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Characterization of a novel human dynein-related gene that is specifically expressed in testis

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Abstract. A novel dynein-related transcript (designated DNEL1) from human adult testis has been identified that can encode a protein with a size of 91087 Da. The complete nucleotide sequence of the open reading frame is the first to be described for a human dynein-related gene. Northern blot analysis of mRNA from 16 different tissues has shown that DNEL1 is expressed specifically in testis. Analysis of somatic cell hybrids has mapped DNEL1 to Chromosome (Chr) 17. Analysis of a panel of 129 whole genome radiaton hybrid clones including 17q22–q25.3 has placed DNEL1 in 17q distal to the ERBA2L locus. DNEL1 shares a high degree of sequence identity and amino acid similarity with the C-terminal region of the outer arm axonemal dynein β-heavy chains derived from sea urchin and other species, but not to any gene encoding dynein intermediate or light chains described to date. The close similarity of DNEL1 to the C-terminal part of the axonemal β-heavy chain may suggest an origin from a common progenitor gene and the testis-specific pattern of expression a possible role in sperm development or motility.

Introduction

Dyneins are molecular motors that form large multi-subunit protein complexes with an estimated molecular mass greater than 1,000,000 Da. They consist of several polypeptides: generally two heavy chains of greater than 400,000 Da, three to five intermediate chains of 60,000–120,000 Da, and four light chains of 19,000–30,000 Da (Witman 1989). Ultrastructural studies indicate that dynein complexes resemble a structure with a globular head (composed predominantly of heavy chains) and stem, the base of which is believed to be attached to microtubules in association with intermediate and light chains (reviewed in Witman 1992). Through ATP hydrolysis, they mediate various motor functions within the cell. Cytoplasmic dyneins are believed to be involved in the motility of a variety of intracellular components such as endocytotic vesicles or condensed chromosomes during mitosis (reviewed in Holzbaur et al. 1994). Axonemal dyneins are involved in the movement of cilia and flagella and are organized into outer and inner arms, which differ in subunit composition and distribution along the outer microtubule doublets (reviewed in Witman 1992). Defects in human axonemal dynein complexes detected by electron microscopy have been shown to be associated with Kartagener's syndrome, which is characterized by recurrent respiratory tract infections, immotile sperm, and situs inversus (reviewed in Afzelius and Mossberg 1989).

This report presents the characterization of a human dynein-related gene (designated DNEL1) identified through isolation of a testis-expressed sequence tag (EST 192, accession numbers Z21174 and Z21269) as part of a program to analyze genetic expression during spermatogenesis (Affara et al. 1994). It is the first complete human dynein-related cDNA sequence with close similarity to axonemal dynein heavy chains (Ogawa 1991; Gibbons et al. 1991; Mitchell and Brown 1994) and may represent a novel class of dynein genes or a potential human axonemal dynein gene. The open reading frame codes for a protein that has a molecular mass of 91,087 Da, which is similar in size to intermediate dynein chains but shows no homology to any gene encoding intermediate or light chains described in any other species so far (Vaughan and Vallee 1995). The gene has a high degree of identity and similarity to the C-terminal region of the outer arm axonemal dynein β-heavy chains derived from sea urchins and *Chlamidomonas* (Ogawa 1991; Gibbons et al. 1991; Mitchell and Brown 1994). DNEL1 has been mapped to Chr 17 and is expressed specifically in testis. The close similarity of DNEL1 to the C-terminal region of the β-heavy chains may indicate that these genes share a common ancestral origin.

Materials and methods

Southern blot analysis. Monochromosome hybrid cell lines containing a single human chromosome in a mouse or hamster cell line were obtained from National Institute of General Medical Science, Coriell cell repository (see Coriell catalog for details), Camden, N.J., with the exception of hybrids for the X (HORL9X) and Y (7631) Chrs that were from cell culture stocks (Dept. of Pathology, University of Cambridge). DNA from the cell lines and total human DNA prepared from male peripheral blood (used as a control) were prepared as described elsewhere (Kunkel et al. 1977). For Southern blot analysis, 8 μg of DNA was digested with *Eco*RI (Boehringer Mannheim) in a buffer provided by the manufacturer. Fragments were resolved through 0.8% agarose and blotted onto a nylon membrane (Amersham, Hybond N+) according to the manufacturer's instructions. cDNA probe 192 containing part of the DNEL1 gene was labeled with ^{32}P-dCTP by random oligonucleotide priming (Feinberg and Vogelstein 1983) and hybridized to genomic DNA from the panel of somatic cell hybrids at 65°C in 10% dextran sulfate, 5 × Denhardts reagent, 1% SDS, 0.08 M NaH_2PO_4, 0.42 M Na_2HPO_4, and 100 μg/ml salmon sperm DNA as described in Sambrook et al. (1989).

