**Novel 44-Kilodalton Subunit of Axonemal Dynein Conserved from *Chlamydomonas* to Mammals**

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Cilia and flagella have multiple dyneins in their inner and outer arms. *Chlamydomonas* inner-arm dynein contains at least seven major subspecies (dynein a to dynein g), of which all but dynein f (also called dynein II) are the single-headed type that are composed of a single heavy chain, actin, and either centrin or a 28-kDa protein (p28). Dynein d was found to associate with two additional proteins of 38 kDa (p38) and 44 kDa (p44). Following the characterization of the p38 protein (R. Yamamoto, H. A. Yanagisawa, T. Yagi, and R. Kamiya, FEBS Lett. 580:6357–6360, 2006), we have identified p44 as a novel component of dynein d by using an immunoprecipitation approach. p44 is present along the length of the axonemes and is diminished, but not absent, in the *ida4* and *ida5* mutants, both lacking this dynein. In the *ida5* axoneme, p44 and p38 appear to form a complex, suggesting that they constitute the docking site of dynein d on the outer doublet. p44 has potential homologues in other ciliated organisms. For example, the mouse homologue of p44, NYD-SP14, was found to be strongly expressed in tissues with motile cilia and flagella. These results suggest that inner-arm dynein d and its subunit organization are widely conserved.

Ciliary and flagellar beating is driven by axonemal dyneins that are contained in the inner and outer dynein arms. Several lines of evidence have indicated that the outer-arm and inner-arm dyneins are functionally distinct and differ totally in their subunit organization and arrangement in the axoneme (for a review, see reference 8). In *Chlamydomonas reinhardtii*, a model organism for cilia/flagellar studies, the outer-arm dynein exists as a single assembly composed of three distinct force-producing heavy chains (*M*, >500,000) and several intermediate and light chains. In contrast, inner-arm dynein exists as multiple species that can be further classified into a double-headed type and a single-headed type. A single species of dynein called subspecies f, or dynein II, belongs to the two-headed type and is composed of two heavy chains, three intermediate chains (molecular mass, 97 to 140 kDa), and several light chains. In contrast, six major and three minor species belong to the single-headed type (T. Yagi and R. Kamiya, unpublished data), which has a simpler organization, with a single heavy chain and a few light chains. The six major dyneins of the single-headed type are called subspecies (or dynein) a, b, c, d, e, and g. Although the detailed subunit composition remains to be clarified, a feature common to all six of these dyneins is that each one contains actin and either centrin or a 28-kDa protein (p28) (in subspecies a, c, d, and g) or a 28-kDa protein called p28 (in subspecies a, c, c, d, and d) (18, 25). These three proteins have been identified in other organisms, such as the sea urchin and human (4, 11, 19), indicating that the general design of single-headed inner-arm dyneins is conserved among various organisms.

In a previous study, we identified the major subspecies of inner-arm dynein by ion-exchange chromatography and also noticed that the fraction of inner-arm dynein d contains two proteins of about 44 kDa and 38 kDa in addition to actin and p28 (7). None of these proteins were present in the corresponding fraction from the *ida4* mutant, which has a mutation in the p28 gene and lacks dyneins a, c, and d (7), or from the *ida5* mutant, which has a mutation in the gene for conventional actin and lacks dyneins a, c, d, and e (12, 13). It seemed likely that the 44- and 38-kDa proteins were subunits of dynein d. We have recently cloned the cDNA of the 38-kDa protein and shown that it is actually associated with isolated dynein d. This protein has been registered in the flagellar proteome database (17) as FAP146 and has been described as a zinc finger-like flagellum-associated protein (24).

In the present study, we cloned and sequenced the cDNA of the 44-kDa protein (p44). Our results suggest that it functions together with p38 in the docking of dynein d to the outer doublet microtubules. Homologues of this protein, as well as those of p38, are found in a wide variety of organisms with motile cilia and flagella, indicating that dyneins structurally related to dynein d are widely conserved and serve some unique functions in cilia and flagellar motility.

