Molecular analysis of a cytoplasmic dynein light intermediate chain reveals homology to a family of ATPases

Sharon M. Hughes, Kevin T. Vaughan, Jonathan S. Herskovits and Richard B. Vallee*

Cell Biology Group, Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545, USA

*Author for correspondence

SUMMARY

Cytoplasmic dynein is a multi-subunit complex involved in retrograde organelle transport and some aspects of mitosis. In previous work we have cloned and sequenced cDNAs encoding the rat cytoplasmic dynein heavy and intermediate chains. Here we report the cloning of the remaining class of cytoplasmic dynein subunits, which we refer to as the light intermediate chains (LICs: 53-59 kDa). Four LIC electrophoretic bands were resolved in purified bovine cytoplasmic dynein preparations by one-dimensional gel electrophoresis. These four bands were simplified to two bands (LIC53/55 and LIC57/59) by alkaline phosphatase treatment. N-terminal amino acid sequence was obtained from a total of 11 proteolytic peptides generated from both LIC53/55 and LIC57/59. Overlapping cDNA clones encoding LIC53/55 were isolated by oligonucleotide screening using probes based on the LIC53/55 peptide sequence. The cDNA sequence contained a 497 codon open reading frame encoding a polypeptide with a molecular mass of ~55 kDa. Each of the LIC53/55 peptides was found within the deduced amino acid sequence, as well as four of the LIC57/59 peptides. Analysis of the LIC53/55 primary sequence revealed homology with the ABC transporter family of ATPases in the region surrounding the P-loop sequence element. Together these data identify the LICs as a novel family of dynein subunits with potential ATPase activity. They also reveal that the complexity of the LICs is due to both post-translational modification and the existence of at least two LIC polypeptides for which we propose the names LIC-1a and LIC-2.

Key words: cytoplasmic dynein, ATPases, ABC transporters, motility, microtubule motors

INTRODUCTION

Cytoplasmic dynein is a minus end-directed microtubule-associated motor protein (Paschal and Vallee, 1987). It has been implicated in retrograde axonal transport and movements of organelles, such as lysosomes and endosomes, towards the minus ends of microtubules (Paschal and Vallee, 1987; Schnapp and Reese, 1989; Schroer et al., 1989; Lacey and Haimo, 1992; Lin and Collins, 1992). The subcellular distribution of certain organelles, such as the Golgi apparatus, has also been attributed to cytoplasmic dynein (Corthesy-Theulaz et al., 1992). Immunological studies (Pfarr et al., 1990; Steuer et al., 1990) and in vitro reconstitution of chromosome-associated movements (Hyman and Mitchison, 1991) have also suggested that cytoplasmic dynein is associated with kinetochores, indicating that it may play a role in chromosome movement during mitosis.

Cytoplasmic dynein has been found to be structurally and biochemically related to axonemal dyneins (Paschal et al., 1987; Lye et al., 1987; Shpetner et al., 1988; Vallee et al., 1988), the ATPases responsible for flagellar and ciliary movements (Gibbons and Rowe, 1965). Despite the similarities between axonemal and cytoplasmic dyneins, there are also many differences, the most striking involving subunit composition. All dyneins contain high molecular mass heavy chains (HCs) which have multiple P-loop consensus sequences (Gibbons et al., 1991; Ogawa, 1991; Koonce et al., 1992; Mikami et al., 1993; Zhang et al., 1993). Whether or not all of these domains are functional in ATP binding is not yet known. Cytoplasmic dynein also contains, along with the heavy chains, at least three electrophoretic species at 74 kDa, and a group of four species with apparent molecular masses of 53, 55, 57 and 59 kDa (Paschal et al., 1987). Axonemal dyneins show greater subunit complexity, with a variety of intermediate chains (ICs) ranging from 69 to 120 kDa, and numerous light chains of unknown function with molecular masses of 10-20 kDa (Pfister et al., 1982; Piperno and Luck, 1979; Tang et al., 1982; reviewed by Holzbaur and Vallee, 1994). Recently, the cytoplasmic dynein 74 kDa subunits were cloned and shown to be homologous to a 70 kDa intermediate chain (IC70) of Chlamydomonas flagellar outer arm dynein (Paschal et al., 1992). The 70 kDa axonemal subunit has been shown by immunoelectron microscopy to be located at the base of the dynein molecule and is believed to be involved in binding the outer arm dynein to the axonemal A subfiber microtubule (King and Witman, 1990). By comparison, the 74 kDa cytoplasmic dynein IC has been postulated to be involved in attaching the cytoplasmic protein to organelles and kinetochores.