Mapping with the whole genome radiation hybrid panel. Fifty nanogram of DNA from 129 hybrid cell lines were screened for the presence or absence of the DNEL1 gene by use of the PCR and primers M1 and M2. Reactions were performed in duplicate in a final reaction volume of 30 μl in Hybaid microtiter plates and cycled in a Hybaid Omnigene PCR machine. Each plate contained four controls: water, DNA from the two parent hybrid cell lines, and DNA from the Chr 17 only somatic cell hybrid line PCTBA1.8. The final concentrations in the reaction mix were: 25 mM TAPS pH 9.3, 50 mM KCl, 1 mM DTT, 2 mM $MgCl_2$, 0.05% W1 detergent, 100 ng of each primer, 125 μM each dATP, dGTP, dCTP, dTTP, and 0.15 U Taq polymerase (NBS). The cycling conditions were: 94°C for 5 min, 35 cycles of 94°C for 0.5 min, 59°C for 0.5 min, 72°C for 1 min, and a final extension at 72°C for 10 min. The PCR products were separated on a 3% agarose gel run in 1 × TAE buffer and visualized by staining with ethidium bromide and excitation with ultraviolet light. The primer sequences for the

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DNEL1 gene were M1: 5′ GCAGAGCCACCGAGAAAATA 3′ and M2: 5′ GCTCATGGTTTCTTACCGTTCT 3′. The data were analyzed with the RHMAP programs (Boehnke 1992; see also Walter et al. 1994) to determine two-point LOD scores and marker order with respect to markers already mapped in this region (see Foster et al. 1996 for detailed analysis of this radiation hybrid panel and marker order determined on Chr 17).

Northern blot analysis. cDNA probe 192 labeled with ^{32}P-dCTP was hybridized to multiple tissue Northern blots (MTN and MTNII, Clontech, Palo Alto, CA) under the conditions described in the Clontech manual. Low stringency washes were performed by washing the blots in 1 × SSC/0.1% SDS at room temperature. Washes of higher stringency were performed in the same solution at 65°C. Blots were exposed to Kodak XAR5 or XLS X-ray film at −70°C.

Reverse transcription PCR (RT-PCR). Total RNA was prepared from human adult testis with the Tri reagent kit and protocol supplied by Molecular Research Center Oxford, Ltd. RT-PCR was performed with the kit supplied by Promega (Madison, Wis.). The primers used were: R1: 5′ ACAAGAAAGGTCAGGGTGGC 3′; R2: 5′ TTCTCTTTCTCAGGACATTCGG 3′; R3: 5′ GAAAATCAACGAGGCCCGAG 3′; R4: 5′ TTTTCTCGGTGGCTCTGCA 3′; R5 5′TCAGGGTAATTTTGAATCCAT 3′. The PCR with primers R1 and R2 was prepared as described above. The annealing temperature was at 65°C. A fragment amplified by R3 and R4 primers was amplified in a PCR reaction that contained 16 mM $(NH_4)_2SO_4$, 670 mM Tris-HCl pH 8.8, 0.01% Tween-20, 3 mM $MgCl_2$, 418 μM deoxynucleotides, 100 ng of each primer, and 1.2 U Bio-X-Act™ polymerase (Bioline, London, UK). Amplification conditions were as follows: 94°C for 3 min, 25 cycles of 94°C for 0.5 min, 60°C for 0.5 min, 68°C for 5 min, and final extension at 68°C for 10 min.

Full-length cDNA sequence and sequence analysis. DNA from clone 192 was amplified by PCR and cloned into the pSPORT vector with the CloneAmp pAMP1 system (Life Technologies, Gaithersburg, WA) and the protocols in the manufacturer's manual. Several clones were sequenced on both strands with the ABI 373A automated sequencer and PRISM dye-deoxy terminators (Applied Biosystems), applying standard cycle-sequencing protocols. The cDNA clone was extended towards the N-terminus with 5′-Race-Ready™ cDNA (Clontech). Extensions of the parent clone sequence towards the C-terminus were obtained by using successive steps of PCR amplification against vector primers to walk through two adult testis cDNA libraries (Affara et al. 1994 and Clontech commercial adult testis cDNA library manual). Two nested specific primers and a general primer based on the sequence of the vector were used at each step. PCR fragments were then cloned into the pSPORT vector as described above and amplified by PCR with pSPORT specific primers. Because part of the cloning strategy involved a multistep PCR walk along a cDNA, care has been taken not to jump between related dynein sequences. Several clones were sequenced for each extension. Only clones that contained identical nucleotide sequence as the clone that they were extended from over at least 80–100 bp of overlap were used. Sequences were aligned and manipulated with GCG (University of Wisconsin; Devereux et al. 1984) and Staden (1990) program packages. DNA and inferred protein sequences were used to search the databases by the Fasta algorithm (Pearson and Lipman 1988).

Results

Expression pattern of dynein-related transcripts from human adult testis. Initially, a 966-bp expressed sequence tag (EST) with 74% identity to the sea urchin dynein β-heavy chain was identified as a consequence of systematic partial sequence analysis of a human adult testis cDNA library (Affara et al. 1994). This clone (clone 192; accession numbers Z21174 and Z21269) was used for Northern blot analysis of poly A+ mRNA from a range of 16 human tissues (Fig. 1). Under low-stringency wash conditions (1 × SSC/0.1% SDS at room temperature, Fig. 1a), Northern analysis with clone 192 detected several transcripts of approximately between 1 and 9 kb in a number of different tissues; these may represent different members of the dynein gene family. Following high-stringency washes (1 × SSC, 0.1% SDS at 65°C), only a 3.2-kb

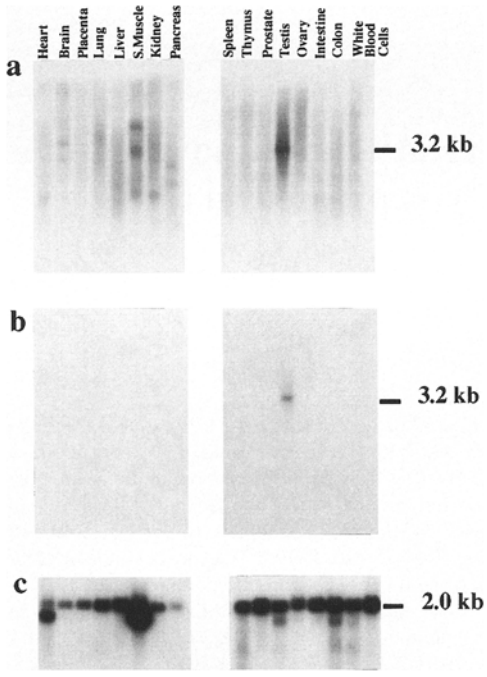

Fig. 1. Expression pattern of dynein-related gene DNEL1 in poly A+ mRNA from 16 different human tissues. **(a)** Northern analysis with clone 192 and low-stringency wash conditions (1 × SSC/0.1% SDS at room temperature). **(b)** Northern analysis with the same probe where blots were washed at higher stringency. Under these conditions (1 × SSC, 0.1% SDS at 65°C), only a testis-specific 3.2-kb transcript can be detected. **(c)** Expression of β-actin, used as a positive control to indicate the presence of RNA in each track.

testis-specific transcript remained (Fig. 1b). The size of the transcript detected by clone 192 indicates that it does not encode a dynein heavy chain. Sequence analysis (see below) demonstrates that the most closely related heavy chain genes show 74% identity to the gene represented by clone 192. At this level of identity, cross-hybridization to similarly related human heavy chain genes will not occur under the hybridization conditions used for the Northern blots (5 × SSPE, 50% formamide and 42°C). Thus, it is not surprising that heavy-chain transcripts are not readily detected in these blots with probe 192. It is unlikely that the discrete 3.2-kb band represents a degradation product of a large transcript; smearing would be expected if this were the case. The same blots were used to detect transcripts of 9 kb in testis and brain RNA that represent the complete open reading frame of a member of the kinesin gene family (Furlong et al. 1996), indicating the existence of large intact RNA species in these mRNA populations.

Chromosome map location of transcript detected by clone 192. The assignment of the testis-specific transcript to Chr 17 was achieved by analysis of a panel of monochromosome somatic cell hybrids (Fig. 2a and b). Human specific fragments are present in the track carrying DNA from the hybrid cell line containing human Chr 17 (marked with black arrow heads). Cross-hybridization to mouse and hamster genomic DNA suggests that the sequence is conserved at least between these species. Four human genomic *Eco*R1 fragments of 18 kb, 14 kb, 11 kb, and 3 kb are detected by the 966-bp clone 192 probe (which lacks *Eco*R1 sites), indicating that at least three introns must interrupt the cDNA sequence in this part of the gene.

