Ultrastructural Analysis of the Dynactin Complex: An Actin-related Protein Is a Component of a Filament That Resembles F-actin

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Abstract. The dynactin complex visualized by deep-etch electron microscopy appears as a short filament 37-nm in length, which resembles F-actin, plus a thinner, laterally oriented filament that terminates in two globular heads. The locations of several of the constituent polypeptides were identified on this structure by applying antibodies to decorate the dynactin complex before processing for electron microscopy. Antibodies to the actin-related protein Arpl (previously referred to as actin-RPV), bound at various sites along the filament, demonstrating that this protein assembles in a polymer similar to conventional actin. Antibodies to the barbed-end actin-binding protein, capping protein, bound to one end of the filament. Thus, an actin-binding protein that binds conventional actin may also bind to Arpl to regulate its polymerization. Antibodies to the 62-kD component of the dynactin complex also bound to one end of the filament. An antibody that binds the COOH-terminal region of the 160/150-kD dynactin polyepitides bound to the globular domains at the end of the thin lateral filament, suggesting that the dynactin polypeptide comprises at least part of the sidearm structure.

The dynactin complex, an activator of cytoplasmic dynein-mediated vesicle movement on microtubules in vitro, is composed of at least nine polyepitides (Gill et al., 1991; Schroer and Sheetz, 1991). These components cosediment at 20 S on sucrose gradients, copurify on ion exchange columns, and antibodies to the individual polyepitides of the complex immunoprecipitate all the other components (Gill et al., 1991; Paschal et al., 1993). The largest polyepitide of the dynactin complex from chick embryo brain is pl60/pl50 dynactin (Gill et al., 1991); cDNAs encoding homologues of this protein have been obtained from chicken (Gill et al., 1991), rat (Holzbaur et al., 1991), and Drosophila melanogaster (Swaroop et al., 1987). Analysis of the dynactin polyepitides in chicken tissues reveals the existence of at least two electrophoretically distinct forms (Gill et al., 1991). The Drosophila homologue is the product of the essential Glued gene (Swaroop et al., 1987), mutations in which cause death during embryogenesis. The original Glued allele displays a dominant phenotype, exhibiting defects in neuronal development and eye morphogenesis (Meyerowitz and Kankel, 1978; Garen and Kankel, 1983).

The most abundant polyepitide in the dynactin complex is an actin-related protein originally named actin-related protein from vertebrates (actin-RPV)1 (Lees-Miller et al., 1992); the human homologue of this protein is referred to as centrinactin (Clark and Meyer, 1992). These proteins comprise one subgroup, the Arpl class, of the recently discovered family of actin-related proteins (Frankel et al., 1994; Fyrberg et al., 1994). To date, five distinct classes of actin-related proteins have been distinguished on the basis of their predicted amino acid sequences. All these proteins show moderate similarity to conventional actins. Members of each class from different species are highly homologous (70–80 % identical; Fyrberg et al., 1994) but the different classes of actin-related proteins are no more similar to each other than they are to actin, suggesting that each plays a distinct role in the cell. The identification of Arpl as a major component of the dynactin complex allows us to consider how this vertebrate actin-related protein might be involved in regulating dynein-mediated, microtubule-based motility.

The primary sequences of the different actin-related proteins allow us to make predictions about their biochemical properties. When compared to the actin crystal structure (Kabsch et al., 1990), the most highly conserved sequences of all actin-related proteins map to regions of the molecule that form the nucleotide and divalent cation binding sites (Holmes et al., 1993). Sequences that are less conserved between actin-related proteins and conventional actin are predicted to lie on the protein surface, in regions that in conventional actin are important for polymerization and for interaction with actin-binding proteins (Kabsch et al., 1990). Whether or not the actin-related proteins bind nucleotide or associate as polymer in vivo is not known; however, Arpl synthesized in vitro binds to columns of immobilized ATP and GTP and copellets with native brain actin under condi-

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1. Abbreviations used in this paper: actin-RPV, actin-related protein from vertebrates; AMP-PNP, 5′adenyl-imidodiphosphate; OMDR, optical memory disk recorder; STEM, scanning transmission electron microscopy.
tions that favor polymerization of conventional actin (Melki et al., 1993).

As a first step to understand how the dynactin complex stimulates dynein-mediated motility, and, in particular, how Arpl contributes to this process, we determined the fine structure of the dynactin complex and mapped the locations of several of its components on this structure. To do this, purified dynactin complexes, with and without bound antibodies, were adsorbed to mica flakes, freeze-dried, and rotary replicated with platinum (Heuser, 1983, 1989). These experiments revealed that the dynactin complex has two major structural domains: a 37-nm long 10-nm wide filament and a projecting sidearm containing a thin, 24-nm long filamentous extension. The 37-nm long filament strongly resembles a short polymer of conventional actin. Decoration of the dynactin complex with antibodies to several of its polypeptide components revealed that the 37-nm long filament binds antibodies to Arpl, antibodies to capping protein, a barbed-end actin-binding protein, and antibodies to the 62-kD component of the complex. The globular heads at the end of the thin lateral filament bind antibodies to COOH-terminal domain of the 160/150-kD dynactin subunit.

