Cytoplasmic Dynein Intermediate-Chain Isoforms with Different Targeting Properties Created by Tissue-Specific Alternative Splicing

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The intermediate chains (ICs) are the subunits of the cytoplasmic dynein that provide binding of the complex to cargo organelles through interaction of their N termini with dynactin. We present evidence that in *Drosophila*, the IC subunits are represented by at least 10 structural isoforms, created by the alternative splicing of transcripts from a unique *Cdic* gene. The splicing pattern is tissue specific. A constitutive set of four IC isoforms is expressed in all tissues tested; in addition, tissue-specific isoforms are found in the ovaries and nervous tissue. The structural variations between isoforms are limited to the N terminus of the IC molecule, where the interaction with dynactin takes place. This suggests differences in the dynactin-mediated organelle binding by IC isoforms. Accordingly, when transiently expressed in *Drosophila Schneider*-3 cells, the IC isoforms differ in their intracellular targeting properties from each other. A mechanism is proposed for the regulation of dynein binding to organelles through the changes in the content of the IC isoform pool.

Cytoplasmic dynein is a multisubunit complex composed of two heavy chains, three intermediate chains (ICs), several light ICs, and one light chain (11, 20). It acts as a minus end-directed microtubule motor, participating in a number of events including anterograde organelle movement (1, 6, 22), mitosis (25), nuclear migration (28), slow axonal transport in nervous tissue (7), and transport from nurse cell cytoplasm to oocytes in *Drosophila* ovaries (12). These events call for binding of the dynein complex to multiple target organelles in the cell. Regulation of this binding is also required to enable relocation of the dynein between targets during the cell cycle and development of the organism. One of the proposed mechanisms implemented in the cell cycle-dependent regulation of dynein binding is through the phosphorylation of the subunits of the dynein complex (17).

Although the heavy chain comprises the catalytic dynein subunit and is capable by itself of the ATP-dependent moving force production on the microtubules (14), the presence of other subunits is apparently required for dynein function in vivo. For one class of these so-called accessory subunits of cytoplasmic dynein, the IC subunits, a key role in linking cytoplasmic dynein to the intracellular targets was suggested and then proved (20, 23). In particular, the N-terminal part of IC is directly involved in binding to the organelles (23) through the interaction with p150/*Glued*, the major component of the dynactin complex (26). Dynactin, also a multisubunit complex, is an activator of dynein in vitro (9) and is required for dynein function in vivo (4, 15, 16). Dynein and dynactin are colocalized in the cell, and overexpression of components of the dynactin complex disrupts dynein binding to organelles (5, 8).

Considering dynactin as a dynein "receptor" or at least a modulator of dynein binding, the interaction of dynein ICs with dynactin is likely to be the point where the regulation of dynein binding takes place. A number of IC isoforms were detected, and the content of IC isoform pool is highly regulated (21). The complexity of IC isoforms is due to the expression of a family of structurally different polypeptides, some of which are further modified by phosphorylation (21, 26). The structural differences are limited to the N-terminal part of the ICs, in the region essential for dynactin binding (26). The phosphorylation is strongly suggested to occur in the same region which contains the serine-rich domain. Thus, the observed complexity of ICs presumably provides a diversity in dynactin-mediated dynein binding to organelles. This means that changing the content of the IC isoform pool would result in relevant changes in dynein targeting.

The mechanism for generating the structural complexity of ICs has been unclear. Alternative splicing of a limited number of transcripts was suggested (26) but never shown directly. In this paper, we demonstrate that in *Drosophila*, the structural IC isoforms are created by the alternative splicing of transcripts from a single-copy *Cdic* gene. The isoforms differ in the polymorphic region located near the N terminus of IC. The exact positions of the polymorphic regions differ in ICs from *Drosophila* and rats, suggesting independent evolution of IC isoform complexity in the ancestry of distant orders.

The splicing pattern of *Cdic* and therefore the content of the IC isoform pool appear to be tissue specific. In addition to the constitutive set, tissue-specific IC isoforms are present in ovaries and neural tissue, where tissue-specific kinds of dynein-dependent transport take place. The IC isoforms differ in their intracellular targeting properties, thus providing the mechanism for developmental regulation of dynein binding to organelles by changing the content of the IC isoform pool.

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MATERIALS AND METHODS

RNA isolation and Northern analysis. Total RNA was isolated from various developmental stages and from adult body parts of *Drosophila melanogaster* and from adults of *D. simulans* with Trizol (Gibco-BRL). Poly(A)$^+$ RNA was purified from the total RNA preparations with Poly(A)-Tract magnetic particles (Promega Corp).

For Northern analysis, 10 μg of total RNA or 2 μg of poly(A)$^+$ RNA was electrophoresed through a 1% agarose–formaldehyde gel and transferred onto a Hybond N membrane in 10× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH$_2$PO$_4$, and 1 mM EDTA [pH 7.7]). Single-stranded ^{32}P-labeled RNA probes were generated by T7 RNA polymerase from the pTZ19R-based plasmid containing the sequence of exon 6 (probe A in Fig. 2). Random priming with the Prime-It system (Stratagene) was used to generate ^{32}P-labeled DNA probes from the same fragment A or from the fragment representing the first 680 bp of *Cdic* cDNA (probe B in Fig. 2). Hybridization procedures were as described previously (19).

Southern hybridization with oligonucleotides. Reverse transcription-PCR (RT-PCR) products corresponding to IC isoforms were separated in a 3% agarose–Tris-borate-EDTA (TBE) gel and transferred to a Nybond N membrane in 0.5 M NaOH–1 M NaCl by capillary blotting. The membrane was neutralized in 1 M ammonium acetate, air dried, and baked for 90 min at 80°C. The following primers, covering specific variable exon junctions, were synthesized (see Fig. 7): "iso2" (5′-TTATTATGATGAATAC-3′), covering the v2/v3 junction specific for *Cdic*2 and *Cdic*5; "iso2‴" (5′-CGGCGATGCTCATGCT-3′), covering the 4/v2 junction specific for *Cdic*1, *Cdic*2, and *Cdic*5; "iso3" (5′-CGG CGATGATGAATAC-3′), covering the 4/v3 junction specific for *Cdic*3; "iso4" (5′-CGGCGATGTGCTTGCA-3′), covering the 4/v4 junction specific for *Cdic*4; and "iso5" (TATATGGAGGACTGGT-3′), representing exon v1, specific for *Cdic*5. The primers were labeled with ^{32}P by T4 DNA kinase and hybridized with the membrane in 4× SSPE–1% Sarkosyl, 0.1% each Ficoll-400, polyvinylpyrrolidone, and sodium pyrophosphate for 2 to 14 h at the following temperatures: "iso2" at 42°C; "iso2‴" and "iso4" at 55°C; and "iso3" and "iso5" at 50°C. The membrane was washed in 100 mM sodium phosphate (pH 8.0)–1% Sarkosyl–1 mM EDTA three times for 15 min at room temperature and then once in 4× SSPE–1% Sarkosyl for 20 min at hybridization temperature.

DNA cloning and sequencing. cDNA clones were obtained by screening a λZAP cDNA library made from poly(A)$^+$ RNA from *D. melanogaster* ovaries (supplied by Stratagene Corp.). Individual lambda clones were converted into the plasmid form by in vivo excision, and the inserts were transferred into the vector pSP72 and sequenced with an ABI 373A automated DNA sequencer after saturation with gamma-delta transposon insertions (24). Sequence data were analyzed with Sequencher software (GeneCodes Corp.).

The 5′- and 3′-RACE (rapid amplification of cDNA ends) PCR products were generated with the Marathon system (Clontech), using female poly(A)$^+$ RNA as a template, and were sequenced after T-A cloning in the pCRII vector (Invitrogen).

A *D. melanogaster* P1 genomic library was screened by a PCR-based assay as described previously (10). The P1 clone containing the cytoplasmic dynein IC genes was subcloned in the λSCAN vector (18), and the subclone of interest was transferred into the vector pSP72 and sequenced as described above.