**MATERIALS AND METHODS**

Strains and culture. The following *Chlamydomonas reinhardtii* strains were used: 137c (wild-type) and the *oda1* (lacking outer-arm dynein) (10), *ida3* (lacking inner-arm dynein f) (9), *ida4* (lacking inner-arm dyneins a, c, and d) (9), *ida5* (lacking inner-arm dyneins a, c, d, and e) (12), and *ida6* (lacking inner-arm dynein e) (12) mutants. Cells were grown in liquid Tris-acetic acid-phosphate (TAP) medium with aeration on a cycle of 12 h of light and 12 h of darkness.

Isolation of axonemes. Flagella were isolated by the dibucaine method of Witman (21) and were demembranated to yield axonemes by extraction with 0.2% Nonidet P-40 in HMDEK solution (30 mM HEPES, 5 mM MgSO₄, 1 mM dithiothreitol, 1 mM EGTA, and 50 mM potassium acetate [pH 7.4]).

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Crude dynein extract and isolation of dynein. Crude extracts containing various dyneins were obtained by high-salt extraction of wild-type or ida5 axonemes as described elsewhere (23) and were fractionated into individual dynein species by chromatography on a Uno Q ion-exchange column (Bio-Rad). We used a Uno Q column instead of a Mono Q column to achieve better separation of dynein d.

Sucrose density gradient centrifugation. A crude dynein extract from wild-type or ida5 axonemes was layered on top of a 4.3-M linear 5 to 20% sucrose density gradient that was prepared in HMDEK solution containing 0.2 mM protease inhibitor (Pefabloc). The gradients were centrifuged in a Hitachi RPS55T-2 swing rotor at 180,000 × g for 5.5 h at 4°C. Catalase (11.45S, aldolase (78S), bovine serum albumin (BSA) (4.45S), and RNase A (28) were also centrifuged as sedimentation coefficient markers on separate sucrose gradients prepared in HMDEK solution. Thirty fractions (400 μl) each were collected.

Protein identification. The p44 protein band of dynein d was separated by sodium dodecy1 sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently excised from the gels and digested with trypsine. The peptide mixture was then eluted and analyzed using an OMALDI-Qq-TOF MS/MS QSTAR Pulsar i (Applied Biosystems). The data were then searched to find the unirefindt genome database (JGI, version 2.0; http://genome.jgi-psf.org/chlr2/chlr2.home.html) using the Mascot search algorithm (http://www.matrixscience.com) to identify the genomic sequences that encode each peptide.

Determination of the cdna sequence of p44. The sequences at the 5′ and 3′ ends of the p44 cdna were obtained following ENFL (rapid amplification of cdna ends) PCR. The primers were used as follows: for 5′ RACE, forward primer AUAP (5′-GGCCACCGCTGCACTGAC-3′) and reverse primer p44-R1 (5′-GGACGAGACCAGAGCTCC-3′); for nested PCR, forward primer AUAP and reverse primer p44-R9 (5′-GTTTGTTGTTAGGGCTGAA-3′); for 3′ RACE, forward primer p44-F1 (5′-GAAAGCTGTCAGAATTCCTAC-3′) and reverse primer AUAP; and for nested PCR, forward primer p44-F2 (5′-GGCTGCAGAATTCCTAC-3′) and reverse primer AUAP. The resulting sequence of the 3′ RACE was confirmed using primers p44-F5 (5′-GAACCTCCACACAGTGTTCTGAGAC-3′), p44-F9 (5′-GGTTGTTTCTGAGTGTAGTCTG-3′), and p44-R6 (5′-CTTCCGCTCTGTTGCACTATTGAACAG-3′).

The cdna used had been synthesized using Superscript III from poly(A) RNA trapped on poly(T)-anchored magnetic beads (Dynabeads Oligo(dT)25; Dynal Biotech). Total wild-type RNA that was used for the isolation of poly(A) RNA was prepared from acid guanidinium thiocyanate-phenol-chloroform extraction.

Northern and Southern blot analyses. To determine the size of the p44 transcript and also to detect its up-regulation after deflagellation, total RNA was isolated from wild-type cells 45 min after deflagellation. The RNA samples were analyzed by Northern blotting using the 608 bp fragment of p44-cDNA as a probe. To determine the number of gene copies, DNA was isolated from the wild-type strain and digested with NotI, SacI, and Sall. The DNA samples were analyzed by Southern blotting using the same probe as that used for Northern blotting.