Materials and Methods

Materials

Taxol was provided by Dr. Nancita Lomax at the National Cancer Institute or was purchased from Calbiochem (La Jolla, CA). The MAPS II kit and reagents for polycrylamide gel electrophoresis and the Bradford protein assay were from BioRad Co. (Richmond, CA). Ampholytes were obtained from Pharmacia LKB Biotechnology (Piscataway, NJ). Immunoblot reagents were purchased as a kit from Troplix (Danvers, MA). Ultrapure Tris and sucrose were obtained from ICN Biomedicals (Cleveland, OH). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Fertilized chicken eggs were obtained from Spaafs, Inc. (Reinholds, PA). The MonoQ anion exchange column was from Pharmacia LKB Biotechnology.

Purification of Dynactin Complex, Dynein, and Actin

Dynactin complex and cytoplasmic dynein were purified as previously described (Schorer and Sheetz, 1991) with the following minor modifications. Protein was isolated from 40 gm of embryonic chick brains or from 2 x 10^9 chick embryo fibroblasts (cultured in roller bottles). Endogenous microtubules in the high speed supernatant were augmented with phosphocolluiose-purified bovine brain microtubules (final concentration 0.25 mg/ml). Binding to microtubules was induced by addition of 40–50 U hexokinase, 400–500 mg glucose and 1 mM MgCl2, 0.1 mM phenylmethyl sulfonyl fluoride (AMP-PPN). Microtubule pellets were washed by resuspending in homogenization buffer containing 1 mM GTP followed by pelleting. Microtubule-binding proteins were eluted with 10 ml of 0.2 mM sodium vanadate and 2 mM ATP. The vanadate/ATP release was layered onto two 34 ml, 5–20% sucrose gradients and centrifuged in a SW28 rotor at 4°C for 17 h at 28,000 g. MonoQ chromatography was as described previously except the salt gradient was modified to optimize protein concentration as well as separation of dynein and dynactin complex. From 40 gm brains, the average yield of dynactin complex was ~500 µg in a total of three one ml fractions. Conventional actin was obtained from the MonoQ column, eluting before both dynein and dynactin complex (Schorer and Sheetz, 1991). Capping protein was purified from chicken pectoral muscle as described (Caldwell et al., 1989a). Purified proteins were stored on ice.

Velocity Sedimentation of MonoQ-purified Proteins

Dynein or dynactin complex (0.5 ml) was overlaid on a 5-ml gradient of 5–20% sucrose in 35 mM K-Pipes, pH 7.2, 5 mM MgSO4, 1 mM EDTA, and 0.5 mM PMSF (PME' buffer). The samples were centrifuged for 4 h at 45,000 rpm in a SW30 rotor. Eleven 0.5-ml fractions were collected from the bottom.

Antibodies

Monoclonal antibodies (mAb) 45A, 62B, C4 (Lessard, 1988; generously provided by Dr. J. Lessard) and 150.1 (Steuer et al., 1990) were used in the form of IgG purified from ascites fluid by protein A affinity chromatography with the MASP II kit (BioRad Co.). mAb H111, specific for the α-subunit of capping protein, was purified on a Protein A column as described (Hug et al., 1992). Goat anti-skeletal muscle capping protein antibodies (Casella et al., 1989) and antibodies specific for the β-subunit of capping protein were affinity purified on skeletal muscle capping protein-Sepharose and Sepharose conjugated with bacterially expressed β-subunit fusion protein, respectively (Schafer et al., 1992). The capping protein β-subunit antibodies are a mixture of IgGs and IgMgs. Anti-tropomyosin monoclonal antibody C1 (Hegmann et al., 1988) was a gift of Dr. J. Lin. Anti-fimbrin antibodies were gifts of Drs. T. Bretscher (Tilney et al., 1989) and M. Mooseker (Heintzelman and Mooseker, 1990). Anti-fascin antibodies were a gift from Dr. FumioMatsumura (Yamashiro-Matsumura and Matsumura, 1985). Anti-tropomodulin was a gift from Dr. W. Fowler (Fowler et al., 1993).

Monoclonal antibodies 45A and 62B: Female Balb/c mice were immunized with 15 µg purified dynactin complex in RIBI adjuvant (RIBI, Inc., MT) at three week intervals until they were seropositive at a 1:10,000 dilution. Hybridomas were prepared as described (Harlow and Lane, 1988) using Sp2/0 as a fusion partner. Antibodies were screened by ELISA using purified dynactin complex or ATP release, and then by immunoblots on SDS gels. mAbs 45A and 62B were found to be IgGs by radial immunodiffusion using a monoclonal isotyping kit (The Binding Site, La Jolla, CA).