The regional assignment on Chr 17 of the transcript represented by clone 192 was investigated through the analysis of a whole genome radiation hybrid mapping panel (129 clones in to-

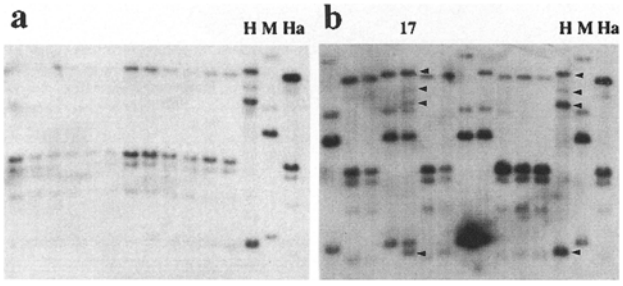

Fig. 2. Mapping of the DNEL1 transcript represented by clone 192. Parts **(a)** and **(b)** show the mapping of DNEL1 to Chr 17 by the Southern analysis of a panel of genomic DNAs (digested with *Eco*RI) from monochromosome somatic cell hybrids. Panel **(a)** left to right represents Chrs 1–12, and panel **(b)** Chrs 13–22, X, and Y. H, M and Ha are control tracks that contain genomic DNA prepared from human male peripheral blood, mouse liver, and CHO cell line respectively. The hybrids containing chromosomes 1–12, 14, 15, 18, 19, 22, X, and Y are on a hamster background, and those containing chromosomes 13, 16, 17, 20 and 21 on a murine background. The lane carrying Chr 17 is highlighted, and the arrowheads flag the human DNA fragments.

tal) including the interval 17q22–q25.3 described and characterized in detail by Foster and associates (1996). Groups of markers showing linkage to the gene represented by clone 192 were subjected to a maximum likelihood multipoint analysis to determine the most probable order of markers in this region. The PCR primers M1 and M2 (located at the 3′ end of the gene; designated DNEL1 and marked in Fig. 4) generate a human-specific DNEL1 fragment of 104 bp from the hybrid genomic DNA. Two-point linkage analysis with the program RH2PT indicated linkage of DNEL1 to at least one member (GAA) of the group of markers (GAA, D17S784, ACTG1, D17S836, and ERB2L) lying distal to the TK gene at a lod score of greater than 3.0 (3.18; Foster et al. 1996). Two-point linkage analysis does not determine marker order. To determine the order of this group of markers, including DNEL1, the RH mapping results were analyzed with the program RHMAXLIK (Boehnke 1992). The most likely order of markers by use of the unequal-retention model places the DNEL1 gene distal to the marker ERBA2L (Fig. 3). The next most probable order shown in Fig. 3 is 257-fold less likely than the order 1. Any other order is more than 10,000-fold less likely than order 1.

Determination of the complete open reading frame for transcript 192. The complete sequence of the DNEL1 transcript was obtained by 5′ and 3′ RACE extension of the original 192 clone as described in the Materials and methods. Figure 4 is a schematic summary of the extensions used to define the sequence of the transcript, indicating the relative position of the initiation and stop codons. The DNEL1 sequence is shown aligned against

Most likely locus order

Rank	Log10 like. diff.	Like. ratio	Breaks	Locus order
1	0	1.0	162	TK - D17S836 - D17S784 - GAA - GACT - ERB2L - **DNEL1**
2	2.4110	257.6	166	TK - D17S836 - D17S784 - GAA - ERB2L - GACT - **DNEL1**

Fig. 3. The most probable order of gene DNEL1 in relation to markers in distal 17q. This order was derived from the analysis of a panel of 129 radiation hybrids using the program RHMAXLIK. The order 1 is 257-fold more likely than the order 2. Any other order is >10,000-fold less likely than the order 1. TK = thymidine kinase; D17S836 = microsatellite marker AFM163yg1; D17S784 = microsatellite marker AFM004xg3; GAA = acid alpha-glucosidase; ACTG1 = cytoskeletal gamma-actin; ERB2AL = avian erythroblastic leukemia viral locus. Two-point analysis has shown linkage of DNEL1 to GAA at a LOD score of 3.18.

