1)). Antiparallel actin dimers have been identified as a precursor to actin filament polymerization by covalent cross-linking during polymerization induced with divalent cations (5). A gelsolin-actin complex capable of nucleating filament growth at the slow growing, pointed end of filaments has also been shown by covalent cross-linking to contain two actin subunits in the antiparallel configuration (6). The assumption of an antiparallel configuration of subunits is based on evidence that Cys-374 in the C terminus of actin is the only residue involved in the cross-linking reaction. In contrast, when polymerization is complete, intrafilament cross-linking yields a parallel dimer. More recently, electron microscopy has revealed that newly formed actin filaments show evidence of incorporation of antiparallel dimers. This incorporation results in a branched filament network, implying that the dimers have nucleating activity (7). Interestingly, analysis of a Listeria model of cell motility using high-resolution laser tracking provides evidence that filaments elongate in 5.4-nm steps, consistent with in vivo incorporation of dimeric actin (8).

In the current work, we provide evidence that polylysine nucleates actin polymerization by enhancing the stability of an antiparallel dimer and that the production of antiparallel dimer induced by polylysine and other nucleation-promoting agents correlates well temporally with filament nucleation. We show that the polylysine-induced dimer accumulates in the presence of latrunculin A, generating a homogeneous complex that can be crystallized. An x-ray structure of an antiparallel dimer is reported that correlates well with the available biochemical data for the solution dimer.

**Experimental Procedures**

**Actin Polymerization and Covalent Cross-linking—**Polymerization of pyrene-labeled rabbit muscle actin‡ (4% of actin was labeled except when detecting excimer, in which case 74% was labeled) was measured at 22 °C using a PBI spectrofluorimeter with excitation at 366 nm and emission at 387 nm. Actin in buffer G (0.1 mM CaCl₂, 0.2 mM dithiothreitol, 0.2 mM ATP, 0.01% sodium azide, and 5.0 mM Tris, pH 7.8) was converted to Mg²⁺-actin by the addition of 125 μM EGTA and 50 μM MgCl₂ 15 min prior to the initiation of polymerization. For covalent cross-linking, actin, polylysine, and latrunculin A were mixed at the indicated concentrations. Polylysine (Sigma) with low polydispersity protein such as gelsolin, which can stabilize dimeric actin, or by a protein complex such as Arp2/3 that is thought to contain two actin-like molecules constrained in an orientation that promotes nucleation (3, 4). Antiparallel actin dimers have been identified as a precursor to actin filament polymerization by covalent cross-linking during polymerization induced with divalent cations (5). A gelsolin-actin complex capable of nucleating filament growth at the slow growing, pointed end of filaments has also been shown by covalent cross-linking to contain two actin subunits in the antiparallel configuration (6). The assumption of an antiparallel configuration of subunits is based on evidence that Cys-374 in the C terminus of actin is the only residue involved in the cross-linking reaction. In contrast, when polymerization is complete, intrafilament cross-linking yields a parallel dimer. More recently, electron microscopy has revealed that newly formed actin filaments show evidence of incorporation of antiparallel dimers. This incorporation results in a branched filament network, implying that the dimers have nucleating activity (7). Interestingly, analysis of a Listeria model of cell motility using high-resolution laser tracking provides evidence that filaments elongate in 5.4-nm steps, consistent with in vivo incorporation of dimeric actin (8).

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Crystal Structure of an Antiparallel Actin Dimer

(weight-average $M_w$/number-average $M_n$ is 1.1) had an average of 24 lysine residues. Phenylenediamine (PDM) was dissolved in dimethylformamide at 5 mM and diluted in 10 mM sodium borate, pH 9.2, immediately prior to use. Cross-linking was initiated by the addition of equal volumes of actin and PDM to achieve a final molar ratio of either 0.51 or 4.1 PDM/actin as previously described (5) so as to optimize the yield of antiparallel or intrafilament dimer, respectively.

