

REVIEW

Primary Structure and Function of a Dynein Motor Molecule

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INTRODUCTION

Gibbons and Rowe [1] were the first to describe the microtubule-associated motor protein known as dynein, which they found when they isolated an ATPase from *Tetrahymena* cilia. The axonemal dyneins are contained in the outer and inner arms that project from the peripheral doublet microtubules, and they form cross bridges between adjacent doublet microtubules. The ATP-driven cross-bridge cycles generate the sliding between microtubules that gives rise to both flagellar and ciliary movements [2]. The outer and inner arm dyneins are different in terms of their peptide compositions. Both the dyneins are multimeric proteins and are composed of heavy chains with ATPase activity, which are assumed to be motor peptides, and several accessory peptides. The molecular mass of the heavy chains in the dyneins (~500 kDa) is 2.5-fold larger than that of the myosin heavy chain and 4.5-fold larger than that of the kinesin heavy chain, the other well-known motor molecules. Progress in analyzing the structure and function of dynein heavy chains was hindered by the lack of primary sequence information, which was due to the difficulties encountered in attempts to clone a cDNA that encodes such a large peptide. Nevertheless, the sequence was avidly pursued by many groups for quite sometime. Finally, Gibbons *et al.* [3] and Ogawa [4] simultaneously determined the complete amino-acid sequence of a dynein motor molecule: the well-characterized β heavy chain of sea-urchin axonemal dynein. The

present review will be limited to a description of the "brave new world" of the sea-urchin axonemal dynein motor molecule, as revealed by molecular cloning.

THE DYNEIN MOTOR MOLECULE

When demembrated sea-urchin sperm are extracted with high salt, the flagellar beat frequency of extracted sperm is only about half that of control sperm that have not been exposed to high salt [5]. Examination by electron microscopy revealed that extraction with high salt removes most of the outer arms from the doublet microtubules, leaving the inner arms apparently intact. The outer arms can be purified as ATPase-containing particles with an *S* value of 21 (referred to as the outer-arm dynein or 21S dynein) by centrifugation through a sucrose density gradient. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) resolved the outer-arm dynein into at least nine different peptides: α and β heavy chains (DaHC and D β HC); three intermediate chains (IC1-3); and at least four light chains (LCs) [6]. Exposure to a low-salt medium converts 21S dynein into three smaller fractions: one containing the D β HC/IC1 complex; one containing aggregates of DaHC; and one containing IC2 and IC3 [7].

Sale *et al.* [8] were able to examine isolated outer-arm dynein by the quick-freeze, deep-etch technique. Replicas revealed that the 21S particles were composed of two globular heads jointed by two irregularly shaped stems that made contact along their length. One head was pear-shaped and the other was spherical. The stems were decorated

with a complex of bead-like particles. The $D\beta$ HC/IC1 complex, obtained as described above, contained only single-headed molecules with single stems. These heads were predominantly pear-shaped. Sale *et al.* concluded that each head is formed by a heavy chain, that the pear-shaped head contains the $D\beta$ HC, and that the spherical head contains the $D\alpha$ HC. Three intermediate chains might decorate the stem that is joined to each head. The position *in situ* of LCs in the outer arm has not been described. Sale *et al.* also observed *in situ* the outer-arm dynein of demembrated sperm. When frozen in reactivation buffer in the absence of ATP, each arm consists of a large globular head that is attached to the A-subfibers of doublet microtubules *via* distally skewed subunits and is attached to the B-subfibers by a slender stalk. In the presence of ATP, this head shifts its orientation such that it can be seen to be constructed from two globular domains. One interpretation of these observations is that these structural changes represent distinct states of a cyclic cross-bridge cycle. The subfractionated samples of the outer-arm dynein were assessed by a translocation assay *in vitro*, in which putative motor protein was allowed to adsorb to a glass coverslip, and microtubules were then applied together with ATP. The "gliding" movement of microtubules under such conditions can be examined by video-enhanced contrast-differential interference contrast (VEC-DIC) microscopy [9]. This system was originally introduced to monitor the activity of microtubule-associated motor protein in cytosolic extracts of squid giant axons, with the resultant discovery of kinesin [10, 11]. The motor proteins, when properly oriented on a

coverslip, can interact with a microtubule in such a way that they generate force along it, causing the microtubule to glide along the glass surface. Motors that are not properly oriented, rather than retarding the microtubule, seem unable to interact with it and have no apparent effect on the net production of force. Sale and Fox [12] observed that microtubules also glide on coverslips coated with just the $D\beta$ HC/IC1 fraction. Neither the $D\alpha$ HC nor IC2/IC3 fractions were associated with gliding of microtubules.

PROTEOLYTIC AND PHOTOLYTIC ANALYSES

The functional substructure (site of hydrolysis of ATP within the molecule) of $D\beta$ HC was revealed by a classical approach rather than by molecular cloning. Ogawa [13] first obtained a tryptic fragment with ATPase activity from a low-salt extract of dynein and named it fragment A. Fragment A is a molecule of about 360–400 kDa in its native form and it can be separated into two peptides, designated f2 (190 kDa) and f3 (135 kDa), by SDS-PAGE [14]. Since f2 and f3 remain associated with each other during native PAGE, it is possible that the corresponding two regions of $D\beta$ HC could be folded back on each other *via* intramolecular interactions. Ow *et al.* [15] established the principal pathway for tryptic cleavage of $D\beta$ HC in a low-salt buffer, as shown in Figure 1. They isolated fragment B (also known as f1 peptide, 130 kDa) which is detached from fragment A during digestion. $D\alpha$ HC did not generate a stable tryptic fragment. This result suggests that the two heavy chains that make up the outer arms are structurally

