

# 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 Arp1 (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 Arp1 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 polypeptides 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.

**T**HE dynactin complex, an activator of cytoplasmic dynein-mediated vesicle movement on microtubules *in vitro*, is composed of at least nine polypeptides (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 polypeptides of the complex immunoprecipitate all the other components (Gill et al., 1991; Paschal et al., 1993). The largest polypeptide of the dynactin complex from chick embryo brain is p160/p150 dynactin (Gill et al., 1991); cDNAs encoding homologues of this protein have been obtained from chicken (Gill et al., 1991), rat (Holzbaier et al., 1991), and *Drosophila melanogaster* (Swaroop et al., 1987). Analysis of the dynactin polypeptides 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 polypeptide in the dynactin complex is an actin-related protein originally named actin-related protein from vertebrates (actin-RPV)<sup>1</sup> (Lees-Miller et al.,

1992); the human homologue of this protein is referred to as centractin (Clark and Meyer, 1992). These proteins comprise one subgroup, the Arp1 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 Arp1 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, Arp1 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'-adenylyl-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 Arp1 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 Arp1, 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 polyacrylamide 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 Tropix (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 Spafas, 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 (Schroer and Sheetz, 1991) with the following minor modifications. Protein was isolated from 40 gm of embryonic chick brains or from  $2 \times 10^9$  chick embryo fibroblasts (cultured in roller bottles). Endogenous microtubules in the high speed supernatant were augmented with phosphocellulose-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 5'adenyl-*imidodiphosphate* (AMP-PNP). 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  $\sim 200 \mu\text{g}$  in a total of three one ml fractions. Conventional actin was obtained from the MonoQ column, eluting before both dynein and dynactin complex (Schroer 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  $\text{MgSO}_4$ , 1 mM EGTA, and 0.5 mM EDTA (PMEE' buffer). The samples were centrifuged for 4 h at 45,000 rpm in a SW50 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 MAPSII kit (BioRad Co.). mAb 1B11, specific for the  $\alpha$ -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  $\beta$ -subunit of capping protein were affinity purified on skeletal muscle capping protein-Sepharose and Sepharose conjugated with bacterially expressed  $\beta$ -subunit fusion protein, respectively (Schafer et al., 1992). The capping protein  $\beta$ -subunit antibodies are a mixture of IgGs and IgMs. Anti-tropomyosin monoclonal antibody CG1 (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. Fumio Matsumura (Yamashiro-Matsumura and Matsumura, 1985). Anti-tropomodulin was a gift from Dr. V. Fowler (Fowler et al., 1993).

Monoclonal antibodies 45A and 62B: Female Balb/c mice were immunized with 15  $\mu\text{g}$  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 20S dynein. mAbs 45A and 62B were found to be IgG; 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 PMEE' buffer to yield  $\sim 150 \text{ mM}$  KCl. Dynactin complex and purified IgGs were mixed at a 1:1 ratio by mass (5  $\mu\text{g}$  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  $\text{MgCl}_2$ , and 3 mM EGTA 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 Incorp., Mountain View, CA). Copies of the images were obtained using a Kodak Colorease 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 (Towbin 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 (AMPPD or CSPD from Tropix, Inc., Danvers, MA) or chromogenic substrate. Samples were assayed for protein by the method of Bradford (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-Triton-X-100 sample buffer containing ampholytes.

### Quantitative Immunoblotting of Actin

Five twofold serial dilutions of dynactin complex (0.14–2.24  $\mu\text{g}$ ) 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

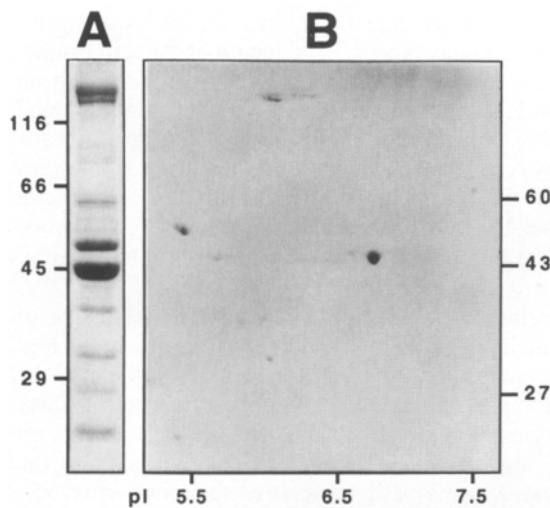
were visualized using chemiluminescence. X-ray films were scanned on an LKB Ultrosan laser densitometer (Pharmacia/LKB Biotechnology, Piscataway, NJ). An actin standard curve was generated by plotting peak intensities vs actin concentration; the standard curve was linear over 3 or 4 of the 5 points. 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 NH<sub>2</sub>-terminal sequencing as described below.

### Peptide Sequencing

Peptides from p160/p150 dynactin and p32 (capping protein  $\beta$  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  $\beta$ -subunit,  $\sim 4 \mu\text{g}$  protein (8 bands) were digested with  $0.8 \mu\text{g}$  endoproteinase GluC. For p160/p150 dynactin,  $\sim 25 \mu\text{g}$  protein (6 bands; no attempt was made to separate the two isoforms as they yielded identical peptide maps) was digested with  $2 \mu\text{g}$  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 NH<sub>2</sub>-terminal microsequencing on an ABI sequencer at the Protein/Peptide Laboratory at the Johns Hopkins Medical School.