Analytical Ultracentrifugation—Actin, polylysine, and latrunculin A were mixed at the stated concentrations and centrifuged in a Beckman XLA analytical ultracentrifuge at 14,400 rpm (for sedimentation equilibrium) or at 53,000 rpm (for sedimentation velocity) in buffer G plus 125 μM EGTA, 2 mM MgCl₂, and 40 mM KCl. Samples of 110 μl reached equilibrium in 36 h at 4 °C. Buffer density was 1.0015, and the partial specific volume for the complex was assumed to be that of actin alone, 0.72 ml/g (9). For sedimentation velocity, 400-μl samples were loaded into double sector cells at 20 °C. Absorbance scans were obtained at 12-min intervals at 290 nm. Sedimentation coefficients were calculated using the second-moment analysis method (10). Theoretical sedimentation coefficients were calculated using HYDROPRO (11) using the atomic coordinates of the antiparallel dimer or of a compact dimer in which two subunits interact via DNase I binding loop contacts with subdomains 1 and 3 as shown in Fig. 6A.

Structure Determination and Refinement—The structure was determined using molecular replacement (14), using the actin coordinates taken from the actin-gelsolin segment 1-latrunculin A structure as the search model (15). The cross-rotation function search was calculated on a 3° grid with a radius of 40 Å in the 20–3.5 Å resolution range. This search produced a clear solution with the two highest peaks, 11.74 and 10.30, which results in a Matthews coefficient ($V_M$) of 103.03, and 126.96 Å³ of eight actin crystals. Data were indexed using HKL software (12) with a correlation coefficient of 0.362 and an R-factor of 0.462 at x = 0.105, y = 0.465, and z = 0.006, and the second G-actin as having rotation angles (θ, ϕ, and χ) of 12.25, 55.09, and 179.50°; with a correlation coefficient of 0.395 and an R-factor of 0.462 at x = 0.105, y = 0.465, and z = 0.006, and the second G-actin as having rotation angles (θ, ϕ, and χ) of 159.15, 85.79, and 100.36°, with a correlation coefficient of 0.362 and an R-factor of 0.475 at x = 0.276, y = 0.276, and z = 0.276. The F-actin monomer was selected as a starting point for refinement because the crystallographic conditions (3 mM MgCl₂) strongly favored conversion of G- to F-actin. A second translation function search for actin dimer composed of the two independent actin monomers had a correlation coefficient of 0.680 and an R-factor of 0.337 at the position x = 0.348, y = 0.276 and z = 0.773. The resulting actin dimer model was refined using crystallography NMR software version 1.1 (17). Initially, rigid body refinement was carried out with each monomer defined as an independent rigid body. Procedures carried out with CNS included simulated annealing using a maximum likelihood target function, restrained individual B-factor refinement conjugate gradient minimization, and bulk solvent correction. The model was refined to a crystallographic R-factor of 0.192 and Rmerge of 0.252 for all data (50–3.5 Å) (5% of the data were sequestered before molecular replacement and were used for calculation of the Rmerge value in CNS refinement). The atomic coordinates and structure factors have been deposited with the Protein Data Bank.

2 The abbreviations used are: PDM, phenylenediamine; MARCKS PSD, phosphorylation site domain of myristoylated alanine-rich protein kinase C substrate; r.m.s., root mean square.

FIG. 1. Nucleation of actin polymerization by polylysine. A, time course of 4% pyrene-labeled rabbit muscle Mg²⁺-actin polymerization with polylysine in 40 mM KCl and 2 mM MgCl₂. The final extent of polymerization is similar in all samples. B, a log-log plot of the time required for actin to become 50% polymerized as a function of actin concentration in the presence of 5 μM polylysine or 5 μM jasplakinolide. For data satisfying nucleation-elongation kinetics, a shift to the left indicates an increase in the rate constant for nucleation, and the slope of each best fit line is equal to one-half the size of the nucleus (18).