Bacterial expression of a partial amino acid sequence of p44. Amino acid residues 34 to 235 of p44 were expressed by amplifying the coding region of the cdna by PCR with primers p44-NF (CTGGATCCAAGCTGACAACCTCA-3′) and p44-NR (GAAGATCTCGTGTCTGACACCCTGAAGAG-3′) which contained recognition sites for BamHI and EcoRI, respectively (underlined). The PCR product was ligated to the BamHI and EcoRI sites of the bacterial expression vector pCold. The resulting fusion protein contained a His tag sequence at its N terminus. Expression of the fusion protein was induced by the addition of isopropyl-p-D-thiogalactopyranoside to a logarithmically growing culture of Escherichia coli to a final concentration of 0.2 mM. Almost all of the expressed protein was contained in inclusion bodies.

Polyclonal antibody production. To produce a p44-specific antibody, the insoluble recombinant p44 protein was sequentially washed with phosphate-buff ered saline (PBS) containing 1% Triton X-100, 0.5 M urea, 2 M urea, and 4 M urea, and the resulting pellet was used as an antigen to immunize two rabbits. Antibodies were affinity purified by using recombinant p44 blotted onto polyvinylidene difluoride membranes (16).

Immunoblotting. Immunoblotting was carried out using standard procedures. Immunoreactive bands were detected using an alkaline phosphatase-conjugated secondary antibody and a BCIP (5-bromo-4-chloro-3-indolylphosphate)-NBT (nitroblue tetrazolium phosphate) solution kit (Kirkegaard & Perry Labora tories, Inc., Gaithersburg, MD) or a horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and a TMB (3,3′,5,5′-tetramethylbenzidine peroxidase) substrate kit (Vector Laboratories, Inc., Burlingame, CA). The primary-antibody dilutions were used as follows: for the affinity-purified anti-p44 antibody, 1:50 to 1:100; for the anti-p38 antibody, 1:50 to 1:100; for the anti-actin antibody, 1:50; and for the p28 antisemur, 1:5,000.

Secondary goat anti-rabbit antibody was used at a dilution of 1:250 to 1:500. Immunoprecipitation of p44. Immunoprecipitation from crude dynein extracts of the wild type or the ida5 mutant was performed using the affinity-purified anti-p44 antibody or anti-p44 antisemur. Protein A beads (Roche Diagnostics GmbH, Mannheim, Germany) were added to the mixture of the antibody, the crude dynein extract (0.5 to 1 mg/ml), and IP buffer (10 mM HEPES [pH 7.5], 5 mM MgCl2, 1 mM dithiothreitol, 0.1 mM EDTA, 25 mM KCl, 1 mM NaN3, 75 mM NaCl, 0.05% Triton X-100, 0.5% Pefabloc, 3% BSA), and the mixture was left standing for 1 h at 4°C. The precipitates were washed three times with IP buffer without BSA, and the samples were boiled and processed for SDS-PAGE.

Immunofluorescence microscopy. Immunofluorescence microscopy was performed using standard procedures. Nucleoflagellar apparatuses (NFA) (22) isolated from the oda1 strain were fixed with 2% paraformaldehyde for 10 min at room temperature, followed by treatment with cold acetone (−20°C). Fixed samples were stained with the affinity-purified anti-p44 antibody diluted 1:10 in immunofluorescence blocking buffer and an Alexa Fluor 488-labeled anti-rabbit immunoglobulin G (IgG) antibody diluted 1:50 in immunofluorescence blocking buffer.

Electron microscopy. Immunoelectron microscopy (immuno-EM) of whole-mount embryos was performed according to the method of Johnson (6) with modifications. Antibodies isolated from the wild-type and ida5 strains were suspended in the HMDEK solution containing 1 mM ATP before being absorbed onto carbon-coated Ni grids. Grids were briefly dried to render the axonemes splashed, rinsed with blocking solution (0.5% BSA plus 1% fish gelatin in PBS), and then incubated for 1 h in an anti-p44 antibody diluted 1:5 to 1:10 in blocking solution. The grids were washed with blocking solution four times. After the wash, specimens were layered with anti-rabbit IgG conjugated with 10-nm-diameter colloidal gold diluted 1:10 to 1:25 in the blocking buffer. Grids were finally washed with PBS and distilled water and were negatively stained with 1% uranyl acetate.