Plasmid constructs. The green fluorescence protein (GFP) fusion expression plasmids were made by inserting the *Cdic* open reading frames (ORFs) upstream of the GFP ORF in pGreenLantern (Gibco-BRL). ORFs containing the specific *Cdic* isoforms were amplified by PCR with the corresponding cDNA clones as the templates and with the primers DIC-F (5′-GGTACCAGCTAATCGCCCC GAGAAATGGAT-3′) and DIC-LL (5′-GGCGGCCGCGTTCATCTTGATCT CGCTAAG-3′). The N-terminal domains were amplified with the primers DIC-F and DIC-R (5′-GCGGCCGCACGCACCACGAACCGCTGGAAG-3′). PCR products were cloned in the vector pCRII and transferred into the pGreen-Lantern as *Kpn*I-*Not*I fragments. Partial digestions with *Not*I were used when necessary.

All the PCR fragments used for plasmid construction were generated with a polymerase mixture possessing proofreading activity (Elongase; Gibco-BRL), and their sequence was checked after cloning in the pCRII vector.

Cell culture transfections. The *D. melanogaster Schneider*-3 cell culture was maintained in *Drosophila* Shields and Sang M3 medium (Sigma) supplemented with 10% insect medium supplement (Sigma) at room temperature. For transfection, the cells were plated on chamber slides (Falcon) at a density of 5×10^5 to 7×10^5 cells/ml and the next day were transfected with the Lipofectin reagent (Gibco-BRL). A 1.5-μg portion of DNA and 9 μl of Lipofectin were used per 5×10^5 to 7×10^5 cells. After 6 to 12 h, the medium was changed to M3 supplemented with 10% insect medium supplement; the cells were then allowed to grow for another 1 or 2 days and fixed for 10 min with 3.7% paraformaldehyde at room temperature. The cellular content was stained with propidium iodide. Alternatively, for Golgi-specific staining, the cells were permeabilized with 0.2% Triton X-100 for 15 min at room temperature and incubated with 20 μg of rhodamine-labeled *Lens culinaris* lectin per ml (Sigma). Lysosome-specific staining was obtained by in vivo incubation of cells with 50 nM LysoTracker DND-99 (Molecular Probes) for 2 h before fixation. The cells were mounted in Permount medium and imaged in a laser scanning microscope (Axiovert 100TV; Zeiss). The images were processed with Adobe Photoshop software.
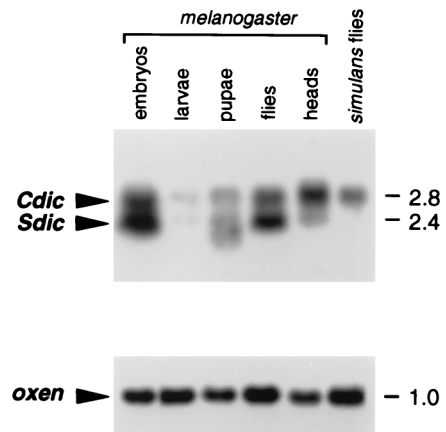

FIG. 1A. Dynein IC transcripts in *Drosophila*. Samples (10 μg) of total RNA isolated from various developmental stages of *D. melanogaster*, as indicated at the top, along with RNA samples from the heads of *D. melanogaster* adults or *D. simulans* adults, were separated in a 1% agarose–formaldehyde gel. Hybridization with probe A (Fig. 2) revealed two major bands, corresponding to the *Cdic* and *Sdic* transcripts. Only the *Cdic* transcripts were detected in *D. simulans*. (B) Control hybridization with the probe for the constitutively expressed gene *oxen* (1a) shows sufficient RNA loading on all lanes. The numbers on the right indicate the sizes of transcripts in kilobases.

RESULTS

Multiple RNAs code for the cytoplasmic dynein IC proteins. In a previous study, we characterized a tandem repeat in cytological region 19F of *D. melanogaster* (2). The unit of this repeat is 7.2 kb long and contains a fragment of the *annexin* X gene. It also contains a long ORF coding for a polypeptide with high similarity to the dynein IC proteins. Using a fragment of this ORF as a probe (probe A in Fig. 2), we were able to detect two bands on a Northern blot, one of 2.4 kb and one of 2.8 kb (Fig. 1).

Using the same probe, we isolated numerous cDNA clones from a *D. melanogaster* λZAP library. Six overlapping clones were sequenced, and the sequences were aligned, resulting in a composite cDNA sequence possessing an ORF for dynein IC polypeptide (Fig. 2). This composite sequence, however, obviously lacked both the 3′ and 5′ ends of the transcript, since neither a poly(A) tail nor a methionine initiation codon was detected. The 3′ end of the RNA was unambiguously mapped by performing 3′-RACE and sequencing several cloned PCR products. Mapping the 5′ end by 5′-RACE led to the description of two major classes of RNAs suggested from Northern analysis. The sequences of the 5′-RACE products could easily be sorted in two subsets, the long and short subsets. Aligning the short 5′ ends with the composite cDNA resulted in a 2.4-kb sequence, apparently representing a 2.4-kb RNA. The same alignment with the long 5′ ends produced a 2.8-kb sequence corresponding to the larger RNA.

The 70-kDa polypeptides encoded by the long, 2.8-kb mRNAs have extensive homology to the cytoplasmic dynein ICs from rats and *Dictyostellium discoideum*. The major stretch of homology covers more than 400 amino acids in the C-terminal part of molecule, which is 61% identical (79% similar) to the rat homolog and 50% identical (69% similar) to the protein from *Dictyostellium discoideum*. Included in the C-terminal region are four WD-40 repeats (see Fig. 6; a fifth repeat, described in reference 27, is very degenerate and is not shown
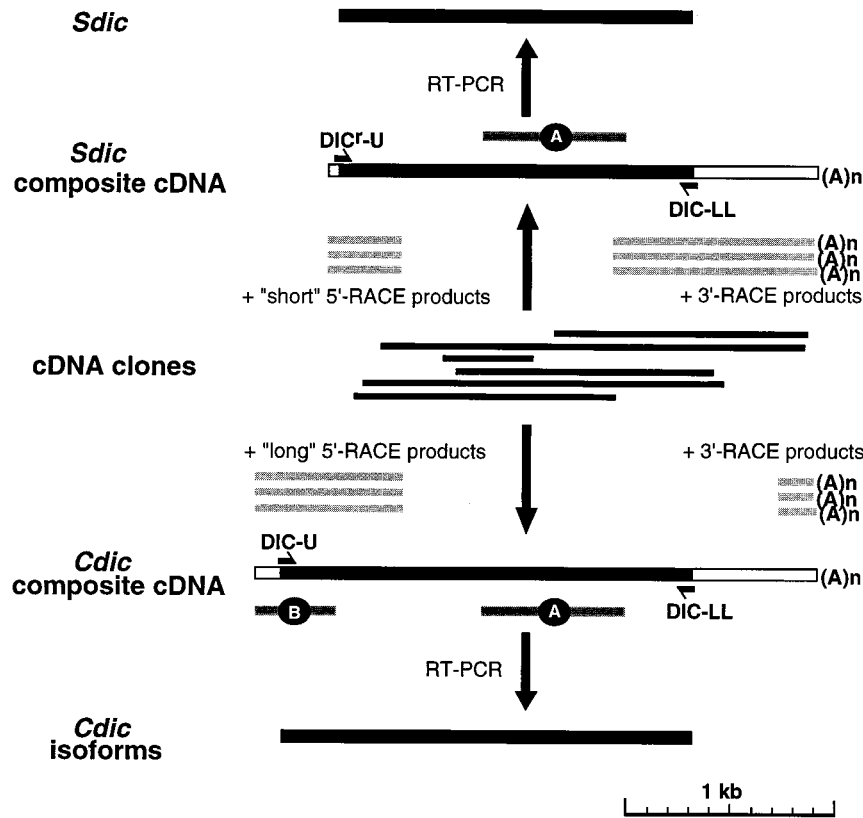
FIG. 2. Cloning and characterization of *Cdic* and *Sdic* cDNAs. λZAP clones are indicated by thin black bars, and RACE products are indicated by shaded bars. In the composite cDNAs, coding regions are black. The positions for the DIC-U and DIC-LL primers, used to amplify cDNAs for *Cdic* isoforms, and for the DIC^r-U and DIC-LL primers, used for *Sdic*, are shown. A and B are the *Cdic*/*Sdic*-specific and *Cdic*-specific probes, respectively.

here). This set of repeats is extremely strongly conserved among all dynein ICs and probably accounts for the interaction with other dynein subunits.

An additional feature characteristic for the cytoplasmic dynein ICs is the presence of a coiled-coil domain at the N terminus followed by a serine-rich domain. Both these domains were detected in the 70-kDa polypeptide. Analysis of the alignment of this polypeptide with other cytoplasmic dynein ICs revealed another conserved stretch of amino acids in the N-terminal region, called PPE/TQT (Fig. 3).