**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 gels are indicated on the sides. Conventional actin is a minor spot with an apparent pI of 5.6 (to the right and below the predominant p50 spot, pI 5.5; see also Fig. 3 in Lees-Miller et al., 1992).

## Results

### Arp1 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 Arp1 with pI  $\approx 6.8$ ; minor components are conventional actin (Lees-Miller et al., 1992) and an unidentified 45-kD protein with pI  $\approx 6.7$ . Densitometric analysis of two-dimensional gels showed the stoichiometry of Arp1 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 2:1.5:1:1:1:1 (Table I). The relative stoichiometry of the major components (p160/p150, p50 and Arp1/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  $1.11 \pm 0.12$  mD (mean  $\pm$  SD;  $n = 151$ ).

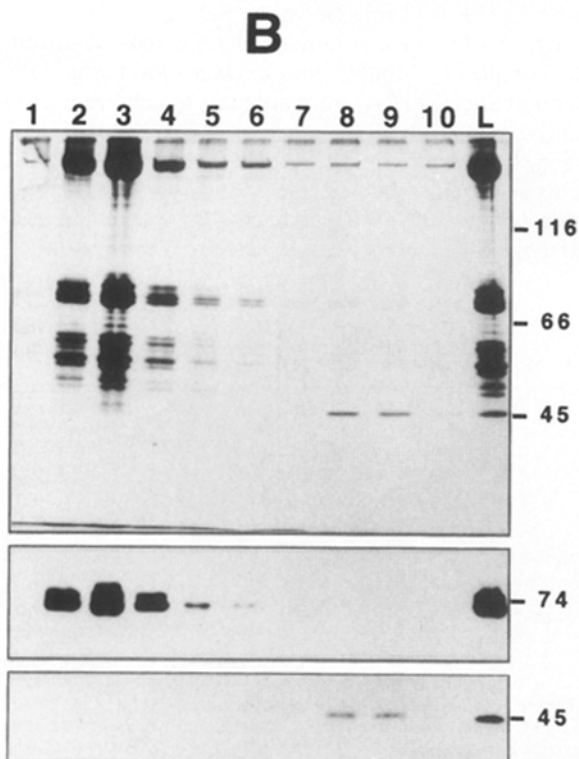
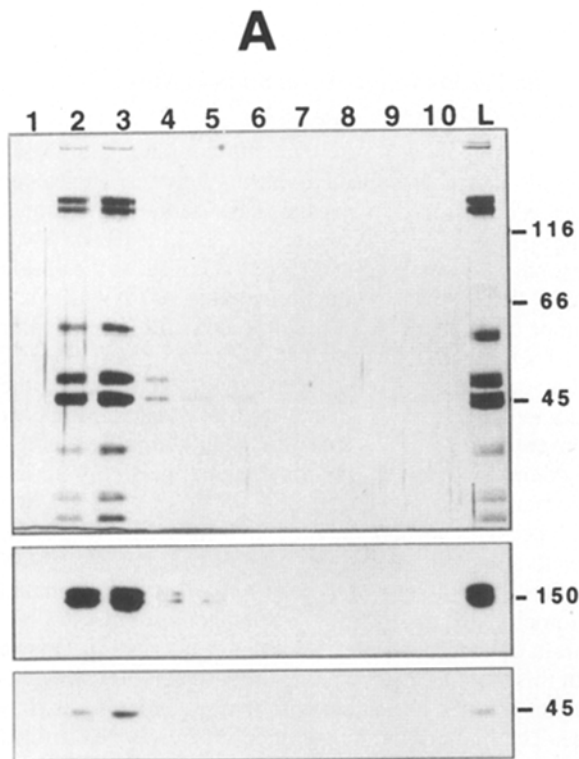
Although most of the conventional actin is separated from dynactin complex by MonoQ ion-exchange chromatography (Schroer and Sheetz, 1991) trace amounts of actin remain in purified dynein and dynactin complex preparations (Fig. 1 B and Fig. 2 A, bottom panel, lane L; Lees-Miller et al., 1992). To determine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

**Table I. Stoichiometry of Dynactin Complex Subunits**

Subunit	1A	1B	2A	2B	3A	3B	Mean $\pm$ SD
p160	0.78	0.68	0.92	0.80	0.74	0.72	$0.80 \pm 0.08$
p150	1.22	1.32	1.08	1.20	1.28	1.28	$1.20 \pm 0.08$
p62	1.41	0.96	0.75	0.31	0.93	0.74	$0.89 \pm 0.33$
p50	4.73	5.51	4.41	4.61	4.72	4.83	$4.70 \pm 0.35$
Arp1/actin	8.27	12.43	8.89	10.52	12.40	12.83	$10.21 \pm 1.82$
p37	0.32	0.29	0.80	1.07	0.74	0.68	$0.65 \pm 0.27$
p32	0.37	0.31	0.50	0.36	1.27	1.75	$0.66 \pm 0.55$
p27	NA	NA	1.05	0.49	0.64	1.54	$0.86 \pm 0.47$
p24	NA	NA	1.03	1.02	0.60	1.71	$1.00 \pm 0.46$

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 Arp1 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.