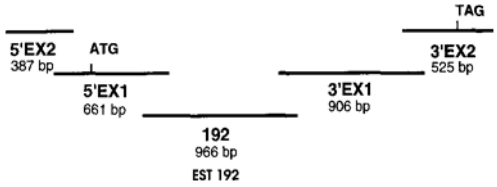

Fig. 4. Schematic representation of the cDNA PCR extensions used to define the transcript detected by clone 192.

the sea urchin axonemal β-heavy chain nucleotide sequence (Fig. 5A), and the sequence of the original EST192 is shown underlined. Each 5′ or 3′ extension of the transcript was determined from the analysis of several independent overlapping clones to ensure accuracy of sequence. Searches of the GenBank, EMBL, and Swissprot databases for sequence matches at the DNA and protein levels showed a high degree of identity to dynein β-heavy chains of two species of sea urchins: *Anthocidaris crassipina* (71% identity in 2661-bp overlap; Ogawa 1991); and *Tripeneustres gratilla* (70% identity in 2691-bp overlap; Gibbons et al. 1991) and to the β-heavy chain of *Chlamidomonas reinhardtii* (59% identity in 988-bp overlap; Mitchell and Brown 1994). The amino acid sequences showed a lower percentage of identity to cytoplasmic dynein of *Drosophila melanogaster* embryos (27%; Li et al. 1994) and *Dictiostelium discoideum* (24%; Koonce et al. 1992).

Definition of the start of the longest open reading frame by determining the in-frame initiator methionine that best matches the Kozak consensus (Kozak 1986) shows that DNEL1 encodes a protein of size similar to dynein intermediate chains (see Fig. 6). However, no sequence similarity to any known intermediate or light chains including rat intermediate chain isoforms of cytoplasmic dyneins (Vaughan and Vallee 1995) was found, indicating that DNEL1 may represent a different class of dynein-related genes. The sequence alignment to the β-heavy chain continues to be conserved (at both the DNA and protein level) for the next 250 bp further 5′ to the initiator methionine, but is not associated with an in-frame ATG codon. The amino acid similarity continues in this region at a level of 82% identity. The sequence then diverges rapidly (25% identity), as would be expected for a 5′ untranslated region (5′ UTR), and contains three in-frame stop codons. The poly A tail at the end of the 3′ untranslated region (3′ UTR) has been reached, and two consensus poly A addition signals (AATAAA) starting 22 bp and 179 bp after the termination codon are present in the sequence.

RT-PCR of mRNA derived from human adult testis has confirmed that this sequence forms a contiguous transcript. First, the primers R1 and R2 (underlined in Fig. 5A) have been used to amplify the 5′ end of the gene (from within the coding region to the extreme 5′ end), and sequence analysis of the RT-PCR product shows a 100% match with the gene sequence derived from analysis of cDNA extensions. Second, the primers R3 and R4 were used to amplify a fragment of the correct size that covers the entire open reading frame from the same adult testis RT-PCR product. This confirms that the sequence presented in Fig. 5A represents a co-linear transcript.

The primers R1 and R5 were used to perform further analysis of the 5′ end of the transcript. PCR analysis of genomic DNA and RT-PCR from human adult testis RNA with these primers generates fragments of 700 and 300 bps respectively, indicating the presence of an intron in the 5′ end of the gene. Sequence analysis of the 700-bp genomic product has shown that a 416-bp intron interrupts the gene at the point where the homology with the sea urchin dynein β-heavy chain breaks down between nucleotides 271 and 272. From the sequence analysis of this genomic DNA, no other introns have been found in the terminal region of the transcript. The intron is defined by consensus splice sites (see Fig. 5B).

