Microtubule-Nucleus Interactions in *Dictyostelium discoideum* Mediated by Central Motor Kinesins†‡

Irina Tikhonenko,1 Dilip K. Nag,1,2 Douglas N. Robinson,3 and Michael P. Koonce1,2*

Division of Translational Medicine, Wadsworth Center, Albany, New York 12201-0509;1 Department of Biomedical Sciences, School of Public Health, University at Albany, Albany, New York 12201-0509;2 and Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

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Kinesins are a diverse superfamilly of motor proteins that drive organelles and other microtubule-based movements in eukaryotic cells. These motors play important roles in multiple events during both interphase and cell division. *Dictyostelium discoideum* contains 13 kinesin motors, 12 of which are grouped into nine families, plus one orphan. Functions for 11 of the 13 motors have been previously investigated; we address here the activities of the two remaining kinesins, both isoforms with central motor domains. Kif6 (of the kinesin-13 family) appears to be essential for cell viability. The partial knockdown of Kif6 with RNA interference generates mitotic defects (lagging chromosomes and aberrant spindle assemblies) that are consistent with kinesin-13 disruptions in other organisms. However, the orphan motor Kif9 participates in a completely novel kinesin activity, one that maintains a connection between the microtubule-organizing center (MTOC) and nucleus during interphase. kif9 null cell growth is impaired, and the MTOC appears to disconnect from its normally tight nuclear linkage. Mitotic spindles elongate in a normal fashion in kif9−/− cells, but we hypothesize that this kinesin is important for positioning the MTOC into the nuclear envelope during prophase. This function would be significant for the early steps of cell division and also may play a role in regulating centrosome replication.

Directed cell migration, organelle transport, and cell division involve fundamental motilities that are necessary for eukaryotic cell viability and function. Much of the force required for these motilities is generated through the cyclical interactions of motor proteins with the cell cytoskeleton. Microtubules (MTs) and actin filaments provide structural support and directional guides, and all eukaryotic organisms have diverse, often extensive families of motors that carry out different tasks. Functional studies have revealed that many of the motors work in combination with others, and that the individual deletion of a single motor activity often is insufficient to produce a defect that substantially impairs cell growth or function. The latter phenomenon is particularly evident in some organisms with simple motor families (14, 42). By contrasting homologous motor functions between simple and complex systems, we hope to learn the details of how each motor is custom-tuned for specific tasks.

*Dictyostelium discoideum* is a compact amoeba that exhibits robust forms of motility common to nearly all animal cells, with speeds that frequently exceed corresponding rates in vertebrate cell models (25, 33, 54). Since *Dictyostelium* possesses a relatively small number of motor proteins (13 kinesin, 1 dynein, and 13 myosin isoforms [23, 24, 26]), it combines advantages of terrific cytology with straightforward molecular genetics and thus represents an excellent model to investigate individual and combined motor protein actions. To date, 11 of the 13 kinesin motors have been analyzed functionally (5, 17, 18, 30, 42, 46, 51, 60). Only 1 of these 11 motors, