Dynactin 3D structure: Implications for assembly and dynein binding

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Key words: dynein, microtubule, actin, single particle analysis, electron microscopy, 3D EM

Abstract

The multisubunit protein complex, dynactin, is an essential component of the cytoplasmic dynein motor. High resolution structural work on dynactin and the dynein/dynactin supercomplex has been limited to small subunits and recombinant fragments that do not report fully on either ≈ 1 MDa assembly. In the present study, we used negative stain electron microscopy and image analysis based on random conical tilt reconstruction to obtain a three-dimensional structure of native vertebrate dynactin. The 35 nm long dynactin molecule has a V-shaped shoulder at one end and a flattened tip at the other, both offset relative to the long axis of the actin-related protein (Arp) backbone. The shoulder projects dramatically away from the Arp filament core in a way that cannot be appreciated in 2D images, which has implications for the mechanism of dynein binding. The 3D structure allows the helical parameters of the entire Arp filament

core, which includes the actin capping protein, CP, to be determined for the first time. This structure exhibits near identity to F-actin and can be well fitted into the dynactin envelope. Molecular fitting of modeled CP-Arp polymers into the envelope shows that the filament contains between 7 and 9 Arp protomers and is capped at both ends. In the 7-Arp model, which agrees best with measured Arp stoichiometry and other structural information, actin capping protein (CP) is not present at the distal tip of the structure, unlike what is seen in the other models. The 3D structure suggests a mechanism for dynactin assembly and length specification.

Introduction

Dynactin's role as an important cofactor for cytoplasmic dynein is clear (reviewed in (1, 2)), but dynactin structure and its full range of functions remain enigmatic. Its distinct binding activities for dynein (3-5), microtubules (6, 7), dynein cargoes (8, 9) and regulators (10) allow dynactin to target and tether dynein to a wide range of cellular components. The dynactin particle has an unusual subunit stoichiometry and a highly asymmetric appearance when viewed in 2D (11). Ongoing structural and biophysical work has yielded important new insights into how the dynein mechanoenzyme works, both by itself (reviewed in (12-14)) and in conjunction with another cofactor, Lis1 (15). Deep understanding of the three-dimensional structure of dynactin, a fundamental component of the large and elaborate dynein motor assembly, will be essential for any future analysis of the organization and mechanical operation of the dynein supercomplex.

Vertebrate dynactin, like most species, has a mass of $\approx 1.1 - 1.2$ MDa and contains components not present in budding yeast (16). Structural analysis remains limited to particles isolated from native sources because dynactin's unusual subunit stoichiometry and the insolubility and unknown stoichiometry of its major constituent, Arp1, has precluded reconstitution from a recombinant source. This makes subunit and domain mapping based on tag localization unfeasible. Biochemical dissection and antibody labeling combined with electron microscopy have provided a good sense of subunit organization in the intact particle (11, 17). This approach, unfortunately, remains limited to the small pool of available reagents that bind epitopes exposed on the native particle surface.

The 11 distinct polypeptide constituents of vertebrate dynactin are assembled into a roughly L-shaped structure. The longer part, which mediates cargo interactions, is a ≈ 37 nm actin-like filament whose major component is the actin-related protein, Arp1. The filament has been estimated (based on densitometry of Coomassie blue-stained gels) to contain between 7 and 12 monomers of three actin-related proteins (Arps) along with the heterodimeric actin capping protein, CP, p62, p27 and p25 (the

subunits not found in budding yeast dynactin). In addition to several Arp1 protomers, the filament contains one Arp11, and, mysteriously, a single actin (Schafer et al., 1994). Antibody labeling of dynactin particles with affinity-purified, polyclonal IgMs have allowed CP to be localized at the end of the actin-like filament nearest the shoulder, identifying this as its plus or “barbed” end. Monoclonal antibodies to p62 label the other end of the filament (11, 17). Arp11 also maps to this site because it binds p62 directly (17). The primary sequence of Arp11 predicts the lack of much of a part of actin (subdomain 2) that is required for subunit addition at the filament minus or “pointed” end. Together, these findings have led to the assumption that Arp11 caps the pointed end of the Arp filament.

Dynactin’s conspicuous shoulder (the short part of the “L”) bears a thin, mobile arm. The shoulder/arm assembly comprises the critical dynein-binding p150^{Glued} component (reviewed in (1, 2)) plus two other subunits, dynamitin (p50) and p24, in a 2:4:2 complex (17). The arm, p150^{Glued} AA 1 - ≈350 (5) is visible on individual particles (11, 17) but not in averaged images (18, 19) because it adopts a range of angles relative to the Arp filament. p150^{Glued} binds microtubules via a small globular domain near its N-terminus. Work using recombinant peptides has mapped the dynein binding site to p150^{Glued} AA 415-530 (5) suggesting dynein binds the shoulder near the base of the arm although this has not been verified. The shoulder is tethered to the Arp filament by direct interactions between Arp and dynamitin AA 1-90 (20). p150^{Glued} AA 1005-1019 has been proposed to bind Arp1 directly (21), suggesting an additional mechanism of shoulder docking.

High resolution structural information on dynactin remains limited to crystal structures of small components and domains (p27, the p150^{Glued} CAP-Gly domain, and CP (10, 22, 23)). An understanding of dynactin as an intact complex remains elusive. The goal of the present study was to obtain a 3D structure of the entire holodynactin particle. This is not straightforward, because dynactin adsorbs onto EM substrates in a single highly preferred orientation (11, 18), which severely limits the angles from which it can be observed and hampers 3D reconstruction. EM analysis has allowed individual subunits of the actin-like filament to be discerned (18, 19) but the structure presented in the only published 3D reconstruction (19) lacked half of dynactin’s mass, the shoulder and the pointed-end binding complex. The structure of the capped Arp filament proposed in that study was later found to be incompatible with the known structure of the CP-F-actin complex (24), which further limits interpretation.

We present here a 3D dynactin structure reconstructed from EM images of negatively stained specimens subjected to random conical tilt. The structure, which is compatible with all published 2D structures (11, 17, 18, 25), reveals important new information. The shoulder is found to protrude dramatically from the

rest of the structure, suggesting a mode of dynein binding in which the shoulder hooks on the dynein tail, positioning the Arp filament between dynein's motor domains. Fitting of modeled Arp polymers of varying length bound to CP allows the range of filament lengths to be refined to between seven and nine Arp protomers (Arp1 plus Arp11 and actin). When the 7-mer filament, which agrees best with compositional analysis and the difference map, is fitted into the dynactin envelope, CP does not locate to the tip of the structure but is instead buried, contrary to what is commonly believed. CP is positioned on the particle surface at the tip in the alternative models. In all models, the shoulder and pointed end volumes at the ends of the structure appear as conspicuous, distinct entities, in contrast to the featureless filament reported previously (19).

Results and Discussion

Overall features

Freshly prepared chick embryo brain dynactin was used for 3D reconstruction because the particles appeared more homogeneous than available preparations of porcine or bovine brain dynactin (18). The chick brain dynactin preparation is highly pure and exhibits proper subunit stoichiometry (Fig. 1A). To get around the problem of preferred orientation, samples were negatively stained and subjected to random conical tilt (RCT) analysis (Fig. 1B-E). Images of a total of 3637 molecules (a standard number of particles for RCT analysis (26)) were centered and aligned based on cross-correlation and back-projected into 3D using SPIDER and Eos (26). The resolution of the 3D structure is 3.4 nm (Fig. S1A) based on the Fourier shell correlation at 0.5 cutoff (27). The raw, averaged, and re-projected images are well-matched (Fig. S1B - D) giving confidence to the quality of the reconstruction. Fig. 2 and Movie 1 show the 3D structure rotated about the x- and y-axes.

In 2D, dynactin is an elongated, highly asymmetric molecule whose two ends appear conspicuously different. The 3D structure reveals additional asymmetry (Fig. 2, Movie 1). Dynactin roughly resembles a human right hand that lands palm down on the EM substrate with the thumb corresponding to the shoulder and the fingertips to the pointed end structure. The exposed side ("dorsal face") of the shoulder is convex and relatively smooth, whereas the "palmar" face has two deep clefts (Fig. 2E, arrowheads). Overall the shoulder appears as a large protrusion with deep, conspicuous grooves on either side. The opposite end of the dynactin particle is dramatically tapered relative to the central Arp filament core.

The structure of the dynein/dynactin supercomplex is completely undefined. The dynein tail and shoulder/arm complex are both essentially dimeric structures, with the dynein tail comprising two protomers each of the heavy chain (N-terminal portion), intermediate chains, light intermediate chains

and three different light chains, and dynactin's shoulder/arm complex comprising two protomers each of p150^{Glued} and p24 complexed with four dynamitin protomers. Small fragments of the dynein intermediate chain and p150^{Glued} have been demonstrated to form a tetrameric complex (5) making it highly likely that the interaction between holodynein and holodynactin exploits the pseudo two-fold symmetry of the dynein tail and dynactin shoulder. The assembled dynein/dynactin super-complex, however, will not possess pseudo two-fold symmetry owing to dynactin's intrinsic asymmetry. The hook-like appearance of the dynactin shoulder suggests a novel mode of dynein binding in which dynein straddles dynactin with the motor domains positioned on either side of the shoulder protrusion. An interlocked binding mode would provide dynein with physical purchase on dynactin that might contribute to the interaction under load, compensating for the relatively low measured affinities of the intermediate chain/p150^{Glued} interaction (5).

Structure of the Arp polymer

Dynactin's structural core is a short, regular polymer of Arp1 capped by CP at the barbed end and Arp11 at the pointed end (11, 17). It contains a single actin monomer. As for all Arps, the Arp1 primary sequence predicts a canonical actin fold. Arp1, the most similar actin-related protein to actin, is the only Arp that forms filaments. Most of the residues involved in actin-actin contacts are conserved and Arp1 can copolymerize with actin (28, 29). Negative stain EM images of filaments assembled from purified native Arp1 suggest Arp1 filaments have highly similar structures to actin (30).

We determined and refined the helical parameters of the Arp filament using 2D images of dynactin reprojected from the 3D structure to generate a 3D model of the Arp polymer (see Methods; Supplemental Figure 1). Like F-actin, dynactin's Arp filament can be described as either two long-pitch right-handed helices with radii of 1.4 nm that wind around each other, or as a single left-handed helix with a helical pitch of ≈ 5.9 nm and a rotational angle between adjacent subunits of 169.5° (Figure S2). These parameters are similar to the 1.8 nm and 168° values obtained previously based on an averaged 2D image (18). Verification of these parameters in a 3D structure, and determination of filament handedness (which is not possible based on a 2D average), confirms that the Arp1/Arp11/actin polymer has a nearly identical structure to F-actin (31-34).

The Arp filament is estimated to contain between 7 and 12 protomers. The 3D dynactin structure provided a means to obtain a more precise estimate. To do this, we first calculated an Arp1 homology model based on the G-actin structure and then used the EM structure of the CP-F-actin complex (24) as a template to generate models of capped Arp polymers of varying length. These complexes have a dramatically

different structure from the 3D structure proposed earlier (19), before the CP-F-actin complex structure was available. To identify the best range of model polymers to fit, we performed quantitative densitometric analysis of Coomassie blue-stained gels to determine the stoichiometry of dynactin's Arp components (Figure S3). This revealed two bands, a major band comprising Arp1 plus actin and a minor, slightly higher M_r band of Arp11, in a molar ratio of 6.5 ± 0.7 to 1 (mean $\pm$ S.D.; $n = 7$). This indicates that the Arp polymer contains a total of 7, 8 or 9 protomers, (i.e., 5/6/7 Arp1 plus 1 actin and 1 Arp11), with 7 or 8 being most likely.

We then fit the modeled polymers into the dynactin envelope. In agreement with the measured Arp stoichiometry, polymers longer than 9 subunits did not fit within the envelope (Figure S4), allowing Arp stoichiometries of 10, 11, or 12 to be excluded. This again indicates that the 10 Arp protomer dynactin structure proposed earlier (19) is probably incorrect. In all fits, some Arp1 loops extend outside the 3D envelope, but these correspond to loops that are flexible in actin and thus not seen in the 3D reconstruction because they are averaged out. The 7-subunit (5 Arp1) model fit the 3D structure well (Fig. 3A-C, Movie 2). The 8-subunit polymer (6 Arp1) could be fit in two different orientations, rotated 168° about the filament long axis (Fig. 3 D-I). In one orientation (Fig. 3 D – F), one-third of the CP α subunit, including the rigid β sheet, was positioned outside the 3D envelope, so this model was excluded from further consideration. In the other orientation, the 8-subunit filament was readily accommodated in the 3D envelope (see also Movie 3). A 9-subunit model also fit within the envelope (Fig. 3J-L; Movie 4), however a stoichiometry of 8 Arp1 plus actin : 1 Arp11 is on the outer limit of our densitometric quantitation (Figure S3).

The 2D average shows a conspicuous gap in density near the shoulder beyond which the lobes of density do not follow the helical symmetry of the rest of the polymer, suggesting these are not part of the Arp filament (18). Such a structure is consistent with the 7-protomer model but not the 8- and 9-mer structures whose polymer helices continue uninterrupted across the gap (between protomers 1 and 3 in Figure 3G and J). It is possible that dynactin contains a 8 or 9 protomer polymer but that its structure is distorted in this region due to association with shoulder components (e.g., dynamitin (20)) or because of a sample preparation artifact, so these models cannot be rigorously excluded. However, the stoichiometry and volume-based mass estimates (see below) strongly favor the 7-mer filament, which contains 5 Arp1 protomers complexed with 1 conventional actin and 1 Arp11, so this is our working model (cartooned in Figure 5).

Structures of the shoulder and pointed end volumes

Dynactin masses of 952 kDa, 995 kDa, 1039 kDa were calculated based on the 7, 8 and 9 protomer polymers (5/6/7 Arp1, 1 Arp11, 1 actin; Table I). All are within experimental error of our initial 1100 – 1200 kDa estimate based on scanning-transmission EM (11). Subtraction of these modeled filaments from the 3D envelope yields difference maps (“shells”) that correspond to the shoulder and pointed end structures (Fig. 4). Distinct volumes are present at the ends of the filament (shoulder, yellow; pointed end structure, white). The shoulder resembles an arrowhead that is offset relative to the Arp filament. The two end volumes make minimal contact with one another, with the closest point of approach lying roughly in the middle of the molecule (arrows).

Biochemically isolated shoulder/arm comprises two p150^{Glued} protomers, four dynamitin protomers and two p24 protomers, for a total predicted Mr of $\approx$ 495 kDa (17). The N-terminal p150^{Glued} arm (AA 1 - $\approx$ 350; mass $\approx$ 75 kDa) is highly mobile and does not contribute to the volume of the averaged image. This yields $\approx$ 420 kDa of mass attributable to the shoulder in the 3D reconstruction. This value agrees very well with the shoulder mass estimated from the volume of the 7 Arp model (Table I). A $\approx$ 420 kDa mass is also in reasonable agreement (10% error) with mass estimates based on the 8 and 9 Arp models.

Inspection of the contacts between the shoulder and Arp filament provides insight on the protein-protein interactions that support shoulder docking. Although the shoulder makes intimate contact with CP, biochemical interactions between CP and shoulder subunits have never been reported. The shoulder is also proximal to multiple Arp protomers. Biochemical work has shown that two dynamitin protomers are tightly and directly associated with the Arp filament via their disordered N-termini (35, 36) (37). We propose that interactions between two dynamitin protomers and two Arp protomers is a major mechanism of shoulder anchoring. This would leave the N-termini of the other two dynamitin protomers free to interact with extrinsic binding partners. The shoulder-filament link may be further stabilized via the C-terminal portion of p150^{Glued} which is required for stable association of p150^{Glued} with the Arp filament (38). This part of p150^{Glued} contains a motif similar to the actin-binding site of villin (21).

The pointed end structure (Fig. 4, white) “caps” the other end of the Arp filament. Its dimpled appearance in the reconstruction is reminiscent of the structure of isolated pointed end complex particles imaged via rotary shadow EM (17). Starting with a tapered distal tip (Fig. 2D, Fig. 4 B, F, J), the pointed end structure widens and extends along the Arp filament. The sequence-based mass of the corresponding components (p62, p27, p25) is $\approx$ 94 kDa. This agrees best with volume-based mass estimates based on the 7 and 9-mer models (Table I). The volume of the flattened tip that extends beyond the end of the filament

($\approx 45 - 54$ kDa; Table I, Figure 5) readily accommodates the ≈ 41 kDa p27/p25 dimer, which is a paralleloid prism with dimensions of $\approx 4 \times 6 \times 4$ nm (10). The 2D averaged image exhibits a conspicuous gap in density in this region that may correspond to the p27/p25 dimer interface (18).

Our molecular fitting suggests two possible positions for CP, one buried within the structure (7-mer, Fig. 3 A-C, Figure 5) and the other exposed at the distal tip (Figs. 3D-F and G-I). Decoration of individual dynactin particles with polyclonal IgMs to CP (11) indicated that some CP epitopes, which are unknown due to the polyclonality of the antibody, are exposed on dynactin's surface. This has led to the long-standing assumption that CP occupies the extreme end of the dynactin structure, a model that served as the basis of the previous 3D reconstruction (19). The shielded position of CP in the favored 7-Arp model (Figure 5) would provide very limited access to the F-actin uncapping protein CARMIL (39-41) and contribute to dynactin's long half-life in vivo (42). To distinguish between the surface and buried positions of CP we attempted to decorate dynactin with the CP β mAb, 3F2 (43), but this effort was unsuccessful because the Ab only recognizes the denatured epitope.

Proposed mechanism of Arp filament length specification

The remarkably uniform size of the Arp1 filament indicates that dynactin assembly involves a mechanism for specifying polymer length (11, 18). Previous models have favored a molecular ruler analogous to tropomyosin, possibly p150^{Glued}, that binds a specific number of Arp protomers (17, 21). Another candidate is dynamitin, which binds Arp1 (37) and, in yeast, Arp11 (44). With a stoichiometry of 4 protomers per dynactin particle, dynamitin could possibly scaffold several Arp protomers, but our work indicates that only two dynamitin protomers are tightly bound to the Arp1 filament (35, 36), limiting this possibility. Each end structure appears to make contact with multiple Arp protomers. We propose that dynactin assembly involves formation of two subcomplexes comprising each end structure plus a small Arp1 oligomer. These are joined to form the intact complex in a process that might allow an actin monomer to be incorporated at the junction. This model takes into account the observations that Arp1 forms stable dimers and trimers (30) and that it can copolymerize with G-actin (28, 29). Furthermore, it offers an unexpected answer to the long-standing questions of why dynactin includes a single monomer of conventional actin and where it is located. In conclusion, the 3D dynactin structure presented here provides a fresh perspective on a possible dynein binding mechanism and assembly pathway and will be a solid basis for further high resolution analysis of dynactin alone and the dynein-dynactin supercomplex.

Acknowledgements

We thank Prof. Masayuki Kajitani (Teikyo U) for purified TMV. This work was supported by an ERATO

grant from JST (Japan Science and Technology Agency; YM), a Grant-in-Aid for Scientific Research (S)-20227008 from JSPS (Japan Society for the Promotion of Science; YM and AN), and by a Special Fund from the Takeda Science Foundation (YM). HI was partly supported by HFSP (ref: RGP9/2008-C) and BBSRC (ref: BB/K000705/1) grants to Dr. Stanley Burgess (University of Leeds) and under the International Collaborative Research Program of the Institute for Protein Research, Osaka University. TAS was supported by NIH grant GM044589.

Materials and Methods

Dynactin purification

Dynactin was purified via microtubule-affinity as in (18) with minor modifications (see Supp. Meth.).