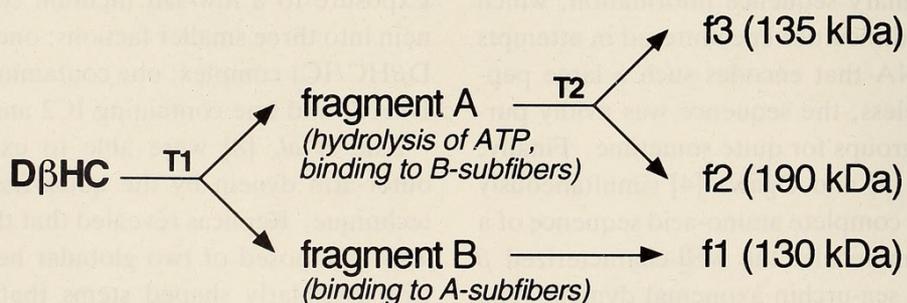


FIG. 1. Principal pathway of tryptic cleavage of $D\beta$ HC. T1 indicates the early cleavage and T2 indicates the subsequent cleavage.

different from one another.

The outer-arm dynein has two microtubule-binding sites. The ability of isolated dynein to rebind to the extracted axonemes was reported by Ogawa and Mohri [16]. Functional recombination of isolated outer arms revealed that the outer arms bind to the A-subfibers in a salt-dependent manner [17]. This type of binding ability of the outer arms was not associated with fragment A [13]. Fragment B may contain the binding site for A-subfibers [15]. However, the ability of fragment B to rebind to the extracted axonemes has not yet been demonstrated. The outer arms can associate with the adjacent B-subfibers in an ATP-dependent manner, as described above [8]. Since fragment A has ATPase activity and is slightly activated by the B-subfiber fraction [13], outer-arm dynein may interact with the B-subfibers through the fragment A moiety of $D\beta HC$ in a ATP-dependent manner.

Irradiation at 365 nm of $D\beta HC$ in the presence of Mg-ATP and a low concentration of vanadate (V_i) cleaved $D\beta HC$ at a single site termed the V1 site and ATPase activity decayed in a biphasic manner [18]. Because vanadate can potentially suppress the activity of dynein ATPase, probably *via* occupation of a site that is normally reserved for the γ -phosphate of ATP, the V1 site probably lies in the hydrolytic domain of the $D\beta HC$. Irradiation in the presence of Mn^{2+} ions and of a higher concentration of V_i resulted in cleavage of $D\beta HC$

at a single site, designated V2, but this cleavage at the V2 site was not correlated with any direct effect on ATPase activity [19]. The peptides produced by sequential cleavage at the V2 site and then the V1 site indicated that the two sites are separated by a region of 100 kDa along the length of the $D\beta HC$. The ATP-hydrolysis pocket of the central domain might be composed of the γ - P_i -binding V1 site and the purine-binding V2 site. The $D\beta HC$ can be covalently modified by reaction with the hydrolyzable photoaffinity analog of ATP, 8-azido adenosine 5'-triphosphate (8- N_3 ATP), which is hydrolyzed by fragment A at about 10% of the rate of hydrolysis of ATP [15]. The V2 site was found to be close to the locus of attachment of 8- N_3 ATP, which may correspond to the purine-binding region of the ATP-hydrolytic site on the $D\beta HC$. Mocz *et al.* [20] proposed a map of the sites of tryptic and photolytic cleavage on the $D\beta HC$, as shown in Figure 2.

HUNTING FOR A GENUINE CLONE

A much more direct approach to the analysis of the functional site is provided by the molecular cloning of the gene for $D\beta HC$. Garber *et al.* [22] claimed initially that they had isolated cDNAs for the dynein heavy chain from trout testis that predicted an extensive, carboxy-terminal, α -helical coiled-coil domain. Because of incomplete characterization, it is unknown which of the several

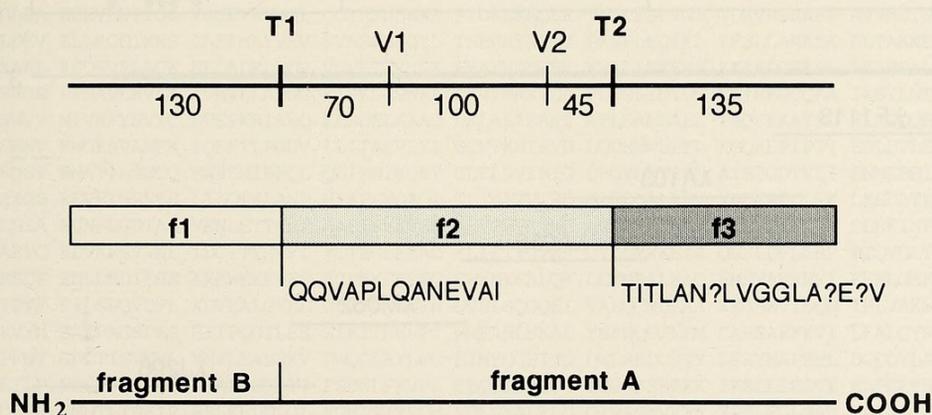


FIG. 2. Tryptic (T) and photolytic (V) sites within $D\beta HC$. The original map proposed by Mocz *et al.* [20] has been revised [21]. Numbers below the top map represent molecular masses in kDa, as determined by SDS-PAGE. The second map shows the positions of three tryptic fragments in the molecule. The amino-acid sequences of the f2 and f3 peptides are also shown. The bottom map shows that fragment A is located on the carboxy-terminal side of the molecule adjacent to fragment B.

heavy chains of trout dynein [23–25] these clones might encode. Mitchell [26] isolated genomic clones of α and β heavy chains from *Chlamydomonas*. However, none of these clones have yet been sequenced. Foltz and Asai [27] characterized a cDNA that encodes a portion of sea-urchin ciliary D β HC. Although four independent criteria suggest that their clone encodes a portion of D β HC, their identification of immunoreactive clones from expression libraries cannot be taken as proof that the cDNA clone of interest has been isolated.

