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# Kinesin motors and microtubule-based organelle transport in *Dictyostelium discoideum*

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## Abstract

Movement of membrane cargoes and chromosomes is driven by kinesin and dynein motors in most eukaryotic cells. In this review, we describe the known kinesin and dynein genes in *Dictyostelium*. *Dictyostelium* primarily utilizes two conventional kinesins, an Unc104/KIF1 kinesin, and cytoplasmic dynein to transport membrane organelles within its cytoplasm. We describe how the biological functions of these motors has been dissected through a combination of biochemical to genetic approaches.

## Introduction

In eukaryotic cells, the long-range transport of membrane organelles and transport vesicles depends upon the microtubule cytoskeleton along which cargo is conveyed in a directional manner (Hirokawa *et al*., 1998). The transport is mediated by molecular motor proteins that convert energy generated by ATP hydrolysis into movement along microtubules. Two distinct types of microtubule-dependent motors have been characterized: the kinesin family with over 20 members in typical higher eukaryotes, the majority of which move towards the microtubule plus-end, and cytoplasmic dynein, a multisubunit protein complex that moves towards the minus-end. The intracellular functions ascribed to these motors include membrane transport, mitotic spindle morphogenesis, chromosome segregation, and RNA localization.

The kinesin motors share a highly conserved motor domain (~40 kDa) responsible for ATP hydrolysis and microtubule binding which can be either located at the amino-terminus, the carboxy-terminus, or internal within the polypeptide chain. This motor domain is evolutionarily related to the catalytic domains of myosin and G proteins (Vale, 1996). Further diversity among the family members arises from the regions outside the motor domain where protein–protein interactions occur with accessory light chains or other interacting proteins (Goldstein, 2001). The dynein motor domain, on the other hand, is much larger (~400 kDa) and is evolutionarily related to another group of ATPases known as the AAA proteins (Vale, 2000). Several intermediate or light chains adorn the dynein holoenzyme and are thought to be important for cargo recognition and/or regulation (Vallee and Gee, 1998).

*Dictyostelium*, the cellular slime mold, is a simple eukaryote that shares dynamic processes such as intracellular traffic, phagocytosis, and cytoskeletal organization with higher eukaryotes. In this review article, we summarize our knowledge on microtubule-based motors and their implication in membrane trafficking. We first present our studies on *in vitro* transport assay developed in *Dictyostelium*, which in our view is one of the most attractive advantages of this organism. We will then discuss how the combination of *in vitro* transport assays, biochemistry, molecular cloning, and the *Dictyostelium* genome project have provided insight into the family of molecular motors in *Dictyostelium*.

## Microtubule-based transport in *Dictyostelium* and *in vitro* assays

Although relatively few laboratories have chosen to work on microtubule motors in *Dictyostelium*, the organism offers several unique advantages for studying this problem. As discussed in many articles in this issue, *Dictyostelium* is a relatively simple unicellular organism that is amenable to both gene replacement and large scale biochemistry. While the genetic tools are modest compared to budding yeast, *Dictyostelium* contains a microtubule array and transport system that is much more akin to that of higher eukaryotes compared to *S. cerevisiae*. In budding yeast, the microtubule network is used almost exclusively for mitosis and spindle positioning, and the six kinesins and cytoplasmic dynein appear to be dedicated to these processes. Myosin motors, on the other hand, are used for intracellular transport of membrane vesicles and mRNAs. In *Dictyostelium*, the microtubules are organized as a radial array emanating from a single microtubule-organizing center (Roos *et al*., 1984), which is very similar to the pattern found in most higher eukaryotic cells. Besides the morphological similarities, *Dictyostelium* also uses γ-tubulin and conserved centrosomal factors to organize the microtubule array (Gräf *et al*., 2000). By light microscopy, numerous vesicles can be observed moving on these microtubule tracks, as motion halts after addition of nocodazole (Roos, 1987). Moreover,