The function of the 53-59 kDa cytoplasmic dynein subunits, which we have termed light intermediate chains (LICs)
(Hughes et al., 1993), is unknown. The present study was initiated to understand the basis for the electrophoretic complexity of these poly peptides and their relationship to other dynein subunits. We report here the primary structure of a 55 kDa cytoplasmic dynein LIC, along with evidence that the electrophoretic complexity of these poly peptides is due to the existence of multiple isoforms and to phosphorylation. We have found that the 55 kDa LIC is unrelated to previously characterized axonemal and cytoplasmic dynein ICs and LCs. Furthermore, we find that the poly peptide contains a P-loop element which has homology to a family of ATPases, the ABC transporter proteins.

MATERIALS AND METHODS

Protein chemistry

dytoplastic dynein was purified from calf brain matter as previously described (Paschal et al., 1991), except that sucrose density gradients were prepared in 100 mM Tris-HCl, pH 8.0, 4 mM MgCl2. For peptide sequencing, the sucrose gradient fractions containing cytoplasmic dynein were electrophoresed on a 7% polyacrylamide gel as described (Laemmli, 1970). The protein was then transferred to PVDF (Millipore, Bedford, MA) in 10 mM CAPS, 10% MeOH for 45 minutes at 50 volts. The blot was stained with Coomassie Brilliant Blue R and the 53/55 kDa and 57/59 kDa doublets were excised for sequencing. The protein was subjected to in situ digestion with either trypsin or endoproteinase Glu-C. The peptides were eluted from the blot and separated by HPLC (Hewlett Packard 1090M) with a C8 microbore column (Aebersold, 1989; Fernandez et al., 1994). Automated peptide sequencing was performed on an Applied Biosystems model 492 Procise sequencing system. All peptide sequence was determined in the Worcester Foundation for Experimental Biology Protein Chemistry Facility.

Alkaline phosphatase treatment of cytoplasmic dynein was carried out in 100 mM Tris-HCl, pH 8.0, 4 mM MgCl2, 0.1 mM ZnCl2. Alkaline phosphatase (Boehringer Mannheim Biochemicals, Indianapolis, IN) was added to a final concentration of 20-120 units/ml. Pyrophosphate was added to 50 mM in controls to inhibit dephosphorylation. All reactions were incubated at 37 °C for 2 hours. The alkaline phosphatase treatment was analyzed by SDS-PAGE and 2-D electrophoresis (O’Farrell, 1975) using the Bio-Rad

PAGE and 2-D electrophoresis (O’Farrell, 1975) using the Bio-Rad

effects of alkaline phosphatase treatment were analyzed by SDS-phorylation. All reactions were incubated at 37 ° C for 2 hours. The alkaline phosphatase treatment was analyzed by SDS-PAGE and 2-D electrophoresis (O’Farrell, 1975) using the Bio-Rad

PAGE and 2-D electrophoresis (O’Farrell, 1975) using the Bio-Rad

Table 1. Comparison of rat LIC 53/55 deduced amino acid sequence with the sequences of bovine LIC peptides