Hisanaga *et al.* [28] isolated “cytoplasmic dynein” with a molecular mass and immunogenicity similar to those of axonemal D β HC from unfertilized eggs of the sea urchin. The substructure [29] was also indistinguishable from that of the axonemal D β HC [8]. Ogawa *et al.* [30] showed that D β HCs from sperm and egg cilia may be similar to one another. There is no evidence to suggest that sea-urchin “cytoplasmic dynein” is different from ciliary or sperm D β HC. Recently, Ogawa [31] screened a cDNA library that corresponded to the poly(A)⁺ RNA of unfertilized eggs using an antibody directed against sperm axonemal dynein heavy chains. The cDNA clones (λ J292, λ J296, λ A101, λ A102, λ A103, and λ A104) obtained may encode ciliary D β HC. Fingerprints of fusion protein produced by lysogenic λ J296 were similar to those of authentic 21S dynein from sperm. The Northern blot of poly(A)⁺ RNA revealed that

only two clones (λ J296 and λ A103) could hybridize with an RNA of ~16 kb in length. Since D β HC has an estimated mass of 480 kDa, it could be encoded by poly(A)⁺ RNA of at least 14 kb in length. Thus, the two clones appear to be strong candidates. Finally, the amino-acid sequence deduced from the nucleotide sequence of λ A103 contains one of the ATP-binding motifs (GKT site, see below) and the amino-terminal sequence of the f2 peptide [21]. Thus, these two clones appear to be the first genuine partial clones of cDNA that encodes D β HC.

CONSTRUCTION OF FULL-SIZE COMPLEMENTARY DNA

The λ J296 and λ A103 clones encode the carboxy-terminal and central regions of D β HC, respectively. The missing segments of cDNA can be isolated by making mini cDNA libraries primed with oligonucleotides that are complementary to the 5'-portion of these clones, with subsequent screening with radiolabelled DNA probes. Ogawa [4] has sequenced additional three clones (λ F1113, λ A055, and λ M062). Full-size cDNA was constructed by the overlapping of five clones, as shown in Figure 3. The long reading frame can encode a protein of 4,466 amino-acid residues with an unmodified molecular mass of 512 kDa. The deduced complete amino-acid sequence of D β HC

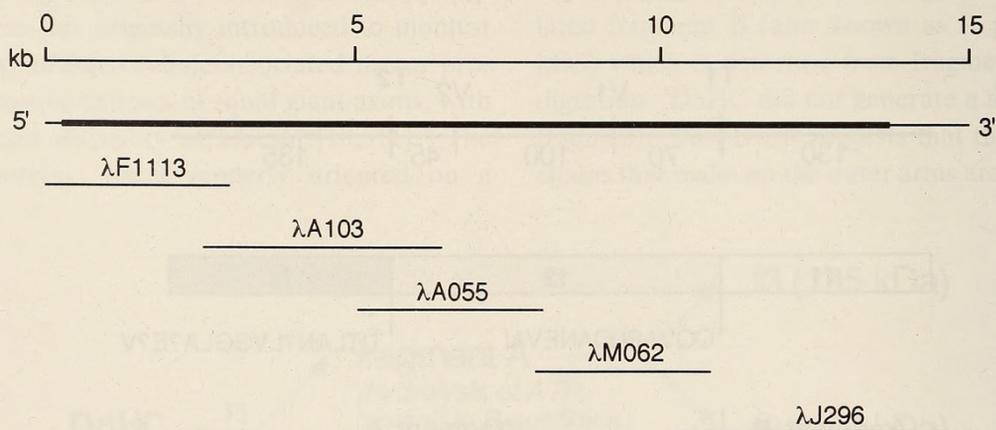


FIG. 3. Five overlapping clones that encode D β HC. The long open reading frame (thick line), flanked by non-coding sequences (thin lines), is shown at the top. Because of multiple allelic variation, the nucleotide sequences of two clones in the overlapping region are different from one another with frequency of one altered base per 100 bases. For overlapping of clones, weight was given to the nucleotide sequences of λ J296 in the case of λ J296 and λ M062, λ M062 in the case of λ M062 and λ A055, λ A103 in the case of λ A055 and λ A103, and λ A103 in the case of λ A103 and λ F1113.

is shown in Figure 4. The sequence was confirmed by the finding of the two amino-terminal sequences of the f2 and f3 peptides of fragment A in the deduced sequence. The former sequence was found at amino-acid residues, 1,192-1,204 and the latter at residues 3,324-3,340. The entire sequence shows no significant similarity to the partial sequences reported by Garber *et al.* [22] for the

trout and by Foltz and Asai [27] for the sea urchin.

