The Molecular Motor Toolbox for Intracellular Transport

Review

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Eukaryotic cells create internal order by using protein motors to transport molecules and organelles along cytoskeletal tracks. Recent genomic and functional studies suggest that five cargo-carrying motors emerged in primitive eukaryotes and have been widely used throughout evolution. The complexity of these "Toolbox" motors expanded in higher eukaryotes through gene duplication, alternative splicing, and the addition of associated subunits, which enabled new cargoes to be transported. Remarkably, fungi, parasites, plants, and animals have distinct subsets of Toolbox motors in their genomes, suggesting an underlying diversity of strategies for intracellular transport.

A cell, like a metropolitan city, must organize its bustling community of macromolecules. Setting meeting points and establishing the timing of transactions are of fundamental importance for cell behavior. The high degree of spatial/temporal organization of molecules and organelles within cells is made possible by protein machines that transport components to various destinations within the cytoplasm.

Landmark discoveries of cytoplasmic transport have been, and continue to be, made through advances in microscopy. Intracellular motion was first observed in the alga Chara by Bonaventura Corti in the late 18th century, and chromosome movements were documented with remarkable accuracy by microscopists in the 19th century. The development of video-enhanced contrast microscopy in the early 1980s enabled the visualization of small membranous organelles (Allen et al., 1982) and large protein complexes (Kozminski et al., 1993). With this clearer view of the cell interior, the tremendous amount of directed cytoplasmic motion became apparent. The use of the green fluorescent protein for tagging organelles, proteins, and RNA led to another wave of discovery of intracellular movement. The list of transported cargoes, which grows larger every year and touches almost every aspect of cell and developmental biology, now includes large membrane organelles (e.g., the Golgi and nucleus), smaller vesicular or tubular intermediates in the secretory and endocytic pathways, a subset of mRNAs, cytoskeletal filaments, proteins building blocks for large macromolecular complexes such as cilia/flagella and centrosomes, and proteins involved in signaling and establishing cell polarity.

The most widely used mechanism for intracellular transport involves molecular motor proteins that carry cargo directionally along a cytoskeletal track (myosins along actin and kinesins and dyneins along microtubules). Recent genomic sequencing projects have uncovered the complete inventories of molecular motors in several organisms. Such data, combined with information from functional studies, are providing clues on the origins of the molecular motors and the intracellular transport strategies employed by various organisms. While prokaryotes contain cytoskeletal filaments, the cytoskeletal motors appear to be an early eukaryotic invention. Several types of cargo-transporting molecular motors emerged in unicellular eukaryotes, and this same ancient "Toolbox" of motors expanded to meet the majority of transport needs of multicellular organisms. These cargo-transporting motors will be the focus of this review. Molecular motors also are used for organizing cytoskeletal filaments (e.g., controlling their dynamics, collecting them into bundles, and causing filament-filament sliding). Intracellular transport also can be driven by attaching cargo to the ends of polymerizing or depolymerizing microtubule or actin filaments. However, these aspects of motor function and cytoskeletal dynamics will not be discussed here.

Prokaryotes Contain Cytoskeletal Filaments Related to Actin and Tubulin

Although simpler in overall design than eukaryotes, prokaryotes also must physically separate replicated DNA and establish a central division plane, and some bacteria have well-defined asymmetric cell shapes. Recently, several of the proteins involved in these processes were discovered to be antecedents of eukaryotic actin and microtubules (van den Ent et al., 2001a). FtsZ, which forms filaments that encircle the middle of the bacterium during septation, shows several striking similarities to tubulin, including a superimposable three-dimensional structure, GTPase activity, and the ability to polymerize in vitro into microtubule-like polymers. FtsZ-like proteins also are found in chloroplasts where they participate in organelle replication. A second group of proteins (MreB, Mbl, and ParM) is related to actin. MreB has a similar three-dimensional structure to actin's and polymerizes in vitro into filaments that are similar but not identical to actin filaments (van den Ent et al., 2001b). In *B. subtilus*, MreB and Mbl form filamentous structures that respectively encircle the middle and the longitudinal cell axes just beneath the membrane (Jones et al., 2001). Loss of either of these proteins (which are not found in spherical bacteria) disrupts the rod-shaped morphology of *B. subtilis*. ParM, on the other hand, serves a different function in segregating replicated plasmids during cell division (Moller-Jensen et al., 2002).

While the existence of a bacterial cytoskeleton is now beyond dispute, the manner in which these filaments perform their duties remains unclear. Depolymerization of FtsZ filaments might drive septation, and the polymerization of ParM has been proposed to drive the separation of replicated plasmids (Moller-Jensen et al., 2002).

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Alternatively, bacterial filaments could undergo force-generating structural transitions (Erickson, 2001) or could serve as passive scaffolds for concentrating molecules (e.g., peptidoglycan synthetic or membrane fusion machineries in the case of FtsZ).

Another possibility is that bacterial filaments serve as tracks for force-generating motors. Comparison of the crystal structures of the structurally related kinesin and myosin motors suggests that these two motor superfamilies originated from a common ancestral protein that might be conceivably lurking in the genome of a modern day prokaryote (Kull et al., 1998). Dynein, on the other hand, belongs to the AAA^+ ATPase superfamily, members of which are present in prokaryotes as well as eukaryotes (Neuwald et al., 1999). However, none of the genes identified as being important for bacterial shape, septation, or DNA segregation encode proteins with sequences that are clearly characteristic of motor ATPases. Thus, while the existence of a prokaryotic cytoskeletal motor remains an open research question, it seems clear that these molecular machines only became abundant and assumed prominent roles in eukaryotic cells.

The Emergence of a Basic "Toolbox" of Cytoskeletal Motors in Early Eukaryotes

In eukaryotes, the cytoskeleton assumed many new roles in addition to cell shape determination and DNA segregation. In comparison with the rapid diffusion of molecules in the bacterial cytosol, diffusional encounters between membrane bounded compartments are slow, and the cytoskeleton evolved roles in facilitating such interactions. In addition, complex macromolecular structures (e.g., flagella) and polarized specializations observed even in single cell eukaryotes necessitated means for delivering and localizing molecules. Rather than evolve multiple filamentous systems for moving different cargoes through polymerization-based mechanisms, eukaryotes emerged with the more efficient and economical solution of utilizing a limited number of tracks (actin and microtubules) and developing a battery of motors, each of which could be designed to carry distinct cargoes and could be subjected to unique regulatory controls.

The genomic inventories of motor proteins have been now uncovered in several diverse organisms (see Table 1). Remarkably, even *Giardia*, a protozoal parasite that is generally placed at the base of the phylogenetic tree for the eukaryotes, contains a rich collection of 25 kinesin genes (Table 1, and H. Elmendorf, S. Dawson, H. Goodson, L. Douglass, A. McArthur, H. Morrison, I. Gibbons, Z. Cande and M. Sogin, unpublished data). Sequence analysis reveals that many of these kinesin genes are unique to *Giardia* and may have arisen by duplication and evolved organism-specific functions. However, single cell eukaryotes also contain the same motor classes that are used by vertebrates for cargo transport (Table 1). This suggests that a basic "Toolbox" of molecular motors appeared in relatively primitive eukaryotes and that this set of motors has been retained and widely used throughout eukaryotic evolution.