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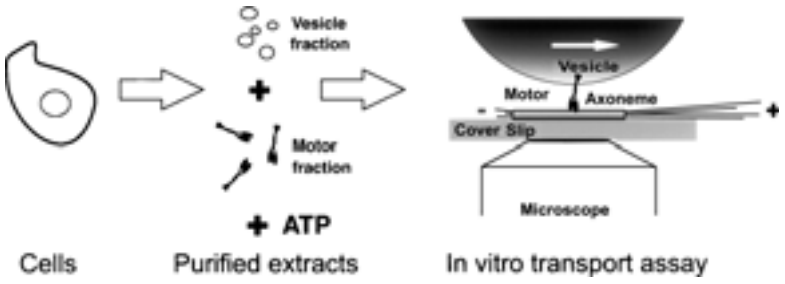


*Fig. 1.* Flow diagram of an *in vitro* membrane motility assay: The diagram demonstrates how motor proteins are assayed *in vitro* for cargo transport properties. Motor and membrane extracts are prepared from axenically grown *Dictyostelium* cells as described (Pollock *et al.*, 1998). Vesicles, motors, and an ATP regenerating system are combined, and the movement of membranes along axonemes and microtubules are observed by video-enhanced differential interference contrast microscopy. The directionality of movement can be determined by the polarity of microtubule growth on the plus (+) and minus (–) end of axonemes.

intracellular trafficking pathways (e.g. endocytosis, secretion) show many similarities to higher eukaryotes (see this volume).

Our laboratory began using *Dictyostelium* as a model system for reconstituting organelle transport along microtubules *in vitro* in order to dissect the process by biochemical methods. *Dictyostelium* has several advantages for such work. The most robust *in vitro*, microtubule-based vesicle movement has been achieved in the squid giant axon; however, the extremely small quantities of this material pose limitations for biochemistry. In contrast, abundant tissue sources such as brain or liver have yielded poor or limited amounts of transport *in vitro*. Using *Dictyostelium*, we have discovered buffer and cell breakage conditions that yielded reproducible and robust membrane transport along microtubules (Pollock *et al.*, 1998). As a substrate for movement, microtubules were nucleated from axonemes so that the plus- and minus-ends of the microtubules could be identified and the direction of organelle movement assessed (Figure 1). The processivity and velocities (up to 2 μm/s) of organelle movement, in general, were similar to what has been observed in living cells. Proteolysis proved to be the most important problem with reconstituting motion, but this could be combated with a cocktail of protease inhibitors and very fresh phenymethyl-sulfonyl fluoride (PMSF).

Using this assay, we showed that an extract made from *Dictyostelium* overexpressing a 380 kDa carboxy-terminal fragment of the dynein heavy chain exhibited impaired minus-end-directed, but not plus-end-directed, microtubule-based motion *in vitro* (Pollock *et al.*, 1998). The result suggested that the altered microtubule patterns observed with 380 kDa overexpressing cells (Koonce and Samso, 1996) might be due to altered activity of native dynein on membranes (possibly the plasma membrane which would impair microtubule interactions with the cell cortex). Moreover, this study demonstrated that it was possible to discover and quantify an organelle transport defect in a mutant cell using the *in vitro* assay. Such quantitative work is much more difficult *in vivo*, where organelle motions are difficult to score. Furthermore, since the directionality of transport is precarious to assess in living cells as the microtubules are curved and the motion is not always radial from the center to the cell surface.

We also used the *Dictyostelium in vitro* assay to biochemically fractionate factors responsible for transporting organelles along microtubules (Pollock *et al.*, 1999). This effort was feasible because the initial extract exhibited robust activity that could be diluted several-fold. In contrast, our laboratory previously attempted to fractionate organelle transport activity from Xenopus oocyte supernatant, but did not succeed because of the difficulty of obtaining sufficiently active and adequate amounts of starting material (unpublished results). Pollock *et al.* used microtubule affinity as a first step in purifying organelle transport factors from *Dictyostelium* high speed supernatants. Minus-end-directed transport (dynein-based) then could be selectively released from the microtubules with 0.3 M KCl (no ATP) and the plus-end-directed transport activity subsequently extracted from microtubules with ATP. This ATP-release fraction was then subjected to column chromatography; each column fraction was combined with a crude membrane vesicle fraction, microtubules and ATP and motion of vesicles along microtubules was scored by video microscopy (Figure 1). Identifying the peak fraction of organelle transport activity was achieved by scoring the number of moving organelles per min. The plus-end-directed transport activity eluted as a single peak from a hydroxyapatite column but split into two fractions in a subsequent MonoQ column. The two peak fractions, which supported movement at slightly different velocities, contained predominant 245 and 170 kDa polypeptides respectively. These polypeptides were microsequenced and discovered to be two members of the kinesin superfamily. The identity and functions of these two kinesins will be further described below.

