We used affinity chromatography to probe for a direct binding interaction between cytoplasmic dynein and dynactin. Purified cytoplasmic dynein was found to bind to an affinity column of p150\text{Glued}, the largest polypeptide in the dynactin complex. To test the specificity of the interaction, we loaded rat brain cytosol onto the p150\text{Glued} affinity column and observed that cytoplasmic dynein from cytosol was specifically retained on the column. Preincubation of the p150\text{Glued} affinity matrix with excess exogenous dynactin intermediate chain resulted in a significant reduction of dynein binding, suggesting that p150\text{Glued} may be interacting with dynein via this polypeptide. Therefore we constructed an affinity column of recombinant dynein intermediate chain and observed that dynein was retained from rat brain cytosol. These results demonstrate that the native dynein and dynactin complexes are capable of direct interaction mediated by a direct binding of the dynein intermediate chain to the p150\text{Glued} component of the dynactin complex. We have mapped the site of this interaction to the amino-terminal region of p150\text{Glued}, which is predicted to form an \(\alpha\)-helical coiled-coil. Regulation of the dynein-dynactin interaction may prove to be key in the control mechanism for cytoplasmic dynein-mediated vesicular transport.

Coordinated trafficking of organelles along microtubules is central to the viability of cell and is powered by the mechanochemical ATPases kinesin and cytoplasmic dynein. While the mechanisms which govern the specificity and regulation of this transport remain to be determined, there is growing evidence for the role of accessory factors in the function of the molecular motors involved. Recently, an integral membrane protein, kinctin, was found to be the essential anchor for kinesin-driven vesicle motility (1, 2). Although no membrane receptor for cytoplasmic dynein has been described yet, a distinct 20 S complex, dynactin, was shown to differentially co-purify with cytoplasmic dynein from a variety of sources (3–5). Also, in an in vitro motility assay the dynactin complex was required by a direct binding of the dynein intermediate chain to p150\text{Glued} component of the dynactin complex. This direct binding between cytoplasmic dynein and dynactin provides evidence in support of involvement of an accessory factor in dynein function. These results

1 The abbreviations used are: Arp1, actin-related protein 1; DHC, dynein heavy chain; DIC, dynein intermediate chain; LIC, light intermediate chain; PIPES, 1,4-piperazinediethanesulfonic acid; BSA, bovine serum albumin; PAGE, polyacrylamide gel electrophoresis.
also suggest that modulation of the dynein-dynactin interaction in vivo may be a key step in the mechanism of regulation of cytoplasmic dynein-mediated organelle trafficking within the cell.

MATERIALS AND METHODS

Cytoplasmic Dynein Binds Directly to the Dynactin Complex

Purified cytoplasmic dynein binds to p150\textsuperscript{Glued}. Although several genetic studies have suggested an in vivo interaction between cytoplasmic dynein and the dynactin complex, evidence showing a direct biochemical interaction between the two has been lacking. In order to investigate a direct binding of cytoplasmic dynein to the dynactin complex, we constructed an affinity column on which an amino-terminal portion (amino acids 133–899 from the rat cDNA clone) was covalently linked to activated CH-Sepharose 4B beads. The 20 S peak fraction resulting from sucrose gradient purification of ATP extracts from a microtubule-enriched fraction of rat brain (lane 1) was loaded on columns constructed of either bacterially expressed fragment (amino acids 133–899) of p150\textsuperscript{Glued} (lanes 2–5) or BSA (lanes 6–9) immobilized on CH-Sepharose 4B beads. The columns were washed with 100 mM NaCl and eluted with 1 ml each of 0.5 and 1.0 M NaCl. The eluted fractions were analyzed by probing with antibodies to DIC. Lane 1, 20 S peak fraction loaded; lanes 2 and 6, flow-throughs; lane 3 and 7, final wash; lanes 4 and 8, 0.5 M NaCl elution; lanes 5 and 9, 1.0 M NaCl elution. Antibody to p150\textsuperscript{Glued} was raised against a recombinant polypeptide which included amino acids 133–899 from the rat cDNA clone (5). The polyclonal antibodies to p150\textsuperscript{Glued} and centrin actin were affinity-purified from rabbit serum against the immunogen polypeptide immobilized on activated CH-Sepharose 4B beads. The DIC antibody used here was a mouse monoclonal IgG, a generous gift of K. K. Pfister (21). The DHC antibody is a rabbit polyclonal generously provided by E. Vaisberg (22).