Immunolabeling of Dynactin Complex

Dynactin complex after MonoQ chromatography was diluted in PME' buffer to yield ~150 mM KCl. Dynactin complex and purified IgGs were mixed at a 1:1 ratio by mass (5 µg of each component) and allowed to incubate for 1 h at room temperature. Samples were diluted in 70 mM KCl, 30 mM Hepes, pH 7.2, 5 mM MgCl2, and 3 mM EDTA to a final volume of 0.5 ml in preparation for electron microscopy (Heuser 1983, 1989).

Electron Microscopy

Protein samples were adsorbed to mica flakes, quick-frozen, freeze-dried and rotary-replicated with platinum as previously described (Heuser, 1983, 1989). To prepare the photomicrographs, the electron microscope negatives were placed on a light box and images of selected molecules were captured with a video camera mounted over the light box and recorded on an optical memory disk recorder (OMDR); prints of the OMDR recordings were obtained using a Sony video printer and compiled as montages. The montages were scanned using an ES-300 Scanner (Epson America, Inc., Torrance, CA) and the contrast and brightness levels of the scanned images were adjusted for optimal contrast using Adobe Photoshop version 2.5 (Adobe Systems Inc., Mountain View, CA). Copies of the images were obtained using a Kodak Colorseam PS printer (Eastman Kodak Co., Rochester, NY).

Gel Electrophoresis, Immunoblotting, and Protein Assay

Samples were analyzed by SDS-PAGE as described (Laemmli, 1970) and gels were stained with Coomassie brilliant blue or silver nitrate (Merril et al., 1981). Immunoblotting was performed as described (Tobin et al., 1979) except that in some cases PVDF membrane (Immobilon P; Millipore Corp., MA) was used instead of nitrocellulose. Alkaline phosphatase–conjugated goat anti–mouse second antibody was detected using a chemiluminescent (AMPD or CSPD from Tropix, Inc., Danvers, MA) or chromogenic substrate. Samples were assayed for protein by the method of Bradford (1976).

Two-dimensional electrophoresis was performed as described (O'Farrell, 1975). For Fig. 1, samples of dynactin complex were solubilized by boiling in SDS sample buffer (Laemmli, 1970), cooled, and mixed with an equal volume of urea sample buffer before being applied to the gel. For Fig. 6, samples were put directly into urea-Trition-X-100 sample buffer containing ampholytes.

Quantitative Immunoblotting of Actin

Five twofold serial dilutions of dynactin complex (0.14–2.24 µg) and a chick brain actin standard (21–335 ng) were run on SDS gels and transferred to PVDF membrane. The blots were probed with mAb C4 and actin bands

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were visualized using chemiluminescence. X-ray films were scanned on an LKB Ultrascan laser densitometer (Pharmacia/LKB Biotechnology, Piscataway, NJ). An actin standard curve was generated by plotting peak intensities vs actin concentration; the standard curve was linear over 3 or 4 of the 5 points. The dilutions of dynactin complex used yielded actin bands whose intensities fell within the linear region of the curve.

**Epitope Mapping of mAb 150.1**

Dynactin peptides were generated by the method of Cleveland (Cleveland, 1983). Briefly, dynactin complex polypeptides were resolved on a 10% polyacrylamide minigel which was lightly stained with Coomassie brilliant blue. The p150 and p160 dynactin bands were excised, equilibrated in gel slice equilibration buffer, loaded on a second gel (15% acrylamide), overlaid with endoproteinase GluC (V8 protease), and then electrophoresed into the gel. As visualized by silver staining, p150 and p160 yielded indistinguishable peptide maps. To determine which peptides bound mAb 150.1, an identical gel was analyzed by immunoblot with mAb 150.1; p150 and p160 also yielded identical peptide maps by this means of analysis. Immunoreactive peptides were then subjected to NH₂-terminal sequencing as described below.

**Peptide Sequencing**

Peptides from p160/p150 dynactin and p32 (capping protein β subunit) were generated by Cleveland mapping as described above. To obtain adequate amounts of protein for sequencing it was necessary to load several bands from the initial preparative gels onto a second, 1.5-mm preparative gel before generating endoproteinase GluC peptides. For capping protein β-subunit, ~4 μg protein (6 bands; no attempt was made to separate the two isoforms as they yielded identical peptide maps) was digested with 2 μg endoproteinase GluC. Peptides were transferred to PVDF membrane and visualized by transillumination or by staining with Ponceau S. Individual bands were excised and subjected to NH₂-terminal microsequencing on an ABI sequencer at the Protein/Peptide Laboratory at the Johns Hopkins Medical School.