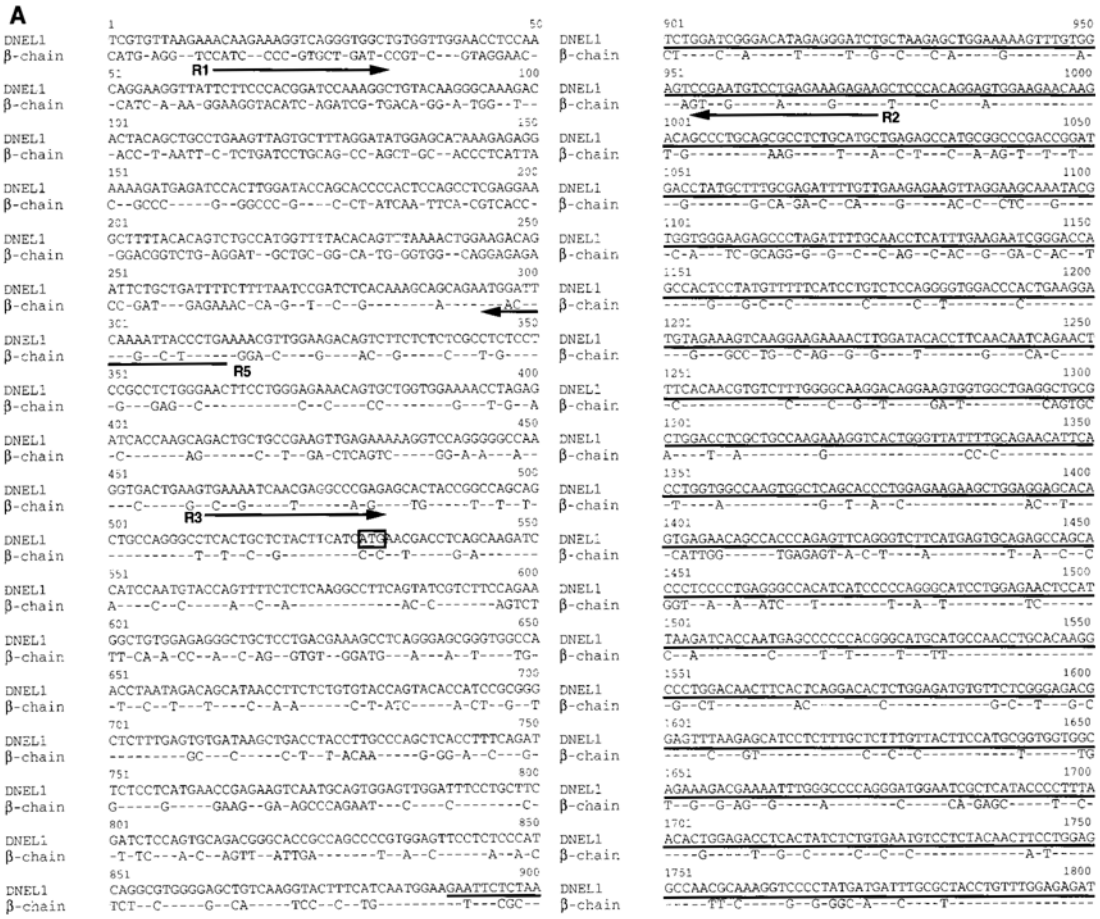

Fig. 5. **(A)** Alignment of the nucleotide sequence of the dynein-related gene DNEL1 to the relevant C-terminal part of the β-heavy chain of sea urchin dynein. The potential start (ATG), stop (TAA), and polyadenylation signal sequences (AATAAA) are boxed. The best match to the Kozak consensus (Kozak, 1986) for DNEL1 is the start codon at position 530 of the nucleotide sequence. Primer sequences used for the analysis of whole genome radiation hybrids (M1 and M2) and for RT-PCR and analysis of genomic DNA (R1 and R2; R3, R4 and R5) are underlined, and the arrowhead indicates the 5′ to 3′ orientation of the primer. The dashes mark sequence identities between the two genes. **(B)** The consensus splice site sequences defining the intron in the 5′ UTR of the DNEL1 gene.

This indicates that the gene has not arisen as a result of retrotransposition of a processed transcript.

Discussion

Despite the close similarity to the sea urchin axonemal β-heavy chain, the size of the messenger RNA detected on Northern blots (~3.2 kb), the transcript defined by sequencing (3.16 kb), and the open reading frame determined by sequence analysis, demonstrates that DNEL1 does not encode a heavy chain gene. A second potential initiation site lies further upstream close to the point where the sequence conservation with the sea urchin gene breaks down. However, this ATG codon is in frame with four stop codons before reaching the ATG associated with the long open reading frame. It has been suggested that the existence of prematurely terminated initiation codons may serve to regulate translation of the genuine open reading frame through competition for ribosomes (Kozak 1986).

High-sequence similarity to the much larger β-heavy chains could alternatively be explained if the transcript represented the C-terminal region of a dynein heavy chain gene that is being transcribed from a cryptic promoter present within an intron. In this case, one would expect to find colinearity at the genomic DNA level between the region of homology to the dynein β-heavy chain sequence and the non-homologous 5′ end of the transcript. PCR analysis from genomic DNA and RT-PCR of testis RNA with primers R1 and R5 has shown the presence of an intron in the 5′ end of the gene at the point where the homology to the dynein β heavy chain ceases. This makes it unlikely that the transcript is initiated from a cyptic promoter within an intron of a β-heavy chain gene. If this intron is also present in β heavy chains, then it would be expected that the most 5′ exon of the transcript should reestablish sequence homology. This is not the case. The consensus splice donor and splice acceptor sites at the beginning and the end of the intron imply a functional splice site at this position and, together with the evidence from Southern analysis of introns in the region covered by the original EST, argue against an origin mediated by retrotransposition. It remains possible that DNEL1 represents a truncated β-heavy chain. However, the truncated gene would have to have become associated with a testis-specific promoter, and if the gene fragment is nonfunctional it is difficult to account for the absence of stop codon and frameshift mutations in the long open reading frame that are usually the hallmarks of pseudogenes.