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>APVVEKREKLLGVPVNGAVAAAGDLTSE</td>
</tr>
<tr>
<td>2</td>
<td>GQSLWSILSE</td>
</tr>
<tr>
<td>3</td>
<td>TVGFHTIPALVVEK</td>
</tr>
<tr>
<td>4</td>
<td>IAILHENTTVKPEDAYEDFIKVPP</td>
</tr>
<tr>
<td>5</td>
<td>GGPSVPSAPSSTGVS</td>
</tr>
<tr>
<td>6</td>
<td>TVLNVQQLEDL</td>
</tr>
<tr>
<td>7</td>
<td>DFQDYIEPFEGQGSSPQR</td>
</tr>
<tr>
<td>8</td>
<td>DAVFIPAGWNEK</td>
</tr>
<tr>
<td>9</td>
<td>NILVFGEGLSGK</td>
</tr>
<tr>
<td>10</td>
<td>NNAASEGVLASFNSNL</td>
</tr>
<tr>
<td>11</td>
<td>DVSSNVAYSVPAGSK</td>
</tr>
</tbody>
</table>

*Sequence 1 is the N-terminal sequence of LIC 53/55. Two different sequences were obtained. Protein with the major sequence at the N terminus was approximately 2.5 times more abundant than protein with the minor sequence.
†Peptides 2 and 5 were generated by endoproteinase Glu-C digestion. All other peptides were generated by digestion with trypsin.
‡Peptide 11 was sequenced from LIC 57/59. The deduced amino acid sequence of LIC 53/55 does not contain any sequences with significant homology.

Another clone, RP1, was subsequently isolated from the same library using probes random-primed from RAT1. Hybridization was performed overnight at 65 ° C in Rapid-hyb (Amersham, Arlington Heights, IL) hybridization buffer. The filters were washed in 2× SSPE, 0.1% SDS, 0.1% pyrophosphate, twice for 30 minutes at room temperature, then 1× SSPE, 0.1% SDS, 0.1% pyrophosphate, 30 minutes 65°C, and 0.7× SSPE, 0.1% SDS, 0.1% pyrophosphate twice for 30 minutes at 65°C.

In order to clone the 5’ end of the cDNA, a restriction fragment of RP1 was used to rescreen the library. Screening was performed using Rapid-hyb as described above. The fifteen clones isolated were all ‘rescued’ using helper phage according to the manufacturer (Strategene). The plasmids were then probed with an oligonucleotide corresponding to the 5’ end of LIC coding sequence from within RP1. Positive clones were sequenced (Sequenase, version 2.0, United States Biochemical, Cleveland, OH), and one was found to contain the N-terminal peptide sequence.

DNA sequencing was completed on both strands using nested deletions (Erase-A-Base: Promega, Madison, WI), convenient restriction sites, and oligonucleotides. All DNA and protein sequence was assembled and analyzed using the GCG DNA analysis programs, including MOTIFS and BESTFIT. The National Center for Biotechnology Information (NCBI) databases were scanned using BLAST (Altschul et al., 1990). The statistical significance of the alignments was determined using RDIF2 (Lipman and Pearson, 1985), and additional homology was examined using the BLOCKS Database Version 7.01 (Henikoff and Henikoff, 1994). The Protein Kinase Catalytic
Domain Database (Hanks and Quinn, 1991) was also scanned for homology using FASTA (Lipman and Pearson, 1988).

Northern blots
A multiple tissue northern (MTN) blot (Clontech Laboratories, Palo Alto, CA) was hybridized with probes random-primed from a BgIII/XhoI fragment of RP1, which contains nearly the entire coding sequence of LIC35/55 and 664 bp of the 3′ untranslated sequence. The MTN was also hybridized with probes from the 3′-untranslated sequence alone. All hybridizations were performed in Rapid-hyb at 65°C overnight. The blots were washed with 2× SSPE, 0.1% SDS, 0.1% pyrophosphate for 15 minutes at room temperature, followed by 0.5× SSPE 0.1% SDS, 0.1% pyrophosphate at 65°C twice for 30 minutes each.