**Results**

**Arpl Is the Predominant Polypeptide in the Dynactin Complex**

The major component of the dynactin complex is a 45-kD polypeptide that is present in approximately ten copies per complex as determined by densitometry of one-dimensional SDS gels (Fig. 1 A, Table I and Gill et al., 1991). Two-dimensional gel electrophoresis (Fig. 1 B) resolves the 45-kD band into three spots: the major spot is Arpl with pI ~ 6.8; minor components are conventional actin (Lees-Miller et al., 1992) and an unidentified 45-kD protein with pI ~ 6.7. Densitometric analysis of two-dimensional gels showed the stoichiometry of Arpl was 9 mol/mol of dynactin complex and conventional actin was estimated at 0.5–1 mol/mol of dynactin complex. Scans of one-dimensional gels allowed the stoichiometry of the p160/p150, p62, p50, p37, p32, p27, and p24 polypeptides to be estimated at 2:1:5:1:1:1 (Table I). The relative stoichiometry of the major components (p160/p150, p50 and Arpl/actin) is 2:5:10, similar to the estimated stoichiometry of 1.5:4:10 for the analogous subunits of bovine brain dynactin complex (Paschal et al., 1993). On the basis of this stoichiometry the mass of the complex is calculated to be 1.2 MD, consistent with that predicted for a 20 S particle. The mass of the dynactin complex was determined by scanning transmission electron microscopy (STEM) analysis to be 1.11 ± 0.12 MD (mean ± SD; n = 151).

Although most of the conventional actin is separated from dynactin complex by MonoQ ion-exchange chromatography (Schroer and Sheetz, 1991) trace amounts of actin remain in purified dynactin and dynactin complex preparations (Fig. 1 B and Fig. 2 A, bottom panel, lane L; Lees-Miller et al., 1992). To determine whether or not conventional actin is a bona fide component of the dynactin complex, MonoQ-purified dynactin complex was subjected to resedimentation

![Figure 1](https://example.com/figure1.png)

**Figure 1.** The polypeptide composition of dynactin complex purified from embryonic chicken brain. Polypeptides were resolved on one dimensional (A) and two-dimensional (B) gels (the SDS gels were 10% acrylamide). The pH gradient is indicated below panel B. Molecular weight markers for the two gel sets were 12 (to the right and below the predominant p50 spot, pI 5.5; see also Fig. 3 in Lees-Miller et al., 1992).

<table>
<thead>
<tr>
<th>Subunit</th>
<th>1A</th>
<th>1B</th>
<th>2A</th>
<th>2B</th>
<th>3A</th>
<th>3B</th>
<th>Mean ± SD</th>
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<tr>
<td>p160</td>
<td>0.78</td>
<td>0.68</td>
<td>0.92</td>
<td>0.80</td>
<td>0.74</td>
<td>0.72</td>
<td>0.80 ± 0.08</td>
</tr>
<tr>
<td>p150</td>
<td>1.22</td>
<td>1.32</td>
<td>1.08</td>
<td>1.20</td>
<td>1.28</td>
<td>1.28</td>
<td>1.20 ± 0.08</td>
</tr>
<tr>
<td>p62</td>
<td>1.41</td>
<td>0.96</td>
<td>0.75</td>
<td>0.31</td>
<td>0.93</td>
<td>0.74</td>
<td>0.89 ± 0.33</td>
</tr>
<tr>
<td>p50</td>
<td>4.73</td>
<td>5.51</td>
<td>4.41</td>
<td>4.61</td>
<td>4.72</td>
<td>4.83</td>
<td>4.70 ± 0.35</td>
</tr>
<tr>
<td>Arpl/actin</td>
<td>8.27</td>
<td>12.43</td>
<td>8.89</td>
<td>10.52</td>
<td>12.40</td>
<td>12.83</td>
<td>10.21 ± 1.82</td>
</tr>
<tr>
<td>p37</td>
<td>0.32</td>
<td>0.29</td>
<td>0.80</td>
<td>1.07</td>
<td>0.74</td>
<td>0.68</td>
<td>0.65 ± 0.27</td>
</tr>
<tr>
<td>p32</td>
<td>0.37</td>
<td>0.31</td>
<td>0.50</td>
<td>0.36</td>
<td>1.27</td>
<td>1.75</td>
<td>0.66 ± 0.55</td>
</tr>
<tr>
<td>p27</td>
<td>NA</td>
<td>NA</td>
<td>1.05</td>
<td>0.49</td>
<td>0.64</td>
<td>1.54</td>
<td>0.86 ± 0.47</td>
</tr>
<tr>
<td>p24</td>
<td>NA</td>
<td>NA</td>
<td>1.03</td>
<td>1.02</td>
<td>0.60</td>
<td>1.71</td>
<td>1.00 ± 0.46</td>
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Three different preparations of dynactin complex (samples 1, 2, and 3) were analyzed by SDS-PAGE. For each sample, two different volumes of the same protein sample were analyzed (A = 2X; B = 1X). The gels were stained with Coomassie brilliant blue and the intensities of the individual bands determined by densitometry. The integrated values were divided by the molecular weight of each subunit. For each of the six scans the values obtained for the p150 and p160 subunits were averaged; this number was used to normalize the values obtained for all nine bands. As these were one-dimensional gels, the calculated stoichiometry of the 45-kD band represents both Arpl and conventional actin. The stoichiometries listed in the Results represent the integral values that are most consistent with these values and the mass of the complex as determined by STEM. Differences between the estimated stoichiometries and the values listed above most likely reflect the variability of Coomassie binding to the different proteins in the dynactin complex.