Based upon genome comparisons and functional data derived from several organisms, I place five types of motors in the cargo-transporting Toolbox (Figure 1). Three of these are microtubule plus-end-directed kinesins: conventional kinesin (also called kinesin I or KIF5), kinesin II (also called heteromeric kinesin), and Unc104/KIF1 (formerly called monomeric kinesin). Cytoplasmic dynein, which moves toward the microtubule minus end, is another Toolbox motor. The fifth Toolbox motor is the actin-based myosin V motor (the plant version is termed myosin XI). All of these Toolbox motors have been shown unambiguously to transport cargo in both unicellular and metazoan organisms. As is true of the most successful proteins in evolution, these five types of molecular machines have proven to be remarkably versatile, expanding into many niches of intracellular transport in various organisms and different tissues. Additional motors, however, are likely used for cargo transport. For example, other myosin and neuron-specific kinesin classes have been proposed to transport membrane cargo, but their roles in cargo transport are less well documented than the Toolbox motors and they are less widely employed by a diverse range of organisms. For these reasons, this review will focus on the Toolbox motors.

Assignment of motor protein genes to the five Toolbox classes are generally made based upon sequence alignments of the motor domains followed by phylogenetic analyses. Many of the residues that are conserved specifically within a motor class are scattered throughout the motor domain and may be involved, at least in part, in setting the motor's ATPase rate. However, discrete structural elements, particularly the mechanical amplifiers adjacent to the catalytic cores, also are conserved in a unique class-specific manner (see Table 1 legend). For example, class V myosins can be identified by their unusual force-generating lever arm helix, which is the longest among the myosins. The extended length enables the two motor domains of the myosin V dimer to bind simultaneously to an actin filament, a feature that enables myosin V to move processively along actin and carry its cargo for long distances without dissociating (Purcell et al., 2002). The mechanical amplifiers of kinesins (the necks) also show strong class-specific conservation (Vale and Fletterick, 1997) and are important for processive movement by conventional kinesin (Vale and Milligan, 2000). The non-motor "tail" domains also can contain class-specific sequences that participate in cargo binding and/or motor regulation.

Below, I will focus on the five Toolbox motors, discussing their conserved structural features, how they are utilized for cargo transport in lower eukaryotes, and how they evolved new functions in metazoan organisms.

Conventional Kinesin

Conventional kinesin was identified in a biochemical fractionation of squid and mammalian nervous tissue for proteins that generate microtubule-based motility in vitro (Vale et al., 1985). The motor polypeptide (kinesin heavy chain, KHC) contains an N-terminal motor domain, a long coiled-coil stalk interrupted by a central hinge, and a globular tail domain (Figure 1). Conserved sequences in the tail domain may be involved in generating a folded, autoinhibited conformation and/or connecting kinesin to its cargo (Seiler et al., 2000).

In lower eukaryotes, conventional kinesin has been best studied in *Ustilago* (Lehmler et al., 1997) and *Neu-*

Table 1. Genomic Representation of Molecular Motors

Genome	Kinesins								Dyneins					Myosins				
			Cargo Transport															
	Ref.	Total	Conv. Kinesin	Kinesin II	Unc104/KIF1	Mitotic	C-terminal	Other	Ref.	Total	Cytoplasmic	IFT	Axonemal	Ref.	Total	Class V	Class II	Others
Giardia	7	25	1	2	3	4	2	13	7	10	1	1?	8	7	0	0	0	0
Malaria	7	9	0	0	0	3	0	6	7	4–6	1	?	3?	7	6	1	0	5
S. cerevisiae	1	6	0	0	0	4	1	1	1	1	1	0	0	1	5	2	1	2
Drosophila	7	25	1	3	4	11	1	5	7	13	1	1	11	4	13	1	2	10
C. elegans	7	20	1	3	3	5	4	4	7	2	1	1	0	4	17	1	9	7
Arabidopsis	2	61	1?	0	0	14	21	25	6	0	0	0	0	5	17	13	0	4
Ciona	7	31	1	3	7	7	4	9	7	19	1	1	17	7	16	1	6	9
Human	3	45	3	4	8	14	3	13	7	14–15	1	1	13	4	40	3	16	21

Estimated numbers and uses of molecular motor genes in various organisms. This information was extracted from the indicated references: (1) Yeast Protein Data Base (www.proteome.com/YPDhome.html); (2) Reddy and Day, 2001b; (3) Miki et al., 2001; (4) Berg et al., 2001; (5) Reddy and Day, 2001a; (6) Lawrence et al., 2001; and (7) sequence analysis performed by our laboratory group (S. Reck-Peterson and G. Goshima). A detailed analysis of dyneins will be presented elsewhere (S. Reck-Peterson and R.V., unpublished data). Our web-based analysis of *Giardia* (McArthur et al., 2000) in advance of publication of the genome was reported with the permission of M. Sogen (Marine Biological Laboratory) using their www site. This group also will report a more detailed analysis of the molecular motors in *Giardia* (H. Elmendorf, S. Dawson, H. Goodson, L. Douglass, A. McArthur, H. Morrison, I. Gibbons, Z. Cande and M. Sogin, unpublished data). For our blast searches, we searched complete genomic sequences using tblastn with the motor domains of several kinesins and myosins or the first AAA domain of various dyneins. Motor classes were assigned based upon both phylogenetic clustering with known subfamilies as well as the presence of characteristic class-conserved structural elements: the neck and tail domain sequences for conventional kinesin; the neck motif EDPKDALLRF/Y in kinesin II; a conserved loop 3 insertion and the FHA domain for Unc104/KIF1; 6 IQ motifs in the lever arm helix and a conserved DIL domain in the tail of myosin V (the later, however, is not obvious in the assigned malarial myosin V). Cytoplasmic, IFT, and axonemal dynein genes can be distinguished from one another in phylogenetic trees generated from sequence alignments of the first AAA domain. The classification of conventional kinesin in *Arabidopsis*, and cytoplasmic/IFT dyneins in *Giardia* and *Malaria* are tenuous, as reflected by the question marks. Our current analysis shows 4 dyneins in *Malaria*, although two additional predicted open reading frames have dynein-like sequence. Humans have 14 clear dynein genes and a 15th gene that might represent a highly divergent dynein. The mitotic kinesins are assigned based upon genomic, expression, and functional data from many sources and include the chromokinesins/Kid, Eg5/BimC/KSP, Kip3-type, CenpE, and cytokinetic kinesins (e.g. Rab6 kinesin, MKLP1). The microtubule depolymerizing Kin I kinesins are placed in the mitotic category, although they may serve other functions as well. The Ncd-type mitotic motor is placed with the other C-terminal motors in this Table. The C-terminal motor domain kinesins are listed separately, as this subfamily contains motors that appear to perform a mixture of mitotic, cargo-transport, and other functions. Class II myosin include muscle myosin and cytoplasmic myosins involved in cytokinesis.