RESULTS

Actin Filament Nucleation Initiated by an Antiparallel Actin Dimer—Actin filament nucleation is fast in vitro when a short oligomer of polylysine is present (Fig. 1A). The effect of polylysine is very potent relative to molecules such as phalloidin or jasplakinolide, which promote nucleation by binding at the interface of three subunits to stabilize a helical actin trimer (Fig. 1B). Given the enhanced intrinsic stability of trimer relative to dimer, an observed effect greater than that caused by a very high affinity actin-trimer ligand such as jasplakinolide is likely related to modulation of actin dimer formation (9). Indeed, if actin is covalently cross-linked during polymerization induced by polylysine, a substantial yield of antiparallel dimers with high electrophoretic mobility on SDS-PAGE is obtained (Fig. 2A). Consistent with the cross-linking results, polylysine, at saturation, changes the slope of a log-log plot of the time required to reach 50% completion of polymerization versus actin concentration from 2.03 for the control data to 1.17. This is most consistent with a change in nucleus size as defined by Oosawa and Asakura (18) (and also Ref. 19) from four to two subunits, as might be expected if polylysine enhances the stability of dimeric actin (Fig. 1B). In contrast, the potent actin filament stabilizing compound jasplakinolide changed the slope to 1.56, suggesting a nucleus size of 3,
the dimer is homogeneous in solution. Sedimentation equilibrium experiments (performed at 4°C as previously described (21) with a mixture of actin, polylysine, and latrunculin A, at concentrations of 5, 2.5, and 12 µM, respectively) confirm that actin is homogeneously dimeric (Fig. 2B), even in the presence of high concentrations of divalent cations (2 mM MgCl₂). Results of sedimentation velocity experiments (data not shown) performed under identical conditions (except at a temperature of 20°C) also reveal a homogeneous species with sedimentation coefficient (s₂₀,₅₀) of 4.81 ± 0.10 S. Because these results show that the actin is dimeric in solution rather than filamentous, the antiparallel cross-link cannot be the result of interfilament cross-linking in a bundle of filaments, as has been speculated to occur in other conditions (7). Cross-linking and sedimentation equilibrium experiments in the presence of excess latrunculin A showed no evidence of dimer formation in the absence of polylysine.

The finding that an antiparallel dimer was an intermediate species during polylysine-induced actin polymerization was unexpected. Given the parallel orientation of subunits in an actin filament, the antiparallel geometry is paradoxical, with a requisite large change in subunit orientation if both subunits are incorporated into a filament. Moreover, in general, conditions that provoke rapid nucleation, e.g., 2.0 mM MgCl₂, have previously been associated with relatively lower yields of antiparallel dimer (5). Indeed, because of these considerations, it has been speculated that the antiparallel dimer could be an inert product of a bimolecular reaction mechanism that is independent of actin polymerization (7). The evidence that ubiquitous antiparallel dimer formation correlates well temporally with nucleation events and the evidence of incorporation during filament polymerization argue that this is not the case (6, 7). Our new data for polylysine are particularly convincing in this regard, as the apparent abundance of antiparallel dimer during extremely rapid polymerization is difficult to reconcile with the idea that the dimer represents actin diverted to an unpolymerizable state.

For more than 20 years since the discovery of the antiparallel actin dimer (5, 22) there has been no technique to allow for its detection other than covalent cross-linking, a procedure requiring extended incubation times and high pH levels (5). We now find evidence that pyrene-labeled actin forms an excimer in the antiparallel dimer (and not in F- or G-actin), providing a "real-time" assay for monitoring its formation (Fig. 3A). Because the presence of excimer implies that the pyrene fluorophores are less than 18 Å apart (23), this is consistent with the biochemical evidence of antiparallel dimer. Of course, this is not proof that antiparallel dimer is present whenever excimer is detected nor proof of the converse. However, the strong correlation between dimer formation and conditions/times yielding cross-linking of antiparallel dimer suggests that these conclusions are reasonable. Polylysine induces rapid formation of excimer, which decreases in abundance with time (Fig. 3B). The less potent polycation nucleating factor, the phosphorylation site domain of myristoylated alanine-rich protein kinase C substrate (MARCKS PSD), causes accumulation of excimer at a slower rate followed by depletion during polymerization (Fig. 3B). Polymerization of Ca²⁺-actin is accompanied by the formation of a small amount of excimer during the time interval that corresponds to filament nucleation (Fig. 3C), providing the first corroborating evidence of antiparallel dimer formation during in vitro polymerization of Ca²⁺-actin.

Crystal Structure of an Antiparallel Actin Dimer—Dimer homogeneity in the presence of latrunculin A unexpectedly implied that polylysine, even though it strongly promoted two-dimensional polymerization, could be exploited to make crys-

consistent with its known binding location (9).