**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.

**Table II.** Percent Actin (by weight) in the Dynactin Complex

	MonoQ-purified dynactin complex	Resedimented dynactin complex
Experiment 1	4.3 ± 0.5%	3.9 ± 0.4%
Experiment 2	2.8 ± 0.4%	3.2 ± 0.8%

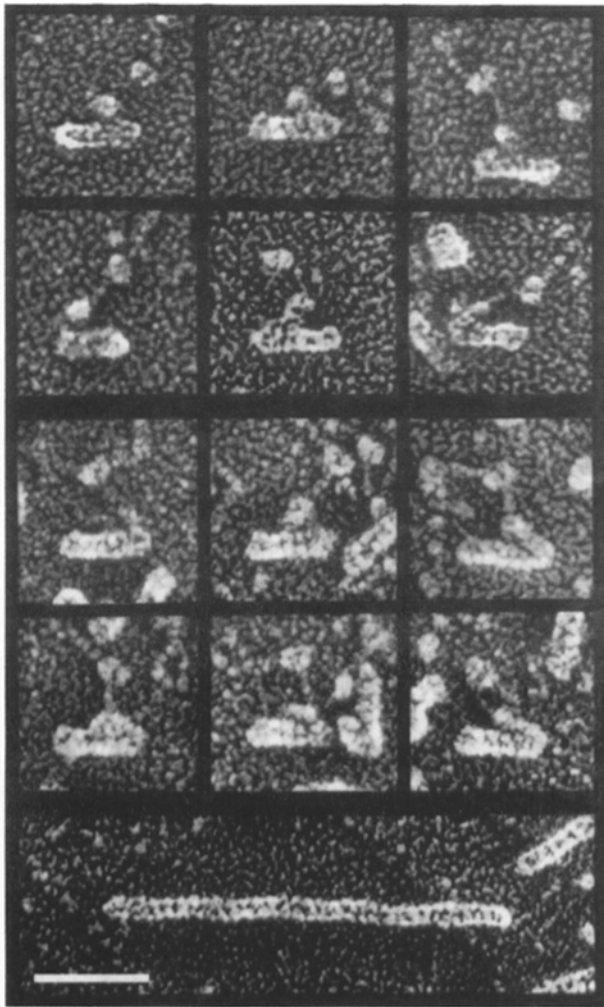
The mass of actin (expressed as mean ± SD) in different preparations of dynactin complex was measured by quantitative immunoblotting (see Materials and Methods for details). The amount of actin is expressed as percent of total protein (wt/wt) in the dynactin complex. The mass of actin in each complex 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.

into a sucrose gradient. Conventional actin cosedimented with the complex (Fig. 2 *A*), suggesting that it is a tightly associated component. In contrast, the small amount of conventional actin present in preparations of MonoQ-purified dynein was separated from dynein and remained in the light fractions of the sucrose gradient after resedimentation (Fig. 2 *B*). Quantitative immunoblot analysis of the purified dynactin complex preparation before and after resedimentation into sucrose gradients demonstrated that each dynactin complex contained one conventional actin molecule (Table II), in agreement with the estimate obtained from densitometry of 2-D gels.

#### ***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-

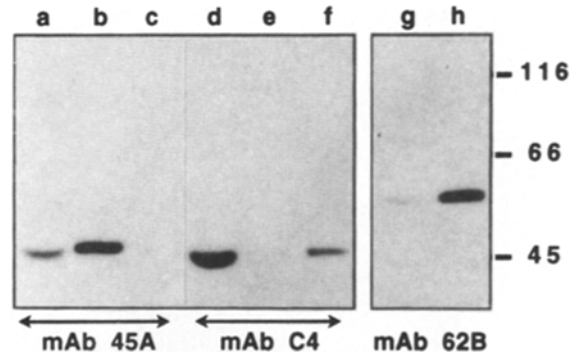


**Figure 3.** Ultrastructure of the dynactin complex and conventional actin filaments observed by electron microscopy. Purified dynactin complex from embryonic chicken brain (top two rows) and chick embryo fibroblasts (middle two rows) was adsorbed to mica flakes, freeze-dried, rotary-replicated with platinum and observed by electron microscopy. The bottom panel shows a filament of conventional actin. Bar, 50 nm.

ments and of dynactin complex filaments:  $9.6 \pm 0.83$  nm (mean  $\pm$  SD,  $n = 4$ ) and  $9.7 \pm 0.65$  nm (mean  $\pm$  SD,  $n = 12$ ), respectively. The length of the filament of the dynactin complex ( $37.2 \pm 2.4$  nm) (mean  $\pm$  SD,  $n = 12$ ), was remarkably constant in several different dynactin complex preparations. The lateral projection was most often situated near one end of the filament; it did not appear to be able to slide along the filament, hence, its location generally could be used to define the polarity of the dynactin complex. In some of the antibody-decorated dynactin complex molecules shown below, the position of the lateral projection was not as clear as in undecorated dynactin complex molecules.