RESULTS

Purified Cytoplasmic Dynein Binds to p150\textsuperscript{Glued}.—Although several genetic studies have suggested an in vivo interaction between cytoplasmic dynein and the dynactin complex, evidence showing a direct biochemical interaction between the two has been lacking. In order to investigate a direct binding of cytoplasmic dynein to the dynactin complex, we constructed an affinity column on which an amino-terminal portion (amino acids 133–899) of p150\textsuperscript{Glued}, the largest polypeptide in the dynactin complex, was covalently linked to activated CH-Sepharose 4B beads. The 20 S peak fraction resulting from sucrose gradient purification of ATP extracts from a microtubule-enriched fraction of rat brain, which is enriched in both cytoplasmic dynein and dynactin, was passed through the p150\textsuperscript{Glued} affinity column (Fig. 1). Following extensive buffer washes, the column was eluted with 0.5 M and 1.0 M NaCl. The resulting fractions were resolved by SDS-PAGE, electroblotted, and then probed for dynein binding to p150\textsuperscript{Glued} using mouse monoclonal antibodies to DIC. Results in Fig. 1 indicate that the 20 S sucrose fraction contains DIC (lane 1), which when loaded on to the p150\textsuperscript{Glued} affinity column (lanes 2–5), binds to the matrix. The DIC was eluted from the column with a step gradient of 0.5 M and 1.0 M NaCl (lanes 4 and 5). A BSA column which was constructed identically did not retain any DIC (lanes 6 and 7). Compared with the amount of dynein loaded (lane 1), there is no DIC present in the flow-through sample from the p150\textsuperscript{Glued} affinity column (lane 2), indicating that all dynein present in the loaded sample bound to the column. Although lane 1 in Fig. 1 shows two bands at ~74 kDa, consistent with the previous observation of Paschal et al. (19), indicating multiple isoforms of dynein intermediate chain, the resolution of these multiple forms on SDS-PAGE is variable (34).

Cytoplasmic Cytoplasmic Dynein Binds to p150\textsuperscript{Glued}.—To further test the specificity of the interaction, we asked whether a p150\textsuperscript{Glued} column could specifically retain cytoplasmic dynein from whole brain cytosol. Rat brain cytosol was loaded on a p150\textsuperscript{Glued} affinity column as well as a control BSA column. The columns were washed extensively with 25 mM NaCl (Fig. 2),...
then eluted with a step gradient of 0.5 M and 1.0 M NaCl. The fractions were resolved by SDS-PAGE and analyzed by Coomassie Brilliant Blue-staining (Fig. 2). The Coomassie Brilliant Blue-stained gel of the eluates shows that out of the whole cytosol, proteins of molecular mass >300, 75, and 50–58 kDa which co-migrate with components of the cytoplasmic dynein complex (DHC, DIC, and LICs), purified according to published methods (18), are specifically retained by the p150^Glued\textsuperscript{Glued}\textsuperscript{Glued} affinity column, whereas they are not retained by the BSA control column.

The identities of the polypeptides specifically retained from cytosol by the p150^Glued\textsuperscript{Glued} column were verified by immunoblotting with antibodies specific for the dynein intermediate chain (Fig. 3A) and heavy chain (Fig. 3B). Both the dynein heavy chain and intermediate chain were retained specifically by the p150^Glued\textsuperscript{Glued} column and not by the control BSA column. Some cross-reactivity of the DIC monoclonal antibody to native p150\textsuperscript{Glued} present in the cytosol is evident by the presence of the doublet at approximately 150/135 kDa in lanes 1 and 2 in Fig. 3A as was observed previously (5).

Comparison of the immunoblots shown in Fig. 3A and B, indicates that comparatively more DIC than DHC was eluted from the p150^Glued\textsuperscript{Glued} affinity column with 0.5 M NaCl. It is likely that this is a fortuitous, due to the higher sensitivity of the anti-DIC antibody as compared with the DHC antibody. Alternatively, it is possible that there may be a limited pool of free dynein intermediate chain in the cytosol, as was observed previously by Paschal et al. (7). This free DIC may bind to dynactin with a lower affinity than does the intact dynein holenzyme, thus eluting at a lower ionic strength from the affinity column.