## Kinesins in *Dictyostelium*

A growing number of kinesin-like proteins has been now reported in *Dictyostelium*. Three kinesin-like proteins have been identified biochemical methods. In addition to the two kinesins purified by Pollock *et al.* (1999), McCaffrey and Vale (1989) identified an 105 kDa

kinesin-like polypeptide that co-purified with microtubules and stimulated microtubule gliding, although the identity of this protein was not pursued further at the level of molecular cloning. Using degenerate PCR primers against conserved sequences in the kinesin motor domain de Hostos *et al.* (1998) identified five developmentally regulated and one constitutively expressed kinesin-related motor, two of which have been completely sequenced. The recent *Dictyostelium* genome sequencing of chromosome 2 (Glöckner *et al.*, 2002) (http://genome.imb-jena.de/*Dictyostelium*/chr2/Chr2map.html) has identified several additional kinesins and will ultimately uncover the complete genomic repertoire. A summary of the kinesins known to date (total of 13) is found in Table 1. Two of these kinesins, DdUnc104 and K7, have also been found in the Japanese cDNA sequencing project (http://www.csm. biol.tsukuba.ac.jp/cDNA/database.html). Some of these kinesins can be tentatively grouped based upon their function in mitosis or organelle transport, and information on these motors is described below.

## Membrane transport motors

The 170 kDa organelle transport kinesin identified by Pollock *et al.* (1999) was later partially cloned, and sequence alignments revealed that it was most similar to conventional kinesin (e.g. mammalian KIF5) (Figures 2 and 3). The sequence similarity extended beyond the N-terminal motor domain into the 'tail' domain region that is conserved in mammalian, invertebrate and fungal conventional kinesins (Figure 2). The sequence is partial and the 3′ end could not be completed due to a long internal polyA region in the coding sequence that was preferred for polyT priming. The *Dictyostelium* 170 kDa kinesin is virtually identical to the 113 amino acid sequence of DdK3 identified by the de Hostos *et al.* PCR screen; therefore we have now renamed DdK3 as KHC, kinesin heavy chain, DdKHC1. Conventional kinesin DdKHC1 is 40–50 kDa larger than most conventional kinesins. Unfortunately with a partial clone in hand, it is not known whether this unique carboxy-terminal region contains any conserved domains that may confer specific biological functions. Biochemically, this conventional kinesin appears to lack associated light chain as is true for the fungal conventional kinesins but unlike the metazoan kinesins (Steinberg and Schliwa, 1995; Grummt *et al.*, 1998; Jeong *et al.*, 2002). The velocity of organelle transport induced by DdKHC1 (1.9 μm/s; Pollock *et al.*, 1999) is much faster than metazoan conventional kinesins (~0.5 μm/s) but similar to fungal conventional kinesins (~2 μm/s). Interestingly in phylogenetic trees of just the motor domain or the whole available coding region, DdKHC1 aligns most closely to the fungal kinesins (Figure 3). Further, DdKHC1 and fungal kinesins may provide some insight into how motor velocity is determined. Little is known about the biological roles of this *Dictyostelium* conventional kinesin. Its ability to induce organelle transport *in vitro* suggests that it is involved in membrane transport, like its fungal and metazoan orthologues. We did not succeed, however, in knocking-out DdKHC1 in *Dictyostelium*.

Interestingly, with the recent completion of the sequencing of chromosome 2 (Glöckner *et al.*, 2002), another full-length conventional kinesin heavy chain of smaller size (110 kDa) was discovered (Table 1). This 110 kDa kinesin is a true conventional kinesin, as it shares high identity in the motor domain as well as the tail region, which contains cargo binding and regulatory sequences (Figure 2). Thus we term this 110 kDa kinesin DdKHC2. Both DdKHC1 and DdKHC2 contain a variation of the IAK motif that has been implicated in down-regulating kinesin's ATPase activity (Hackney and Stock, 2000) Therefore, we suspect that *Dictyostelium* kinesins have a similar ATPase regulatory mechanism to metazoan kinesins. *Dictyostelium* is unusual in having two conventional kinesins. With the exception of mammals, which have a ubiquitously and two neuronally expressed conventional kinesins, all other organisms described to date posses a single conventional kinesin gene. It will be interesting to see whether these *Dictyostelium* conventional kinesins are expressed at the same developmental stages and test for functional redundancy in knock-outs and *in vitro* transport assays.