#### **Arp1 Is a Component of the Actin-like Filament in the Dynactin Complex**

To determine if the actin-like filament contained Arp1, dynactin complexes were incubated with mAb 45A and the sample was examined by electron microscopy (Fig. 5). mAb

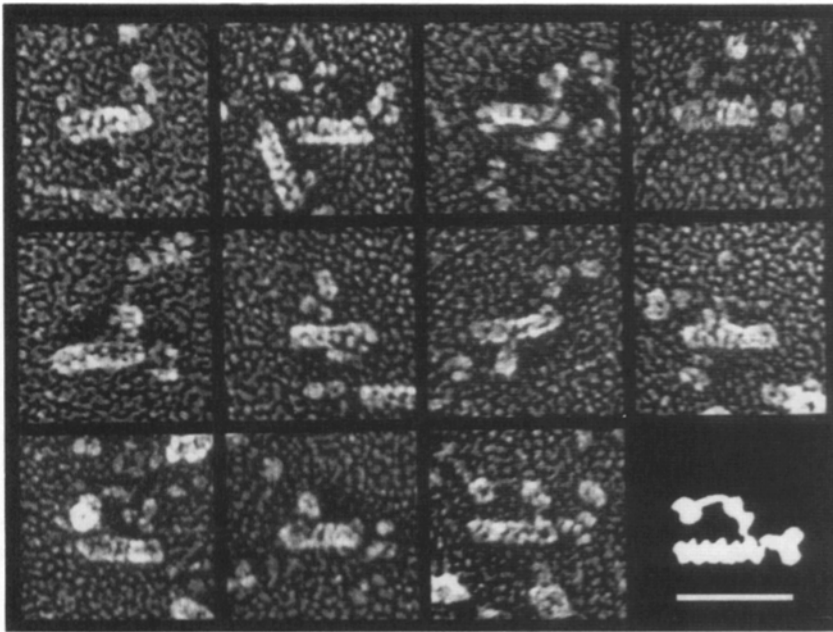


**Figure 4.** Specificity of mAbs to different components of the dynactin complex. mAb 45A is specific for Arp1 and does not react with actin. mAb 62B recognizes the p62 component of the complex exclusively. Immunoblots of embryonic chicken brain high speed supernatant (lanes a, d, and g), purified dynactin complex (lanes b, e, and h), and embryonic chicken brain actin (lanes c and f) were probed with anti-Arp1 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).

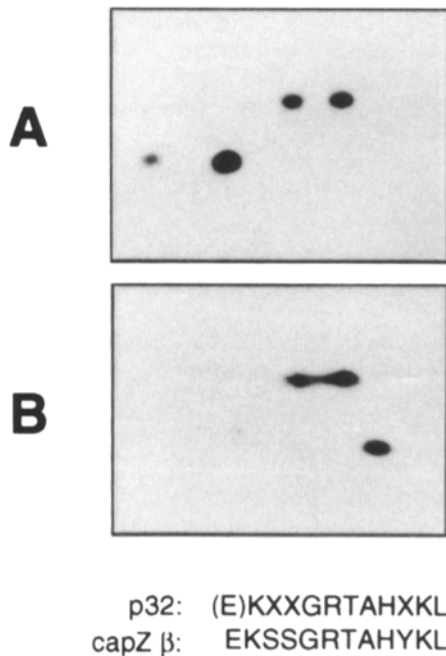
45A recognizes Arp1 exclusively and does not react with conventional actin or other proteins on immunoblots of whole tissue extracts of chick brain (Fig. 4). mAb 45A is an IgG, so it appears as a small, three-lobed structure by freeze-etch electron microscopy (Heuser, 1983, 1989). The antibody decorated 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  $\alpha$  and  $\beta$  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  $\alpha$ - and  $\beta$ -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  $\alpha$ -subunits using mAb 1B11 which is specific for capping protein  $\alpha$ -subunit (Hug et al., 1992); p32 also reacted with polyclonal antibodies specific for the