Semi-quantitative reverse transcription-PCR (RT-PCR) of mouse NYP-SP14 protein. Total RNA was prepared from various tissues using TRizol reagent (Invitrogen). First-strand cdna was generated using the Transcriptor RTase kit (Roche Diagnostics GmbH, Mannheim, Germany). Semi-quantitative PCR was performed using primers mNYP-SP14 F1 (5′-GCGGGCTGAAAAAGAGAGTTGG-3′) and mNYP-SP14 R4 (5′-TGTTCCACGATCTCCTGACAC-3′). The partial sequence of the housekeeping protein NADPH dehydrogenase was amplified as a loading control.

Other methods. SDS-PAGE was performed using 10% or 11% acrylamide gels or 3 to 5% acrylamide gels with a 3 to 8 M urea gradient (5, 14). Gels were stained with Coomassie brilliant blue (CBB) or silver. Protein concentrations were measured using the method of Bradford (1). For the sequence comparison of p44 homologues, data were aligned using ClustalW, and the output was processed with Boxshade (http://www.ch.embnet.org/index.html). Protein motifs were obtained using SMART (simple modular architecture research tool) analysis (http://smart.embl-heidelberg.de/).

Identification of p44. The cdna sequence of Chlamydo monas p44 has been deposited in GenBank with accession number AB533122.

RESULTS

Identification of p44. Crude dynein extracts from wild-type axonemes can be fractionated by ion-exchange chromatography into almost individual outer- and inner-arm dynein species (Fig. 1A). We used SDS-PAGE analysis to confirm the previous finding that a fraction of dynein d contains a 44-kDa band in addition to the three established inner-arm subunits, actin, p38, and p28 (7, 24) (Fig. 1B). In this study we examined this 44-kDa protein. First, we carried out mass spectrometry using isolated protein samples that were cut out of the gels and digested with trypsine. Two partial amino acid sequences (Fig. 2) were determined. A search of C. reinhardtii in the JGI database (release 2.0) revealed that these sequences were present in the genome database (JGI LinkOut, version 2; C_780042, protein ID 169788) but not in the flagellar proteome database (17). The gene that corresponds to the p44 protein is located on the left arm of linkage group VIII. Although the loci of two flagellar mutations, pf3 and oda10, are
Sequence analysis of p44. The genomic sequence of p44 in the JGI database of C. reinhardtii (release 2.0) has gaps at its 3' and 5' ends. Part of the sequence is also present in the JGI database, release 3, but its predicted cDNA sequence appeared somewhat abnormal, suggesting the presence of errors. We therefore carried out 3' and 5' RACE analyses and determined the complete cDNA sequence. The deduced cDNA sequence contained an open reading frame encoding a 408-amino-acid polypeptide with a predicted molecular mass of 44,137.23 Da and an isoelectric point of 5.10 (Fig. 2). Five tetratricopeptide repeat (TPR) motifs and a coiled-coil region were found in its sequence (Fig. 3A). TPR motifs are known to be involved in protein-protein interactions (3). Northern blot analysis revealed that the p44 gene is expressed and is up-regulated upon deflagellation, in accordance with p44 being a flagellar protein (Fig. 4A). Southern blot analysis of the genomic DNA indicated that there is a single copy of the p44 gene in the Chlamydomonas genome (Fig. 4B).

Levels of p44 are diminished but not completely missing in mutants lacking inner-arm dynein d. A polyclonal antiserum was raised against bacterially produced polypeptides that corresponded to amino acid residues 34 to 235 of the p44 protein. The affinity-purified anti-p44 antibody recognized a single band in immunoblots (Fig. 3B). Unexpectedly, we found that p44 is diminished but not completely absent in the axonemes of the ida4 and ida5 mutants, both of which lack dynein d and a few other inner-arm dyneins (Fig. 3C). Comparison of the band densities for these axonemes with those for sequentially diluted wild-type axonemes led to an estimate that the amount of p44 is reduced to 5 to 10% of the wild-type level. This amount is smaller than the amount of p38 in the ida4 and ida5 axonemes, which has been estimated to be 30 to 50% of the wild-type level (24). To identify the flagellar compartment where p44 is localized, isolated flagella were demembranated with 0.2% Nonidet P-40, and the resulting axonemes were further extracted with 0.6 M KCl to remove dyneins. Immunoblot analysis of these flagellar fractions showed that p44 is predominantly present in the KCl-soluble fraction (Fig. 3D).