The 245 kDa polypeptide that stimulates organelle transport in the Pollock *et al.* (1999) assay was cloned and identified as an orthologue of a worm (Unc104) and mammalian (KIF1A) kinesin (Pollock *et al.*, 1999). We termed the 245 kDa kinesin as *Dictyostelium* (Dd) Unc104. In addition to a highly homologous motor domain, DdUnc104 shares a forkhead homology associated (FHA) domain containing and a pleckstrin homology (PH) domain with its metazoan kinesin relatives. However, Unc104 and KIF1A are both monomeric, while hydrodynamic studies revealed that DdUnc104 is a constitutive dimer. This difference is due to an extended coiled-coil region at the C-terminus of the DdUnc104 motor which is absent in Unc104 and KIF1A. DdUnc104, like Unc104 and KIF1A lacks associated light chains. DdUnc104 transports organelles at 2.6 μm/s, making it one of the fastest known kinesin motors and more than 2-fold faster than metazoan Unc104 or KIF1A.

The worm Unc104 and mouse (KIF1A) are both expressed exclusively in the neurons where they transport synaptic vesicle precursors from the cell body to the nerve terminal. DdUnc104 was the first member of the Unc104/KIF1A subfamily to be identified in a unicellular organism, indicating that the membrane transport functions of this kinesin subfamily are ancient and precede the development of a nervous system. A knock-out of the DdUnc104 was performed, and the cells appear normal in morphology, growth rate, and spore formation, although they have a substantial reduction in the number of moving organelles in their cytoplasm (Pollock *et al.*, 1999). The identity of the membranes transported by DdUnc104 is unknown, but mitochondria do not appear to be a cargo, since they move normally in the DdUnc104

*Table 1.* Microtubule-dependent motors in *Dictyostelium*

| Name | Identified | Length (aa) | Subfamily | Protein ID | Function | Assay | References |
|---|---|---|---|---|---|---|---|
| *Kinesins* | | | | | | | |
| DdKHC1 | Transport assay | 1108* | Kinesin heavy chain | AY162212 | Vesicle transport | – | Holleran and Vale (direct submission) |
| DdKHC2 | Genome | 990 | Kinesin heavy chain | Q8T135 | Vesicle transport (?) | – | Glöckner *et al.* (2002) |
| DdUnc104 | Transport assay | 2206 | Unc104/KIF1A | gi:7533190 | Vesicle transport | KO | Pollock *et al.* (1999) |
| DdK2 | PCR | 714 | ncd/Kar3, C-type kinesin | gi:3133267 | Spindle | KO/OE | Iwai *et al.* (2000) and de Hostos *et al.* (1998) |
| DdK4 | PCR | 156 | – | gi:2323468 | – | – | de Hostos *et al.* (1998) |
| DdK6 | PCR | 319 | KIN I | gi:1572665 | – | – | de Hostos *et al.* (1998) |
| DdK7 | PCR | 1254 | – | gi:1526991 | Development | Aggregation | de Hostos *et al.* (1998) |
| DdK8 | PCR | 338 | Chromokinesin | gi:1572667 | – | – | de Hostos *et al.* (1998) |
| Putative 125 kDa kinesin-related protein | Genome | 1224 | – | dd_03187 | – | – | Glöckner *et al.* (2002) |
| Contig-U02542-1 | cDNA | 191 | – | – | – | – | cDNA (Japan Seq. Project) |
| Contig-U05555-1 | cDNA | 116 | – | – | – | – | cDNA (Japan Seq. Project) |
| Contig-U00335-1 | cDNA | 456 | – | – | – | – | cDNA (Japan Seq. Project) |
| Contig-U07506-1 | cDNA | 433 | – | – | – | – | cDNA (Japan Seq. Project) |
| *Dynein* | | | | | | | |
| Dynein heavy chain | Biochemical | 4725 | Cytoplasmic dynein | gi:323077 | Vesicle transport, microtubule array | KO/OE | Koonce and McIntosh (1990), Koonce and Samso (1996), Pollock *et al.* (1998) |
| Dynein intermediate chain | PCR | 652 | Dynein intermediate chain | gi:1706540 | – | – | Trivinos-Lagos *et al.* (direct submission) |
| Contig-U07315-1 | cDNA | 149 | Dynactin | – | – | – | cDNA (Japan Seq. Project) |