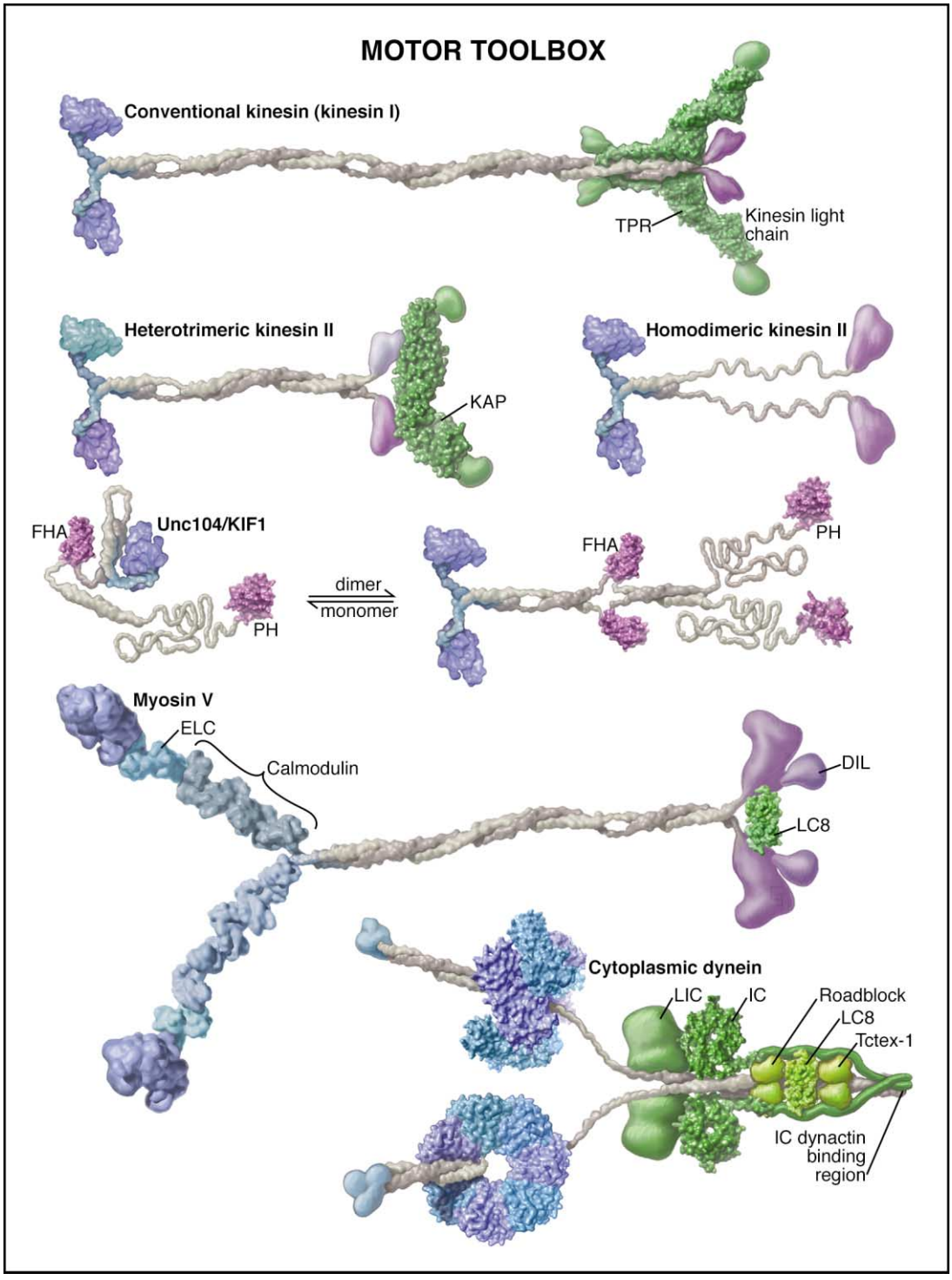

Figure 1. The "Toolbox" of Cargo-Transporting Motor Proteins

Surface features are rendered based upon atomic resolution structures when available (see Supplemental Data for details of figure preparation available at http://www.cell.com/cgi/content/full/112/4/467/DC1) and appear as smooth images for domains of unknown structure. The motor catalytic domains are displayed in blue, mechanical amplifiers in light blue, and tail domains implicated in cargo attachment are shown in purple. Dynein is shown in mixed purple, blue shading to illustrate the distinct domains that comprise the motor head, four of which are likely to be functional ATP binding AAA^{+} domains. The stalks extending from the ring bind to microtubules at their globular tips. Heterotrimeric kinesin II contains two distinct motor subunits, which is reflected in the two different color shadings. Tightly associated motor subunits (light chains) are shown in green. Domains described in the text are labeled. The Unc014/KIF1 motor can exist as a monomer and dimer, as indicated by the equilibrium. Metazoan conventional kinesin (with light chains) and the *C. elegans* Osm-3 motor (shorter than the KIF17 homodimeric kinesin II) are depicted in this figure. Prepared by G. Johnson (graham@fiVth.com).

rospora (Seiler et al., 2000) (two filamentous fungi), and *Dictyostelium* (Klopfenstein et al., 2003), a slime mold. In these organisms, conventional kinesin exists as a homodimer without associated light chains. In filamentous fungi, conventional kinesin transports membrane vesicles to the tips of growing hyphae. *Dictyostelium* has two conventional kinesins, one of which was shown to transport membranes in vitro, but their in vivo roles have not been elucidated.

Metazoan conventional kinesins have been reported to transport numerous membrane cargoes including mitochondria, lysosomes, endoplasmic reticulum, and a subset of anterograde-moving vesicles in axons (Hirokawa, 1998). Metazoan conventional kinesin also transports nonmembranous cargo, such as mRNAs (Brendza et al., 2000) and intermediate filaments (Prahlad et al., 1998). This expanded repertoire of cargoes was made possible by several evolutionary modifications of conventional kinesins. First, metazoans introduced an accessory subunit (kinesin light chains, KLC) that binds to the KHC tail domain. Recent studies revealed that the light and heavy chains mediate distinct cargo interactions. The light chain's tetratricopeptide (TPR) motif region interacts with MAP kinase scaffolding proteins called JIPs (Jun-N-terminal kinase (JNK)-interacting proteins) (Bowman et al., 2000; Byrd et al., 2001; Verhey et al., 2001), the amyloid precursor protein (APP) on axonally transported membrane vesicles (Kamal et al., 2000), and vaccina virus (Rietdorf et al., 2001). In contrast, the tail domain of the heavy chain interacts with the glutamate-receptor-interacting protein (GRIP1) (Setou et al., 2002) and the neurofibromatosis protein (Hakimi et al., 2002).

Conventional kinesins also expanded to three heavy chain genes in vertebrates (Table 1), two of which are expressed primarily in neuronal cells. Vertebrates also have three light chain genes (Rahman et al., 1998 and L. Goldstein, personal communication) that undergo alternative splicing C-terminal to the TPR motifs (Cyr et al., 1991). The light and heavy chains can combine in various permutations (Rahman et al., 1998), potentially creating a variety of different motors. The multiple KHCs and KLCs in vertebrates very likely have distinct cargo recognition and/or regulatory properties, but the distinctions between these isoforms have yet to be uncovered.

Kinesin II

Kinesin II was first identified biochemically in sea urchin eggs and found to contain two distinct motor-containing polypeptide chains that come together to form a heterodimer (Cole et al., 1993) (Figure 1). Heterodimerization is mediated by complementary charge interactions in an extended region of the coiled-coil stalk (De Marco et al., 2001), but the purpose of heterodimer formation (unique in the kinesin superfamily) is unknown. Bound to this motor's tail domain is a tightly associated subunit (called KAP) with an armadillo repeat domain that is known to mediate protein-protein interactions. Because of its three distinct subunits, this motor is referred to here as heterotrimeric kinesin II. Metazoans also have another kinesin II gene (Osm3/KIF17), which current evidence indicates encodes a protein that forms homodimers (Signor et al., 1999b; Setou et al., 2000) and does not have an associated subunit (referred to here as homodimer kinesin II). The heterotrimeric and homodimeric kinesin IIs share a unique sequence (EDPKDALLRF/Y) in the neck region, but the function of this signature sequence is not known.

Heterotrimeric kinesin II is found in two flagellated single cell eukaryotes, *Giardia* and *Chlamydomonas*. In *Chlamydomonas*, a temperature-sensitive mutation in one of the motor subunits (termed Fla10) has provided a wealth of information on the function of this motor. At the restrictive temperature, the flagella shrinks due to the impaired delivery of building blocks (e.g., tubulin, flagellar dyneins, radial spoke proteins) from the base to the tip of the flagella. This "intraflagellar transport" (IFT) occurs by the movement of large protein particles along the axonemal microtubules just beneath the plasma membrane (Rosenbaum and Witman, 2002). The presence of kinesin II genes in *Giardia* suggests that IFT evolved concomitant with the genesis of the axoneme. Other functions for kinesin II have not been described in single cell eukaryotes, and this class of kinesin genes is absent from fungi.