Immunoprecipitation of a p44-containing complex. To define the proteins that are associated with p44, we carried out immunoprecipitation experiments on crude dynein extracts using the anti-p44 antibody and protein A-conjugated beads. In addition to p44, the resultant precipitate from wild-type extracts always contained actin, p38, p28, and a high-molecular-weight protein that was the size of a dynein heavy chain, as detected by gel patterns and specific antibodies (Fig. 5A). SDS-PAGE analysis using 3 to 5% acrylamide gels with a 3 to 8 M urea gradient confirmed that the high-molecular-weight protein was the dynein heavy chain (Fig. 5B). These results indicate that p44 is associated with a complex that contains the dynein heavy chain, actin, p38 and p28, all of which have been previously identified as the components of dynein d. A high-salt extract from ida5 axonemes, which do not contain dynein d, did not yield the dynein heavy chain, actin, or p28 in the precipitate upon addition of the anti-p44 antibody and protein A beads; interestingly, it did yield p38 (Fig. 5A). This suggests that although p44 does not associate with the dynein heavy chain, actin, or p28 in the ida5 axonemes, it forms a complex with p38. Essentially the same result was obtained with the high-salt extract from ida4 axonemes (data not shown).

p44 cosediments with the inner-arm dynein d complex in wild-type axonemal extracts. The p44 molecule in the wild-type axoneme is salt extractable (Fig. 3D) and cosediments with the inner-arm dynein d complex in fractions of 11.3 to 13.0S on 5 to 20% sucrose gradients; it was also salt extractable from the ida5 axoneme. In the latter case, p44 sedimented in a peak at 4.4 to 5.5S, which corresponds to 60 to 120 kDa (Fig. 6). This result suggests that p44 is not contained in a dynein complex, but is present in a smaller complex, in the ida5 axonemes. This complex is likely to contain p38, as indicated by immunoprecipitation experiments and by the fact that part of p38 also sediments in a peak at 4.4 to 5.5S in the ida5 axonemal extract: it is likely that the peak positions of p38 and p44 differ slightly, because p38 is present in a larger amount than p44 and some p38 molecules are present without being associated with p44.

Localization of p44 by immunofluorescence and electron microscopy. Indirect immunofluorescence microscopy was performed on NFA using the anti-p44 antibody. The oda1 strain was used instead of the wild type, so that the bulky outer dynein arms would not block the access of the antibody to the inner dynein arms. The NFA were used instead of whole cells, because the autofluorescence of the chloroplast hindered observation of the basal body. The staining demonstrated that p44 is uniformly localized along the lengths of the axonemes (Fig. 7A). In addition, it is also localized on the basal body. Although the basal-body staining is unexpected, we have observed that an anti-p38 antibody also stains basal bodies and the nucleus (Fig. 7B) (24). In each case, the basal body became stained with two batches of polyclonal antibodies derived from different rabbits but not with control labeling (secondary antibody only). The basal body was also observed in the ida5
FIG. 2. cDNA sequence and predicted amino acid sequence of p44. Five TPR motifs and a coiled-coil region were identified by SMART analysis. Stop codons are marked by asterisks. A *Chlamydomonas* polyadenylation signal sequence (TGTAA) is boxed. The two partial amino acid sequences that were determined by mass spectrometry are underlined. Broken lines indicate TPR motif regions.
mutant. Whether p44 and p38 have functions in the basal body also remains an interesting future problem.