RESULTS
Alkaline phosphatase reduces the complexity of cytoplasmic dynein light intermediate chains
By SDS-PAGE, four bands can be distinguished in the 50-60 kDa size range (Paschal et al., 1987). The four bands appear as two doublets and have been assigned molecular masses of 53, 55, 57 and 59 kDa. In order to examine the complexity of the LICs further, cytoplasmic dynein was subjected to 2-D gel electrophoresis (Fig. 1B). Each of the four electrophoretic species seen by SDS-PAGE resolved into multiple spots.

To determine whether phosphorylation was responsible for the observed electrophoretic complexity, we treated cytoplasmic dynein with alkaline phosphatase. The effect of alkaline phosphatase was analyzed by SDS-PAGE (Fig. 1A) and 2-D gel electrophoresis (Fig. 1C). A clear reduction in the intensity of the 59 kDa band as well as the 55 kDa band was observed with increasing alkaline phosphatase concentration, while the 57 and 53 kDa bands increased in intensity. This change in pattern was also observed by 2-D gel electrophoresis (Fig. 1C), but relatively little effect on the number of spots corresponding to each remaining band was observed.

To learn more about the molecular basis for the complexity of the LICs, we set out to obtain primary sequence data by direct amino acid sequencing and by cDNA analysis. The LIC SDS-PAGE doublets were digested with either endoprotease Glu-C or trypsin, and the resulting peptides were then purified and subjected to microsequencing. Multiple peptides were sequenced from each doublet (Table 1, all except sequence 1). Interestingly, two of the sequences generated from LIC57/59 peptides were related to sequences generated from LIC53/55 peptides. However, differences between sequences from the two doublets were also apparent. These data suggested that LIC53/55 and LIC57/59 represent related but distinct protein isoforms.

The full-length polypeptides were also subjected to N-terminal sequence analysis. The N terminus of LIC57/59 appeared to be blocked, but a mixture of two overlapping sequences was obtained from the N terminus of undigested LIC53/55 (Table 1A, sequence 1).

Using part of the amino acid sequence of peptide 4 of LIC53/55 (Table 1), a 26-mer oligonucleotide was designed for screening a rat brain lambda ZAP II library. Two clones, RAT1 and RAT2, were isolated and found to encode multiple LIC53/55 peptide sequences. One of these clones was used to make random-primed probes to re-screen the library. One 4.4 kb clone, RP1 (Fig. 2), was isolated and sequenced completely. This clone was found to have part of a cDNA coding for malate dehydrogenase fused near the 5′-end of the LIC coding sequence. To obtain the remainder of the LIC sequence, oligonucleotides made from the 5′-most portion of the LIC sequence of RP1 were used to re-screen the library. Out of fifteen clones isolated at this stage, all of them were found to be identical to part of RP1, but only one contained enough 5′ sequence to locate the initiator methionine, previously identified by N-terminal sequencing of the undigested LIC53/55 doublet (see Table 1, sequence 1). Clones contributing to the initial identification of LIC cDNAs and to the final cDNA sequence are presented in Fig. 2.

The 4.4 kb cDNA encodes a protein of 55 kDa (Fig. 3). Each of the peptides sequenced from LIC53/55 was found in the

![Fig. 1. The effect of alkaline phosphatase (AP) treatment on cytoplasmic dynein light intermediate chains. (A) 20 S dynein was treated with 20-120 units/ml of alkaline phosphatase for 2 hours, then run on a 9% SDS-polyacrylamide gel and silver stained. The top of the gel including the cytoplasmic dynein heavy chain is not shown due to intense silver staining. For untreated dynein, the predominant bands are 55 and 59 kDa. With increasing alkaline phosphatase concentration, the 55 and 59 kDa bands shift to 53 and 57 kDa, respectively. The far right lane shows alkaline phosphatase inhibition by 50 mM pyrophosphate. (B) 2-D gel of untreated LICs. (C) 2-D gel of LICs treated with 80 units/ml alkaline phosphatase.](image-url)
deduced amino acid sequence (Fig. 3). Only cDNAs corre-
sponding to the minor N-terminal sequence were found.

All but one of the peptide sequences obtained from
LIC57/59 could be aligned with the LIC53/55 deduced amino
acid sequence (Table 1 and Fig. 3). The peptide data suggest
that our cDNAs encode LIC53/55. This conclusion is based on
the fact that peptide 11 (Table 1C), derived from LIC57/59,
was absent from the deduced amino acid sequence, and peptide
7 (Table 1B) from LIC53/55 is closer to the deduced amino
acid sequence than is the corresponding peptide from
LIC57/59.

Sequence analysis

We compared the deduced amino acid sequence of LIC53/55
with the previously determined sequences of cytoplasmic and
axonemal dynein intermediate and light chains. No clear rela-
tionship was observed with the five known rat cytoplasmic
dynein intermediate chain isoforms (Paschal et al., 1992;
Vaughan and Vallee, 1993), or IC70 (Mitchell and Kang,
1991) and IC78 (C. Wilkerson, personal communication) of
*Chlamydomonas* flagellar outer arm dynein. Despite the size
difference between the LICs and the *Chlamydomonas*
flagellar outer arm dynein light chains (LCs; molecular mass 10-20
kDa) we compared our sequence with the one available light
chain sequence (10 kDa) and found no significant relationship
(S. M. King and R. S. Patel-King, unpublished results).

The deduced amino acid sequence of LIC53/55 was also
analyzed for secondary structure and for the presence of struc-
tural motifs (see Materials and Methods). No substantial blocks
of alpha-helix, coiled-coil alpha-helix, or beta-sheet were
observed. However, the MOTIFS program revealed a P-loop
sequence near the N terminus, from amino acid 60 to 68 sug-
ifying a possible nucleotide-binding site.

While a comparison to the Protein Kinase Catalytic Domain
Database failed to reveal similarity to known kinases, a search
of the protein sequence databases using the BLAST program
revealed short segments of homology with a variety of
proteins, including several nucleotidases. Prominent among
these were members of the effector family of ATPases. Screening
the databases with a 29 amino acid sequence from the P-loop region of LIC53/55 (amino acids 47-76) revealed the highest degree of homology with the ABC
transporters (Table 2). Analysis of the LIC53/55 sequence
using the BLOCKS program, which searches for multiple
blocks of homology among members of multi-gene families,
again revealed a relationship with the ABC transporters.

Recently, Gill et al. (1994) reported the cloning of cDNAs
encoding a 56 kDa chicken brain cytoplasmic dynein subunit
referred to as DLC-A. Comparison of that sequence with
LIC53/55 revealed 64% amino acid identity and 80% similar-
ity including conservative amino acid substitutions (Fig. 4).

We note that amino acid sequences derived from the bovine
LIC57/59 peptides are closer to the chicken DLC-A than to the
amino acid sequence deduced from the LIC53/55 cDNAs. Of
particular interest, peptide 11 (Table 1C), derived from
LIC57/59, is clearly related to amino acids 402-417 of the
DLC-A sequence (Fig. 4), but is absent from the LIC53/55
sequence. Furthermore, the N-terminal sequence obtained from
bovine LIC53/55 is completely absent from DLC-A. These
results suggest that DLC-A is the chicken homologue of
LIC57/59.

Northern blot analysis

To gain further insight into the relationship between LIC
isoforms, northern blot analysis was performed (Fig. 5). LIC
transcripts of 4.4, 3.5 and 2.0 kb were observed. The 4.4 kb

![Fig. 2. Line diagram of LIC53/55 cDNAs. Only the clones contributing to the initial identification of the LIC cDNA (RAT1) and to the final
cDNA sequence (RP1 and FP2) are presented. The broken line at the 5′ end of RP1 indicates the region which was found to encode malate
dehydrogenase rather than LIC53/55. Numbering corresponds to the final nucleotide sequence in Fig. 3.](image-url)
species was found in all tissues examined and was the most prominent species in most tissues. The 2.0 and 3.5 kb transcripts were particularly abundant in testis, although they were detectable in other tissues. The same blot was hybridized with