Based both on sequence homology and structural similarity to the cytoplasmic dynein ICs from rats and *Dictyostelium discoideum*, the 70-kDa polypeptide encoded by the long mRNAs was defined as the *D. melanogaster* cytoplasmic dynein IC (Cdic). In contrast, the 60.4-kDa polypeptide encoded by the short 2.4-kb mRNA lacks the N-terminal coiled-coil and serine-rich domains characteristic of ICs of cytoplasmic dyneins, although it shares most of its sequence with the Cdic polypeptide. It was shown to represent a novel sperm-specific IC subunit of axonemal dynein and was called Sdic (17a).

FIG. 3. Comparison of the sequence of the N-terminal regions of Cdic and other known cytoplasmic ICs from rats (GenBank accession no. U39046) and *Dictyostellium* (accession no. U25116). Conserved amino acids are outlined and presented in the consensus line. Coiled-coil domains, shown by solid boxes, were predicted with the PAIRCOILS (3) and COILS (13) algorithms. The serine-rich domain and PPE/TQT conserved block are outlined by boxes. The positions of variable regions in the rat IC (var-1 and var-2) and the beginning of the variable region in Cdic (var) are indicated. Cdic isoform shown is Cdic5b.

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Sdic cDNA                                                                      5'-------------------------------A-T-T--TCCAAACACAA---
Annx/dyn   GGGAAA-TCTGTTG-------------TG--T-----G-C----C-------------------------------------A-T-T--TCCAAACACAA---
Cdic gene  CAACTGTAGAGACAGATTCCCCATCTACAAGCTTAACATAAAAA.AATATCAGTAGTCAAAATTGGAATCCTGGTTAGAGAGCGTGGCAA..........TTT
Cdic cDNA  ...................................................................................................


Sdic cDNA  -----.....--------T----------TT---TTGAATAAGC----------------G---------------------------T-------C--
Annx/dyn   -----.....--------T----------TT---TTGAATAAGC----------------G---------------------------T-------C--
Cdic gene  TTACAATTCAATTTTAGCATTAGCCGTTTTAAAAA..........TTAGTATTACACAGATTAGACAATGGGCTTAGTACTGATTAAGTTTTAACGATCAATGT
Cdic cDNA  .............386 bp intron.......................................................................