Metazoans also use heterotrimeric kinesin II to power IFT. Mouse knockouts of heterotrimeric kinesin II genes result in ciliary defects, and analyses of these mutant mice have uncovered new functions for cilia in mammals. One consequence of kinesin II knockouts is a developmental defect called situs inversus, a condition in which the heart is frequently on the wrong side of the midline (Nonaka et al., 1998; Marszalek et al., 1999). This phenotype was traced to a failure to form cilia on the embryonic nodal cells; beating of these cilia in normal embryos was subsequently observed and hypothesized to establish a flow and a consequent gradient of a yet undiscovered morphogen involved in left-right axis formation (Nonaka et al., 1998). Immotile or "primary" cilia (which contain the 9 microtubule outer doublets, but lack dynein arms and the central pair) similarly require heterotrimeric kinesin II-mediated IFT, as demonstrated for the cilia that extend from the dendrites of chemosensory neurons in *C. elegans* (Signor et al., 1999b). Mammalian photoreceptor cells also contain an unusual ciliary structure that forms a narrow isthmus between the inner segment (containing the nucleus and Golgi) and the outer segment (containing the light-sensing machinery) (Rosenbaum and Witman, 2002). A Cre-loxP knockout of heterotrimeric kinesin II in these cells revealed that this motor is required for transporting opsins and other components through the connecting cilium and into the outer segment (Marszalek et al., 2000).

In metazoans, heterotrimeric kinesin IIs expanded their cargo-transporting duties beyond IFT to include movement of vesicular cargo and soluble cholinesterase in neurons (Kondo et al., 1996; Ray et al., 1999), pigmented melanosomes in melanophore cells (Tuma et al., 1998), and the adenomatous polyposis colon protein (APC) in tissue culture cells (Jimbo et al., 2002). Like conventional kinesin, the tail domains of the two motor subunits and nonmotor KAP subunit may participate in distinct cargo interactions. However, thus far, only the KAP subunit has been reported to participate in potential cargo interactions (with fodrin, a nonmuscle spectrin, Takeda et al., 2000), the dynactin complex in melanophores (Deacon et al., 2003), and the APC protein (Jimbo et al., 2002). Only one KAP gene has been described,

but it can generate two alternatively spliced isoforms, and APC binds to only one of the isoforms (Jimbo et al., 2002). Vertebrate neurons also have a third motor subunit (KIF3C) that forms heterodimers only with KIF3A, but the function of the KIF3A/C motor is unclear, as KIF3C null mice have no obvious phenotype (Yang et al., 2001). Given the relatively sparse molecular diversity of heterotrimeric kinesin II in metazoans, there are currently few clues on how this motor acquired its additional transport activities, beyond IFT.

Homodimeric kinesin II is only found in metazoans, in contrast to heterotrimeric kinesin II. In *C. elegans*, the homodimeric kinesin II (Osm3) is necessary for maintaining the structure of chemosensory cilia, perhaps by carrying IFT cargo distinct from those moved by heterotrimeric kinesin II (Signor et al., 1999b). Although Osm3 is expressed at low concentrations in other neurons (Signor et al., 1999b), Osm3 mutant worms do not have neuronal phenotypes other than those described for the ciliated chemosensory neurons. In contrast, the mouse homodimeric kinesin II (KIF17) transports NMDA receptor-containing vesicles in dendrites of CNS neurons (Setou et al., 2000), and overexpression of this motor enhances learning and memory in transgenic mice (Wong et al., 2002). A testes-specific isoform of KIF17 (with relatively few amino acid differences in the tail domain) was reported to bind to and control the intracellular localization of a transcriptional factor involved in spermatogenesis (Macho et al., 2002). A role for vertebrate KIF17 in IFT, however, has not yet been described. Whether homodimeric kinesin II evolved distinct roles in *C. elegans* and vertebrates or can execute neuronal, IFT, and testes transport functions in a single organism remains an open question.

Unc104/KIF1

The Unc104 motor was discovered in a mutant screen in *C. elegans*, where null mutations cause paralysis due to a failure to transport synaptic vesicles to the presynaptic terminals of motor neurons (Hall and Hedgecock, 1991). A knockout of the mouse ortholog, KIF1A, produced a similar phenotype (Yonekawa et al., 1998). The Unc104/KIF1 kinesins have two diagnostic class-conserved features: a conserved insertion in loop 3 near the nucleotide binding pocket, and the presence of a fork head homology (FHA) domain (documented in other proteins to binds phosphothreonine) C-terminal to the motor domain (Figure 1). The functions of these highly conserved elements are unknown. An unusual property of the Unc104/KIF1 kinesins is that they are predominantly monomeric (Okada et al., 1995), in contrast to other kinesins, which are dimeric or tetrameric. However, when concentrated in solution or on membranes, Unc104/KIF1 can dimerize via coiled-coil regions adjacent to the motor domain (Figure 1), and dimerization allows the motor to move processively along microtubules like conventional kinesin (Tomishige et al., 2002). The monomer-to-dimer transition may serve to activate Unc104/KIF1A transport in vivo, and the FHA domain, by virtue of its position in between two coiled-coil domains (Figure 1), could be involved in such a regulatory mechanism.

In lower eukaryotes, Unc104/KIF1 kinesins have been best studied in *Ustilago* (Wedlich-Soldner et al., 2002) and *Dictyostelium* (Pollock et al., 1999). In both organisms, gene knockouts of this motor inhibit membrane transport. The tail domains of the *Ustilago* and *Dictyostelium* Unc104/KIF1 motors both contain a pleckstrin homology (PH) domain that binds to phosphoinositol lipids and facilitates membrane attachment (Klopfenstein et al., 2002). Interestingly, *Giardia* contains three Unc104/KIF1 type motors, although their roles are not known.

The cargo transporting roles of Unc104/KIF1-type motors also expanded considerably in metazoans, primarily through gene duplication (7 and 8 genes in *Ciona intestinalis*, a sea squirt, and humans respectively; Table 1). Thus far, other subunits have not been found complexed with the Unc104/KIF1 motor polypeptide. *C. elegans* Unc104, *Drosophila* Klp53D, and mouse KIF1A, by virtue of their C-terminal PH domains, appear to be the closest relatives of the *Dictyostelium* and *Ustilago* motors. Interestingly, while the lower eukaryotic Unc104/KIF1A motors have more general roles in membrane trafficking, the metazoan orthologs have taken on the specialized function of transporting synaptic vesicle precursors in the nervous system (Hall and Hedgecock, 1991; Yonekawa et al., 1998). One of the new metazoan Unc104/KIF1A-type motors (*Drosophila* kinesin-73, *C. elegans* CeKLP-4, mouse KIF13B, and human GAKIN) contains a C-terminal cap-gly domain that is known in other proteins to bind tubulin. An intriguing attribute of GAKIN is its binding to the disc large tumor suppressor (Dlg) protein, a membrane-associated guanylate kinase (MAGUK) (Hanada et al., 2000). This finding raises the possibility that GAKIN-type motors may transport membrane-associated scaffolding proteins to cell contact sites in multicellular organisms.