Electron microscopy

Dynactin (15 $\mu\text{g/ml}$) was mixed with tobacco mosaic virus (30 $\mu\text{g/ml}$) in PMEE buffer (45) in the presence of ~ 30 mM KCl and negatively stained with 1% uranyl acetate on glow-discharged carbon grids. Dynactin images were collected as described (18) with a nominal magnification of 20,000, first with the stage tilted (60°) and then untilted (0°). The EM was equipped with a low dose mode (~ 900 electrons/ nm^2 per image).

Image analysis

Image films were scanned using a film scanner (Z/I Photoscan 2000) at ~ 0.35 nm/pixel on the image scale; images were analyzed using Eos (46) and SPIDER (26). Magnification was calibrated using the 2.3 nm layer line of tobacco mosaic virus (47). The images were low-pass filtered and high-pass filtered at 1.4 nm and 66.67 nm, respectively. The contrast transfer function was corrected by Eos (untilted images) or according to (48) (tilt images). Particles were selected from untilted datasets (in the Web; (26) that exhibited a ~ 40 -nm filament and generally resembled the 2D averaged dynactin image (18). Only particles with the exposed “B” side ($>90\%$; the strongly preferred orientation; (18) were used because “A” side particles were less homogeneous. The tilted images were centered by correlation with compressed images of 2D averaged dynactin (18) at magnification of cosine [tilt angle = 60°] in the direction perpendicular to the tilt axis. The 3D reconstruction of an initial model was done using SPIDER, then refined via projection matching (26) by Eos. The handedness of the Arp polymer was determined as previously described (49)

Fitting of Arp1 filament model bound with CP

The helical parameters of the Arp polymer were determined by fitting helically arranged spheres to five different 2D images reprojected from the 3D image. Reprojection images at -20° , -10° , 0° , $+10^\circ$, and $+20^\circ$ (0° corresponds to untilted images) around the long axis were used for this analysis to minimize effects of flattening (50) and the missing cone problem (51). An Arp1 atomic model was generated using MODELLER (52) based on an F-actin subunit template (32), then helically arranged according to these parameters to generate an Arp1 polymer model. The CP atomic structure (23) was placed on Arp1 polymer models so as to match the CP-actin complex model (24). The resulting CP-Arp polymer was fitted into the density map of the refined 3D dynactin structure using a 3D cross-correlation function. All molecular graphics images were produced using UCSF Chimera (53).

FIGURE LEGENDS

Figure 1. Chick embryo brain dynactin analyzed by SDS-PAGE and negative stain EM. A: A 5%-20% Coomassie blue stained gel of a typical preparation; subunits are labeled. The preparation is highly pure and contains no contaminants. MS on a subset of the very faint bands between p150^{Glued} and p62, which are vastly substoichiometric to p27 and not consistently observed, showed them to be minor degradation products of p150^{Glued}. B, C: Three negatively stained dynactin particles imaged untilted (B) or tilted 60° (C) show the projecting p150^{Glued} arm. The tracings on the right depict the raw images in the left hand panels. (D, E) A typical field on an untilted specimen stage (D) or tilted 60° (E). The tilt axis is indicated by the black line; particles are circled. Bar = 50 nm.

Figure 2. Dynactin 3D structure. The reconstruction (100% volume; low pass filtered at 3.4 nm) was rotated at 45° intervals along its long axis (B, D, E, F) or the perpendicular axis (A, B, C); see also Fig. 2 Movie). B corresponds to the side that is usually exposed (“dorsal” face) and E to the side that adsorbs to the substrate (“palmar” face). Arrowheads in E indicate deep clefts flanking the shoulder that we propose contribute to dynein anchoring. Bar = 10 nm.