Kinesin members where sequence similarities exist spanning over a region of >200 amino acids, have been assigned to subfamily based on a phylogenetic tree. The length of DdKHC1 is most likely a partial sequence (indicated by *) since Northern blot analysis shows a larger fragment. DdKHC2 corresponds to the K3 PCR sequence identified by de Hostos *et al.* (1998). Contig sequences show partial overlap amongst themselves in sequence alignments in the motor domain region suggesting that these cDNA fragments belong to different motors. Dynein and dynein-related proteins are shown below.
Abbreviations: aa, amino acids; OE, overexpression; KO, knock-out.
References: cDNA project Japan, http://www.csm.biol.tsukuba.ac.jp/cDNA/database.html; database of chromosome 2, http://genome.imb-jena.de/dicdi/chr2oracle_table.pl.

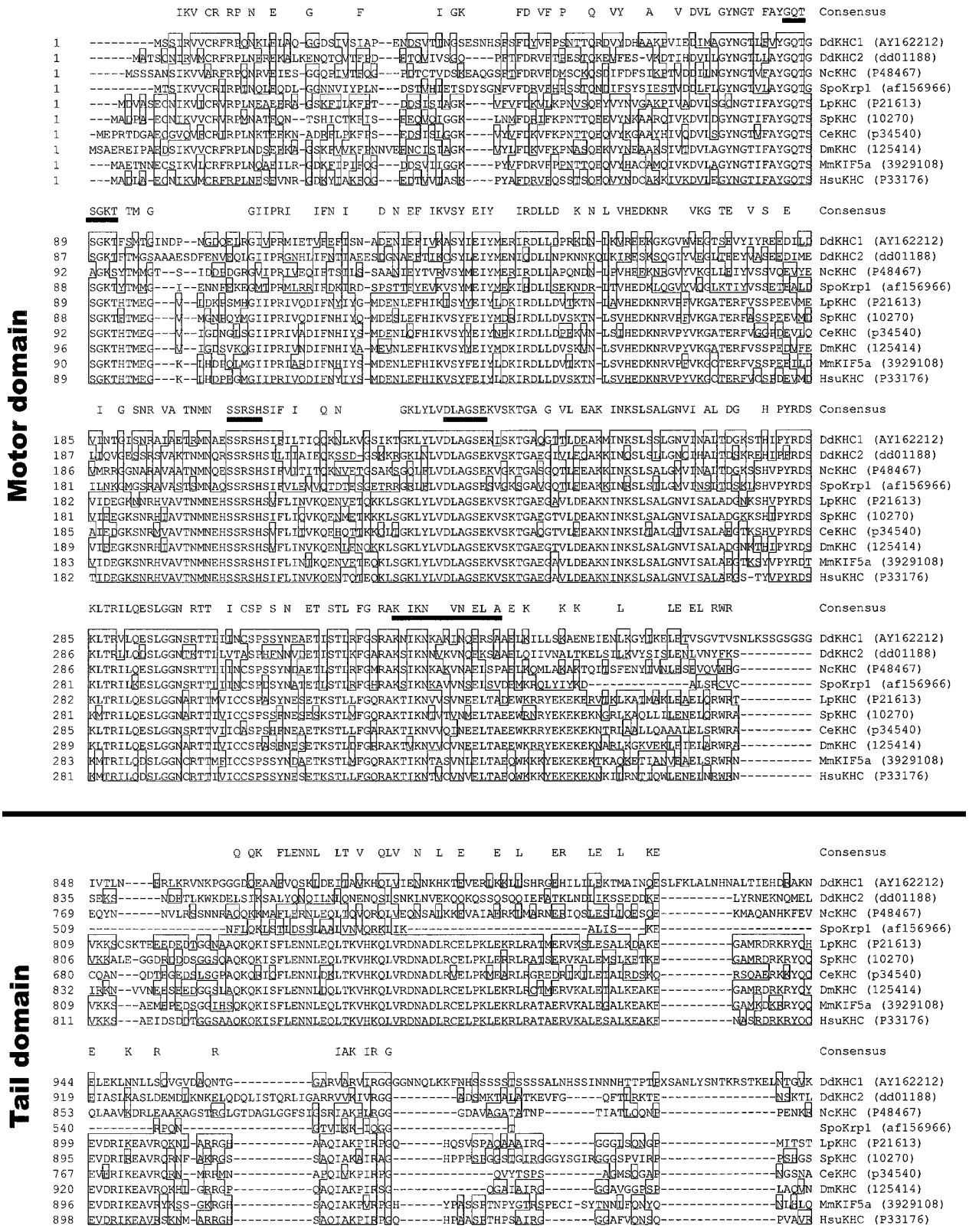


*Fig. 2.* Multiple sequence alignment of conventional kinesin heavy chains: Multiple sequence alignment of kinesin heavy chains in the motor domain region (first 380 amino acids, four upper blocks) and the tail domain (two lower blocks). The coiled-coil stalk domain is omitted in this figure. DdKHC1 and DdKHC2 are truly members of this subfamily as they not only exhibit high homology to the canonical motor domain but also sequence conservation in the tail domain, which allows cargo binding and motor regulation. Consensus positions (minimum of 70% identity) are boxed and the consensus sequence is displayed at the top of each block. Conserved kinesin elements in the motor region of DdKHC1 are underlined in the consensus: P-loop (GQTGSGKT) amino acids 85–92; switch I region (SSRSH) amino acids 204–208, switch II region (DLAGSE) amino acids 234–239, and neck linker (KNIKNKAKINQERSA) amino acids 327–341. Designations in parenthesis indicate accession numbers.