ATP-BINDING SITES

Fragment A corresponds to amino-acid residues 1,192-4,466. In view of the ability of fragment A to hydrolyze ATP, a search was made in this region for the consensus ATP-binding motif

1	MGDVVDARLD	FISEYILKSY	KLKPKDKWTKC	INVEENKILM	LEFLEKADNP	QLVFTVNPAG	LITPSYEFPS	ALKNTKAIYF	IKKGREPVGK
91	DNIKTNLVYG	DLSTYTPLEQL	SALVDEVLP	LLANPRNHEQ	WPVVVSQDVL	RHVHNLKSSV	YVVAGQVKGK	TLPLPLVGS	KVETAGSSE
181	KDDSYDRSLV	HAIESVIIDW	THQIRDLVKR	DSAPLLEGL	NPQPMVEINF	WKAKCENLDC	IFQQLRDPKV	RKMKELLERT	QSSYLPSFNN
271	IERDVEAALT	EAQDINIHLK	PLVYQIESMD	ELEFSDLTPT	LAPILHTVCL	IWSNSDYNT	APRVIVLLQE	ICNLLIDLRC	TFLDPSEIFK
361	LEPEESLEKV	RGALTVLKNW	RELYDEHRAK	LKDYFKDGKE	VKEWEPASPL	VFTRMDNFIR	RIETIQSLFE	TNVEFSKLEK	TEMGSMKGRM
451	LSQQVEKIHE	EFQECAKVFT	ERPVDGLDPT	CQEFLEDYEE	FEKKVFDLDR	RLGSILCQGF	DDCCGLEAAF	KMLDCYGPLL	DRPVIRNDFE
541	CKYPIVLMY	DQELDQSKEI	YDEHMRVEEA	NGNAPLNKMN	PDVAGQLKWS	AQLRDRIKSP	MGSLKMHMEP	TGVRRIESE	DAKVIQKYE
631	EMLNLLNKYE	RKTVFNWTKG	VDEVCKTNLD	QSLITRDDAS	KLIMVNFDPK	LVSVLREVKY	LQIRGEETIP	ESAASIEYEH	ETLRKYVANL
721	DLTVAWYNKV	QVTVLEVEFP	LIEGQLADLD	TLRLQAEADL	NWTSDSVWEY	IQETRDQVRD	LEKRVQQTKD	NVDRIKKIMA	EWTKQPLFER
811	KELKESLLA	LDDRQDRLKK	RYAETTTAGE	KIHSLMKENL	DLFKAESSD	IWKAYVDYVD	DMVIDGFNFC	IHCTLTLYLE	NTDPRHCAAP
901	LPEARLELQV	PDMIFNPSLD	YGIADGFYDL	VEMLISDTYK	MASLVNRLAE	HNGQEHYQAD	LEGMDDLSDV	RNDLMDRVQT	IMTKAQEYRN
991	SPDNYAYLYV	DDRKEFMRQF	LLYNHVLTPTE	EIEAHAEDGV	PECPTLDQF	KEQVDTYEKI	YSEADEIEPE	QVFDWFRVD	SKPFKAALLN
1081	IHKKWSFMFK	QHLIDHVTNS	LSELQEFIKV	GNSGLTKTVE	DGDYNGLVDC	MGHLMVAVKER	QAATDEMFEF	IKQTIELKLT	YDQEMSEEVH
1171	TQLQELPEQV	NNTKKAIAIT	<u>KQOVAPLQAN</u>	<u>EVAIIRRKCT</u>	SPDVRQHEFR	ERFRKEAPFI	FLFDGPIYQL	DKCHSEIYEM	EEHMAKLES
1261	AGLFEVNMMPD	YKQLKACRRE	<u>VRLKGLWDL</u>	<u>IMVVRTSIED</u>	WKTTPWLEIN	VEQMEMDCCK	FAKDIRSLDK	EMRAWDYNG	LDAITVKNMLT
1351	SLRAVSELQN	PAIRERHWQQ	LMAATKVKFT	MDKETLSDL	LALNLHNFED	EVNRIVDKAV	KEMGMEKVLK	ELNITWSSMD	FDYEPHSRTG
1441	ISLLKSNEEL	IETLEDNQVQ	LQNLMTSKHI	AHFLEEVSGW	QKKLSTTDSV	ITIWFEVQRT	WSHLESIFIG	SEDIRNQLPE	DSKRFDGDIT
1531	DFKELAAEME	KTPNVVEATN	KARLFDRLEA	IQGSIVVCEK	ALAEYLETKR	LAFPRFYFVS	SADLLDILSQ	GNNPTQVQRH	LSKLFDNMAK
1621	LKFKQDDEGN	DTKLALGMYG	KEGEYVDFDK	ECECTGQVEV	WLNVRVMDTMR	STVRSQFADA	VVSYEEKPRE	QWLYDYPAQV	ALATTQVWWT
1711	TEVNISFARL	EEGHENSMKD	YNKKQILQLN	TLIGLLIGLK	TKGDRQKIMT	ICTPIDVHARD	VVAMMVLLVK	DSAQAFQWLS	QLRHRWADDD
1801	KHCYANICDA	QFKYSYEYLG	NTPRLVITPL	TDRCYITLTQ	SLHLVMSGAP	AGPAGTGKTE	TTKDLGRALG	IMVYVFNCS	QMDYKSCGNI
1891	YKGLAQGTAW	GCFDEFNRIS	VEVLSVAVQ	VKCVQDAIRD	KKERFNFMG	EISLIPSGVI	FITMNPYAG	RTELPELNKA	LFRPCAMVVP
1981	DFELICEIML	VAEGFLEARL	LARKFITLYT	LCKELLSKQD	HYDWGLRAIK	SVLVVAGSLK	RGDFQRPEQD	VLMRALRDFN	VPKIVSDDTTP
2071	VFMGLIGDLF	PALDVPRRRD	LDFEKVVQVS	TLDLKLQAE	SFVLKVVQLE	ELLAVRHSVF	VIGNAGTGKS	QVLKVLNKTY	SNMKRKPVFI