Sdic cDNA  ---C----.---..............................................................--------------------T---
Annx/dyn   ---C----.---AGGTACTATAA---------------A--------------G-----------------------------------------T---
Cdic gene  ATTGTACTATTG..........CTATTGCCATTTAACCGATTCCTAACTAATACCAATTTCTTATGTGCACCCCCCACCAGCGGCGGAAAGAAACAGCCCCTC
Cdic cDNA  ......................................................................---exon d4------------
```
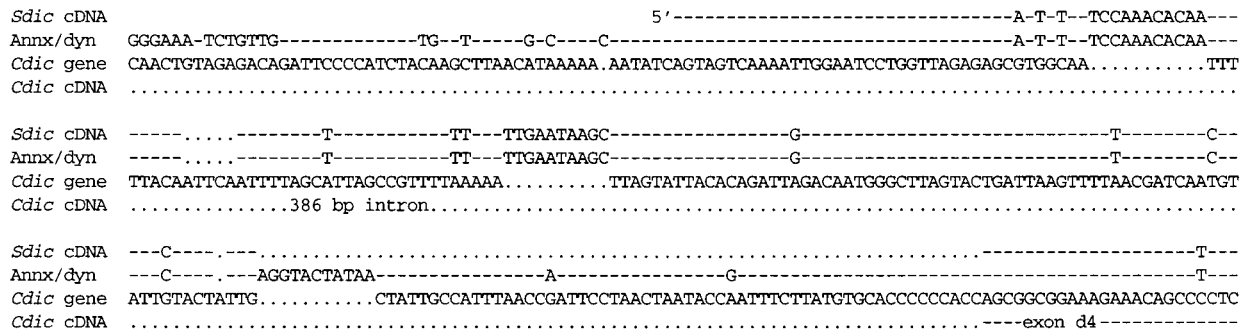
FIG. 4. Sequence comparison shows that *Cdic* cDNA represents the transcripts from the *Cdic* gene and that *Sdic* cDNA corresponds to the transcripts from the annexin-dynein repeat. The entire *Cdic* gene sequence is presented; for cDNAs and the annexin-dynein repeat, only the differences are shown. Gaps introduced in the sequences are marked with dots.

Amplification and sequencing of full-length *Cdic* cDNAs revealed multiple transcripts that differ by small insertions and deletions in the N-terminal part of the ORF and apparently code for the Cdic isoforms.

2.8-kb *Cdic* RNAs are transcribed from the single-copy cytoplasmic dynein IC (*Cdic*) gene. Although all dynein IC cDNAs were isolated with a fragment of the 7.2-kb annexin-dynein repeat, the very 5′ end of the *Cdic* transcripts is not homologous to the repeated unit. This sequence, represented by probe B specific for *Cdic* transcripts (Fig. 2), was found to be unique in the genome on the basis of Southern analysis and mapped by in situ hybridization in the site 19E, i.e., in close vicinity to the annexin-dynein repeat. Northern analysis demonstrated that, as expected, the same *Cdic*-specific probe B hybridized with only the 2.8-kb *Cdic* mRNAs and not with the 2.4-kb *Sdic* mRNAs (data not shown).

When a P1 phage genomic library was screened for the *Cdic*-specific sequence, three clones that also contained the annexin-dynein repeat were obtained. None of these three clones, however, contained the complete *annexin* X gene located at the end of annexin-dynein tandem cluster (2). Considering the length of the tandem repeat (about 10 copies at 7.2 kb each) and the average length of a P1 clone (80 kb), these data suggest that the *Cdic*-specific sequence is located in the vicinity of the tandem cluster of annexin-dynein repeats, at the opposite end from the *annexin* X gene.

Cloning and sequencing of the corresponding genomic region revealed the structure of the gene encoding the *Cdic* transcripts. This *Cdic* gene is located at the 5′ end of the tandem cluster, and its 3′ end is directly fused to the initial 7.2-kb annexin-dynein repeated unit. The exon-intron structure of the 8.3-kb *Cdic* transcription unit was determined by aligning the *Cdic* cDNA sequences to the genomic sequence. A perfect match was obtained between *Cdic* cDNAs and the exons of the *Cdic* gene. A number of significant differences, however, were detected between the *Cdic* genomic sequence and the 2.4-kb *Sdic* cDNA, indicating that, unlike 2.8-kb *Cdic* cDNAs, this one does not represent the transcript from the *Cdic* gene. The true origin of *Sdic* transcripts was exposed, since a perfect match was achieved between the sequences of the *Sdic* cDNA and the annexin-dynein repeat (Fig. 4). The identity of 2.4-kb *Sdic* mRNAs as the transcripts from the annexin-dynein repeat was further supported by the fact that in *D. simulans*, a close relative of *D. melanogaster* that does not have any repeated structure analogous to the annexin-dynein repeat, no 2.4-kb *Sdic* transcripts were found: the only class of dynein mRNAs detected corresponds to the 2.8-kb *Cdic* mRNAs (Fig. 1).

Transcription of *Cdic* changes throughout the development of *D. melanogaster* (Fig. 1). The transcripts are abundant in embryos and adult flies and apparently are up-regulated in the heads of adult flies, but they are hardly detectable in larvae and pupae.

Multiple Cdic isoforms are generated by alternative splicing. Previous data state that all *Cdic* mRNAs are transcribed from the unique *Cdic* gene, even though multiple variants of *Cdic* transcripts were detected. Analysis of the exon-intron structure of the *Cdic* gene demonstrated that these variants, coding for the Cdic isoforms, are created by alternative splicing.

The transcription unit is 8.3 kb long and consists of 10 exons. The first four exons (1 to 4 in Fig. 5) are separated by relatively small introns, as are the last three exons (5 to 7 in Fig. 5). These two groups of exons are separated by 4.2-kb "spacer" region containing three "variable" exons, v1, v2, and v3. Alternative splicing of transcripts leads to the skipping of the variable exons, providing shortened versions of mRNAs apparently carrying the corresponding deletions in the dynein IC ORF (Fig. 5 and 6). Additional polymorphism of the mRNAs is created by using two alternative splice acceptor sites preceding exon v1 and three alternative splice acceptor sites of the intron preceding exon d5, which also results in insertions/deletions in the same region of the dynein IC ORF (Fig. 6). Use of one of the acceptor sites preceding exon v1 leads to the frameshift and premature termination of translation (isoform Cdic5a in Fig. 6). Except for this one, as many as 10 full-sized
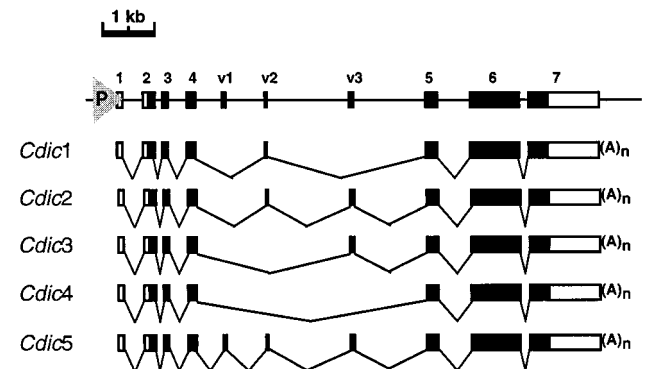

FIG. 5. Exon-intron structure of the *Cdic* gene. The genomic sequence is shown at the top, with exons indicated by boxes. Coding sequences are shown as solid boxes. The promoter is shown as triangle. 1 to 7, constitutive exons present in all *Cdic* mRNAs. v1 to v3, variable exons. Five classes of *Cdic* transcripts are shown below the sequence.

Cdic gene ATCGCAGCAGCTAATCGCCCCGAGAAATGGATCGCAAGGCTGAGCTGGAACGCAAGAAGGCCAAGTTGGCCGCCCTGCGCGAGGAGAAGGATCGCCGGCGTCGCGAAGGAGATCAAGGACATGGA
Cdic cDNAs ATCGCAGCAGCTAATCGCCCCGAGAAATGGATCGCAAGGCTGAGCTGGAACGCAAGAAGGCCAAGTTGGCCGCCCTGCGCGAGGAGAAGGATCGCCGGCGTCGCGAAGGAGATCAAGGACATGGA
           M D R K A E L E R K K A K L A A L R E E K D R R R R E K E I K D M E
                                           exon 2   coiled coil 1

Cdic gene GGAGGCGGCCGGTCGCATTGGCGGCGGAGCAGGCATCGACAAGGATCAGCGCAAGTGGGTT 74 bp intron TCAGGGATCTCGACGAAATGCTGTCATCGCTGGGCGTGGCCCCCGTCT
Cdic cDNAs GGAGGCGGCCGGTCGCATTGGCGGCGGAGCAGGCATCGACAAGGATCAGCGCAAG..........................GATCTCGACGAAATGCTGTCATCGCTGGGCGTGGCCCCCGTCT
           E A A G R I G G G A G I D K D Q R K   D L D E M L S S L G V A P V

Cdic gene CCGAGGTCCTTTCCTCACTCTCCTCCGTCAACTCGATGACTTCGGACAACTCGAACACACAGACCCCCGACGCCAGCCTCCAAGCCACTGTCAATGGCCAGAGGT 386 bp intron CCAC
Cdic cDNAs CCGAGGTCCTTTCCTCACTCTCCTCCGTCAACTCGATGACTTCGGACAACTCGAACACACAGACCCCCGACGCCAGCCTCCAAGCCACTGTCAATGGCCAG..........................