Fig. 3. Primary sequence of LIC53/55. The nucleotide and deduced amino acid sequences of LIC53/55 are shown. cDNA and protein sequence numbering is presented in the left margin. Peptide sequences are underlined and the P-loop consensus sequence is double underlined. A consensus polyadenylation signal was identified at nucleotides 4277-4282 (AATAAA). This sequence can be retrieved from GenBank using Accession number U15138.
22 S. M. Hughes and others

**DISCUSSION**

The LICs represent the least well-characterized components of cytoplasmic dynein. The present study indicates that these polypeptides are related to each other and constitute a new dynein subunit class which is distinct from the heavy, intermediate, and light chains.

We have found that the electrophoretic complexity of the LICs is greater than previously recognized (Paschal et al., 1987). However, our results also indicate that the complexity may derive from extensive modification of as few as two polypeptide isoforms. Alkaline phosphatase treatment substantially simplified the one-dimensional LIC electrophoretic pattern (Fig. 1), suggesting that phosphorylation is responsible for some of the observed complexity. High levels of alkaline phosphatase failed to eliminate all of the 2-D electrophoretic complexity (Fig. 1B,C). This observation may reflect alkaline phosphatase-insensitive phosphorylation, other post-translational modifications, or even greater isoform diversity than that revealed by our peptide and cDNA sequence analysis.

We obtained two distinct N-terminal sequences from Edman degradation of the p53/55 electrophoretic doublet (Table 1). It is unlikely that this heterogeneity contributed to the observed electrophoretic complexity because the two amino acids included in the longer sequence are uncharged. Despite the fact that the shorter sequence was more abundant than the longer sequence, the latter corresponded to the amino acid sequence deduced from cDNA analysis. The shorter sequence could reflect alternative splicing of LIC transcripts; however, the observed heterogeneity seems equally likely to reflect limited proteolysis at the N terminus of the polypeptide.

We obtained two distinct N-terminal sequences from Edman degradation of the p53/55 electrophoretic doublet (Table 1). It is unlikely that this heterogeneity contributed to the observed electrophoretic complexity because the two amino acids included in the longer sequence are uncharged. Despite the fact that the shorter sequence was more abundant than the longer sequence, the latter corresponded to the amino acid sequence deduced from cDNA analysis. The shorter sequence could reflect alternative splicing of LIC transcripts; however, the observed heterogeneity seems equally likely to reflect limited proteolysis at the N terminus of the polypeptide.

The results of the present analysis and that of Gill et al. (1994) are most readily understood in terms of two LIC genes. In the present study peptide sequences derived from LIC53/55 were all found in the deduced amino acid sequence. Amino acid sequences from two LIC57/59 peptides were found to be
related to sequences from LIC53/55 peptides, but the sequence of peptide 7 (Table 1B) is actually much closer to sequences in chicken DLC-A. In addition, sequence from LIC57/59 peptide 11 (Table 1C) was only found in DLC-A, and the amino-terminal sequence of LIC53/55 was unique to the LIC53/55 deduced amino acid sequence. Together these results suggest that each of the two LIC isoforms has been conserved throughout vertebrate evolution. Furthermore, because sequence differences are distributed across the length of the isoforms, it seems more likely that they represent products of distinct genes, rather than being the results of alternative splicing.

We identified three LIC transcripts in rat at 4.4, 3.5 and 2.0 kb at high stringency (Fig. 5), one of which (2.0 kb) was not recognized by a 3′-untranslated probe. In view of its size it is likely that the 4.4 kb species corresponds to LIC53/55. However, the origin of the 3.5 kb species, which also hybridized with the 3′-untranslated probe, is uncertain. We note that the 3′-untranslated sequence for LIC53/55 contains repetitive elements (Fig. 3). Thus, the relationship between the 3.5 kb and 4.4 kb transcripts is uncertain. Gill et al. (1994) described DLC-A cDNAs of 2.4 and 1.6 kb in chicken which were identical except for unusual alternative polyadenylation site usage. They suggested that these sequences correspond to the 2.4 and 1.7 kb transcripts observed in their study, but no evidence is provided to confirm this prediction. Thus, it remains uncertain whether the transcripts observed in our study encode two or three LIC isoforms, and the correspondence between rat and chicken transcripts remains to be resolved.