Sample 1 was analyzed on a 7% gel which did not resolve p27 and p24. NA, not applicable.
Table II. Percent Actin (by weight) in the Dynactin Complex

<table>
<thead>
<tr>
<th></th>
<th>MonoQ-purified dynactin complex</th>
<th>Resedimented dynactin complex</th>
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<tbody>
<tr>
<td>Experiment 1</td>
<td>4.3 ± 0.5%</td>
<td>3.9 ± 0.4%</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>2.8 ± 0.4%</td>
<td>3.2 ± 0.8%</td>
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The mass of actin (expressed as mean ± SD) in different preparations of dynactin complex was measured by quantitative immunoblotting (see Materials and Methods for details). The amount of actin is expressed as percent of total protein (wt/wt) in the dynactin complex. The mass of actin in each complex can be calculated by dividing the mass of the dynactin complex (1.2 mD) by these percentages. This yields an average actin mass of 42,600 D for both the MonoQ-purified and the resedimented dynactin complex, consistent with a stoichiometry of one actin monomer per complex.

Figure 2. Actin is tightly associated with the dynactin complex. Samples of purified dynactin complex (A) and dynein (B) were sedimented into 5-20% sucrose gradients. Fractions were collected from the bottom of the gradient (lanes 1-10 are numbered from bottom to top). The polypeptide compositions of the starting material (lane L) and individual gradient fractions were analyzed by silver staining (top panels) or immunoblotting with anti-dynactin mAb 150.1 (A, middle panel), anti-dynein intermediate chain mAb 70.1 (B, middle panel), or anti-actin mAb C4 (bottom panels). The lanes are identical in all panels.

Dynactin Complex Contains a Filament That Resembles Short Conventional Actin Filaments

The structure of the dynactin complex was analyzed by performing electron microscopy on molecules that had been adsorbed to mica flakes, freeze-dried, and rotary shadowed with platinum (Heuser, 1983, 1989). As seen in Fig. 3, the dynactin complex had two major structural domains: a helical filament, ~10 nm wide and 37-nm long, and a lateral projection composed of a “shoulder” on one side of the filament, from which emanated a thinner filament that terminated in two globular "heads." The length of the thin projection including its globular heads, was 24.3 ± 2.2 nm (mean ± SD, n = 12). A small subset of the molecules contained a second thin projection extending from the globular heads (Fig. 3, 3rd panel in the top 2 rows; Fig. 9 top, top row, 3rd panel). No differences were noted in images of dynactin complex isolated from chick embryo brain (Fig. 3, upper two rows) vs those isolated from chick embryo fibroblasts (Fig. 3, middle two rows).

The structure of the filament closely resembled that of conventional actin filaments visualized by this method (Fig. 3, bottom panel). The most obvious similarity between the dynactin complex filament and F-actin is that both display the continuous, oblique stripes that are thought to represent the helical organization of actin subunits. The pitch of the filament was determined by measuring the length over several oblique striations and dividing by the number of striations in the measured segment to yield the length of a single striation. For conventional actin filaments, the pitch of the helix was 51.0 ± 4.0 Å (mean ± SD, n = 15), close to the value of 59 Å obtained from diffraction analyses (Hanson and Lowy, 1963). The pitch of the helix of the filament of the dynactin complex was 51.3 ± 6.1 Å (mean ± SD, n = 15). Also similar were the widths of conventional actin fila-
Figure 3. Ultrastructure of the dynactin complex and conventional actin filaments observed by electron microscopy. Purified dynactin complex from embryonic chicken brain (top two rows) and chick embryo fibroblasts (middle two rows) was adsorbed to mica flakes, freeze-dried, rotary-replicated with platinum and observed by electron microscopy. The bottom panel shows a filament of conventional actin. Bar, 50 nm.

Figure 4. Specificity of mAbs to different components of the dynactin complex. mAb 45A is specific for Arpl and does not react with actin. mAb 62B recognizes the p62 component of the complex exclusively. Immunoblots of embryonic chicken brain high speed supernatant (lanes a, d, and g), purified dynactin complex (lanes b, e, and h), and embryonic chicken brain actin (lanes c and f) were probed with anti-Arpl mAb 45A (lanes a, b, and c), anti-actin mAb C4 (lanes d, e, and f), or anti-p62 mAb 62B (lanes g and h).