In a series of immuno-EM observations, anti-p44 antibodies conjugated to gold particles specifically labeled outer doublet microtubules of wild-type axonemes (Fig. 7C), while control labeling (secondary antibody only) was almost undetectable (data not shown). The anti-p44 antibody also labeled the outer doublet microtubules of $\text{ida}5$ axonemes, although much less frequently and intensely than the wild-type outer doublets. Staining was not observed after the axonemes had been treated with 0.6 M KCl. Figure 7D shows a rare example of labeled $\text{ida}5$ outer doublets. In both wild-type and $\text{ida}5$ axonemes, the distribution of the p44 signals often showed a periodicity of $\sim 110$ nm (Fig. 7C and D).

p44 exists in various organisms and tissues with motile cilia and flagella. BLAST searches identified putative homologues of p44 in a wide range of ciliated organisms, including Tetrahymena thermophila (BLAST E value, $1e^{-17}$), Trypanosoma brucei ($2e^{-6}$), zebrafish ($8e^{-14}$), mouse ($1e^{-12}$), and human ($9e^{-10}$) (Fig. 8). All of these homologues were registered as proteins of unknown function. *Chlamydomonas* p44 is similar...
to a TPR motif-containing protein, NYD-SP14, which is present in mouse and human. We examined the expression levels of the NYD-SP14 protein in various mouse tissues by semiquantitative RT-PCR (Fig. 9). The NYD-SP14 protein was strongly expressed in the lung, trachea, testis, and oviduct, i.e., tissues with motile cilia and flagella. This result suggests that the mouse NYD-SP14 protein also functions in cilia and flagella, most likely as an inner-arm dynein subunit.

**DISCUSSION**

In this study we have identified a 44-kDa protein (p44) that is associated with inner-arm dynein d and have determined its cDNA and amino acid sequences. Immunoprecipitation experiments with wild-type axonemal extracts detected the heavy chain of dynein d and its previously identified components, i.e., actin, p38, and p28. These results have established that p44 is a subunit of dynein d. Until now, actin, centrin, p38, and p28 have been known to associate with single-headed inner-arm dyneins; p44 is therefore the fifth subunit.

An interesting finding made in this study is that the axonemes of the ida4 and ida5 mutants, which lack dynein d and other inner-arm dyneins, retain a reduced amount of p44. This observation is reminiscent of our previous observation that p38, another dynein d subunit, is present in the axonemes of these mutants (24). The results of immunoprecipitation and sucrose density gradient centrifugation indicated that p44 in the axonemes of the ida5 mutant does not form a complex with a dynein heavy chain but forms a 60- to 150-kDa complex with p38. This complex may involve other proteins as well. We hypothesize that, in the wild-type axoneme, the p38/p44 complex may bind to both dynein d and another, unidentified protein on the outer doublet microtubules, thereby functioning to attach dynein d to specific loci. In the ida4 and ida5 mutants, however, the amounts of p38 and p44 attached to the axoneme differ: the level of p38 is reduced to 30 to 50%, and that of p44 is reduced to 5 to 10%, of the wild-type value. We speculate that this possibly occurs because p38 is bound to the outer doublets more strongly than p44 and because the association between p38 and p44 is not so strong.

Immuo-EM shows that p44 epitopes are localized along outer doublet microtubules at intervals of ~100 nm; this distance is similar to the 96-nm axial repeat of inner dynein arms. Importantly, the ~100-nm interval is also observed for the ida5

![FIG. 6. Western blots of axonemal extracts obtained from wild-type (Wt) and ida5 axonemes fractionated on 5 to 20% sucrose gradients. Fractions were probed with antibodies against p44, p38, and p28. p44 cosedimented with inner-dynein arm d at 11.3 to 13.0S on sucrose gradients of Wt axonemal extracts but sedimented in at 4.4 to 5.5S on sucrose gradients of ida5 mutant axonemal extracts.](image-url)