**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.

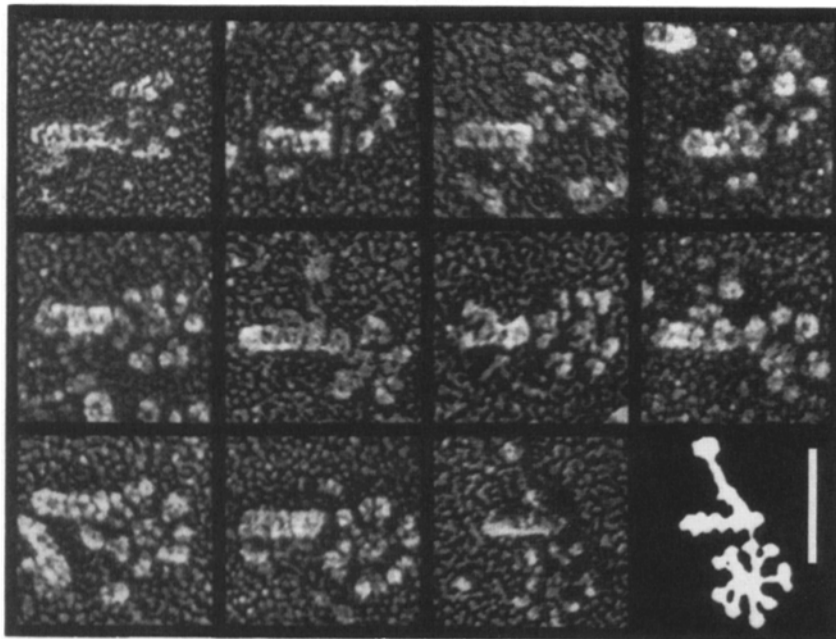


**Figure 6.** Dynactin complex contains actin-capping protein. Purified chicken skeletal muscle capping protein (*A*) and dynactin complex (*B*) were run on 2D gels and subjected to immunoblot analysis with a polyclonal antibody that recognizes the  $\alpha$  and  $\beta$  subunits of capping protein. Only the region of each blot containing the capping protein subunits is shown. The pH range depicted is  $\sim 4.5$ – $6.0$  (left to right). (Bottom) Sequence comparison of p32 and the  $\beta$ -subunit of capping protein. Peptides generated from p32 of the dynactin complex by digestion with endoproteinase Glu-C were subjected to  $\text{NH}_2$ -terminal microsequencing. The  $\text{NH}_2$ -terminal sequence of an internal 18-kD peptide from p32 was identical to amino acids 158–169 of the  $\beta$ -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  $\text{NH}_2$ -terminal residue was made on the basis of the cleavage specificity of the protease.

$\beta$ -subunit of capping protein from skeletal muscle (data not shown). The capping protein  $\beta$ -subunit of the dynactin complex migrated as a single protein of pI 5.9 (Fig. 6 *b*) which is significantly more alkaline than that of the major  $\beta$ -subunit of capping protein from skeletal muscle. Studies to be reported elsewhere demonstrate that the capping protein  $\beta$ -subunit isoform in the dynactin complex is a novel isoform ( $\beta 2$ ) that is related to the  $\beta$ -subunit isoform of skeletal muscle capping protein ( $\beta 1$ ) 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  $\beta$ -subunit was obtained by peptide sequencing (Fig. 6, bottom).

To localize capping protein in the dynactin complex, purified dynactin complexes were incubated with  $\beta$ -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 Arp1.

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-



**Figure 7.** Immuno-decoration of chick embryo brain dynactin complex with affinity-purified antibody specific for capping protein  $\beta$ -subunit. The antibody preparation contains primarily immunoglobulins of the IgM class, which resemble rosettes. Dynactin complexes were incubated with antibody, and then processed for electron microscopy. The panel in the lower right corner is a tracing of the antibody-decorated dynactin complex shown in the panel to the immediate left. Bar, 50 nm.

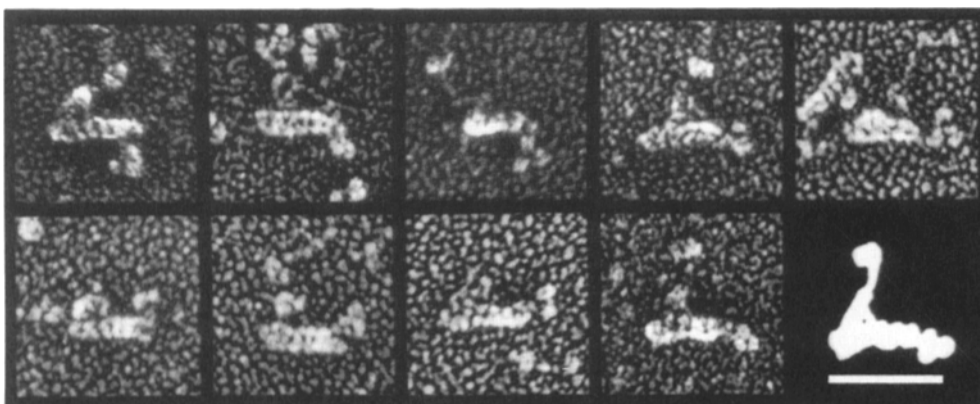
posite end of the filament. The location of the epitope recognized by mAb 62B at the end of the filament opposite that bound by capping protein suggests that p62 may be a pointed-end capping factor and/or may specify the length of the filament. To determine if p62 was similar to any known actin-binding 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***

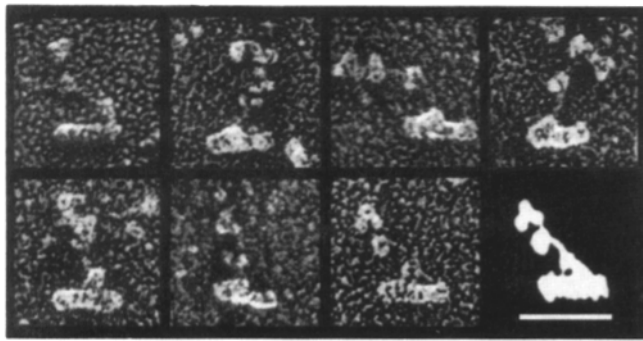
Projecting from each filament is a thin, 24-nm long filament

whose structure resembles a myosin tail or a kinesin stalk, two structures known to be comprised of  $\alpha$ -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  $\alpha$ -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  $\text{NH}_2$ -terminal microsequencing. The sequences derived from both peptides mapped to the COOH-

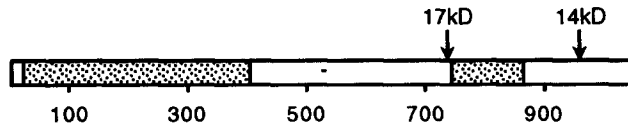


**Figure 8.** Immuno-decoration of chick embryo brain dynactin complex with anti-p62 mAb 62B. Dynactin complexes were incubated with mAb 62B and processed for electron microscopy. The panel in the lower right corner is a tracing of the mAb 62B-decorated dynactin complex shown in the panel to the immediate left. Bar, 50 nm.