Figure 3. Models of CP docked on a modeled Arp1 polymer (see Methods) were fit into the 3D envelope (120% volume; low pass filtered at 3.4 nm). A, B, C: seven Arp protomers (see also Fig. 3 Movie); D, E, F and G, H and I: eight Arp protomers with the core in the two possible orientations, J, K, L: nine Arp protomers. CP α and β subunits are pink and cyan, respectively, Arp11 (the Arp protomer at the filament pointed end) is green, and the remaining Arp protomers are numbered (odd = blue; even = red). A, D, G and J show the dorsal view. B, E, H and K show dynactin rotated about the filament long axis by 85° . C, F, I and L show a view from the barbed end (rotated 104° about the Y-axis, as in Figure 2A). Portions of

CP that extend outside the envelope in D and F are circled. Bar = 10 nm. The gap in density seen in the 2D average (18) is distal to Arp protomer 1 in A, and between Arp protomers 1 and 3 in G and J.

Figure 4. The two end complexes and the Arp polymer core. The volume of the CP-Arp filaments of different lengths (7-mer, A-D; 8-mer, E-H; 9-mer, I-L) were subtracted from the 3D envelope to yield a “shell” structure (i.e., difference map; the pale mesh indicates 100% volume) comprising the other subunits. The CP-Arp filament core is illustrated using ribbon diagrams (as in Fig. 3). A, E, I: dorsal views. The other images are rotated about the long axis in 90° increments (B, F, J: 90°, C, G, K: 180°, D, H, L: 270°). The green space-filled residues on the Arp protomers represent the C-termini (K360 - D366). The point of closest contact between the two end complexes are indicated with arrows. Bar = 10 nm.

Figure 5. Model of dynactin based on the 3D EM structure. The shoulder/arm subunits p150, p50 and p24 are yellow; pointed-end complex subunits p62, p27 and p25 are white. Components of the Arp filament core are labeled. We propose that the N-termini (AA 1-90) of two dynactin protomers are associated with the two Arp1 protomers nearest CP. The location of the projecting arm, which is mobile and not seen in the averaged image, is indicated, with the dotted line indicating its range of movement. The structure cartooned here (5 Arp1, 1 Arp11, 1 actin) fits best with the existing data, although the alternative models cannot be rigorously excluded (see text).

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Table I

Comparison of sequence-based M_r s and volume-based mass calculations for different length Arp filaments

	7-mer	7-mer	8-mer	8-mer	9-mer	9-mer
Structure	Predicted M_r	Volume -based mass	Predicted M_r	Volume -based mass	Predicted M_r	Volume- based mass
Dynactin (including sidearm)	952,325	NA	994,970	NA	1,037,617	NA
Dynactin (no sidearm)	875,968	876 kDa	918,610	919 kDa	961,260	961 kDa
Core (CP-Arp1, Arp11, actin)	364,818	372 kDa	407,464	424 kDa	450,110	481 kDa
“Shell” (no sidearm)	511,150	504 kDa	511,150	495 kDa	511,150	481 kDa
Shoulder (no sidearm)	417,376	418 kDa	417,376	357 kDa	417,376	380 kDa
Pointed end structure (no Arp11)	93,773	86 kDa	93,773	138 kDa	93,773	101 kDa
Pointed end distal tip	ND	45 kDa	ND	ND	ND	54 kDa

Predicted M_r s are from chicken sequences (p27: mouse; see Supplement for accession numbers). Volume-based mass calculations were made for filaments containing 7, 8 or 9 Arps (variable numbers of Arp1 plus one Arp1 and one actin) in which CP is entirely contained within the envelope (as shown in Figure 3A-C and G-L). “Dynactin (no sidearm)” excludes AA 1-350 of p150^{Glued} which comprises the mobile arm (Siglin et al., 2013).

Masses were calculated from 3D volumes set at 100% (1.35 g/cm³ density). NA: By definition, these masses are the same as the predicted M_r s. The mass of the dynactin core was calculated by treating each atom in the modeled CP-Arp filament as a 2.0Å radius sphere. The volume-based mass of the dynactin shell (i.e., the difference map generated by subtracting the core from the entire volume) was estimated by subtracting the core mass from the total mass. The volumes assigned to the shoulder and pointed end structures were assigned based on their location and breaks in envelope continuity. When possible (i.e., for the 7-mer and 9-mer), the pointed end structure was further divided by visual inspection. ND: Cannot be determined based on available information.

Figure 1

Figure 2

Figure 3

Figure 3

Figure 4

Figure 4

Figure 5