Further diversity of Unc104/KIF1-type motors in metazoans is achieved through alternative splicing. This has been best documented for the KIF1B gene, where alternative splicing gives rise to motor isoforms with completely different tail domains (Gong et al., 1999; Zhao et al., 2001). The tail domain of KIF1Bα targets the motor to mitochondria (Nangaku et al., 1994), while the KIF1Bβ tail targets to synaptic vesicle precursors (Zhao et al., 2001). Additional alternative splicing of this gene in the motor domain creates several more variants that may have distinct biophysical attributes (Gong et al., 1999).

Cytoplasmic Dynein

Dynein was originally identified as a force-generating ATPase in *Tetrahymena cilia* (Gibbons and Rowe, 1965), and a cytoplasmic dynein was later discovered to power minus-end-directed motion in nonciliated cells (Paschal et al., 1987). The dynein heavy chain (DHC) contains a large motor domain ($\sim$380 kDa) that is composed of 6 AAA^+ ATPase-like and possibly a seventh domain arranged in a ring (Samso et al., 1998) (Figure 1). The first four AAA^+ domains are thought to bind nucleotide (the first being the main ATP hydrolytic site), and the last two AAA^+ domains do not bind nucleotide and may serve a structural role (dynein structure reviewed in King, 2000). A coiled-coil extends from the motor domain, and a small globular domain at its tip mediates attachment to microtubules. The nonmotor region of the DHC contains coiled-coil sequences for dimerization as well as binding sites for dynein light chains (to be discussed later).

Cytoplasmic dynein participates in many transport activities in lower eukaryotes, most of which occur in

higher eukaryotes as well. Cytoplasmic dynein moves and positions nuclei in fungi as well as in migrating neurons of the mammalian brain (reviewed in Bloom, 2001; Vallee et al., 2001). Cortically localized dynein, by pulling on astral microtubules, also positions the mitotic spindle in budding yeast and rotates the mitotic spindle during asymmetric cell divisions in higher eukaryotes (Bloom, 2001). Cytoplasmic dynein powers minus-end-directed membrane transport in *Dictyostelium* and filamentous fungi (Wedlich-Soldner et al., 2002), as it does in animal cells (Hirokawa, 1998). *Chlamydomonas* also possesses another cargo-transporting DHC that moves IFT particles in the retrograde direction from the axoneme tip to the basal body (Porter et al., 1999; Pazour et al., 1999). This motor is referred to as cytoplasmic dynein 1b or 2. However, as this dynein functions primarily within the axoneme and segregates from cytoplasmic dynein in phylogenetic trees, I refer to this motor as IFT dynein in this review. Cilia-containing metazoan organisms also have a single IFT dynein gene that is needed for axoneme maintenance (Mikami et al., 2002; Signor et al., 1999a), although it might have a minor role in Golgi organization (Grissom et al., 2002).

Cytoplasmic dynein also expanded its roles in metazoans; the huge and growing list of activities attributed to this motor include mRNA localization, intermediate filament transport, nuclear envelope breakdown, apoptosis, transport of centrosomal proteins, mitotic spindle assembly, virus transport, kinetochore functions, and movement of signaling and spindle checkpoint proteins. This enormous breadth of activities is not the result of motor gene expansion, since a single cytoplasmic DHC gene appears to be responsible for virtually all of these transport events. Instead, the association of dynein with its many cargoes appears to be governed by its numerous associated subunits (Figure 1).

The vertebrate cytoplasmic dynein holoenzyme is composed of several tightly associated subunits: the light intermediate chains (DLIC, currently two genes identified with multiple protein isoforms), the intermediate chains (DIC, two genes that give rise to multiple isoforms through alternative splicing and phosphorylation), the Tctex1/rp3 light chains (two genes), the roadblock light chains (two genes), and the LC8 light chains (three genes). The intermediate chain functions as a crucial scaffold in the complex, as it can bind simultaneously to the DHC as well as the three light chain subunits described above and the $p150^{glued}$ subunit of dynactin (Susalka et al., 2002) (Figure 1). The rules for assembling holoenzymes from the multiple types of subunits are not fully understood, although it is likely that several distinct cytoplasmic dynein holoenzymes with different subunit combinations can co-exist within a cell. Accumulating evidence also indicates that many, if not all, of these subunits interact with distinct protein partners, potentially facilitating dynein associations with different cargoes. In addition, subunit isoforms can bind to distinct cargoes (Susalka et al., 2000; Tai et al., 2001; Tynan et al., 2000). For example, the DLIC-1 isoform specifically binds to the centrosomal protein pericentrin (Tynan et al., 2000), and a third, highly divergent DLIC associates specifically with IFT dynein (Grissom et al., 2002; Mikami et al., 2002) and is most likely responsible for docking onto IFT particles.

Although not a constitutively associated subunit, the multisubunit dynactin complex also interacts with cytoplasmic dynein (Hirokawa, 1998). Dynactin appears to play a general regulatory role, since disruption of this complex by overexpression of its dynamitin subunit interferes with most, if not all, transport mediated by cytoplasmic dynein (Burkhardt et al., 1997). Enhancing processive movement of cytoplasmic dynein could represent one such general regulatory action of the dynactin complex (King and Schroer, 2000). However, the dynactin complex also may bind several different protein partners, and by doing so, link dynein to different cargoes. For example, dynactin's actin-related Arp1 subunit binds to Golgi-associated spectrin (Holleran et al., 2001), while the dynamitin subunit binds bicaudal D, a protein that interacts with GTP-loaded Rab6 on membrane vesicles (Hoogenraad et al., 2001). Another important dynein regulatory protein is the lissencephaly-1 (Lis1) protein, which is required for dynein's roles in mitosis and nuclear migration, but not organelle transport (Vallee et al., 2001). Thus, many protein components appear to be involved in dynein-based transport, but much remains to be learned about how each of these components participates in the plethora of activities ascribed to this motor.

Myosin V

The class V myosins were first identified biochemically in vertebrate brain as a myosin-like, calmodulin binding protein and later shown to have motor activity (Cheney et al., 1993). The principal structural/sequence feature that characterizes myosin Vs is a long lever arm helix that is stabilized by binding one essential light chain and five calmodulins (Reck-Peterson et al., 2000) (Figure 1). Myosin Vs have a conserved ~100 residue C-terminal domain (called the dilute, DIL, domain) that is also present in AF6/CNO, a scaffold protein localized at intercellular junctions. Myosin V, at least in vertebrates, binds the same LC8 light chain that is found in cytoplasmic dynein as well as other enzymes such as nitric oxide synthase. This subunit is thought to serve a structural role rather than a cargo binding function.

In lower eukaryotes, the biological functions of myosin Vs have been best studied in *S. cerevisiae* and *S. pombe*. In *S. cerevisiae*, Myo2p delivers various membranes (e.g., secretory vesicles and vacuoles), Kar9 (a protein involved in anchoring microtubles to the bud tip), and Smy1p (a highly divergent kinesin) from the mother to the bud (Reck-Peterson et al., 2000). Elegant mutagenesis experiments discovered that secretory vesicles and vacuole transport utilizes two distinct regions in the Myo2p tail domain (Catlett et al., 2000). The other *S. cerevisiae* class V myosin (Myo4p) transports a subset of mRNAs into the bud (Bertrand et al., 1998). In *S. pombe*, Myo52 (the ortholog of Myo2p in budding yeast) localizes cell wall synthetic enzymes to the tips for polarized growth and orients the mitotic spindle (Win et al., 2002); a deletion of the second *S. pombe* myosin V (Myo51) produces no obvious phenotype. A myosin V-like gene is also present in *Malaria*, but has not been studied.