DNEL1 aligns to the C-terminal region of the sea urchin axonemal dynein β-heavy chain (Ogawa 1991) from residue 3669 and, therefore, does not contain any of the consensus nucleotide binding sites located more N-terminal in β-heavy chain (Walker et al. 1982). It is possible that this dynein related sequence reflects a primordial gene that gave rise to present-day heavy chains through the acquisition of an ATP-binding motif (possibly by exon shuffling) from another gene. Subsequent duplication and diver-

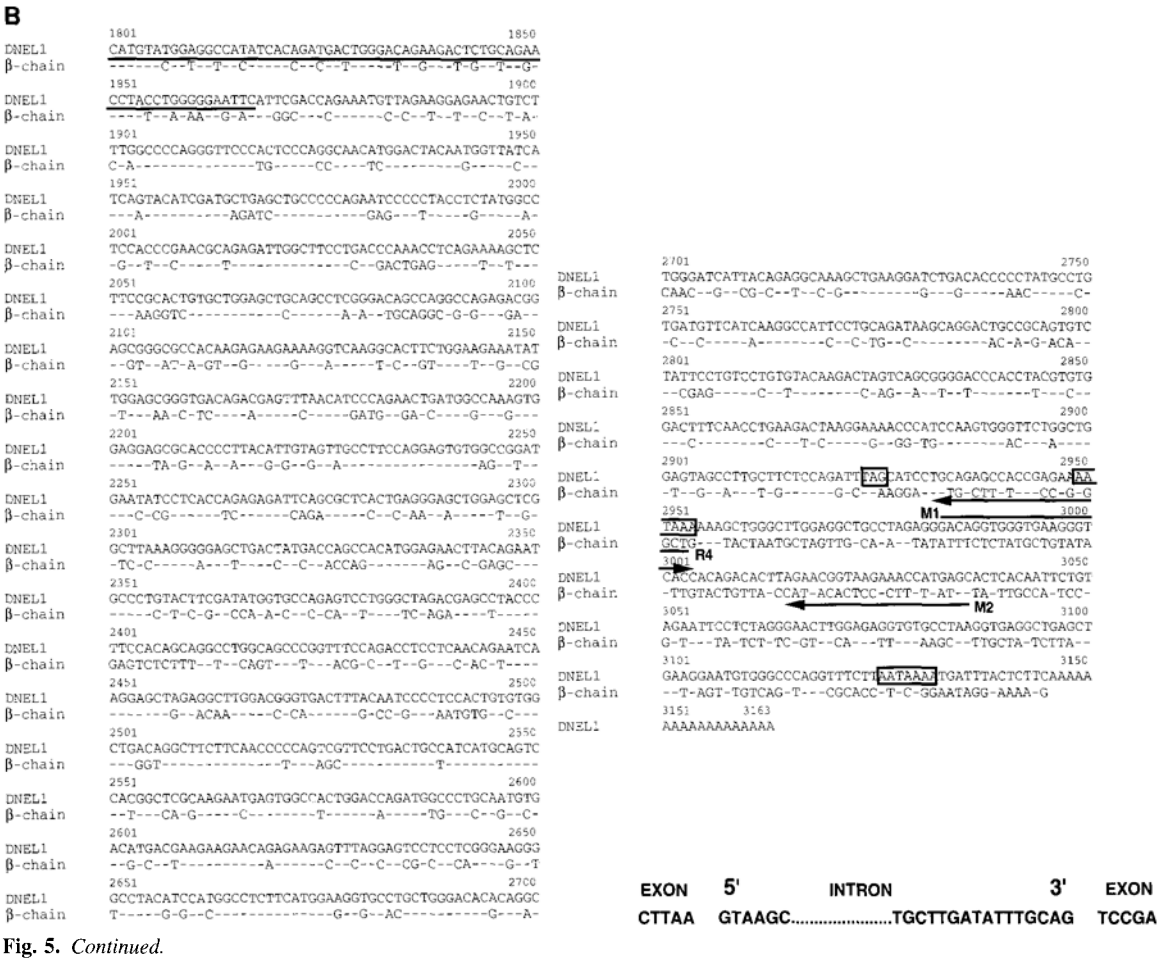

Fig. 5. *Continued.*

gence of the ATP binding domain could then give rise to the current β-heavy chains which contain multiple related but not identical ATP binding motifs. Cross-hybridization of DNEL1 to pig (not shown), mouse, and hamster genomic DNA (shown by hybridization to the control mouse and hamster DNA of the somatic cell hybrid panel; Fig. 2) suggests that the gene is conserved at least across these species.