![FIG. 7. Immunofluorescence micrographs of the NFA of the oda1 mutant (A and B) and immuno-EM of wild-type and ida5 axonemes (C and D). (A) NFA of the oda1 mutant were stained with the anti-p44 antibody, followed by Alexa Fluor 488-labeled anti-rabbit IgG. Flagella are uniformly stained, suggesting that the inner-arm dynein d is uniformly present along the length of the axoneme. (Left) Differential interference contrast images; (right) indirect immunofluorescence micrographs. (B) NFA of the oda1 mutant were stained with the anti-p38 antibody. (Left) Differential interference contrast images; (right) indirect immunofluorescence micrographs. (C and D) Wild-type (C) and ida5 (D) axonemes were detected with the anti-p44 antibody and a 10-nm-gold-labeled secondary antibody and were negatively stained with 1% uranyl acetate. Note that gold particles show an axial spacing of ~100 nm (arrowheads). Bars, 5 μm in panels A and B and 200 nm in panels C and D.](image-url)
mutant, which lacks dynein d. This observation is consistent with the idea that p44 constitutes the docking site of dynein d. p44 has five TPR motifs in its amino acid sequence, as indicated by SMART analysis. The TPR motif, a motif consisting of 3 to 16 tandem repeats of 34 amino acid residues, has been found in >800 kinds of proteins from various organisms ranging from bacteria to humans. It is thought to mediate protein-protein interactions and the assembly of multiprotein complexes. A TPR motif is also found in a kinesin light chain (3). The five TPR motifs in the p44 molecule may well be involved in the interaction between dynein d and another protein, possibly participating in dynein docking. As an alternative possibility, the residual p38 and p44 present in ida5 axonemes may be derived from some minor dyneins or dynein d heavy chains that can be present in very small amounts in the mutants. However, since our sucrose density analysis of the ida5 extract indicated that the majority of p38 and p44 sediment at 4.4 to 5.5S (Fig. 6) and not at >11S, where dynein complexes sediment, it seems unlikely that all of the residual p38 and p44 are present in association with minor dyneins.

BLAST searches have indicated that p44 has putative homologues in organisms with motile cilia and flagella, such as humans and zebrafish, but not in organisms that have only immotile cilia, such as Caenorhabditis elegans. Together with the previous findings that p38 and p28 are conserved in organisms with motile cilia and flagella, our finding suggests that the subunit composition of single-headed inner-arm dyneins is conserved in a wide range of organisms. In accordance with this idea, the mouse homologue of p44, NYD-SP14, is strongly expressed in tissues with motile cilia (Fig. 9). Furthermore, p38 is a homologue of the trypanosome flagellar protein TAX-1, the depletion of which greatly impairs the motility of bloodstream trypanosomes (2). Our hypothesis that p38 and p44 form a docking complex for dynein d suggests that the motility impairment in TAX-1-depleted trypanosomes is caused by the loss of a dynein(s) homologous to dynein d.

FIG. 8. Sequence comparison of potential homologues of p44. The sequences of Chlamydomonas p44 and homologues were aligned using ClustalW, and the output was processed with Boxshade. Characters with black and gray backgrounds represent identical and conservatively substituted amino acids, respectively. TPR motifs in the Chlamydomonas p44 sequence are underlined. The second, fourth, and fifth TPR motifs are conserved in several organisms. However, other organisms have another TPR motif between the fourth and fifth, while Trypanosoma has no TPR motifs as judged by SMART analysis. GenBank accession numbers are as follows: for Chlamydomonas p44, AB353122; for mouse NYD-SP14, NP_898919; for human NYD-SP14, NP_114162; for zebrafish p44, XP_001340503; for Tetrahymena p44, XP_001014763; for Trypanosoma p44, XP_843793.
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FIG. 9. Expression analyses of the mouse homologue of p44, NYD-SP14. (A) Expression levels of mouse NYD-SP14 protein in various tissues, assessed by semiquantitative RT-PCR. The 589-bp fragments of the NYD-SP14 mRNA sequence were amplified. (Upper panel) Amplification with 25 cycles; (lower panel) 35 cycles. NYD-SP14 is strongly expressed in tissues with motile cilia and flagella such as the lung, trachea, testis, and oviduct, suggesting its role in ciliary and flagellar activity. (B) Control experiments amplifying part of the mRNA of a housekeeping protein, NADPH dehydrogenase. (Upper panel) Amplification with 25 cycles; (lower panel) 35 cycles.

Recent phylogenetic analyses of dynein heavy-chain genes from various organisms have shown that the genes for single-headed dyneins can be classified into three groups and that the dynein d heavy chain belongs to a group distinct from the other two groups, which contain all other Chlamydomonas single-headed dynein heavy chains (15, 20; T. Yagi et al., unpublished data). The conserved presence of p38 and p44 suggests that the single-headed dyneins that are associated with these light chains, such as dynein d of Chlamydomonas, constitute a unique subgroup of single-headed dyneins and have unique importance for axonemal activity.