The yield of cross-linked antiparallel dimer is substantially higher when polylysine is used to nucleate polymerization than has been previously reported for other polymer-inducing conditions (5). If cross-linking is performed as the polymerization reaction nears completion then the yield of antiparallel dimer is significantly less, and the major cross-linked product is then an intrafilament dimer with lower electrophoretic mobility. (Lane 7 of Fig. 2A shows the depletion of antiparallel dimer with time.) Both the cross-linking pattern of mature filaments and the normal appearance of negatively stained polylysine-induced filaments (20) suggest that polylysine does not alter filament structure. However, if the actin-monomer sequestering drug latrunculin A (13) is added at the initiation of polymerization, polymerization is arrested at the dimeric stage, and the antiparallel dimer accumulates (Fig. 2A, lane 6). The cross-linking reaction is not 100% efficient, and therefore monomer is expected to be visible on an SDS-denatured gel even if

![Fig. 2. Accumulation of antiparallel actin dimers with latrunculin A. A, SDS-polyacrylamide gel electrophoresis analysis of PDM covalent cross-linking of 3.0 µM Mg²⁺-actin subunits during the first 12 min of polymerization after addition of 2.0 mM MgCl₂ and polylysine. Cross-linking at a 0.5:1 ratio of PDM/actin is optimal for detection of antiparallel dimer with or without polylysine (lanes 1 and 2). Cross-linking at a 4:1 ratio of PDM/actin allows detection of intrafilament dimer (43) with varying polylysine concentrations (lanes 3–5, respectively). When 8 µM latrunculin A is added to prevent polymer formation, cross-linking at a 0.5:1 ratio of PDM/actin yields only antiparallel dimer with 3.0 µM actin and 1.5 µM polylysine (lane 6). Lane 7 is identical to lane 2 except actin was polymerized for 60 min prior to cross-linking. B, sedimentation equilibrium of actin (5 µM) with polylysine (2.5 µM) and latrunculin A (12 µM) in 2 mM MgCl₂ and 40 mM KCl. The solid line shows the best fit to the data assuming actin is dimeric, and the dashed line shows the best fit to monomeric actin. The upper panel shows the distribution of residuals relative to the theoretical curve for dimeric actin.
Crystal Structure of an Antiparallel Actin Dimer

FIG. 3. Pyrene excimer formation during actin polymerization. A, emission spectra (excitation at 343 nm) of four samples of 73% pyrene-labeled MgCl₂–actin (1 μM) immediately before (curves 1–4) or 20 min after (curves 5–8) initiation of polymerization by addition of 2 mM MgCl₂. Sample one (spectra 1 and 5) contained no polylysine and no latrunculin A, sample two (spectra 2 and 6) contained no polylysine and 15 μM latrunculin A, sample three (spectra 3 and 7) contained 5 μM polylysine and no latrunculin A, and sample four (spectra 4 and 8) contained 5 μM polylysine and 15 μM latrunculin A. Inset, expansion of the same spectra between 440 and 550 nm. Spectrum 9 is the difference between spectra 7 and 8. B, time course of excimer formation for 1 μM MgCl₂–actin in the presence or absence of 0.5 μM polylysine or 5.0 μM MARCKS PSD was followed at excitation wavelength 343 nm and emission wavelength 478 nm. The time course of polymerization of the same samples was followed at excitation wavelength 366 nm and emission wavelength 387 nm. C, time course of polymerization (circles) and excimer formation (squares) of 10 μM CaCl₂–actin after addition of 2 mM CaCl₂.

tals of actin. Successful crystallization conditions were as previously reported for a complex of latrunculin A and actin (13). The use of polylysine led to much more rapid crystallization, occurring in as little as a week at 4 °C compared with 3–5 months in the absence of polylysine at this temperature. These crystals diffracted X-rays to 3.5 Å resolution, and the structure was determined with refinement of the data to a crystallographic R-factor of 0.192 and Rfree of 0.252. Consistent with the biochemical data shown in Fig. 2, the structure reported here is that of an antiparallel dimer (Fig. 4). A disulfide bond can be identified between the Cys-374 residues (Fig. 5). In comparison, previously reported crystallographic structures of actin have either required the presence of an actin-binding protein that prevents polymerization (24, 25, 26), or more recently, a model of F-actin (29) and, moreover, is similar to that for profilin-bound actin in the open state, a structure hypothesized to be the precursor complex that adds to the barbed end of growing filaments (30). In light of a previous report that <0.1 kcal/mol might be required for the transition between the two most extreme positions of subdomain 2 shown in Fig. 6C, the full range of depicted conformations are likely represented in solution (30). Our results, therefore, suggest that the DNase I binding loop is likely molded into the observed conformation by its interaction with an antiparallel actin dimer. Alternatively, dimerization, like many other binding events at the C terminus of actin (31), may have induced allosteric changes in the conformation of the DNase I binding loop, but this is unlikely because both DNase I binding loops are not similarly structured in this otherwise symmetrical antiparallel dimer.