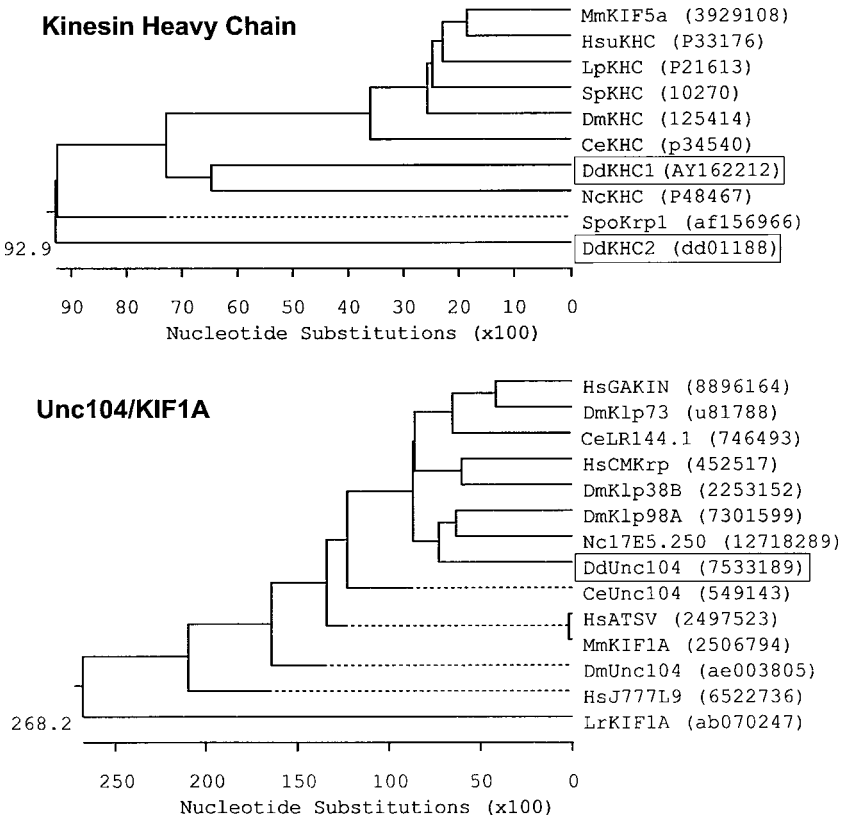


*Fig. 3. Dictyostelium* kinesins in relation to other species: Phylogenetic trees of kinesin heavy chains (top panel) and Unc104/KIF1A family members (bottom panel) were constructed from multiple sequence alignments using the ClustalW algorithm in the DNASTAR program (gap penalty: 10, gap penalty length: 0.1, pairwise alignment matrix: Gonnet250). Note the close relation to fungal kinesins. Dashed lines indicate 'negative' distances due to the balanced, rooted dendrogram, accession numbers are shown in parenthesis. The entire polypeptide chain was used for alignments.

null cell line. The mechanism by which DdUnc104 binds to membrane cargo will be discussed later.

Another kinesin with a possible organelle transport function is K7, a kinesin related protein identified by a PCR-based screen (de Hostos *et al.*, 1998). K7 was completely sequenced and shows highest homology to conventional kinesin in the motor domain, but outside the motor domain, K7 exhibits no sequence identity to any other kinesin or protein in the data bank. Hence, we do not classify K7 as a true conventional kinesin. K7 is a plus-end-directed motor when expressed in *E. coli* and elicits a velocity of 0.14 μm/s. Regarding its biological function, K7 is developmentally expressed, with peaking after 12–16 h following starvation. K7-null cells behave normally throughout development; however, K7-null cells are excluded from the prestalk region when mixed with *Dictyostelium* wild-type cells (de Hostos *et al.*, 1998). K7 may transport a membrane cargo motor, since immunolocalization of K7 labels perinuclear membranous structures. However, functional evidence linking K7 with membrane transport is lacking.

## Possible mitotic kinesin motors

Like fungi, *Dictyostelium* has a 'closed' mitosis in which the nuclear envelop remains intact and a mitotic spindle is formed within the nucleoplasm (Roos and Camenzind, 1981). The roles of kinesins in mitosis have received very little attention compared to many other organism. However, based upon sequence similarities with known mitotic kinesins, a few *Dictyostelium* kinesins are reasonable candidates for playing a role in mitosis. One such kinesin is K6 (de Hostos *et al.*, 1998) which has a motor domain located in an internal position within the polypeptide chain. Other members of this KIN I family have been shown to cause microtubule depolymerization and play important roles in mitosis (Desai *et al.*, 1999).