In metazoans, myosin Vs also are widely used for organelle transport. Documented examples include endoplasmic reticulum movement in squid axoplasm (Tabb et al., 1998), melanosome transport in *Xenopus* melanophores (Rogers and Gelfand, 1998), and the rapid

movement of membranes in plants (the related class XI myosin; Morimatsu et al., 2000). The most detailed understanding of a metazoan myosin V has been obtained in mice, owing to mutations in this gene, which causes pigmentary dilution due to impaired transport of melanosome granules in melanocytes (Reck-Peterson et al., 2000). These "dilute" mice also have nervous system defects that may arise from improper localization of smooth endoplasmic reticulum in dendrites.

Three myosin Vs, which exhibit distinct tissue distributions, appear to contribute to the diversity of cargo transport activities of this motor class in vertebrates. A compelling case for alternative splicing contributing to cargo recognition also has been made for myosin Va (Wu et al., 2002). The transport of melanosomes by this motor has been linked to a specific alternatively spliced exon (exon F) in the myosin V tail that interacts with an adaptor protein (melanophilin) that in turn binds GTP-loaded Rab27a on melanosome membranes (reviewed in Langford, 2002). Mutations in melanophilin and Rab27 produce coat color defects like myosin Va, arguing strongly for the proposed mechanism. Exon B in myosin Va, on the other hand, is neuron-specific and may bind axonal cargo, perhaps via another Rab GTPase.

Versatility of the Motor Toolbox: Filling Distinct Niches in Different Organisms

The genomic data in Table 1, combined with the functional data described above, provides a perspective on how new cargo transporting activities were created during evolution. Unique ("unclassified") motors genes, derived by gene duplication and sequence divergence, are found in all organisms. Some of these motors may transport cargo, although documented examples are few. More commonly, new cargo transporting activities were created through modifications of the Toolbox motors. In some instances, the addition of motor-associated subunits enabled new cargo associations (e.g., conventional kinesin, cytoplasmic dynein), whereas in other cases, gene duplication followed by recombination/divergence of the tail domains expanded the roles of a motor class (e.g., Unc104/KIF1 kinesins and myosin Vs). A single motor gene also can give rise to multiple motors with different cargo specificities by alternative splicing in the tail domain (e.g., vertebrate myosin Va and KIF1B). An expansion of molecular motors through the above mechanisms is likely to be an important component in enabling increasing organismal complexity. For example, *Ciona intestinalis*, a sea squirt that is thought to resemble the ancestral organism that gave rise to the chordate/vertebrate lineage, has significantly more motors than other invertebrates (Table 1) despite having a comparable number of genes (Dehal et al., 2002), and vertebrates further increased their motor inventory.

Despite the conservation of the motor Toolbox throughout eukaryotic evolution, the genomic data in Table 1 shows that different organisms utilize these motors in dramatically different ways. In *Dictyostelium* and filamentous fungi, for example, the majority of organelle transport appears to be driven by kinesins (primarily conventional kinesin and Unc104/KIF1) and cytoplasmic dynein. In *S. cerevisiae*, on the other hand, membranous traffic is driven exclusively by class V myosin, and the cargo-transporting kinesin genes disappeared from the genome.

The motor inventory in *Arabidopsis* reveals an even more remarkable selective use of Toolbox motors. Unlike animal cells, plants primarily use actin filaments for membrane transport, and this is reflected by the considerable expansion of myosin V-type motors (13 genes) in the *Arabidopsis* genome compared with other organisms (Reddy and Day, 2001a). These plant motors are placed in a separate class (class XI) in the literature; however, their long lever arm architecture, conserved DIL domain in the tail, and role in membrane transport arguably place them in the same class as other myosin Vs. *Arabidopsis* contains only one other class of myosin genes (the plant-specific class VIII myosins), and no cytoplasmic myosin II, the motor involved in cytokinesis in fungi and animal cells. Interestingly, *Arabidopsis* possesses more kinesin genes (61) than any other known organism (Reddy and Day, 2001b), yet lacks at least two of the three Toolbox kinesins (kinesin II and Unc104/KIF1). The absence of kinesin II is not surprising, since *Arabidopsis* does not possess ciliated cells, and the membrane transport functions of Unc104/KIF1 may have been taken over by the myosin Vs. A conventional kinesin gene was initially assigned (Reddy and Day, 2001b), but its classification is tenuous from our sequence comparisons, and functional studies will be needed to ascertain if it is a bona fide cargo-transporting motor.

Remarkably, the dynein heavy chain genes are absent from the *Arabidopsis* genome (Lawrence et al., 2001). The situation is not general to all plants, since dynein genes are present in the rice (King, 2002) and tobacco (S. Reck-Peterson and R.V., unpublished data). An *Arabidopsis* dynein gene may yet emerge when remaining sequence gaps are filled. However, the lack of dynactin genes in *Arabidopsis* (Lawrence et al., 2001) is consistent with an absence of cytoplasmic dynein. Given dynein's widespread presence and numerous activities, how has *Arabidopsis* made due without this motor? The answer may lie in the vast expansion of genes encoding C-terminal motor domain kinesins (Kin C motors) in *Arabidopsis* (21 genes compared to 3 in humans) (Table 1). Like dynein, the Kin C motors power minus-end-directed motion along microtubules. One of the Kin C motors (Ncd-type) is involved in mitotic spindle formation. Other Kin C motors may transport membranes, although they appear to play subservient roles to cytoplasmic dyneins in animal cells (Xu et al., 2002). However, in *Arabidopsis*, Kin C motors may have expanded to occupy the transport niches belonging to cytoplasmic dynein in other organisms.

On the opposite end of the spectrum from *Arabidopsis*, the *Giardia* genome contains many dyneins and cargo-transporting kinesins, but no myosins (Table 1). *Malaria*, on the other hand, has 6 myosins (four unusual class XIV myosins involved in host cell invasion; Meissner et al., 2002; one class V myosin, an unclassified myosin, but no cytoplasmic myosin II). *Malaria* lacks identifiable cargo-transporting kinesins. *Malaria* and *Giardia*, therefore, have evolved repertoires of molecular motors that differ dramatically from one another and from most other eukaryotes. These unique motor inven-

tories no doubt reflect the unique cell biologies and life cycles of these organisms.

In summary, cargo transport, as well as other motor functions such as cytoskeletal organization and cytokinesis, have been solved by various unique combinations of kinesins, dyneins, and myosin motors. Unneeded motors appear to have been lost from genomes. The only universally conserved motors in eukaryotes are the mitotic kinesins, and specifically, our analysis indicates that the Eg5/BimC/Ksp-type kinesin and a mitotic KinC-type kinesin may be the only ubiquitous motors in eukaryotes. Thus, the deployment of motors has been much more variable and plastic compared with other highly conserved molecular machines (e.g., polymerases).

Traffic Control: Coordinating the Activities of Multiple Motors on the Same Cargo

To achieve law and order on the intracellular highways, the multiple cargo-carrying motors in a single cell must be regulated. In the majority of animal cells, individual organelles switch frequently between anterograde (microtubule plus-end-directed) and retrograde (minus-end-directed) movement; the relative time spent traveling in these two directions determines the overall steady state distribution of that particular organelle population. This game is not played exclusively by opposite polarity microtubule motors, since regulation of myosin V activity also can influence organelle distribution (Gross et al., 2002a). In most cells, relatively little is known about the regulation and coordination of bidirectional motion. Perhaps the best-studied system is the melanophore, where hormones acting through cAMP-dependent pathways can bias the motion of the pigmented melanosomes in either the anterograde or retrograde direction (Gross et al., 2002a; Rogers and Gelfand, 1998). Other systems that might provide fertile ground for understanding motor coordination are the axon and the flagellum, both of which are highly specialized for long distance transport. As will be described below, individual cargoes move primarily unidirectionally in these extended processes, and a switch in direction occurs when cargoes reach the ends of these elongated structures.