DISCUSSION

Nucleation of actin polymerization by antiparallel dimer as reported here has been previously observed qualitatively (7). The relationship between the crystallographic and solution structures of antiparallel actin dimer is of interest. The disulfide bond that we observe in the crystallographic structure very likely does not form until after crystallization is complete, given that the PDM cross-linking and analytical ultracentrifugation data prove definitively that the dimer exists in solution without a disulfide (the PDM reaction requires a reduced cysteine). The flexibility of the C terminus reported by others may account for the observation that a finite length, covalent cross-linking reagent such as PDM (~12 Å separates the reactive maleimide groups) can cross-link antiparallel dimeric actin in solution, whereas the cysteine residues are fixed in space at a distance of 6.8 Å (Ca to Ca) by the disulfide bond in the crystal structure. Similarly, the ability to form a pyrene excimer implies that the antiparallel dimer can accommodate a bulky fluorescent probe at the subunit interface. The two salt bridges found in the dimer interface provide a thermodynamic rationale for the stability of the dimer in solution in the absence of disulfide, and hydrodynamic data support the contention that the crystal and solution dimers are of similar shape. The expected sedimentation coefficient of the antiparallel actin dimer can be estimated directly from the atomic coordinates (11),
yielding a value of 4.85 S, a result that compares very well with the experimental value of 4.81 ± 0.10 S. In contrast to this end-to-end dimer, a compact side-to-side actin dimer yields a sedimentation coefficient of 5.25 S by the same methodology. Dynamic properties of the antiparallel dimer in solution remain unexamined and add potential complexity to this analysis.

In summary, the crystallographic dimer is probably not identical to that occurring in solution (and, in fact, the flexible C-terminal residues may not have a well defined structure in solution), but the evidence strongly supports the following: 1) that both crystallographic and solution structures are dimeric, 2) that the dimer interface in the crystal is similar to that present in solution, and 3) that the sedimentation coefficient is reasonable for a dimer of the shape predicted by the crystal structure.

When latrunculin A was soaked into gelsolin-actin crystals only minor changes were observed in actin structures involving loops between residues 55–66 and 197–207 in subdomains II and IV, respectively (15). Because of the absence of identifiable alterations that fully explain the inability of actin to polymer-

**Table I**

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**Refinement statistics**

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^a R_sym = Σ|I_obs - I_avg|/ΣI_obs, where the summation is over all reflections.

^b R-factor = Σ|F_obs - F_calc|/ΣF_obs.

^c For calculation of R_free, 5% of the reflections were reserved.

**FIG. 4.** Antiparallel actin dimer. Individual subunits are colored green and purple in ribbon-and-strand representation, parallel to the 2-fold axis (A) and perpendicular to the 2-fold axis (B). An arrowhead indicates the position of the ordered DNase I binding loop of subdomain 2 (only observed in one subunit of the dimer), and an arrow indicates the disulfide bond between the Cys-374 residues (depicted as ball-and-stick). Changes in the actin structure compared with previous reports are limited to the C terminus and subdomain 2. Polylysine is not resolved in the structure. Latrunculin A occupies a position adjacent to ATP, similar to that described for a structure of a gelsolin-actin crystal in which latrunculin A had been soaked into the crystal prior to diffraction data collection (15).