45A recognizes Arpl exclusively and does not react with conventional actin or other proteins on immunoblots of whole tissue extracts of chick brain (Fig. 4). mAb 45A is an IgG, so it appears as a small, three-lobed structure by freeze-etch electron microscopy (Heuser, 1983, 1989). The antibody decorated the filament at several sites along the filament (Fig. 5 A). A preferred location for binding of mAb 45A seemed to be near either end of the filament; however, examples of antibody binding along its side were also observed. This preference for binding near the filament ends may reflect limited access of mAb 45A to epitopes that are masked during filament formation. At higher concentrations of antibody, aggregates of two or more dynactin complexes were often seen, suggesting that mAb 45A could cross-link complexes together (data not shown).

Capping Protein and the 62-kD Protein Are Located at Opposite Ends of the Filament

The observation that the filaments of the dynactin complex are of uniform length suggests that the structure includes proteins that regulate assembly and filament length. Among the nine polypeptides in the dynactin complex are species of 37 kD and 32 kD, approximately the same sizes as the α and β subunits of capping protein, a barbed-end actin-binding protein which, in skeletal muscle, has been referred to as CapZ (Casella et al., 1987). First isolated from Acanthamoeba (Isenberg et al., 1980), capping protein is ubiquitous among eukaryotes and is found in all chicken tissues examined, including brain (Casella et al., 1989; Cooper et al., 1991; Schafer et al., 1992). Capping protein was identified as a component of the dynactin complex in immunoblots of 2D gels probed with polyclonal antibodies that react with both α- and β-subunits of chicken skeletal muscle capping protein (Fig. 6). Two dimensional gels resolve capping protein in the dynactin complex and from chicken skeletal muscle into three major spots. The two 37-kD proteins were confirmed to be α-subunits using mAb 1B11 which is specific for capping protein α-subunit (Hug et al., 1992); p32 also reacted with polyclonal antibodies specific for the

Arpl Is a Component of the Actin-like Filament in the Dynactin Complex

To determine if the actin-like filament contained Arpl, dynactin complexes were incubated with mAb 45A and the sample was examined by electron microscopy (Fig. 5). mAb...
Figure 5. Immuno-decoration of chick embryo brain dynactin complex with anti-Arpl mAb 45A. Dynactin complexes were incubated with the antibody, and then processed for electron microscopy. The panel in the lower right corner is a tracing of the mAb 45A-decorated dynactin complex shown in the panel to the immediate left. Bar, 50 nm.

Figure 6. Dynactin complex contains actin-capping protein. Purified chicken skeletal muscle capping protein (A) and dynactin complex (B) were run on 2D gels and subjected to immunoblot analysis with a polyclonal antibody that recognizes the α and β subunits of capping protein. Only the region of each blot containing the capping protein subunits is shown. The pH range depicted is ~4.5–6.0 (left to right). (Bottom) Sequence comparison of p32 and the β-subunit of capping protein. Peptides generated from p32 of the dynactin complex by digestion with endoproteinase Glu-C were subjected to NH₂-terminal microsequencing. The NH₂-terminal sequence of an internal 18-kD peptide from p32 was identical to amino acids 158–169 of the β-subunit of chicken muscle capping protein (Caldwell et al., 1989b). Ambiguous residues are indicated with an X. The assignment of glutamic acid (E) for the NH₂-terminal residue was made on the basis of the cleavage specificity of the protease.

β-subunit of capping protein from skeletal muscle (data not shown). The capping protein β-subunit of the dynactin complex migrated as a single protein of pl 5.9 (Fig. 6 b) which is significantly more alkaline than that of the major β-subunit of capping protein from skeletal muscle. Studies to be reported elsewhere demonstrate that the capping protein β-subunit isoform in the dynactin complex is a novel isoform (β2) that is related to the β-subunit isoform of skeletal muscle capping protein (β1) by an alternative splice in the 3'-region of the mRNA (Schafer, D. A., Y. O. Korshunova, T. A. Schroer, and J. A. Cooper, manuscript submitted for publication). Additional verification that p32 was a capping protein β-subunit was obtained by peptide sequencing (Fig. 6, bottom).