           S E V L S S L S S V N S M T S D N S N T Q T P D A S L Q A T V N G Q
                         serine-rich   exon 3

Cdic gene CAGCGGCGGAAAGAAACAGCCCCTCAACCTAAGCGTCTACAATGTGCAGGCTACGAACATTCCACCAAAAGAGACACTGGTCTACACAAAGCAGACCCAGACGACCAGTACCGGAGGCGGAAACGGC
Cdic cDNAs .AGCGGCGGAAAGAAACAGCCCCTCAACCTAAGCGTCTACAATGTGCAGGCTACGAACATTCCACCAAAAGAGACACTGGTCTACACAAAGCAGACCCAGACGACCAGTACCGGAGGCGGAAACGGC
           S G G K K Q P L N L S V Y N V Q A T N I P P K E T L V Y T K Q T Q T T S T G G G N G
                                 exon 4   PPE/TQT

Cdic gene GATGGTG 383 bp intron ACAGTTCTTTCTTGCCACTCCTCGCCTCTGTCAGGATATATGGAGGACTGGTGGCGTCCACGTAAAGGTA 885 bp intron CAGCTCATGCTACGGATTAT
Cdic1a GATG..CTCATGCTACGGATTAT
Cdic1b GATG..CTCATGCTACGGATTAT
Cdic1c GATG..CTCATGCTACGGATTAT
Cdic2a GATG..CTCATGCTACGGATTAT
Cdic2b GATG..CTCATGCTACGGATTAT
Cdic2c GATG..CTCATGCTACGGATTAT
Cdic3a GATG..
Cdic3b GATG..
Cdic4   GATG..
Cdic5a GATG..............GTTCTTTCTTGCCACTCCTCGCCTCTGTCAGGATATATGGAGGACTGGTGGCGTCCACGTAAAG...................CTCATGCTACGGATTAT
Cdic5b GATG..............................GATATATGGAGGACTGGTGGCGTCCACGTAAAG...................CTCATGCTACGGATTAT
           D   G Y M E D W W R P R K   A H A T D Y
peptide DIC5a D   GSFLPLLASVRIYGGLVAST *
                                              exon v1   exon v2

Cdic gene TATGGTG 1257 bp intron AAGATGAATACAATCTTAATCCGGGTTTAGAGTGGGAGGATGAATTCACAGGTA 1493 bp intron CAGTGCTTGCATTTGATGCCCAAGGAGACGACGA
Cdic1a TATG..TGCTTGCATTTGATGCCCAAGGAGACGACGA
Cdic1b TATG..GAGACGACGA
Cdic1c TATG..ACGACGA
Cdic2a TATG..................ATGAATACAATCTTAATCCGGGTTTAGAGTGGGAGGATGAATTCACAG..............................GAGACGACGA
Cdic2b TATG..................ATGAATACAATCTTAATCCGGGTTTAGAGTGGGAGGATGAATTCACAG..............................ACGACGA
Cdic2c TATG..................ATGAATACAATCTTAATCCGGGTTTAGAGTGGGAGGATGAATTCACAG..............................TGCTTGCATTTGATGCCCAAGGAAACGACGA
Cdic3a ..................ATGAATACAATCTTAATCCGGGTTTAGAGTGGGAGGATGAATTCACAG..............................GAGACGACGA
Cdic3b ..................ATGAATACAATCTTAATCCGGGTTTAGAGTGGGAGGATGAATTCACAG..............................ACGACGA
Cdic4   ....TGCTTGCATTTGATGCCCAAGGAGACGACGA
Cdic5a TATG..................ATGAATACAATCTTAATCCGGGTTTAGAGTGGGAGGATGAATTCACAG..............................ACGACGA
Cdic5b TATG..................ATGAATACAATCTTAATCCGGGTTTAGAGTGGGAGGATGAATTCACAG..............................ACGACGA
           Y   D E Y N L N P G L E W E D E F T   V L A F D A Q G D D E
                                        exon v3   variable region v4

Cdic gene AGAGAGCTCACTGCAGAACCTGGGCAACGGATTCACCTCCAAGCTGCCACCGGGCTATCTCACCCACGGCCTGCCCACCGTTAAGGACGTCGCCCGGCCATCACACCACTCGAGATCAAGAAGGAG
Cdic cDNAs AGAGAGCTCACTGCAGAACCTGGGCAACGGATTCACCTCCAAGCTGCCACCGGGCTATCTCACCCACGGCCTGCCCACCGTTAAGGACGTCGCCCGGCCATCACACCACTCGAGATCAAGAAGGAG
           E S S L Q N L G N G F T S K L P P G Y L T H G L P T V K D V A P A I T P L E I K K E
                                                   exon 5

Cdic gene ACTGAAGTGAAGAAGGAGGGTGAGT 378 bp intron TCCAGTCAACGAGCTGTCCGAGGAGCAGAAGCAGATGATCATACTGTCGGAGAACTTCCAGCGGTTCGTGGTGCGTGCCGGCCGC
Cdic cDNAs ACTGAAGTGAAGAAGGAG..........................GTCAACGAGCTGTCCGAGGAGCAGAAGCAGATGATCATACTGTCGGAGAACTTCCAGCGGTTCGTGGTGCGTGCCGGCCGC
           T E V K K E   V N E L S E E Q K Q M I I L S E N F Q R F V V R A G R
                                                  coiled coil 2   exon 6

Cdic gene GTCATCGAACGGGCCCTCTCGGAGAATGTGGACATATACACGGACTACATCGGCGGCGGCGACAGCGAGGAGGCGAACGACGAGCGATCGCATGCGCGGCTCTCGCTGAACCGCGTCTTCTACGACG
Cdic cDNAs GTCATCGAACGGGCCCTCTCGGAGAATGTGGACATATACACGGACTACATCGGCGGCGGCGACAGCGAGGAGGCGAACGACGAGCGATCGCATGCGCGGCTCTCGCTGAACCGCGTCTTCTACGACG
           V I E R A L S E N V D I Y T D Y I G G G D S E E A N D E R S H A R L S L N R V F Y D

Cdic gene AGCGCTGGTCGAAGAACCGCTGCATCACCAGCATGGACTGGTCCACCCACTTCCCCGAGCTGGTGGTGGGCTCGTACCACAACAACGAGGAGAGTCCGAACGAGCCGGACGGCGTGGTGATGGTGTG
Cdic cDNAs AGCGCTGGTCGAAGAACCGCTGCATCACCAGCATGGACTGGTCCACCCACTTCCCCGAGCTGGTGGTGGGCTCGTACCACAACAACGAGGAGAGTCCGAACGAGCCGGACGGCGTGGTGATGGTGTG
           E R W S K N R C I T S M D W S T H F P E L V V G S Y H N N E E S P N E P D G V V M V W

Cdic gene GAACACCAAGTTCAAGAAGAGCACGCCCGAGGACGTCTTCCACTGCCAGAGCGCGGTGATGTCCACCTGCTTTGCCAAGTTCAATCCCAACCTGATCCTCGGCGGCACCTATTCGGGCCAGATTGTG
Cdic cDNAs GAACACCAAGTTCAAGAAGAGCACGCCCGAGGACGTCTTCCACTGCCAGAGCGCGGTGATGTCCACCTGCTTTGCCAAGTTCAATCCCAACCTGATCCTCGGCGGCACCTATTCGGGCCAGATTGTG
           N T K F K K S T P E D V F H C Q S A V M S T C F A K F N P N L I L G G T Y S G Q I V
                                                WD40 repeat #2

Cdic gene CTGTGTGGGACAATCGCTGCAGAAGCGCACGCCCATACAGCGCACGCCCCTCAGTGCCGGCGGCGCACACGCATCCCGTCTACTGCCTCCAAATGGTGGGCACCCAGAATGCGCACAACGTCATCTCCA
Cdic cDNAs CTGTGTGGGACAATCGCTGCAGAAGCGCACGCCCATACAGCGCACGCCCCTCAGTGCCGGCGGCGCACACGCATCCCGTCTACTGCCTCCAAATGGTGGGCACCCAGAATGCGCACAACGTCATCTCCA
           L W D N R V Q K R T P I Q R T P L S A A A H T H P V Y C L Q M V G T Q N A H N V I S
                                                                       WD40 repeat #3

Cdic gene TATCCTCGGACGGCCAAGCTGTGCTCCTGGTCGCTGGACATGCTGTCGCAACCACAGGACACGCTGGAGCTGCAGCAGCGCCAGTCGAAGGCCATTGCCATTACATCGATGGCCTTCCCGGCCAACGA
Cdic cDNAs TATCCTCGGACGGCCAAGCTGTGCTCCTGGTCGCTGGACATGCTGTCGCAACCACAGGACACGCTGGAGCTGCAGCAGCGCCAGTCGAAGGCCATTGCCATTACATCGATGGCCTTCCCGGCCAACGA
           I S S D G K L C S W S L D M L S Q P Q D T L E L Q Q R Q S K A I A I T S M A F P A N E

Cdic gene GATCAATAGCCTGGTGATGGGCAGTGAGGACGGCTACGTCTACTCCGCCTCGCGCCACGGCCTGCGCTCGGGGGTCAACGAGGTTTACGAACGCCATCTTGGCCCTATCACTGGCATATCCACGCAC
Cdic cDNAs GATCAATAGCCTGGTGATGGGCAGTGAGGACGGCTACGTCTACTCCGCCTCGCGCCACGGCCTGCGCTCGGGGGTCAACGAGGTTTACGAACGCCATCTTGGCCCTATCACTGGCATATCCACGCAC
           I N S L V M G S E D G Y V Y S A S R H G L R S G V N E V Y E R H L G P I T G I S T H

Cdic gene TACAACCAGCTGTCGCCGGACTTTGGCCACCTCTTCCTAACCTCGTCCATTGACTGGACCATCAAGCTCTGGTCGCTAAAGGT 67 bp intron ACTCTTTCAGGACACAAAGCCGCTGTAC
Cdic cDNAs TACAACCAGCTGTCGCCGGACTTTGGCCACCTCTTCCTAACCTCGTCCATTGACTGGACCATCAAGCTCTGGTCGCTAA..........................AGGACACAAAGCCGCTGTAC
           Y N Q L S P D F G H L F L T S S I D W T I K L W S L   K D T K P L Y
                         WD40 repeat #4   exon 7

Cdic gene TCCTTTGAGGACAACTCCGACTACGTGATGGACGTCGCCTGGTCGCCCGTGCATCCCGCACTCTTCGCCGCCGTCGACGGCAGCGGCCGCCTGGATCTGTGGAACCTCAACCAAGACACGGAGGTGC
Cdic cDNAs TCCTTTGAGGACAACTCCGACTACGTGATGGACGTCGCCTGGTCGCCCGTGCATCCCGCACTCTTCGCCGCCGTCGACGGCAGCGGCCGCCTGGATCTGTGGAACCTCAACCAAGACACGGAGGTGC
           S F E D N S D Y V M D V A W S P V H P A L F A A V D G S G R L D L W N L N Q D T E V
                         WD40 repeat #5

Cdic gene CGACCGCCTCGATTGTCGTGGCGGGAGCACCAGCCCTTAACCGCGTCTCTTGGACCCCATCCGGTCTGCACGTGTGCATCGGCGACGAGGCCGGCAAGCTGTACGTGTACGACGTGGCCGAGAACCT