2161	DLNPKAVTND	ELFGIINPAT	REWKDGLFSV	IMRDSMNIETH	DGPKWIVLDG	DIDPMWIESL	NTVMDDNKVL	TLASNERIPL	TPSMRLLFEI
2251	SHLKTATPAT	VSRAGILYIN	PSDLGWNPIV	TSWIDTREVQ	SERANLTIIF	DKYLPPLLDT	LRIRFKKIIP	IPEQSMVQML	CYLLLECLLTP
2341	ENTPADCPKE	LYELYFVFAS	IWAFGGSMFQ	DQLVDYRVEF	SKWITEFKT	IKFPNQGTVF	DYYIDQESK	FLWSEKVP	FELDPEIPMQ
2431	AVLVHTNETT	RVRPFMDLLM	ERGRPVMLVG	NAGLQKSLV	GDKLSNLGED	SMVANVPFNY	YTTSEMLQRV	LEKPLEKKAG	RNYGPPGTTK
2521	LVYFIDDMNM	PEVDTYGTVQ	PHTLIRQHMD	YKHWDRAKL	TLKEIHKCQY	VSCMNPSTGS	FTINSRLQRH	FCVFALSPFG	QDALSTIYNS
2611	ILSQHLANIA	VSNALQKLS	TVVSATLDLH	KKVAQSFLPT	AIKFHYVFNL	RDLSNVFQGL	LYSGSDLLKS	PIDFARLWMH	ECQRYVGDKM
2701	INDQDIEAF	KLVPFYAKKF	FEDVDEEALK	AKPNIHCHFA	TGIDGPKYMP	CATWPELNKI	LVEALDTYNE	INAVMNLVLF	EDAMQHVCR
2791	NRILESPRGN	ALLVGVGGSG	KQSLARLASY	ISSLEVFQIT	LRKGYGIPDL	KLDLATVCMK	AGLNKIGTVP	LMTDAQVSD	KFLVLINDLL
2881	ASGEIPDLFA	DVEVENIIGG	VRNEVKMGML	QDTRENCWKF	FIDRLRRQLK	TVLCFSPVGT	TLRVRSRKFP	AVVNCTSIDW	FHEWPQEALV
2971	SVSKRFLDEV	ELKGDIKNS	IAEFMAYVHV	SVNESSKQYL	TNERRNYT	PKSFLEQIKL	YESLLAMKSK	ELTAKMERLE	NGLTKLQSTA
3061	QQVDDLKAKL	ASQVEVLAQK	NEDADKLIVQ	VGVEVEKVS	EKATVDDEEK	KVAIINEEVS	KKAKDCSEDL	AKAEPALLAA	QEAINTLNKN
3151	NLTELKSFSG	PPSAVLKVA	AVMVLAPNG	KIPKDRSWKA	AKVVMNKVDA	FLDSLINYDE	ENIHENCQKA	IKEYLNDPEF	EPEYIKGKSL
3241	AAGGLCSWVV	NIVKFNVYCV	DVEPKRIALQ	KANDELKAAQ	DKLALIKAKI	AELDANLAE	TAQFEKATSD	KLKQEQEAEA	TSRTITLANR
3331	<u>LVGGLASENV</u>	<u>RWGEAVANFK</u>	<u>IQEKTLPGDV</u>	<u>LLITAFVSYI</u>	<u>GCFTKNYRVD</u>	<u>LQDRMWLPFL</u>	<u>KSQKDPITPIT</u>	<u>EGLDVLSTML</u>	<u>DDADIAVWNN</u>
3421	EGLPSDRMST	ENATILSNQC	RWPLMIDPQL	QGIIKWKQKY	GDELRVIRIG	QRGYLDTIEN	AISSGDTVLI	ENMEESIDPV	LDPVLRNNTI
3511	KKGRYIKIGD	KEVEYNPEFR	LILQTKLANP	HYKPEMQAQT	TLINFTVTRD	GLEDDQLLANV	VAQERPDEK	LKSDLTQKQN	DFKILLKELE
3601	DNLLSRLSSA	EGNFLGDTAL	VENLETTKRT	AAEISVKVEE	AKVTEVKINE	ARELYRPA	RASLLYFILN	DLNKINPIYQ	FSLKAFNTVF
3691	SLSIARAEP	EDVKERVNL	IDCITYSVFI	YTRRLGFEAD	KLIFTTQVAF	QVLLMKKEIA	QNELDFLRRF	PIQVGLTSPV	DFLTNSAWGA
3781	IKSLSAMEDF	RNLDRDIEGS	AKRWKFFVES	ECPEKEKFPQ	EWKNKSAALQK	LMMRALRAD	RMSYAVRNFI	EELKGSKYVE	GRQVEFAKSY
3871	EETDPATPVF	FILSPGVDP	KDVEALGKKL	GFTFDNNTNFH	NVSLGGQVEI	VAEQCMDLAA	KEGHVWILQN	IHLVAKWLST	LEKKLEQYSI
3961	GSHEYSRYVM	SAEPAGSPES	HIIPQGILES	SIKITNEPPT	GMFANLHKAL	YNFNQDTLEM	CAREAEFKVI	LFALCYFHAV	VGERQKFGPQ
4051	GWNRSYFNT	GDLTISVNVL	YNLEANSKV	PWQDLRYLFG	EIMYGGHITD	DWDRRLCRTY	LEEYMAPEML	DGDLYLAPGF	PVPPNSDYKG
4141	YHQYIDEILP	PESPYLYGLH	PNAEIGFLTT	ESDNLFKVVV	ELQPRDAGG	GGGSSSREEK	IKSLDDEIVE	KLPEEFNME	IMGKVEDRTP
4231	YVVVAFQCE	RMNTLTSEIR	RSLKELDLGL	KGELTTPDM	EDLSNALFLD	QIPASWVKRA	YPSLFGLSAW	YADLLQRIKE	LEQWADFAL
4321	PNVVWLGFFF	NPQSFLTAIM	QSMARKNEWP	LDKMCLQCVD	TKKNKEDFSS	APREGSVVHG	LFMEGARWDT	QTNMIADARL	KELAPNMPVI
4411	FIKAIIPVDKQ	DTRNIYECVP	YKTKQRGPTF	VWTFNLKSKE	KAAKWTLAVG	ALLLQV			