In the flagellum, IFT particles move in continuous streams in the anterograde and retrograde directions along a unipolar array of outer doublet microtubules (Figure 2; Supplemental Movie S1 available at http://www.cell.com/cgi/content/full/112/4/467/DC1). The anterograde and retrograde particles are distinct species: retrograde particles are smaller, more numerous, and move at faster velocities compared with the anterograde particles (Iomini et al., 2001). The lack of back-and-forth motion of IFT particles suggests that either kinesin II or IFT dynein is active on a particular IFT particle, but not both. Moreover, IFT dynein requires heterotrimeric kinesin II to reach the flagellar tip, indicating that it travels as an inactive passenger on anterogradely moving IFT particles (Iomini et al., 2001). Collectively, these observations suggest that heterotrimeric kinesin II delivers IFT particles to the flagellar tip, the IFT particles are then remodeled with some of the protein cargo extracted for incorporation into the axoneme; subsequently, IFT dynein is activated and kinesin II is downregulated in order to recycle the IFT particles back toward the cell body (Iomini et al., 2001). A similar process must then occur at the flagellar base, where new cargo is loaded and heterotrimeric kinesin II and IFT dynein are activated and inactivated respectively. This recycling process can occur for several hours in the absence of protein synthesis (Piperno and Mead, 1997), and some unknown accounting mechanism must keep the flux of material balanced in the two directions. These observations suggest that the regulation of opposite polarity microtubule motors appears to be restricted to specialized "turnaround" zones located at the base and tip of the axoneme.

The nerve axon also is packed with cargo moving either toward the nerve terminal or the cell body along a unipolar microtubule array (Figure 2; Supplemental Movie S2 available at above website). Most of the small organelles travel unidirectionally with infrequent reversals in direction (although mitochondria behave differently, undergoing frequent back-and-forth movement). A subpopulation of cytoplasmic dynein localizes to anterograde-moving vesicles (Li et al., 2000; Susalka et al., 2000), suggesting that it travels (presumably in an inactive form) on anterograde cargo. These observations suggest a similar model to that described above for IFT. Membrane cargo travels unidirectionally using kinesin motors to the nerve terminal; once at this "turnaround" zone, cytoplasmic dynein and kinesin become activated and repressed respectively. A zone for switching the direction of organelle movement also may be created when axonal transport is blocked at a site of nerve injury. After a delay, many vesicles accumulating on the anterograde side of the block reverse their direction and travel back to the cell body (Smith, 1988), where they may instruct the nucleus to respond to the injury. This reversal may be mediated, at least in part, by the recruitment of additional cytoplasmic dynein onto these vesicles (Li et al., 2000).

The microscopic observations of cargo transport in axons and flagella raise a number of similar questions. How do the opposite polarity motors, kinesin and dynein, coordinate their activities? What kind of machinery processes the incoming cargo and switches motor direction at the "turnaround" zones? Molecular answers to these questions are beginning to emerge but are far from complete. A communication mechanism between motors is suggested by findings in several systems showing that inhibition of either kinesin or dynein function impairs both directions of travel (Brady et al., 1990; Deacon et al., 2003; Gross et al., 2002b; Martin et al., 1999). The molecular basis of this cross-talk between the opposite polarity motors is unknown. However, a recent study revealed that dynactin (traditionally thought to only regulate dynein) is required for both anterograde and retrograde movement of *Xenopus* melanosomes (Deacon et al., 2003). At a molecular level, Deacon et al. discovered that heterotrimeric kinesin II and cytoplasmic dynein bind to an overlapping site on the $p150^{glued}$ subunit of dynactin; competition for this dynactin binding site might be involved in coordinating the activities of these motors. It will be interesting to determine whether dynactin plays a similar role in IFT or axonal transport.

Little is known about the molecules that signal to

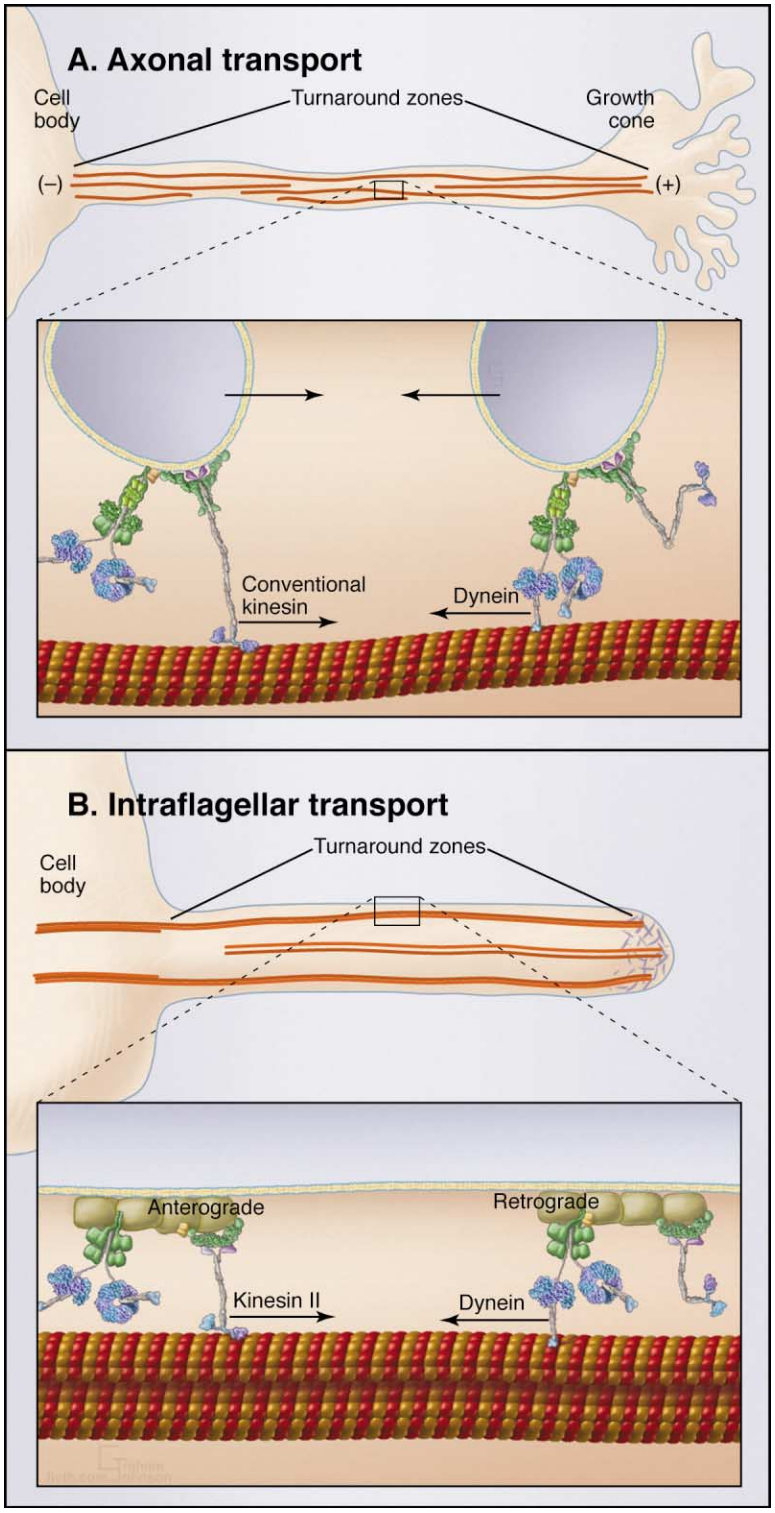

Figure 2. Coordination of Opposite Polarity Motors in Axonal and Intraflagellar Transport