17 kD peptide: (E)GEYDADRPQ  
 dynactin AA 730 - 739: EGEYDADRPQ

14 kD peptide: (E)LAGLKPLQVAKVXL  
 dynactin AA 944 - 956: ELAGLKPLQVAKVSL



**Figure 9.** (Top) Immuno-decoration of chick embryo brain dynactin complex with anti-dynactin mAb 150.1. Dynactin complexes were incubated with mAb 150.1 and processed for electron microscopy. The panel in the lower right corner is a tracing of the mAb 150.1-decorated dynactin complex shown in the panel to the immediate left. Scale bar, 50 nm. (Bottom) Epitope mapping of mAb 150.1. Two immunoreactive peptides (17kD and 14kD) generated by endoproteinase Glu-C digestion of the p160/p150 dynactin subunits were subjected to NH<sub>2</sub>-terminal microsequencing and found to match sequences in the COOH-terminal one-third of the dynactin molecule (AA = amino acid). Ambiguous residues are indicated with an X. The assignments of glutamic acids (E) for the NH<sub>2</sub>-terminal residues were made on the basis of the cleavage specificity of the protease. The schematic representation of the p150 dynactin amino acid sequence indicates the locations of the two domains that are predicted to fold into  $\alpha$ -helical coiled-coils (shaded).

terminal one-third of the molecule (Fig. 9); the NH<sub>2</sub> terminus of the smaller of the two peptides corresponds to amino acid 944 of the p150 dynactin primary sequence. This part of the dynactin molecule includes a divergent region that is predicted to have a globular structure adjacent to a predicted coiled-coil domain (Fig. 9; Gill et al., 1991). Taken together, the epitope mapping studies suggest that the extreme COOH termini of the p160/p150 polypeptides correspond to the small globular heads at the end of the sidearm, while the adjacent predicted coiled-coil domain may form the thin, 24-nm sidearm.

## Discussion

The dynactin complex, a multiprotein complex whose major component is the actin-related protein, Arp1, provides an opportunity to study the structural properties of this class of protein. Electron microscopic analysis of freeze-dried complexes absorbed to mica flakes reveals that they are composed of a 37-nm long, 10-nm wide filament with a laterally oriented projection from which emanates a thin, 24-nm long

filament that terminates in a pair of small globular heads. The filament resembles conventional actin filaments visualized by this method. Occasionally, we observed filaments that had no apparent lateral projections, but it was difficult to be certain that they were not hidden beneath the filament or were torn off before adsorption to the mica. Moreover, STEM analyses gave consistent measurements of the mass of the dynactin complex, suggesting that the molecules are of uniform size and contain both the filament and the side projection.

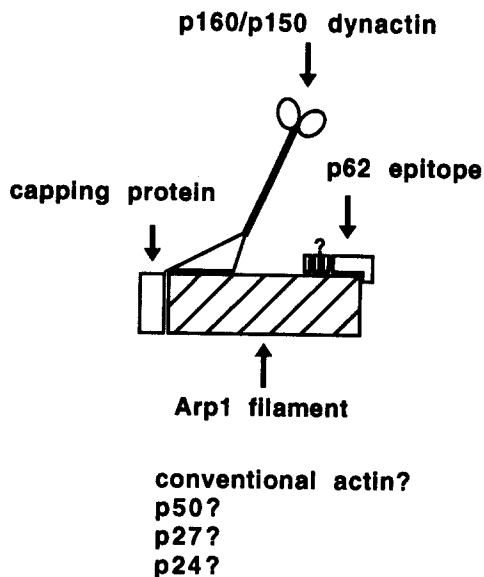
We have localized several polypeptides in the dynactin complex using a battery of polyclonal and monoclonal antibodies to decorate the structure. Antibodies to Arp1 bind the filament, suggesting that this actin-related protein assembles into a polymer. One end of the filament appears to contain the barbed-end actin-binding protein, capping protein. The other end is decorated with antibodies to the p62 polypeptide of the dynactin complex. The globular heads at the end of the lateral projection bind an antibody to the COOH terminus of the p160/p150 dynactin polypeptide. Since the primary sequence of p160/p150 predicts that regions of the molecule fold as a coiled-coil structure, this protein may also form the thin, 24-nm long sidearm that links the globular heads to the actin-like filament. A model of the structure of 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 Arp1 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 Arp1 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





**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 Arp1, one molecule of conventional actin, one molecule of capping protein ( $\alpha/\beta$  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 Arp1 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 p160/p150 dynactin polypeptide; this region of the molecule is predicted to fold into a globular structure. The composition of the shoulder (*triangle*) is unknown.