The testis-specific expression of DNEL1 may reflect a specialized function in this tissue. One possibility is a role in flagellar function of the sperm. It is interesting to note that in *Drosophila melanogaster,* dynein heavy chain-related sequences have been mapped to a fertility locus (Gepner and Hays 1993) which when deleted leads to loss of the outer dynein arms in sperm flagella (Hardy et al. 1981). In humans, defects in dynein arms have been detected by electron microscopy in Kartagener syndrome patients characterized by the triad of chronic respiratory tract infections, sinusitus, situs inversus and immotile sperm leading to infertility (Afzelius and Mossberg 1989). In 50% of cases, situs inversus can also be present. One would, therefore, expect the gene(s) involved in this syndrome to be expressed quite widely. Narayan and colleagues (1994) have suggested a chromosomal location of 14qter for this syndrome based on the mapping of a locus that causes situs inversus in 50% of iv/iv mice to a syntenic region of Chr 12 (Brueckner et al. 1989). However, in contrast with Kartagener individuals, the respiratory epithelium, sperm, and cilia are structurally normal in the iv mouse (Handel and Kennedy 1984) and therefore it is not a very good model for the disease. Furthermore, the dynein heavy chain mapped by Narayan and coworkers (1994) is cytoplasmic and is unlikely to be involved in flagellar and ciliary defects. Studies in patients indicate that there is very considerable phenotypic heterogeneity in Kartagener syndrome as, for example, structurally normal respiratory cilia may be found in otherwise

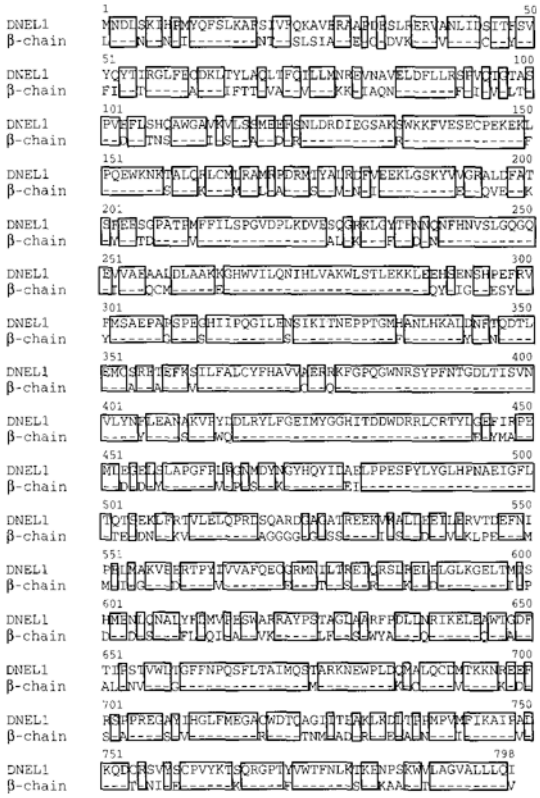

Fig. 6. Alignment of the deduced amino acid sequence of DNEL1 against the relevant part of a β-heavy chain from a sea urchin axonemal dynein (Ogawa 1991). Identities are indicated by a dash and are boxed.

typical Kartagener syndrome patients (Schidlow and Katz 1983). It is probable that the phenotypic heterogeneity reflects the involvement of several genes that are important in ciliary and flagellar function, including different members of the dynein gene family. Specificity of expression in one cell and tissue type could explain the presence of only some of the features of the syndrome in certain patients and thus does not necessarily exclude DNEL1 from being a candidate for aspects of the Kartagener phenotype affecting sperm function. The fine mapping of DNEL1 in relation to already mapped markers in distal Chr 17 can now be exploited to test for linkage in families segregating Kartagener syndrome by use of closely linked microsatellite markers.

Involvement in flagellar movement would imply that the DNEL1 protein is localized to the sperm tail. The absence of ATP-binding sites in DNEL1 would suggest that any role in flagellum movement is not due to a motor function, but rather a structural contribution to the dynein complexes found in axonemes. However, it is also possible that DNEL1 may have a function in the morphological changes of spermatids during spermatogenesis. As spermatogenesis proceeds, the spherical nucleus alters shape and becomes the sperm head under the influence of a microtubule structure known as a manchette, which is associated with cytoplasmic dyneins (Yoshida et al. 1994). This would suggest a cytoplasmic location for the DNEL1 protein. Studies on antibodies raised against DNEL1 will be required to determine precisely the localization of this protein in the cell and dynein complexes and will help to provide an insight into its function.

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