K2 (de Hostos *et al.*, 1998) has been recently sequenced and characterized (Iwai *et al.*, 2000), and it has its motor domain located at the C-terminus of the polypeptide chain. Many KIN C motors, which display minus-end-directed motility, play roles in mitosis and meiosis, although some motors in this family are expressed in neurons. K2-null cells are indistinguishable from wild-type cells in growth and development; however overexpression of K2 results in a partial mitotic arrest. Further supporting a role in mitosis K2-GFP localizes to the mitotic spindle (Iwai *et al.*, 2000).

## Other putative kinesins

We also assembled a list of partial *Dictyostelium* kinesins sequences that have been obtained from ge-

nomic sequencing and submitted to databases (Table 1). Blast homology searches of tentatively annotated kinesin sequences revealed however that only a subset have significant homology to kinesins upon closer inspection using our own sequence alignments. We have identified four additional kinesins although the short regions of homology do not permit the assignment to a particular subfamily.

## The mechanism of membrane binding by DdUnc104

DdUnc104 has undergone the most extensive analysis of any *Dictyostelium* kinesin motor to date. Studying the motor/cargo interaction *in vitro* revealed that DdUnc104 transports membranes, at least in part, through a direct lipid interaction (Klopfenstein *et al*., 2002). The pleckstrin homology domain in DdUnc104's carboxy-terminal region binds preferentially to phosphatidylinositol-(4,5)-bisphosphate ($PIP_2$) with lower specificity for other acidic phospholipids. *In vitro* transport assays have shown that transport of native *Dictyostelium* vesicles can be competed with either exogenously added PH domain or masking the $PIP_2$-headgroups. It is likely that a combination of lipids and accessory proteins contribute to optimal cargo binding given that proteolytic digest of cytosolic-exposed vesicle proteins also decreases transport efficiency. Interestingly, DdUnc104 also moves protein-free liposomes suggesting that this novel motor–lipid interaction is a bona-fide mechanism for cargo binding. The *in vivo* relevance of lipid binding by the DdUnc104 PH domain is currently under investigation.