FIG. 4. Deduced amino-acid sequence of sea-urchin *Anthocidaris crassispina* axonemal D β HC. The amino-acid residues that have been confirmed by direct sequencing are underlined and putative-ATP-binding motifs are indicated by filled circles. Amino acids marked with filled squares define the sites of trypsin cleavage.

GXXXXGK(T/S), where X is any amino acid. This motif was found at three positions in the f2 peptide as follows, between residues 1,192 to 3,323: beginning at Gly 1,852 (termed the GKT site); at Gly 2,133 (the GKS1 site); and at Gly 2,460 (the GKS2 site). The sequence GXXXXSGK is also accepted as an ATP-binding sequence of adenylate kinases [32], and this sequence was also found in the f2 region, beginning at Gly 2,805 (the SGK site). Therefore, molecular cloning of D β HC has revealed the presence of four putative ATP-binding sites in the middle region of the molecule.

Since the amino-terminal sequence of the f2 peptide begins at residue 1,192, the T1 site can be identified on the carboxy side of Lys 1,191. According to the map of D β HC (Fig. 2), the γ -P_i-binding site (V1 site) is separated by a region of 70 kDa from the T1 site in the carboxy-terminal direction. There are 660 amino-acid residues between Gln 1,192 of the T1 site and Gly 1,852 of the GKT site, and this distance is equivalent to a peptide of 73 kDa. Thus, the GKT site corresponds to the V1 site revealed by photocleavage of D β HC and may be able to catalyze the hydrolysis of ATP. The binding site for 8-N₃ATP (V2 site) is separated by a region of 170 kDa from the T1 site in the carboxy-terminal direction. There are 1,603 amino-acid residues between Gln 1,192 of the T1 site and Gly 2,805 of the SGK site, and this distance is equivalent to a peptide of 177 kDa. Thus, the SGK site corresponds to the V2 site. Since fragment A can hydrolyze 8-N₃ATP at about 10% of the rate of hydrolysis of ATP, the SGK site may also be able to catalyze the hydrolysis of ATP. The presence of two GKS sites was not predicted by the photocleavage of D β HC. The sites have sequences that are very similar to one another. The ATP-dependent *ClpA* protease of *E. coli* has also two ATP-binding motifs which are very similar to one another [33]. Thus, the sequence

similarity between the two GKS sites in the molecule may not be a coincidence, but may represent proof of two functional sites for hydrolysis of ATP. Fragment B does not have any ATPase activity [15]. The sequence AXXXXGKT, beginning at Ala 154, appears to be a modified nucleotide-binding motif, as found in the GTPase superfamily [34]. At the present time, however, it is uncertain whether fragment B has the ability to bind GTP and catalyze its hydrolysis.

The position of the ATP-binding motif on a motor molecule may be related to the directionality of movement along a microtubule. Both dynein and kinesin are microtubule-motor proteins and they move in opposite directions along a microtubule. The striking difference between the amino-acid sequences of both motor proteins is reflected in differences between the positions of the ATP-binding motifs on their heavy chains; the motif is located at the amino terminus of the kinesin heavy chain [35] and in the midregion of D β HC. The product of the *claret* (or *ncd*) gene belongs to the kinesin superfamily. It is noteworthy that the ATP-binding motif is located at the midregion of this gene product [36, 37] and the molecule moves toward the microtubule's minus end [38, 39], a direction characteristic of dynein [40].

POLYMORPHISM

The nucleotide sequence shows two types of polymorphism (Fig. 5). When λ F1113 was isolated, 14 additional shorter cDNAs were also obtained. Two clones, λ F1106 and λ F1114, were sequenced and their cDNAs overlapped the sequence of λ F1113. Fifteen bases common to both λ F1106 and λ F1113, which encode five amino acids, were absent in the sequence of λ F1114 (possibly as a result of alternative splicing). Furthermore, the underlined nucleotide sequence in

Residue number	609	610	611	612	613	614	615	616	617
	H	P	T	G	V	R	R	I	L
λ F1113	CAT	CCG	ACC	GGT	GTC	AGG	AGA	ATT	TTG
λ F1106	CAT	CCG	ACC	GGT	GTC	AGG	AGG	ATT	TTG
λ F1114	CAT	CCG	---	---	---	---	---	ATT	TTG

Fig. 5. Polymorphism of cDNA clones that encode D β HC.