**FIG. 5.** Close-up view of the dimer interface at the barbed ends of each of the two subunits. Each monomer contributes two parallel helices to this interface. A, the four small helices are stabilized by two intersubunit salt bridges between residues Glu-361 and Arg-372. B, disulfide bond between the Cys-374 residues is revealed in a stereo plot of the 2F_o - F_c electron density contoured at 1.2σ and centered on a Cys-374 residue with slab thickness of 30 Å.

sis. In summary, the crystallographic dimer is probably not identical to that occurring in solution (and, in fact, the flexible C-terminal residues may not have a well defined structure in solution), but the evidence strongly supports the following: 1) that both crystallographic and solution structures are dimeric, 2) that the dimer interface in the crystal is similar to that present in solution, and 3) that the sedimentation coefficient is reasonable for a dimer of the shape predicted by the crystal structure.
The crystallographic contact reveals a large surface of interaction between the DNase I binding loop of subdomain 2 and subdomains 1 and 3 of another subunit (subdomain nomenclature is defined in Fig. 4B). This interaction involves the same surface as the site of longitudinal interaction between subdomain 3 and the DNase I binding loop that occurs along the long pitch axis of F-actin in the Lorenz model of the filament, a model based on low resolution diffraction data from F-actin gels (16, 32). Interestingly, the extended loop in our crystal structure reveals a much more extensive surface of interaction than that of the filament model (32). This is significant because a structure derived from crystals of profilin-actin contains actin-actin contacts of the DNase I binding loop with subdomain 4 and with the N terminus, and both contacts are distinct from those of the Lorenz model. The large surface area of the contacts in the profilin-actin crystal compared with those of the Lorenz model has led to controversy regarding the model’s validity (26). Normal mode refinements in F-actin structure support the conclusion that the DNase I binding loop cannot be uniquely oriented given available data (29), but similar to the crystallographic contact reported here, the refined filament structure includes long pitch axis subunit contacts with subdomain 1 at residues Ser-350 and Thr-351 (Fig. 6B). These results suggest that specific features of the crystallographic contact might be incorporated into the current model of F-actin without significant repositioning of the DNase I loop, resulting in more extensive long pitch helical contact and lower global free energy.

The crystallographic contact between the DNase I binding loop and subdomains 1 and 3 provides the basis for a hypothesis that explains the paradox regarding participation of an antiparallel actin dimer in the helical polymerization of parallel subunits. The extensive surface of interaction and hydrophobic burial suggests that this contact could reflect an authentic solution interaction. Because of stabilization of the mobile DNase I binding loop of a third actin subunit at the interface between the two subunits of the dimer, the association of a monomer with antiparallel dimer in this configuration is expected to be more favorable than the association of two parallel monomers, assuming otherwise similar binding surfaces. The stability conferred to the DNase I binding loop by its sandwiched position is apparent in the electron density map of the dimer in which the non-stabilized loop, in contrast, is disordered. Because the monomer-antiparallel dimer contact contains parallel-oriented subunits, this provides a mechanism to augment the association of dimeric subunits with parallel orientation. Although the orientation between parallel subunits in the crystal structure differs significantly from that of existing F-actin models, flexibility in the DNase I binding loop may stabilize polymerization. Crystals grown in the presence of latrunculin A (as reported here) similarly do not reveal significant changes in actin subunit structure that can be attributed to latrunculin binding. In the absence of atomic resolution data for F-actin (and data describing the free energy profile that restricts dynamic fluctuations in that structure), speculation that any small conformational change in the actin subunit might prevent polymerization is not verifiable. The ability of latrunculin A to prevent actin polymerization (but not antiparallel dimer formation) remains unexplained.
permit extensive realignment. Rotation of the DNase I loop-dimer of dimers with one antiparallel actin dimer outlined in red. B, illustration showing one subunit binding to an antiparallel actin dimer with the same orientation as in the dimer of dimers. (The ghosted image of a second subunit is shown only for comparison with A. The monomer is stabilized by the same crystallographic contacts as those occurring between antiparallel dimers.) The subunit then pivots on its DNase I binding loop in the direction of the blue arrow so as to reach the position shown by the subunit at the termination of the arrow. C, elongation at the barbed end of a filament following nucleation by an antiparallel dimer. As previously observed in samples viewed by electron microscopy (7), one subunit of the antiparallel dimer is not incorporated into the filament.