filament. Together, nine molecules of Arp1, plus one molecule each of conventional actin, capping protein ( $M_r = 64$  kD), and p62 would provide a protein mass equivalent to  $\sim 12$  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 p160/p150 dynactin polypeptide. The predicted secondary structure of the p160/p150 polypeptide includes two  $\alpha$ -helical, coiled-coil domains,  $\sim 50$  nm and 20 nm in length, interspersed with globular domains (Gill et al., 1991). The two molecules of dynactin present in each complex (two p160, two p150, or one each p160/p150) 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, p160/p150 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 Arp1. This is the first evidence that an actin-related protein assembles into a polymer similar in dimensions to the short, conventional actin filaments associated with the erythrocyte membrane skeleton (Pinder and Gratzner, 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 dynein 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).

Arp1 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 Arp1 is present in a 20 S particle (Paschal et al., 1993; Schroer, T. A., unpublished observations); Arp1 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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## References

- Bennett, V. 1989. The spectrin-actin junction of erythrocyte membrane skeletons. *Biochim. Biophys. Acta.* 988:107-121.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248-254.
- Caldwell, J. E., S. G. Heiss, V. Mermall, and J. A. Cooper. 1989a. Effects of CapZ, an actin capping protein of muscle, on the polymerization of actin. *Biochemistry.* 28: 8506-8514.
- Caldwell, J. E., J. A. Waddle, C. J. A. Cooper, J. A. Hollands, S. J. Casella, and C. J. F. Casella. 1989b. cDNAs encoding the  $\beta$  subunit of Cap Z, the actin-capping protein of the Z line of muscle. *J. Biol. Chem.* 264:12648-12652.
- Casella, J. F., S. J. Casella, J. A. Hollands, J. E. Caldwell, and J. A. Cooper. 1989. Isolation and characterization of cDNA encoding the alpha subunit of Cap Z(36/32), an actin-capping protein from the Z line of skeletal muscle. *Proc. Natl. Acad. Sci. USA.* 86:5800-5804.
- Casella, J. F., S. W. Craig, D. J. Maack, and A. E. Brown. 1987. CapZ(36/32), a barbed end actin-capping protein, is a component of the Z-line of skeletal muscle. *J. Cell Biol.* 105:371-379.
- Clark, S. W., and D. I. Meyer. 1992. Centractin is an actin homologue associated with the centrosome. *Nature (Lond.).* 359:246-250.
- Cleveland, D. W. 1983. Peptide mapping in one dimension by limited proteolysis of sodium dodecyl sulfate-solubilized proteins. *Methods Enzymol.* 96:222-229.
- Cooper, J. A., J. E. Caldwell, D. J. Gattermeir, M. A. Torres, J. F. Amatruda, and J. F. Casella. 1991. Variant cDNAs encoding proteins similar to the alpha subunit of chicken CapZ. *Cell Motil. Cytoskeleton.* 18:204-214.
- de Cuevas, M., T. Tao, and L. S. Goldstein. 1992. Evidence that the stalk of *Drosophila* kinesin heavy chain is an alpha-helical coiled coil. *J. Cell Biol.* 116:957-965.
- Fowler, V. M., M. A. Sussmann, P. G. Miller, B. E. Flucher, and M. P. Daniels. 1993. Tropomodulin is associated with the free (pointed) ends of the thin filaments in rat skeletal muscle. *J. Cell Biol.* 120:411-420.
- Frankel, S., M. B. Heintzelman, S. Artavanis-Tsakonas, and M. S. Mooseker. 1994. Identification of a divergent actin-related protein in *Drosophila*. *J. Mol. Biol.* 235:1351-1356.
- Fyrberg, C., L. Ryan, M. Kenton, E. Fyberg. 1994. Genes encoding actin-related proteins of *Drosophila*. *J. Mol. Biol.* In press.
- Garen, S. H., and D. R. Kankel. 1983. Golgi and genetic mosaic analyses of visual system mutants in *Drosophila melanogaster*. *Dev. Biol.* 96:445-466.
- Gill, S. R., T. A. Schroer, I. Szilak, E. R. Steuer, M. P. Sheetz, and D. W. Cleveland. 1991. Dynactin, a conserved, ubiquitously expressed component of an activator of vesicle motility mediated by cytoplasmic dynein. *J. Cell Biol.* 115:1639-1650.
- Hanson, J., and J. Lowy. 1963. The structure of F-actin and of actin filaments isolated from muscle. *J. Mol. Biol.* 6:46-60.
- Harlow, E., and D. Lane. 1988. *Antibodies: a laboratory manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 726 pp.
- Hegmann, T. E., J. L.-C. Lin, and J. J.-C. Lin. 1988. Motility-dependence of the heterogeneous staining of culture cells by a monoclonal anti-tropomyosin antibody. *J. Cell Biol.* 106:385-393.