The Dynactin Intermediate Chain Mediates the Dynactin-Dynein Binding—Potentially, the interaction between dynein and dynactin could be mediated by the binding of p150^Glued\textsuperscript{Glued} to the DHC, DIC, or to the dynactin LICs. However, by analogy to the intermediate chain of flagellar outer arm dynein which may mediate the ATP-insensitive binding to A subunit microtubule (22), the DIC of cytoplasmic dynein may localize to the base of the dynein and mediate the interaction of motor with its cargo. This binding of cytoplasmic dynein to the organelle may be direct (23) or may be mediated by a vesicle bound receptor. Dynactin may function to link dynein to the membrane, as p150^Glued\textsuperscript{Glued} has been suggested to localize to organelles and vesicles within the cell, based on its punctate staining pattern by immunocytochemistry (6, 7). Also, Fath et al. (24) have reported that p150^Glued\textsuperscript{Glued} co-purifies with isolated Golgi-enriched preparations from intestinal epithelial cells.

To test whether the dynactin-dynactin interaction is mediated by an interaction between p150^Glued\textsuperscript{Glued} and DIC we prepared p150^Glued\textsuperscript{Glued} affinity beads as before but preincubated the matrix with 2.5 M excess of bacterially expressed DIC before loading the cytosol onto the column (Fig. 4). A control column was pretreated with 2.5 M excess BSA. If DIC mediates the dynactin-dynactin interaction, then excess DIC should block the binding sites on p150^Glued\textsuperscript{Glued}, and therefore dynein should no longer bind to the column. When the salt eluates from DIC-treated or BSA-treated p150^Glued\textsuperscript{Glued} affinity columns were probed for DHC, we observed a significant reduction (~80%, as judged by comparative densitometry) in the observed binding of DHC to the DIC-treated column, whereas the BSA-treated p150^Glued\textsuperscript{Glued} column showed high levels of binding (compare lanes 4 and 5 with lanes 8 and 9). This result suggests that DIC mediates the binding of cytoplasmic dynein to the p150^Glued\textsuperscript{Glued} component of the dynactin complex.

The Dynactin Complex Binds to Dynactin Intermediate Chain—Although we demonstrated that exogenous DIC blocks dynein binding to the p150^Glued\textsuperscript{Glued} column, we cannot exclude the possibility that dynactin-dynactin binding may also be mediated by components other than DIC within the cytoplasmic dynein complex. To test the hypothesis that p150^Glued\textsuperscript{Glued} may directly bind to DIC, we constructed affinity columns of bacterially expressed DIC. Whole brain cytosol was loaded onto an affinity column of bacterially expressed DIC. The column was washed extensively and then eluted with 0.5 and 1.0 M NaCl. The resulting fractions were resolved by SDS-PAGE and probed for the binding of dynactin complex to DIC by Western blot using antibodies to p150^Glued\textsuperscript{Glued} and centrinactin. Because p150^Glued\textsuperscript{Glued} and centrinactin are bona fide components of the dynactin complex, the presence of both components would suggest that the intact dynactin com-
Cytoplasmic Dynein Binds Directly to the Dynactin Complex

C. M. Waterman-Storer, S. Kuznetsov, S. Karki, G. M. Langford, D. G. Weiss, and E. L. F. Holzbaur, submitted for publication.

**Fig. 4.** The dynein intermediate chain blocks dynein-dynactin binding. p150

A

Glued

affinity beads were preincubated in 0.3 mg/ml bacterially expressed DIC (lanes 2–5) or 1 mg/ml BSA (lanes 6–9). After mild washing (50 mM NaCl), whole brain cytosol was loaded and processed as described in the legend to Fig. 3. The eluates were probed for DHC with anti-DHC antibodies. Results show that excess DIC blocks dynein binding to p150

B

Glued

affinity column. Lane 1, cytosol-loaded; lanes 2 and 6, flow-throughs; lanes 3 and 7, final wash; lanes 4 and 8, 0.5 mM NaCl elution; lanes 5 and 9, 1.0 mM NaCl elution.