Experimental data consistent with a nucleus size of 2–5 actin subunits have been reported by others (19, 33, 34). Part of this discrepancy can be explained by differences in the definition of the nucleus (35, 36). A recent theoretical thermodynamic analysis by Sept and McCammon (2) did not consider possible conformational transitions between oligomeric actin structures. In the absence of this consideration, the effective nucleus size is defined by assumptions implicit in the structural model of the nucleus, and more specifically, by the number of different types of actin-actin contacts in the helical oligomer. Given a model (2) employing two different possible actin-actin intrafilament contacts, the addition of a fourth subunit to a trimer would represent the same free energy change as that associated with the addition of another subunit to a long filament (except for very small differences in change in entropy as estimated in Ref. 37). Theoretical log-log plots of actin polymerization (as in Fig. 1B) based on this model would yield a slope of 2, consistent with the experimental data (2, 36). However, conformational transitions were introduced by Oosawa and Asakura (18) as alternative pathways for nucleus assembly for helical polymers. To the extent that F-actin and G-actin subunit structures differ, the assumption of some conformational transition during nucleus formation is a necessity. The conformational transition implied by the diagram in Fig. 7B, like those introduced by Oosawa, may alter both the effective size of the nucleus and the expected dependence of the nucleation rate on the concentration of actin.

Given the results reported here, it is likely that mutations in the C terminus of actin would result in an aberrant formation of an antiparallel dimer. The severe nucleation defect in a yeast Cys-374 to Ser actin mutant (38) and the lethality of a yeast C-terminal-truncated mutant (39) are therefore consistent with a significant role for the antiparallel dimer as a filament precursor for in vitro nucleation and in vivo function. The abnormalities are not otherwise likely explained by disruption of intrafilament contacts between actin subunits because the Lorenz model shows no actin-actin contacts at the C terminus (32). Similarly, a filament model based on the crystallographic interface of profilin-actin crystals will not contain actin-actin contacts involving the C terminus because profilin occupies that surface (26). Allosteric effects originating at (or failing to originate at) the C terminus could provide an alternative explanation for the behavior of these mutants (31).

Covalently cross-linked intrafilament dimers are excellent nuclei for seeding actin polymerization (40). However, the formation of dimers with subunit contacts in the same configuration as intrafilament subunits is thermodynamically unfavorable (2, 19), as might be expected so as to suppress spontaneous nucleation in vivo and ensure ordered polymer formation productive for cell motility. With intrafilament dimer formation intrinsically unlikely, why might the antiparallel pathway to filament polymerization be useful? The antiparallel dimer, with its unique configuration, may be more amenable to specific regulation than a dimer that looks exactly like part of an actin filament. In fact, actobindin from *Acanthamoeba castellanii* is a specific inhibitor of nucleation that has been postulated to induce accumulation of a non-helical actin dimer (41, 42). Also, prior data imply that the antiparallel dimer may facilitate filament branching in conjunction with nucleation (7), which may be related to the mechanism of Arp2/3-mediated nucleation, either by accumulation of actin subunits in an antiparallel conformation or by an antiparallel arrangement of the actin-related subunits in the active complex. Finally, an antiparallel dimer may participate more specifically in nucleation than a comparable intrafilament dimer, which, depending on how it is stabilized by other actin-binding proteins, might be expected to be as competent for elongation or capping as for nucleation.

There are several questions associated with the model in Fig. 7. However, it should be remembered that no alternative explanation for paradoxical nucleation by antiparallel dimer has been proposed despite more than 20 years of investigation (22). Most notably, actin-actin crystallographic contacts may not be predictive of solution interactions. There is at present no evidence that the DNase I loop binds to antiparallel dimer in solution as seen in the crystallographic contact. More generally, there is also no evidence that a third subunit binds to the dimer, with Fig. 2B showing that there is no self-association of dimers at concentrations of up to ~10 μM. However, it should be noted that even if the equilibrium dissociation constant was in the range of 1–3 μM, this mechanism would still be 1000-fold more effective at creating a linear dimer than through an intrafilament dimer mechanism (2). There is also the concern that limitations in the range of mobility of the DNase I binding loop could prevent the conformational reorganization depicted in Fig. 7B, but this is tempered by the absence of proof that the solution structure of an actin filament is identical to any of the proposed filament models.

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Crystal Structure of an Antiparallel Actin Dimer

Polylysine Induces an Antiparallel Actin Dimer That Nucleates Filament Assembly: CRYSTAL STRUCTURE AT 3.5-Å RESOLUTION
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