In both transport systems, kinesin motors carry cargo (membrane organelles in the axon and submembranous protein particles in the flagellar axoneme) along a unipolar array of microtubule toward the plus ends. Dynein is carried along with this anterograde cargo in a repressed form, and reversals in the direction of movement are infrequent. At a "turnaround" zone at the tip of these structures, dynein is activated and kinesin is repressed, and the processed cargo then can be transported back toward the cell body. The opposite activation/inactivation of the motors is believed to occur at the base near the cells body. Molecules that mediate the coordination of these opposite polarities motors (here depicted as hypothetical yellow proteins located near the motors at the site of cargo attachment) as well as the switching mechanisms at the "turnaround" zones remain to be elucidated. Movies of axonal and intraflagellar transport are provided in the Supplemental Data available at http://www.cell.com/cgi/content/full/112/4/467/DC1. Prepared by G. Johnson (graham@fivth.com).

motors at the "turnaround" zones, although protein kinases and G proteins represent candidates. Rab GTPases have been found to regulate myosin V (Langford, 2002) and dynein (Jordens et al., 2001; Short et al., 2002), and a small GTPase, IFT27, is a core component of the IFT particles, although its role has not been elucidated (J. Rosenbaum, personal communication). Localization of guanine nucleotide exchange or hydrolysis factors (GEFs or GAPs) to "turnaround zones" could potentially activate or repress motors that require small GTPases. JNK kinases also have emerged as potential regulators of conventional kinesins, since kinesin light chains were shown to bind to JNK kinase scaffolding proteins (the JIPS) (Bowman et al., 2000; Byrd et al.,

2001; Verhey et al., 2001). Kinesin transport is needed for JIP localization (Byrd et al., 2001; Verhey et al., 2001), suggesting that kinesin may be used to localize JNK kinases to nerve terminals where they could phosphorylate their target proteins. A second possibility, however, is that motors themselves are targets for phosphorylation, and the JNK kinases may control the balance of kinesin- and dynein-based membrane transport, perhaps even in response to cell stress or nerve injury.

Human Disease and Intracellular Transport

In addition to their importance for cell biology, studies of intracellular transport and the Toolbox motors are beginning to shed light on several human diseases and suggest therapeutic opportunities.

Diseases Associated with Impaired Cargo Transport

Motor and sensory neurons, with their extremely long axons, might be expected to be particularly sensitive to defects in intracellular transport. This notion first received validation in *Drosophila*, where loss-of-function alleles of conventional kinesin cause motor neuron dysfunction (Hurd and Saxton, 1996). At a subcellular level, these kinesin mutations caused abnormal accumulations of organelles, presumably as the result of impaired transport. The phenotype of these mutant flies suggested that perhaps some human neurodegenerative diseases also might arise from motor protein mutations. Recently, two such examples have been reported. A loss-of-function mutation in the motor domain of the KIF1B gene was found in a subset of families with Charcot-Marie-Tooth type 2A disease (Zhao et al., 2001), and a mutation in the neuronal-specific conventional kinesin KIF5A gene was identified in patients with hereditary spastic paraplegia (Reid et al., 2002). Griscelli's syndrome, a recessive human disease characterized by pigmentation dilution (analogous to the dilute mouse) as well as immunodeficiency and neurological disorders, has been linked to mutations in myosin V and its membrane docking partner Rab27a (Anikster et al., 2002).

In addition to motor protein mutations, protein aggregation, prevalent in many human neurodegenerative diseases, can impede axonal transport. Superoxide dismutase mutants that produce aggregation and cause amyotrophic lateral schlerosis impair axonal transport when engineered into transgenic mice (e.g., Warita et al., 1999). Overexpression of amyloid precursor protein (Gunawardena and Goldstein, 2001) or tau (Stamer et al., 2002), both of which give rise to protein aggregates in patients with Alzheimer's disease, also preferentially inhibit anterograde axonal transport. Whether defective axonal transport is an early trigger for human neurodegenerative disorders or is an end stage consequence of this process, however, requires further investigation.

Motile and primary (immotile) cilia represent other structures that are highly reliant upon motor-driven cargo transport. Interruption of intraflagellar transport by mutations or deletions of motor or IFT particle proteins in mice gives rise to several physiological defects including left-right asymmetry defects (situs inversus) (Marszalek et al., 1999; Nonaka et al., 1998), photoreceptor cell degeneration and death (Marszalek et al., 2000; Pazour et al., 2002), and polycystic kidney disease (due to loss of primary cilia in kidney epithelial cells) (Pazour et al., 2000). While many ciliary diseases have been described in humans, none yet have been linked to defects in IFT. However, this situation is likely to change as several human diseases (e.g., polycystic kidney diseases and retinopathies) come under closer scrutiny.

Targeting Motors for Pharmacological Therapy

The motors also might constitute future targets for pharmacological therapy. The ability to inhibit a specific molecular motor in cells with a small molecule has been demonstrated for the mitotic kinesin Eg5/Ksp (C. Beraud et al., submitted; Mayer et al., 1999). Small molecule inhibitors of Eg5/Ksp with low nanomolar affinity have anti-tumor activity (C. Beraud et al., submitted), and one such agent has entered phase I clinical trials for cancer. In most cases, inhibition of cargo transporting motors would not be desirable. However, one could imagine certain circumstances where a therapeutic benefit might be derived. For example, impairing motor-driven delivery of MHC-peptide complexes to the surface of dendritic cells (Boes et al., 2002; Chow et al., 2002) or the cell surface delivery of cytotoxic granules in T cells could provide a strategy for immunosuppression. In addition, inhibition of highly divergent, "organism-specific" motors in fungi and parasites could be used to treat infections (Meissner et al., 2002). Drugs that activate cargo-transporting motors also might be beneficial in neurodegenerative diseases, especially as small effects might help to restore a misbalance between anterograde and retrograde transport.

Targeting the cargo binding mechanism of molecular motors offers other potential therapeutic strategies. For example, several viruses (e.g., neurotrophic viruses and HIV) hijack microtubule-based transport systems. As mechanisms for motor-virus attachments become elucidated (Rietdorf et al., 2001), it might be possible to interfere with these associations and provide therapeutic benefit. Adopting the strategy of viruses, a therapeutic agent with an aptamer that binds to a motor tail domain might be localized and concentrated intracellularly (e.g., near the nucleus or within the cell body of neurons), which might result in improved efficacy or therapeutic index of certain agents. The feasibility of modulating cargo binding in such a manner, however, awaits a deeper understanding of this aspect of motor biology.

Conclusions

Fifteen years ago, only a few molecular motors were known. In contrast, complete inventories of molecular motors are now available in a number of diverse organisms. While these remarkable accomplishments have answered many questions, the genomic inventories also have exposed many areas of ignorance. The unusual collections of motors in *Arabidopsis*, *Giardia*, and *Malaria* certainly highlight how little we know about intracellular transport in plants and parasites compared with animal cells. Understanding motors in such organisms is likely to provide general insights into how transport processes are used in biology. Moreover, we now have a glimpse of the many motor isoforms that were created by gene duplication, alternative splicing, and unique associated subunits. What is still missing is a global understanding of how these and other mechanisms guide

motors to the vast number of possible cargoes and how cells adjust motor activities to achieve the correct distributions of intracellular organelles and proteins. Clearly, such issues constitute the next frontier of research. Unraveling these secrets will be facilitated by carefully examining how evolution has conserved or varied different regions of motor proteins and linking this information to the biological roles of motors in different organisms.

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