Cdic cDNAs CGACCGCCTCGATTGTCGTGGCGGGAGCACCAGCCCTTAACCGCGTCTCTTGGACCCCATCCGGTCTGCACGTGTGCATCGGCGACGAGGCCGGCAAGCTGTACGTGTACGACGTGGCCGAGAACCT
           P T A S I V V A G A P A L N R V S W T P S G L H V C I G D E A G K L Y V Y D A A E N L

Cdic gene GGGCGCAGCCATCGCGCGCGACGAATGGTCGCGGTTCAACACCCATCTTAGCGAGATCAAGATGAACCAGAGCGATGAGGTCTAGGACGATATAGTTAACTGGTAGTTGAGAGTCGGTACCCACTTTTGCT
Cdic cDNAs GGGCGCAGCCATCGCGCGCGACGAATGGTCGCGGTTCAACACCCATCTTAGCGAGATCAAGATGAACCAGAGCGATGAGGTCTAGGACGATATAGTTAACTGGTAGTTGAGAGTCGGTACCCACTTTTGCT
           A Q P S R D E W S R F N T H L S E I K M N Q S D E V *

Cdic isoforms are generated by alternative splicing of *Cdic* transcripts.

The content of the Cdic isoform pool is tissue specific. The representation of the Cdic isoforms in the total Cdic pool was determined by two methods. First, RT-PCR was performed on the mRNA isolated from the embryos and from the adult flies by using the primers flanking the Cdic ORF, as shown in Fig. 2. PCR products were cloned, and a number of randomly picked clones were sequenced, resulting in the data presented in Table 1. Second, RT-PCR was carried out on the RNA isolated from embryos and adult females and also from the dissected female parts (ovaries, midguts, heads, and torsos) by using the primers var-U (5′-GCAGGCTACGAACATTCCA-3′) and var-L (5′-GCAGGCCGTGGGTGAGATA-3′), positioned close to the variable region of the ORF. PCR products were purified and subjected to 10 cycles of mock sequencing with the *Taq* polymerase in the presence of deoxynucleoside triphosphates and primer var-L labeled with ^{32}P by T4 DNA kinase. The reaction products were separated in the sequencing gel and detected by autoradiography (Fig. 7A). The gel image was analyzed with BioMax 1D software (Kodak), producing the quantitative data in Table 2. Females were used here to avoid interference with the testis-specific expression of the *Sdic*. In males, similar representation of isoforms was detected in the midgut, torso, and head.

The data presented in both tables are in good agreement, indicating that the bulk of *Cdic* mRNAs is represented by three isoforms, Cdic1a, Cdic2a, and Cdic2b. They usually make up over 70% of *Cdic* mRNAs and are constitutively expressed in flies and embryos and in all tested body parts. The other eight isoforms usually make up to less than 30% of the pool. Only one of these "minor" isoforms, Cdic2c, is constitutively expressed at low levels. All the others demonstrate tissue specificity, being differentially expressed in the fly body. In particular, it appears that the isoforms Cdic1b and/or Cdic4, Cdic1c, and Cdic3a are up-regulated in the ovaries and Cdic5a and Cdic5b are overexpressed in the head and, to a lesser extent, in the torso. In these cases, the representation of some of the tissue-specific isoforms in the organ (17% of Cdic1b/Cdic4 in the ovary and 23% of Cdic5b in the head) is at the same level as that of major constitutively expressed isoforms.

Since isoforms Cdic1b and Cdic4 have exactly the same length of variable region, they could not be resolved by gel electrophoresis. To discriminate between these two isoforms and to confirm the identity of the RT-PCR products seen in the sequencing gel, they were separated in a 3% agarose gel, transferred to the nylon membrane, and hybridized to the labeled oligonucleotides specific to the particular isoforms. As seen in Fig. 7B, Cdic4, as well as Cdic3 and Cdic1b/Cdic1c, is abundant in ovaries, while Cdic1a and Cdic2a/Cdic2b are constitutively expressed. As expected, Cdic5a and Cdic5b are overexpressed in head and torso.

Cdic isoforms differ in intracellular distribution. Structural variations between Cdic isoforms are limited to the N-terminal region involved in the interaction with dynactin and binding of the dynein complex to the organelles. We suggested that these variations may impel the differences in the targeting properties of Cdic isoforms. To check whether this may be true, Cdic isoforms were expressed in *Drosophila Schneider*-3 cell culture,

and their intracellular distribution was analyzed. Since *Schneider*-3 cells possess only the constitutive set of endogenous Cdic isoforms (Fig. 7A), we excluded the tissue-specific isoforms from this analysis. To visualize the molecules in the cell, the polypeptides were fused at the carboxyl terminus to the green fluorescent protein (GFP).

When overexpressed at high levels, fusion proteins uniformly stained the cytoplasm. At low levels of expression, however, pronounced differences in the intracellular distribution of the fusion proteins were observed (Fig. 8). One of the ovary-specific isoforms, Cdic1c, was still distributed more or less diffusely throughout the cytoplasm, apparently reflecting the lack of ovary-specific target in the cultured cells. This isoform was chosen as a control, in contrast to the constitutive isoforms, which should have the relevant targets in the cultured cells, and clearly demonstrated different intracellular localizations.

Fusion proteins corresponding to the constitutively expressed isoforms Cdic2a and Cdic2b possessed perinuclear localization, which is tighter for Cdic2b. The aggregation of Cdic2b fusion is more proximal than the perinuclear structures stained for Golgi (Fig. 9A) and most likely represents binding to the nuclear envelope. For the isoform Cdic1a, numerous small aggregations were detected and were distributed in the cytoplasm and around the nucleus. In the case of the isoform Cdic2c, the protein accumulated in one local area, as in Fig. 8, or in several patches often clustered in a sector of cytoplasm. In all cases, distribution of the fusion proteins was strictly different from the strong nuclear localization of GFP tag expressed alone (Fig. 8).

In the cells overexpressing Cdic fusion proteins at a high level, morphological changes which would result from competitive inhibition of dynein binding to organelles may be expected. In particular, the distribution of lysosomes in the cytoplasm was checked for all constitutive isoforms, and only in the case

TABLE 1. Representation of isoforms in *Cdic* cDNA clones[a]

Cdic isoform	% of *Cdic* cDNAs (no. of clones) in[b]:	
	Embryos	Flies
1a	38 (19)	45 (27)
2a	14 (7)	27 (16)
2b	20 (10)	8 (5)
2c	2 (1)	0 (0)
1b	8 (4)	8 (5)
4	8 (4)	3 (2)
1c	0 (0)	2 (1)
3a	6 (3)	2 (1)
3b	2 (1)	0 (0)
5a	0 (0)	2 (1)
5b	2 (1)	3 (2)
Total	50	60

[a] cDNAs for *Cdic* isoforms were amplified as shown in Fig. 2, and PCR fragments were cloned. A total of 50 randomly chosen clones were sequenced for cDNA from embryos, and 60 clones were sequenced for flies.

[b] For each isoform, the percent representation in the cDNA pool is shown, as calculated from the number of relevant clones detected (in parentheses).