- Heintzelman, M. B., and M. S. Mooseker. 1990. Assembly of the brush border cytoskeleton: changes in the distribution of microvillar core proteins during enterocyte differentiation in adult chicken intestine. *Cell Motil. Cytoskeleton.* 15:12-22.
- Heuser, J. E. 1983. Procedure for freeze-drying molecules adsorbed to mica flakes. *J. Mol. Biol.* 169:155-195.
- Heuser, J. 1989. Protocol for 3-D visualization of molecules on mica via the quick-freeze, deep-etch technique. *J. Electron Microsc. Tech.* 13:244-263.
- Holmes, K. C., C. Sander, and A. Valencia. 1993. A new ATP-binding fold in actin, hexokinase and Hsc70. *Trends Cell Biol.* 3:53-59.
- Holzbaur, E. L. F., J. A. Hammarback, B. M. Paschal, N. G. Kravitt, K. K. Pfister, and R. B. Vallee. 1991. Homology of a 150K cytoplasmic dynein-associated polypeptide with the *Drosophila* gene *Glued*. *Nature (Lond.).* 351:579-583.
- Hug, C., T. M. Miller, M. A. Torres, J. F. Casella, and J. A. Cooper. 1992. Identification and characterization of an actin-binding site of CapZ. *J. Cell Biol.* 116:923-931.
- Isenberg, G., U. Aebi, and T. D. Pollard. 1980. An actin-binding protein from *Acanthamoeba* regulates actin filament polymerization and interactions. *Nature (Lond.).* 288:455-459.
- Kabsch, W., H. G. Mannherz, D. Suck, E. F. Pai, and K. C. Holmes. 1990. Atomic structure of the actin:DNase I complex. *Nature (Lond.).* 347:37-44.
- Kuznetsov, S. A., G. M. Langford, and D. G. Weiss. 1992. Actin-dependent organelle movement in squid axoplasm. *Nature (Lond.).* 356: 722-725.
- Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680-685.
- Lees-Miller, J. P., D. M. Helfman, and T. A. Schroer. 1992. A vertebrate actin-related protein is a component of a multisubunit complex involved in microtubule-based vesicle motility. *Nature (Lond.).* 359:244-246.
- Lessard, J. L. 1988. Two monoclonal antibodies to actin: one muscle selective and one generally reactive. *Cell Motil. Cytoskeleton.* 10:349-362.
- Lippincott-Schwartz, J. 1993. Bidirectional membrane traffic between the endoplasmic reticulum and Golgi apparatus. *Trends Cell Biol.* 3:81-88.
- Lorenz, M., D. Popp, and K. C. Holmes. 1993. Refinement of the f-actin model against x-ray fiber diffraction data by the use of a directed mutation algorithm. *J. Mol. Biol.* 234:826-836.
- Lowy, S., and C. Cohen. 1962. Studies on the structure of myosin. *J. Mol. Biol.* 4:293-308.
- Melki, R., I. E. Vainberg, R. L. Chow, and N. J. Cowan. 1993. Chaperonin-mediated folding of vertebrate actin-related protein and  $\gamma$ -tubulin. *J. Cell Biol.* 122:1301-1310.
- Merrill, C. R., D. Goldman, S. A. Sedman, and M. H. Ebert. 1981. Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. *Science (Wash. DC).* 211:1437-1438.
- Meyerowitz, E. M., and D. R. Kankel. 1978. A genetic analysis of visual system development in *Drosophila melanogaster*. *Dev. Biol.* 62:112-142.
- O'Farrell, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* 250:4007-4021.
- Paschal, B. M., E. L. F. Holzbaur, K. K. Pfister, S. Clark, D. I. Meyer, and R. B. Vallee. 1993. Characterization of a 50-kDa polypeptide in cytoplasmic dynein preparations reveals a complex with p150<sup>GLUED</sup> and a novel actin. *J. Biol. Chem.* 268:15318-15323.
- Pinder, J. C., and W. B. Gratzer. 1983. Structural and dynamic states of actin in the erythrocyte. *J. Cell Biol.* 96:768-775.
- Schafer, D. A., M. S. Mooseker, and J. A. Cooper. 1992. Localization of capping protein in chicken epithelial cells by immunofluorescence and biochemical fractionation. *J. Cell Biol.* 118:335-346.
- Schroer, T. A., and M. P. Sheetz. 1991. Two activators of microtubule-based vesicle transport. *J. Cell Biol.* 115:1309-1318.
- Steuer, E. R., T. A. Schroer, L. Wordeman, and M. P. Sheetz. 1990. Cytoplasmic dynein localizes to mitotic spindles and kinetochores. *Nature (Lond.).* 345:266-268.
- Swaroop, A., M. Swaroop, and A. Garen. 1987. Sequence analysis of the complete cDNA and encoded polypeptide for the *glued* gene of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA.* 84:6501-6505.
- Tilney, M. S., L. G. Tilney, R. E. Stephens, C. Merte, D. Drenckhahn, D. A. Cotanche, and A. Bretscher. 1989. Preliminary biochemical characterization of the stereocilia and cuticular plate of hair cells of the chick cochlea. *J. Cell Biol.* 109:1711-1723.
- Towbin, H., S. T. Staehelin and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350-4354.
- Yamashiro-Matsumura, S., and F. Matsumura. 1985. Purification and characterization of an f-actin-bundling 55-kilodalton protein from HeLa cells. *J. Biol. Chem.* 260:5087-5097.