As discussed previously, one unique feature of DdUnc104 compared to metazoan Unc104/KIF1A is the presence of a long coiled-coil stalk at the carboxy-terminus (Pollock *et al*., 1999). One hypothesis for this divergence is that regulation of cargo transport occurs by different mechanisms. Our studies show that liposome transport by worm Unc104 is triggered in highly cooperative manner with the clustering of $PIP_2$ in the membrane. The worm Unc104 becomes a fast processive motor when two monomers come together into a dimeric species (Tomishige *et al*., 2002). Thus, we suggested that concentration of worm Unc104 within $PIP_2$-containing lipid domains triggers motor dimerization which then allows for processive vesicle motion (Klopfenstein *et al*., 2002). In contrast to the results with worm Unc104, liposome transport frequency powered by DdUnc104 increases in a quasi-linear manner with increasing $PIP_2$ concentration (Klopfenstein *et al*., 2002). Thus, in contrast with metazoan Unc104 motors where the monomer–dimer transition may be important for regulation, the constitutive dimeric DdUnc104 might be controlled through post-translational modifications or accessory regulators. Further experiments addressing motor regulation are needed to test such hypotheses.

### *Dictyostelium dynein*

*Dictyostelium* has a single heavy chain gene (Koonce *et al*., 1992) in contrast to higher eukaryotes which have multiple isoforms. A knock-out of the *Dictyostelium* dynein gene has been attempted, but not achieved. This suggests that dynein is an essential gene in this *Dictyostelium*, most likely because it is required for mitosis. As another means of exploring the function of cytoplasmic dynein, Koonce and Samso (1996) overexpressed a 380 kDa fragment containing the ATPase sites of the dynein motor domain. This overexpression resulted in a collapse of the interphase microtubule network, which might be interpreted to suggest that dynein links the plus-ends of microtubules to the cortex, as described now in other organisms. *Dictyostelium* dynein also transport membrane organelles to the microtubule minus-end in the *in vitro* assay system. Interestingly, however, a highly purified dynein fraction does not stimulate organelle movement, suggesting that a co-activator is needed (Pollock *et al*., 1999). The identity of this factor(s) could be determined by biochemical complementation. Ma and Chisholm (2002) also tagged the dynein intermediate chain with GFP (IC-GFP) to monitor the dynein-dependent movement of membrane cargo. The IC-GFP shows fast linear movements both towards the microtubule organizing center and in opposite direction to the periphery of the cell with frequent reversals of direction.

Despite the general domain organization amongst motor proteins, there are structural features of dynein that set it apart from the other motors and much of this information has emerged from studies of *Dictyostelium* dynein. The 3D-reconstruction of EM images of a 380 kDa heavy chain fragment exhibits 7–8 globular domains of 13.5 nm in length oriented in a ring-like structure encircling a central cavity (Samso *et al*., 1998). The microtubule binding site has been mapped at the tip of a long projection of dynein heavy chain (termed the B-link) to three clusters of amino acids important for the physical contact with microtubules; two of these fall within a region sharing sequence homology with MAP1B (Koonce and Tikhonenko, 2000). The globular lobes of the head ring contain 6 AAA domain that can bind ATP with different affinity which could be a putative mechanism of motor regulation (reviewed in Asai and Koonce, 2001). These structural and enzymatic features are different from those used by the other motors, suggesting that dynein produces movement by a distinct mechanism.

## Conclusions and outlook

With the sequencing project under its way, we are expecting to learn more about the kinesin motor family and their representatives in *Dictyostelium*. One area that has received little attention thus far is the role of these microtubule-based motors in the developmental process

of *Dictyostelium*. Surprisingly, several kinesin motors are strongly upregulated during development (de Hostos *et al*., 1998) but their functions are not clear. Tagging these kinesins with GFP facilitates determining and analyzing the dynamics of their subcellular localization throughout the cell cycle and development. This should be an interesting area of study and will perhaps shed light on other uses of motors in the development of other organisms.

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