FIG. 6. Sequence alignment of coding regions of the *Cdic* gene with *Cdic* cDNAs. The genomic sequence is at the top, with the gaps introduced in place of introns. cDNA sequences are below, shown as a single line in "constitutive" regions where they are identical and shown individually in the variable region. A conceptual protein sequence is shown at bottom; for the isoform Cdic5a, a frameshifted translation of exon v1 is included (peptide 5a). Coiled-coil domains, outlined as black boxes, were predicted with the PAIRCOILS (3) and COILS (13) algorithms. In the N-terminal region, serine-rich domain and the PPE/TQT conserved region are boxed. In the C-terminal region, four of five WD-40 repeats (27) are underlined.
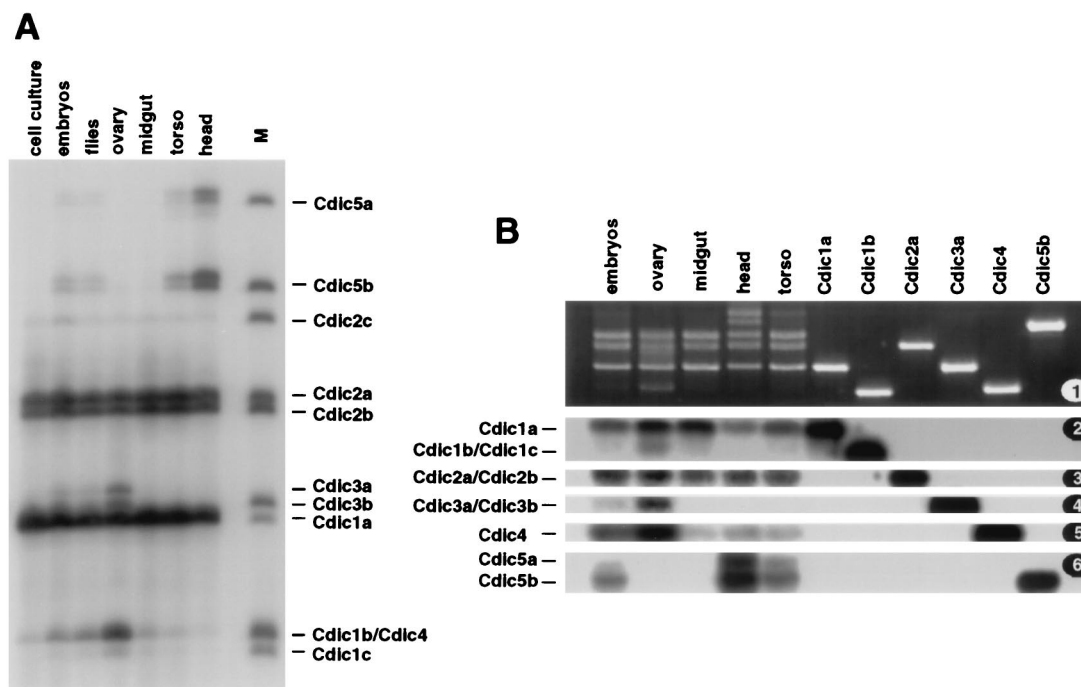
FIG. 7. Tissue specificity of *Cdic* isoforms. RT-PCR fragments were amplified across the variable region of *Cdic* transcripts. (A) PCR fragments were labeled at one DNA strand with ^{32}P and separated in a 5% acrylamide sequencing gel. The source of the RNA is indicated at the top. Lane M contains marker fragments generated from the of cDNA clones representing *Cdic* isoforms. (B) PCR fragments were separated in a 3% agarose gel (1) and, after Southern transfer, hybridized with oligonucleotides "iso2" (2), "iso2′′′" (3), "iso3" (4), "iso4" (5), and "iso5" (6). The source of the RNA is indicated at the top; individual cDNA clones *Cdic*1a, *Cdic*1b, *Cdic*2a, *Cdic*3a, *Cdic*4, and *Cdic*5b were used to generate the marker fragments in the six right-hand lanes.

of the Cdic2c fusion was redistribution of lysosomes observed. This effect has been proved to be indicative of disruption of the dynein IC-dynactin interaction and, therefore, of inhibition of dynein binding (5). In 65% of transfected cells, lysosomes were aggregated in one local area, as seen in Fig. 9B, or along the periphery of one side of the cell. This was found in only 12% of the untransfected cells, in which much more random and mostly juxtranuclear distribution was usually observed.

Since interaction with dynactin seems to be limited to the N-terminal portion of dynein ICs (26), we tested whether fusion proteins containing only this N-terminal part of ICs can possess isoform-specific intracellular distribution. For N-Cdic2b, perinuclear localization was still observed, but it was not as specific as that with the complete DIC2b fusion (Fig. 8). Overexpression of the N-Cdic2c protein caused lysosomal aggregation in 30% of transfected cells—somewhat greater than the 12% in nontransfected cells, but far less than the 65% in cells expressing the complete Cdic2c fusion (Fig. 9). This may explain the differences in intracellular localization between Cdic2c and N-Cdic2c; while the first usually accumulated in a local area of cytoplasm, presumably binding to the lysosomal aggregates, the localization of the other suggests binding to the normally distributed lysosomes (Fig. 8).

DISCUSSION

Alternative splicing of Cdic transcripts generates the diversity of cytoplasmic dynein ICs. Although the isoform complexity of dynein ICs has been studied for a long time (21, 26), the molecular basis for it has been unknown, since no gene has been cloned to date. However, the suggestion has been presented that in the rat, the structural isoforms are produced by alternative splicing of transcripts from two highly homologous genes (26).

Our data directly demonstrate that in the *D. melanogaster*

species group, the Cdic diversity is created by alternative splicing of transcripts from a unique gene. Alternative splicing of exons and multiple splice acceptor sites are used, resulting in both length and amino acid sequence variability in the short

TABLE 2. Representation of isoforms in *Cdic* mRNAs from different sources[a]

Cdic isoform	% of *Cdic* mRNA in[b]:					
	Embryo	Fly	Ovary	Midgut	Head	Torso
Constitutive						
1a	41 ± 3	54 ± 4	43 ± 1	51 ± 1	36 ± 6	49 ± 5
2a	27 ± 4	28 ± 5	22 ± 1	24 ± 5	21 ± 1	27 ± 4
2b	15 ± 2	20 ± 4	11 ± 2	16 ± 3	11 ± 1	17 ± 2
2c	4 ± 1	4 ± 1	6 ± 3	4 ± 1	3 ± 2	2 ± 2
Ovary-specific						
1b+4	10 ± 4	8 ± 2	17 ± 2	2 ± 1	ND	ND
1c	3 ± 1	1 ± 1	3 ± 2	ND	ND	ND
3a	4 ± 1	2 ± 1	8 ± 2	ND	ND	ND
Head-specific						
5a	ND[c]	1 ± 1	ND	ND	11 ± 4	2 ± 1
5b	3 ± 2	3 ± 1	ND	ND	23 ± 3	6 ± 1

[a] *Cdic* RT-PCR products amplified from the RNA extracted from indicated sources were end-labeled with ^{32}P and separated in a sequencing gel, as shown in Fig. 7. The gel was exposed to Biomax-MR film (Kodak), the film was scanned, and the image was analyzed with Biomax-1D software (Kodak) to quantitate the intensity of the bands.

[b] Representation of the isoform in the total mRNA pool was calculated by normalizing the intensity of relevant band by the total intensity of all specific bands on the lane. The numbers indicate the mean and standard error of results obtained in three experiments.

[c] ND, nondetectable.

Full-size IC:GFP fusion
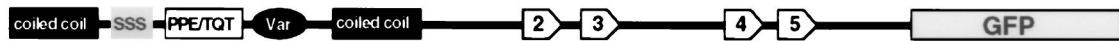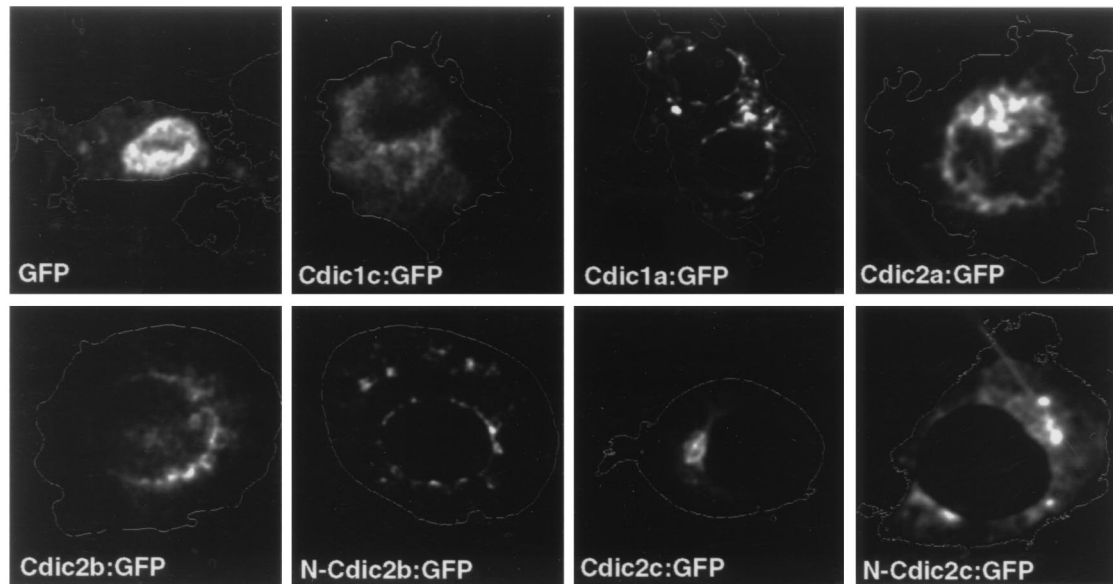

N-terminal IC:GFP fusion

FIG. 8. Localization of the Cdic-GFP fusion proteins in cultured *Schneider*-3 cells. A schematic representation of the full-size fusion and N-terminal fusion proteins is shown at the top. Cells were transfected with plasmids expressing fusion proteins under the control of the cytomegalovirus promoter and stained with propidium iodide. Staining of the cellular content with propidium iodide was detected in the rhodamine channel, and the image was converted to the contour of the cell. GFP fluorescence was detected in the fluorescein isothiocyanate channel and pseudocolored in white. IC isoforms are indicated; for example, Cdic1a:GFP is a full-size fusion of Cdic1a isoform with GFP, and N-Cdic2b and N-Cdic2c are the N-terminal fusions. The localization of the GFP expressed alone is shown for comparison.

region near the N terminus of the polypeptide. As many as 10 isoforms were detected and characterized.

Comparison of the N-terminal part of *Drosophila* Cdic polypeptides with the cytoplasmic dynein ICs from rat revealed that the presence of the variable region created by the alternative splicing is conserved. This does not apply, however, to the location of this region: there are two variable regions in the rat polypeptide, flanking the serine-rich domain, and only one variable region in the *Drosophila* polypeptide (Fig. 3). Moreover, the position of the single variable region in the *Drosophila* protein does not correspond to the position of any of the two regions in rats, since these two are located on the different side of a well-conserved PPE/TQT block of amino acids. Therefore, it may be suggested that the mechanism of alternative splicing, generating the diversity of the N termini of dynein ICs, evolved independently in *Drosophila* and rats. This finding emphasizes the importance of IC heterogeneity for dynein functions and raises further questions about the implications of this heterogeneity.