In view of our evidence that the 53 and 55 kDa LIC electrophoretic species are generated from a common polypeptide by post-translational modification, we propose the name LIC-2 for the polypeptide. Similarly, we propose the name LIC-1 for the larger polypeptide corresponding to the 57 and 59 kDa electrophoretic species.

**Relationship to other dynein polypeptides and ATPases**

The present study, together with that of Gill et al. (1994), appears to complete the molecular cloning of the known cytoplasmic dynein subunits. The complex array of cytoplasmic dynein subunits (Paschal et al., 1987) can now be seen to represent only three polypeptide classes. Whether axonemal dyenines will prove to have components related to the LICs, and whether cytoplasmic dyenine will prove to have components related to the axonemal LCs, remains to be determined. However, it is clear that molecular analysis of the numerous dyenine subunits which have been identified biochemically (reviewed by Holzbaur and Vallee, 1994) will likely be required to determine their structural and functional interrelationships.

We observed LIC-2 to contain a P-loop element near the N terminus of the polypeptide. While such elements are found in many nuleotidases, it is difficult to assess whether or not they are functionally significant. This issue is particularly acute for the dyenines, which contain from four to five P-loop sequences per heavy chain. It is not known if more than one of these is functionally important (reviewed by Holzbaur and Vallee, 1994).

However, in the case of the LICs we have detected additional homology beyond the P-loop sequence with a subset of ATPases, the ABC transporters (also called traffic ATPases). Most of these proteins are involved in regulation of cell surface channels. The LICs contain only a portion of the conserved ABC transporter sequence; thus, we feel it is unlikely that they are closely related to the ABC transporters in function. However, the extended homology between the LICs and the ABC transporters around the P-loop region strongly suggests some common feature in their mechanisms of action, presumably ATP binding and hydrolysis. Future work in our laboratory will be directed at testing the LICs for enzymatic activity.

**Potential roles for the LICs in cytoplasmic dynein function**

An intriguing question is the possible role for an additional ATPase in the dynein complex. One interesting possibility is in the regulation of organelle and kinetochore binding. Recent work has suggested that the link between cytoplasmic dyenine and other subcellular structures may be quite complex and subject to an elaborate regulatory mechanism involving an interaction with an additional polypeptide complex, the dynactin (or Glued) complex (Holzbaur et al., 1991; Schroer and Sheetz, 1991; Gill et al., 1991; Paschal et al., 1993). A direct interaction between cytoplasmic dyenine and dynactin has not yet been observed. Nonetheless, in a search for subcellular receptors for cytoplasmic dyenine, we have found evidence for a direct interaction between the cytoplasmic dyenine ICs and p150Glued (K. T. Vaughan, E. L. F. Holzbaur and R. B. Vallee, unpublished observations). Curiously, p150Glued has also been found to have a microtubule-binding site (Waterman-Storer et al., 1993), which would be predicted to interfere with dyenine-mediated motility rather than to stimulate it. Together these data suggest that the dyenine-p150Glued interaction must be regulated.

We suggest that a possible role for the LICs is in regulating these interactions. Perhaps the LICs serve to order a series of interactions between the two complexes and the cellular substrates for dyenine-mediated motility (such as organelles). In this case, the LICs may behave as low turnover, kinetic ‘switches’, ordering the steps in a complex pathway.

The authors thank the Keck Foundation for supporting the Worcester Foundation for Experimental Biology Protein Chemistry Facility, and Curt Willkerson and Steve King for comparing unpublished sequences to our LIC sequence. A preliminary report of this study has been presented (Hughes et al., 1993).

**REFERENCES**


(Received 9 August 1994 - Accepted 9 September 1994)