Figure 5 differed between λ F1106 and λ F1113 (possibly as a result of multiple alleles). The latter type of polymorphism occurs at a rate of about one base per 100 bases in the two overlapping clones but, so far, no substitutions of amino acids have been found. Since the full-size cDNA was constructed by overlapping of the present five clones, which include λ F1113, the amino-acid sequence of D β HC described here is just one possible sequence, and slightly longer and shorter versions may also be present in the sea urchin.

SECONDARY STRUCTURE

The secondary structure of D β HC was analyzed by Dr. Ken Nishikawa of the Protein Engineering Research Institute, Osaka, Japan (Fig. 6). There are two long α -helix-dominant regions (termed $\alpha 1$ and $\alpha 2$) in the sequence, suggesting that the D β HC is composed of three large β -structure-dominant domains (termed the N, M, and C domains) separated by these regions. The M domain is split by short α -helix-dominant regions into four smaller β -structure-dominant regions, and the C domain is similarly split into three smaller region. Although analysis of secondary structure predicts that the $\alpha 1$ region is rich in α helix, there are no long hydrophobic heptad repeats without interruption, as

are found in the α -helical coiled-coil regions of filamentous motor proteins such as myosin and kinesin. The $\alpha 2$ region contains two heptad repeats, which are predicted to be largely α -helical, at amino-acid residues 3,028–3,153 and 3,234–3,338 with interruptions, as shown in Figure 7. In particular, two leucine heptad repeats at residues 3,028–3,083 and 3,262–3,303 could favour the formation of a leucine zipper structure, with resultant generation of a large globular structure from the M and C domains. This leucine zipper structure may explain why f2 and f3 peptides remained together in fragment A during tryptic digestion of D β HC, while f1 was detached, as described above.

MODEL OF THE STRUCTURE

Figure 8 shows a model of structure of D β HC, as deduced from the predictions about secondary structure and the proteolytic analysis of the authentic protein. Quick-freeze deep-etch electron microscopy of the D β HC/IC1 complex revealed that the complex is composed of a pear-shaped head and an irregularly shaped stem, while the base looks like a small globular bead [8]. According to this structural model, the N domain may correspond to the base, the $\alpha 1$ region to the irregularly shaped stem, and the associated M and

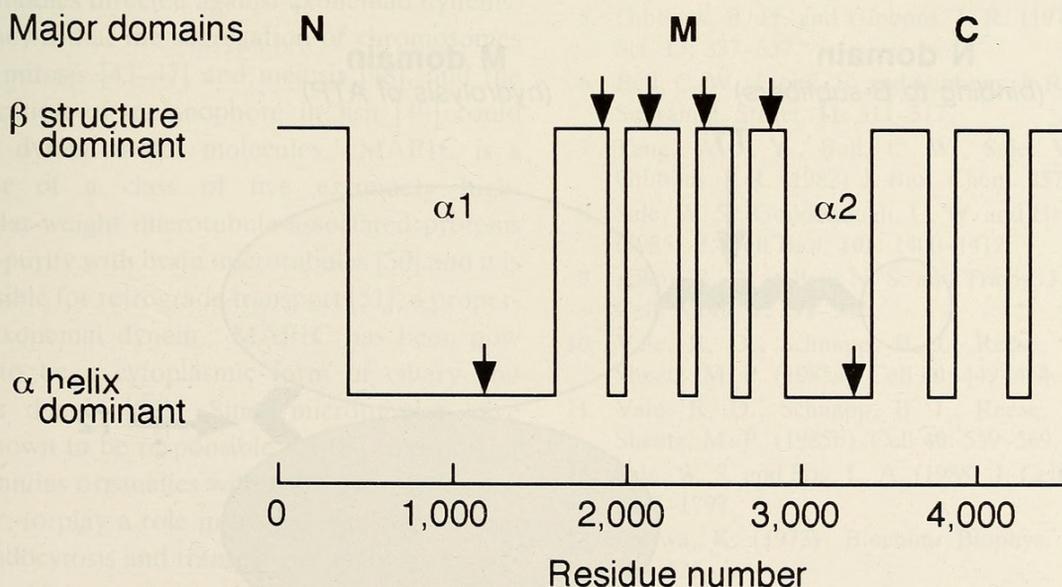


FIG. 6. Predicted secondary structure of the D β HC. The four arrowheads in the M domain indicate the GKT, GKS1, GKS2, and SGK sites of the ATP-binding motifs, from the left. The arrowheads in the $\alpha 1$ and $\alpha 2$ regions indicate the T1 and T2 sites, respectively.

C domains to the pear-shaped head. As described above, it has also been documented that the outer arm *in situ* attaches to the B-subfibers of adjacent outer-doublet microtubules *via* a slender stalk. This slender stalk is seen neither in the isolated outer arms nor in the D β HC/IC1 complex. It is possible that the slender stalk corresponds to the $\alpha 2$ region of the present structural model.