The tissue specificity of splicing alters the content of Cdic isoform pool. Although at least 10 isoforms are created by alternative splicing, only 4 are represented in consistent amounts in all tested tissues. These are referred to as constitutive, and

in some tissues, for example in the midgut, or in cultured cells, they make up all the content of isoform pool. This means that these four Cdic isoforms provide at least the requisite set of the dynein-mediated activities. It still is not apparently enough in the other cases, requiring more specialized actions, since upregulation of other isoforms was observed in nervous tissue (in particular, in the head) and ovaries. This up-regulation clearly reflects changes in the splicing pathways and leads to a lowered percent representation of constitutive isoforms, since the tissue-specific isoforms contribute a significant proportion (up to a quarter) of the total pool. This, however, does not seem to cause depletion of the constitutive isoforms, since at least in the head it is compensated for by the overall increase in expression of Cdic (Fig. 1).

In neurons, a specific and very active process of axonal transport takes place, and it has been shown to be cytoplasmic dynein dependent (7). In the ovaries, extensive transport takes place from the nurse cell cytoplasm to the oocyte, and this transport has also been shown to depend on cytoplasmic dynein (12). It seems than wherever specific transport takes place, specific Cdic isoforms are added to the basic set. Also, since the specificity of these kinds of transport mainly concerns the type of cargo organelle to be moved, it seems very likely that

A

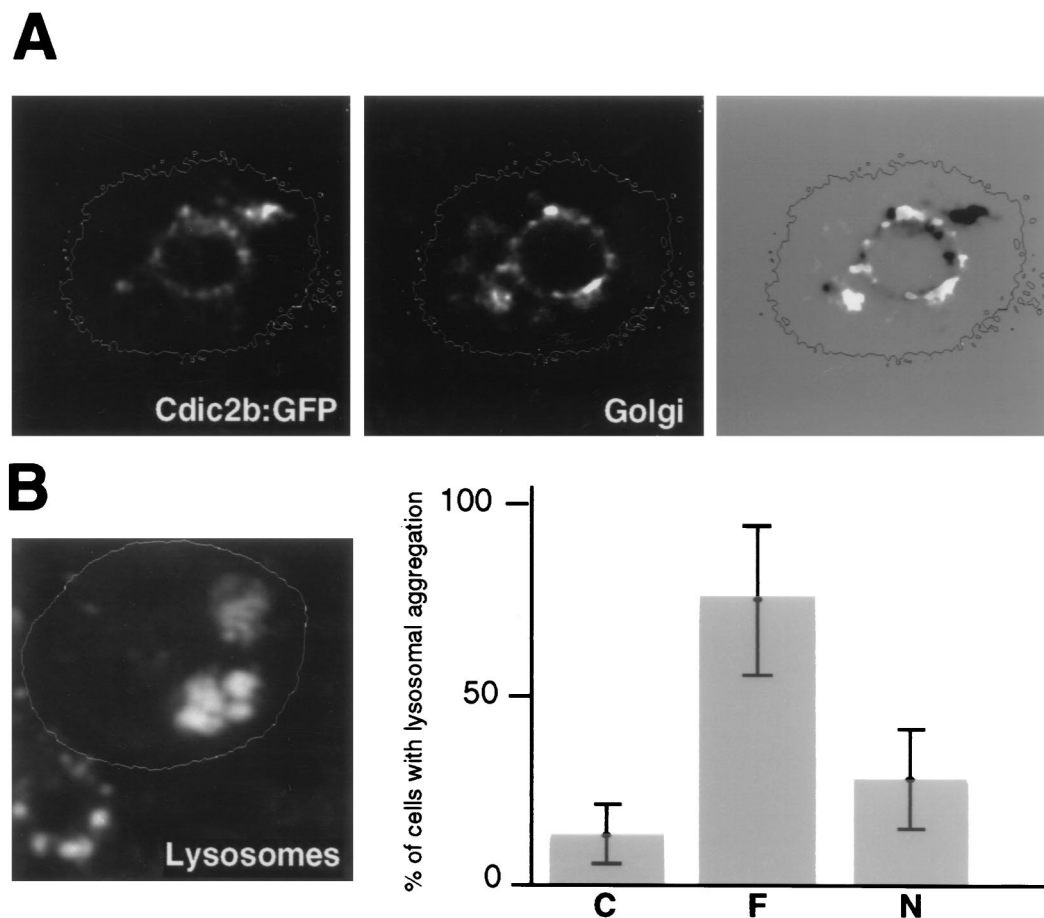Cdic2b:GFP   Golgi

B

Lysosomes

FIG. 9. (A) Tight association of the Cdic2b fusion protein with the nucleus. In this case, Golgi staining mostly reveals the perinuclear elements. On the right, the GFP fluorescence image was converted to the negative and overlaid on the Golgi staining image. Note that the GFP fusion protein is located more proximal than the Golgi elements. (B) Redistribution of the lysosomes in the cells overexpressing the Cdic2c:GFP fusion protein. On the left, two transfected cells are contoured. Staining with Lysotracker DND-99 revealed aggregation of the lysosomes in transfected cells, in contrast to the random and mostly juxtranuclear distribution in nontransfected cell in the left lower corner. On the right, the frequency of the lysosomal aggregation in cells expressing Cdic2c:GFP (F) or N-Cdic2c:GFP (N) versus nontransfected cells (C) is shown. Error bars represent 95% confidence intervals.

the specific Cdic isoforms provide targeting of dynein complex to these peculiar cargoes.

Cdic isoforms differ in intracellular targeting. Considering the multiplicity of intracellular targets for dynein binding and the diversity of IC isoforms, it seems very likely that different IC isoforms provide targeting of the dynein complex to distinct organelles. If this were the case, the constitutive IC isoforms would furnish the targeting to organelles regularly bound to dynein, for example, lysosomes, Golgi complex, nuclei, and mitotic chromosomes. Tissue-specific IC isoforms, in turn, would provide dynein binding to cargo organelles that are specific to the tissue and are not regularly present or are not bound with dynein in other tissues.

When IC isoforms fused to the GFP at the C terminus were expressed in the cultured cells, they possessed different patterns of intracellular distribution. Although it is not clear whether these patterns reflect the distribution of endogenous ICs, it is certain that they are due to the variable N termini of ICs, since all other parts of the expressed fusion proteins are identical.

For the fusion proteins corresponding to the tissue-specific Cdic1c, a uniform diffuse distribution in the cytoplasm or aggregation around small, unidentified structures was observed (data not shown). This pattern, which may reflect the absence

of tissue-specific targets for these ICs in the cytoplasm of cultured cells, is clearly different from that observed for the constitutive ICs. An apparent binding to the nuclear envelope was detected for Cdic2b. Overexpression of the Cdic2c fusion protein caused relocation of the lysosomes. Considering that this effect was not detected for other isoforms, Cdic2c could be called the lysosome-specific IC isoform.

It has been demonstrated that targeting of the organelles by ICs requires the N-terminal portion of the molecule (23). We checked whether this N-terminal portion of IC, fused to GFP, is capable of the specific targeting observed for the full-size IC fusion. This was somewhat true for isoform Cdic2b, although the pattern was not as well defined as with the full-size fusion protein. Overexpression of the N-terminal fusion for isoform Cdic2c had little effect on the lysosome relocation, compared to that of the full-size protein. This implies that the C-terminal part of the IC molecule, which has been considered to be a domain responsible for interaction with other components of the dynein complex, is also important for the organelle targeting. It is conceivable that the incorporation of the ICs in the dynein complex plays a substantial role. Resolution of this question requires additional experiments that include localization of endogenous IC isoforms in fly tissues. This would also

provide additional data on the specificity of organelle targeting by isoforms.

The data presented here support a model in which alternative splicing generates a number of cytoplasmic dynein IC isoforms that differ in their targeting properties. These IC subunits mediate dynein binding to organelles, enabling various types of minus-end directed transport along microtubules. The default splicing pattern provides isoforms necessary for dynein-mediated "housekeeping" activities necessary for maintaining any cell or tissue, including anterograde movement and positioning of lysosomes and Golgi complex, mitosis, and nuclear migration. Tissue-specific alteration in the splicing supplies additional IC isoforms, capable of targeting cargo organelles that are subject to unusual tissue-specific transport. This mechanism provides a long-term modulation of the dynein binding to organelles in the development of organism, in addition to more swift regulation by phosphorylation of dynein subunits described previously (17).

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