To localize capping protein in the dynactin complex, purified dynactin complexes were incubated with β-subunit-specific polyclonal antibodies and processed for electron microscopy as above. Fig. 7 shows that the antibodies, which are primarily IgMs, and hence, appear as pentagonal rosettes (Heuser, 1983, 1989), bound only to one end of the filament. Most often, this was the end nearest the lateral projection. However, in some of these immune complexes, the lateral projection appeared displaced centrally or was not visible, in which case assignment of the polarity of antibody binding was not possible. Of a total of 14 antibody-decorated molecules, 8 had IgM bound at the end nearest the lateral projection, 2 had IgM bound at the end furthest from the lateral projection and in 4 molecules an assignment could not be made. Thus, images of a majority of the immune complexes suggested that the capping protein is associated with the end of the filament nearest the lateral projection. Since capping protein is known to bind to the barbed end of conventional actin filaments (Caldwell et al., 1989a; Casella et al., 1989), we speculate that capping protein in the dynactin complex is bound to the analogous end of a filament of Arpl.

Monoclonal antibodies to p62 decorated the end of the filament furthest from the lateral projection (Fig. 8). No molecules were observed that bound the antibody at the op-
posite end of the filament. The location of the epitope recognized by mAb 62B at the end of the filament opposite that bound by capping protein suggests that p62 may be a pointed-end capping factor and/or may specify the length of the filament. To determine if p62 was similar to any known actin-binding protein, antibodies to several actin-binding proteins were tested for reactivity with dynactin complex on immunoblots. Polyclonal antibodies to human tropomodulin, a 40-kD pointed-end capping protein that binds tropomyosin and actin (Fowler et al., 1993) failed to react with any component of the dynactin complex. Likewise, antibodies to chicken non-muscle tropomyosin (Hegmann et al., 1988), fascin (Yamashiro-Matsumura and Matsumura, 1985), and fimbrin (Tilney et al., 1989; Heintzelman and Mooseker, 1990) did not react with any component of the dynactin complex (but did react with their respective antigens in either chicken or human whole tissue extracts; data not shown).

**The Globular Heads on the Lateral Projection Are Comprised of the p160/p150 Dynactin Polypeptide**

Projecting from each filament is a thin, 24-nm long filament whose structure resembles a myosin tail or a kinesin stalk, two structures known to be comprised of α-helical coiled-coils (Lowey and Cohen, 1962; de Cuevas et al., 1992). On the basis of its primary sequence, p160/p150 dynactin is predicted to fold into two long α-helices that contain the hydrophobic heptad repeats that characterize coiled-coil structures (Fig. 9 C; Gill et al., 1991; Holzbaur et al., 1991). To determine if p160/p150 dynactin comprises a part of the 24-nm long sidearms, dynactin complexes were incubated with the anti-p160/p150 dynactin polypeptide monoclonal antibody mAb 150.1 (Steuer et al., 1990) and processed for electron microscopy as above. The antibody bound to the globular heads at the end of the 24-nm long sidearms (Fig. 9). Dynactin complexes that were cross-linked via their heads by the antibody were occasionally observed (data not shown).

The epitope bound by mAb 150.1 was localized near the COOH terminus of the dynactin polypeptide. Peptide maps were probed with mAb 150.1 and two immunoreactive peptides were analyzed by NH₂-terminal microsequencing. The sequences derived from both peptides mapped to the COOH-
adjacent predicted coiled-coil domain may form the thin, 24-nm long sidearm that links the globular heads to the actin-like filament. A model of the structure of the dynactin complex, based on the ultrastructural and biochemical data presented here and on the known biochemical activities of some of the components, is shown in Fig. 10.

We propose that nine molecules of Arpl and one molecule of conventional actin form a polar filament with capping protein bound to the barbed end of the filament. Capping protein presumably stabilizes the barbed end of the filament. Whereas the freeze-etch technique used here permits the detection of gelsolin at one end of conventional actin filaments, it has failed to reveal bound capping protein (Heuser, J.E., and J. A. Cooper, unpublished observations). Consistent with this, no structural features are visible on either end of the filament that allow us to distinguish capping protein or p62 from other filament components. The location of the epitope of p62 recognized by mAb 62B is at the opposite end of the filament from capping protein, suggesting that p62 may stabilize the pointed end of the filament. Alternatively, or in addition, p62 may be involved in determining the length of the filament, which appears to be invariant. As its primary sequence has not yet been determined, we are unable to speculate on p62 structure; it may be a globular protein associated with the end of the Arpl filament or an elongated protein bound along the filament length. The mAb 62B binding localization does not distinguish between these possibilities.

Each dynactin complex contains one mole/mole of conventional actin which is most likely incorporated into the filament. Unfortunately, we could not determine the location of conventional actin within the dynactin complex because suitable antibodies were not available. Actin may not be randomly distributed along the filament but its location may instead be specified by another component of the complex. Perhaps capping protein associates with the single conventional actin molecule to cap the barbed end of the actin-like filament.