**Fig. 5.** The dynactin complex binds to dynein intermediate chain. Whole brain cytosol (lane 1) was loaded on a column of bacterially expressed dynein intermediate chain (lanes 2–5) or BSA (lanes 6–9) immobilized on CH-Sepharose 4B beads. The columns were washed with 50 mM NaCl and eluted with 1 ml each of 0.5 and 1.0 mM NaCl. The eluates were analyzed by probing with either anti-p150

A

Glued

antibodies (A) or anti-centractin antibodies (B). Lane 1, cytosol-loaded; lanes 2 and 6, flow-throughs; lanes 3 and 7, final wash; lanes 4 and 8, 0.5 mM NaCl elution; lanes 5 and 9, 1.0 mM NaCl elution. A and B are separate blots but have the same samples. CENT, centractin or Arp1.

The results presented in this paper clearly demonstrate that native cytoplasmic dynein binds to column-immobilized p150

A

Glued

and that the dynactin complex binds to column-immobilized DIC. We also show that this interaction is mediated by the direct binding of the DIC to p150

B

Glued

since we could effectively block dynein binding to the p150

A

Glued

column by excess exogenous DIC. A biochemical interaction between polypeptides of cytoplasmic dynein and the dynactin complex has also been observed by Vaughan and Vallee,3 using the solid-phase blot overlay method.

We have mapped the DIC binding domain to the amino-terminal half of p150

A

Glued

between amino acids 133 and 899. This region is predicted to form an extended a-helical coiled coil (5). Recent results from our laboratory demonstrate that the p150

A

Glued

component of the dynactin complex binds to both the microtubule and to centractin (27), an actin-related protein which is a major stoichiometric component of the dynactin complex (6–8, 11, 12). Taken together, these data suggest that p150

A

Glued

is a multifunctional polypeptide with at least three interacting domains as depicted in Fig. 6A.

One perplexing observation is that although dynein and the dynactin complex biochemically interact, as we have demonstrated here, they do not co-precipitate when antibodies to components of either complex are used (7). However, this apparent discrepancy may result from the blocking of the sites of interaction by the immunoprecipitating antibodies. Recently Waterman-Storer et al.4 observed that two distinct polyclonal antibodies to p150

A

Glued

blocked the interaction between dynein and dynactin. This lends support to the idea that antibodies used previously to co-precipitate dynein and dynactin interfered with the dynein-dynactin interaction and hence led to the failure to co-precipitate.

The observation that the binding of cytoplasmic dynein to

---

3 Vaughan, K. T., and Vallee, R. B. (1995) J. Cell Biol. in press.

4 C. M. Waterman-Storer, S. Kuznetsov, S. Karki, G. M. Langford, D. G. Weiss, and E. L. F. Holzbaur, submitted for publication.
p150\textsuperscript{Glued} is mediated by DIC is interesting in view of the functional homology of this polypeptide to the 70-kDa intermediate chain of flagellar outer arm dynein from Chlamydomonas. Studies on flagellar outer arm dynein suggest that the 70-kDa intermediate chain is involved in the structural, ATP-insensitive binding of axonemal dynein to the A subfiber microtubule (22). Paschal et al. (19) have therefore predicted that the cytoplasmic DIC may function in an analogous manner to attach cytoplasmic dynein to organelles or kinesinches. Since p150\textsuperscript{Glued} is localized to the membranous structures in the cytoplasm, it is possible that the dynactin complex on the surface of membranous structures targets the binding of the dynein motor via the interaction between p150\textsuperscript{Glued} and DIC.

It has been demonstrated recently that p150\textsuperscript{Glued} binds to microtubules independently of its association with cytoplasmic dynein (27). This microtubule-binding motif shares homology with a similar motif in the microtubule-organelle linking protein CLIP-170 (27, 28). CLIP-170 has been proposed to act as a docking protein for the binding of membranous vesicles to the microtubule (28). By analogy with CLIP-170, we speculate that p150\textsuperscript{Glued} may function to target organelles and vesicles to the microtubule and subsequently allow cytoplasmic dynein to bind. Fig. 6B depicts a model in which p150\textsuperscript{Glued} is simultaneously linked to the microtubule, the dynein intermediate chain, and dynactin. Alternatively, a continuous, but weak, interaction with the microtubule (K\textsubscript{d} = 10 \mu M; Ref. 27) during vesicle translocation may prevent diffusion of the vesicle during that stage of the dynein cross-bridge cycle when both heads are predicted to be detached (see Ref. 35 for kinetics of axonemal dynein). In the model shown in Fig. 6B, dynactin may be a structural link to the organelle, potentially via the cortical cytoskeleton.