A NEW FAMILY OF MICROTUBULE-BINDING MOTOR PROTEINS

As described above, D β HC appears to have two types of microtubule-binding site in the molecule. It may form a stable complex with the A-subfibers of axonemal doublet microtubules in the fragment B region. MAP2 [41] and tau [42] proteins form stable complexes with microtubules; they have regions of homologous sequence at their carboxy termini, where there are three 18-residue repeats

	<i>d e f g a b c</i>	<i>d e f g a b c</i>	<i>d e f g a b c</i>
3,028	I K L Y E S L	L A M K S K E	L T A K M E R
3,049	L E N G L T K	L Q S T A Q Q	V D D L K A K
3,070	L A S Q E V E	L A Q K N E D	A D K L I Q V
3,091	V G V E T E K	V S K E K A T	V D D E E K K
3,112	V A I I N E E	V S K K A K D	C S E D L A K
3,133	A E P A L L A	A Q E A L N T	L N K N N L T
3,234	Y I K G K S L	A A G G L C S	W V V N I V K
3,255	F Y N V Y C D	V E P K R I A	L Q K A N D E
3,276	L K A A Q D K	L A L I K A K	I A E L D A N
3,297	L A E L T A Q	F E K A T S D	K L K C Q Q E
3,318	A E A T S R T	I T L A N R L	V G G L A S E

FIG. 7. Hydrophobic heptapeptide repeats in the $\alpha 2$ region. The numbers on the left indicate the residue number from the deduced amino-acid sequence of D β HC. There is a disruption of the repeats from Glu 3,154 to Glu3233. Bold letters indicate hydrophobic amino acids.

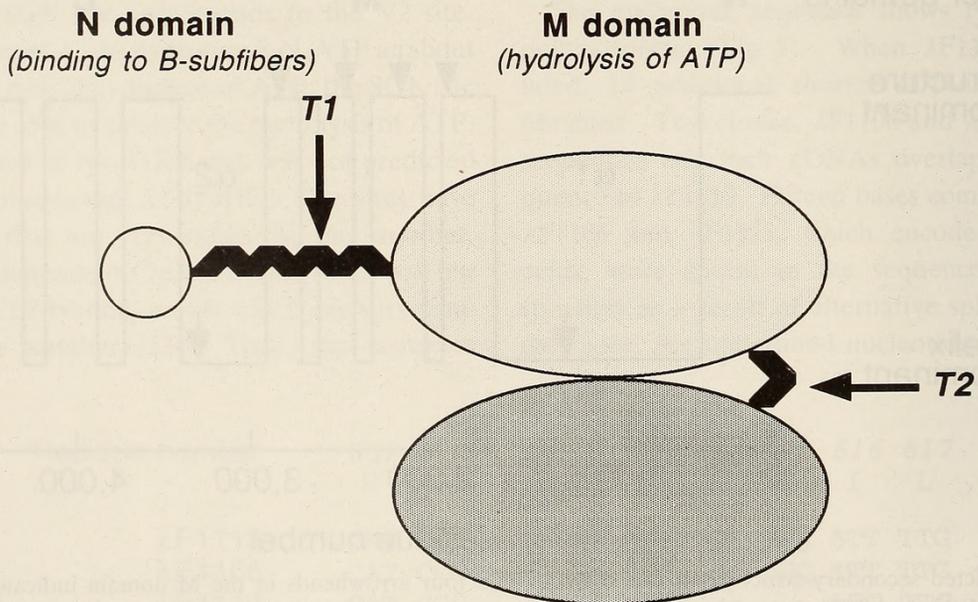


FIG. 8. A model of the structure of D β HC. T1 and T2 indicate the principal sites of trypsin cleavage.

that have been proposed as the microtubule-binding site. These repeated sequences are not found in the N domain of the D β HC sequence. D β HC transiently associates with the B-subfibers of the adjacent doublet microtubules during the ATP-hydrolytic cycle. Members of the kinesin superfamily of microtubule motor proteins also associate transiently with microtubules during the ATP-hydrolytic cycle. They share a region of sequence homology that extends from the ATP-binding site towards the carboxy-terminal end of the molecule, and this sequence has been suggested to constitute the site of ATP-dependent binding to microtubules [35]. This sequence homology is not found in the C domain, which extends from the M domain (multiple ATP-binding sites) toward the carboxy-terminal end of the molecule, or anywhere else in the amino-acid sequence of D β HC. Thus, D β HC seems to be a member of a new family of microtubule-binding motor proteins with unique microtubule-binding sequences that are unlike those of MAP2, tau, or members of the kinesin superfamily.

DYNEIN SUPERFAMILY

It is natural to speculate that dynein may also be involved in motile functions associated with cytoplasmic microtubules. Immunological studies, using antibodies directed against axonemal dyneins, have shown that the segregation of chromosomes during mitosis [43–47] and meiosis [48], and the translocation of melanophore in fish [49] could involve dynein motor molecules. MAP1C is a member of a class of five extremely high-molecular-weight microtubule-associated proteins that co-purify with brain microtubules [50] and it is responsible for retrograde transport [51], a property of axonemal dynein. MAP1C has been now found to be a cytoplasmic form of ciliary and flagellar dynein [52]. Since microtubules have been shown to be responsible for the transport of membranous organelles within the cytoplasm and, thereby, to play a role in axonal transport, secretion, endocytosis and transcytosis, cytoplasmic dynein could have very general functional role in cells. Isolation of a clone for cytoplasmic dynein is now the goal of many groups.

Axonemal and cytoplasmic dyneins may constitute a superfamily of force-generating proteins, with each member possessing a conserved force-generating domain joined to a different "tail" that confers specific attachment properties. The outer- and inner-arm dyneins attach to different sites on axonemal doublet microtubules, while cytoplasmic dyneins interact with organelles and chromosomes. Structural and enzymatic studies suggest that the motor domains of the dyneins are similar to one another [see ref. 53 for a review]. Therefore, it is likely that the members of the dynein superfamily share a common motor domain that is linked to a distinct tail with unique binding properties in each case.

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