The length of 37 nm measured for the filament is close to that predicted for a polymeric structure containing the proteins determined here to be present in the dynactin complex.
together. nine molecules of Arpl, plus one molecule each of conventional actin, capping protein (M, = 64 kD), and p62 would provide a protein mass equivalent to ~12 actin monomers (assuming that capping protein and p62 are each the approximate dimensions of an actin monomer). Twelve molecules of conventional actin would form a 33-nm filament (at 2.75 nm/monomer as determined by Lorenz et al., 1993). As the platinum rotary shadowing method tends to inflate molecular dimensions by 2–4 nm (Heuser, 1983), a 33-nm long filament would appear close to 37 nm long when visualized by this technique. It is also possible that other dynactin complex subunits lie within the filament, contributing to the observed length.

The mAb 150.1 decoration results provide some insight into the structure of pl60/pl50 dynactin polypeptide. The predicted secondary structure of the pl60/pl50 polypeptide includes two α-helical, coiled-coil domains, ~50 nm and 20 nm in length, interspersed with globular domains (Gi1 et al., 1991). The two molecules of dynactin present in each complex (two pl60, two pl50, or one each pl60/pl50) may form a dimer in which the 50-nm coiled-coil is contained within the filament or the shoulder of the lateral projection while the 20-nm coiled-coil extends to form the thin, projecting sidearm. That an epitope recognized by mAb150.1 lies within a predicted globular domain is consistent with its binding to the globular heads at the end of the dynactin sidearm.

The locations of the three remaining components of the dynactin complex have not been determined. The abundance of p50, present in four-to-five moles/mole dynactin complex, suggests that this protein may form the shoulder of the lateral projection; unfortunately, the dynactin complex could not be decorated with the p50 antibodies currently available. When dynactin complex is treated with KI, pl60/pl50 dynactin and some p50 remain tightly associated (Eckley, D.M., and T. A. Schroer, unpublished observations) suggesting that p50 may interact directly with the dynactin polypeptides. The identities of the two smallest components of the dynactin complex, p27 and p24, are not known, and antibodies are not yet available.

What does the ultrastructure of the dynactin complex tell us about its function in vivo? The most interesting finding is the presence in the complex of the short, actin-like filament composed primarily of Arpl. This is the first evidence that an actin-related protein assembles into a polymer similar to conventional actin. The Arpl filament is similar in dimensions to the short, conventional actin filaments associated with the erythrocyte membrane skeleton (Pinder and Gratzer, 1983; Bennett, 1989). Since dynactin complex has been shown to stimulate cytoplasmic dynein-mediated vesicle motility along microtubules in vitro (Gill et al., 1991; Schroer and Sheetz, 1991), perhaps it functions to regulate the interaction of dynactin with the surface of motile organelles. Alternatively, the dynactin complex may be a component of a vesicle-associated “membrane cytoskeleton” that stimulates dynein-based vesicle motility. A similar role has been proposed for the coatomer complex in regulation of the interaction of membranes with microtubules and motors (Lippincott-Schwartz, 1993).

That the dynactin complex contains an actin-like filament suggests binding to a member of the myosin superfamily. Such an interaction might involve the conserved motor head domain or the widely divergent tail domain. If the dynactin complex were to interact with both myosin motors and with cytoplasmic dynein, it might serve to couple the movement of intracellular organelles along the actin- and tubulin-based cytoskeletal systems (Kuznetsov et al., 1992).

Arpl and other actin-related proteins are present in cells at concentrations significantly lower than conventional actin. Biochemical fractionation of brain tissue indicates that all cytosolic Arpl is present in a 20 S particle (Paschal et al., 1993; Schroer, T. A., unpublished observations); Arpl and other dynactin complex subunits localize to membrane vesicles by immunofluorescence (Gill et al., 1991; Paschal et al., 1993; Meads, T., and T. A. Schroer, unpublished observations). Other actin-related proteins may constitute distinct structural or regulatory elements that govern different cell functions, perhaps in specific subcellular locations. This behavior can be contrasted with that of conventional actin, which assembles into a variety of dynamic and stable structures that are distributed throughout cytoplasm. It remains to be determined whether or not other actin-related proteins have the capacity to polymerize into filaments, form other types of protein complexes, or whether they function as monomers within cells.

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Figure 10. Schematic representation of dynactin complex structure based on the ultrastructural and biochemical characterization reported here. Dynactin complex is comprised of two major structural domains: a short, actin-like filament and a lateral projection comprised of a shoulder with a thin projection that ends with two globular heads. The filament is likely composed of nine molecules of Arpl, one molecule of conventional actin, one molecule of capping protein (α/β dimer), which is situated at one end of the filament, and one molecule of p62, which contains an epitope situated at the opposite end of the filament. We do not know whether p62 is also associated with the length of the Arpl filament, and if so, how far the protein extends. The globular heads at the end of the thin projection contain the COOH-terminal region of pl60/pl50 dynactin polypeptide; this region of the molecule is predicted to fold into a globular structure. The composition of the shoulder (triangle) is unknown.
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