Now that our results establish a direct binding between the cytoplasmic dynein and dynactin complexes through DIC and p150\textsuperscript{Glued}, it will be important to determine how the interactions among dynein, dynactin, the microtubule, and the cellular cargo are regulated if we are to understand the molecular mechanism of dynein-dynactin function. How p150\textsuperscript{Glued} that is bound to the microtubule in an ATP-insensitive manner would facilitate dynein-based motility if it is simultaneously bound to both dynein and microtubule is an important issue and raises the possibility that interaction of dynactin to either the microtubule or DIC is transient and regulatory. Phosphorylation of p150\textsuperscript{Glued} may regulate dynein function by altering the affinity of the polypeptide for either the microtubule or the DIC. Farshori and Holzbaur\textsuperscript{5} have shown that p150\textsuperscript{Glued} is differentially phosphorylated in response to cellular effectors that have been reported to increase cellular vesicle transport (29). CLIP-170 has been shown to dissociate from microtubules upon phosphorylation (30) and by analogy the interaction of p150\textsuperscript{Glued} to microtubules may also be regulated in a similar manner to allow transport of organelles along microtubules by cytoplasmic dynein. Alternatively, phosphorylation of p150\textsuperscript{Glued} may regulate its binding to dynein. In this context it is interesting to note that Lin and Collins (31) and Lin et al. (32) have demonstrated that okadaic acid (an inhibitor of phosphatases 1 and 2a) causes redistribution of cytoplasmic dynein from lysosomes to the cytosol. On the basis of our current results, this redistribution could be due to phosphorylation of p150\textsuperscript{Glued} which induces the dissociation of DIC from p150\textsuperscript{Glued} and therefore the release of cytoplasmic dynein from the organelles.

Only very recently have genetic and biochemical studies provided useful insights into the interaction between cytoplasmic dynein and its proposed activator, the dynactin complex, although we do not as yet understand the mechanism by which dynactin regulates dynein activity. In this paper we have described a system where the dynein-dynactin interaction can be observed in vitro and have identified the components involved in this interaction. These results now provide insight into the cellular basis for the lethal defect observed in the Glued mutation in Drosophila, as dynactin may be an essential component of the retrograde transport mechanism.

Acknowledgments—We thank K. T. Vaughan and R. B. Vallee of the Worcester Foundation for Experimental Biology for providing the DIC expression clone, K. K. Pfister and E. A. Vaisberg for their generous gifts of dynein intermediate chain and dynein heavy chain antibodies, respectively, M. K. Tokito for expert technical assistance, C. M. Waterman-Storer and E. Holleran for their helpful discussion, and the Drug Synthesis and Chemistry Branch of the National Cancer Institute, Bethesda, MD for the gifts of taxol.

REFERENCES

1. Toyoshima, I., Yu, H., Steuer, E. R., and Sheetz, M. P. (1992) . Cell Biol. 118, 1121–1131.
2. Kumar, J., Hanry, Y., and Sheetz, M. P. (1995) Science 267, 1834–1837.
3. Paschal, B. M., Shpetner, H. S., and Vallee, R. B. (1987). Cell Biol. 105, 1273–1282.
4. Steuer, E., Wordeman, L., Schroer, T. A., and Sheetz, M. P. (1990) Nature 345, 266–268.
5. Holzbaur, E. L. F., Hammerback, J. A., Paschal, B. M., Kravit, N. G., Pfister, K. K., and Vallee, R. B. (1991) Nature 351, 579–583.
6. Gill, S., Schroer, T. A., Solia, I., Steuer, E. R., Sheetz, M. P., and Cleveland, D. W. (1991) J. Cell Biol. 115, 1639–1650.
7. Paschal, B. M., Holzbaur, E. L. F., Pfister, K. K., Clark, S., Meyer, D., and Vallee, R. B. (1993) J. Biol. Chem. 268, 15318–15323.

\textsuperscript{5} P. Farshori and E. L. F. Holzbaur, manuscript in preparation.
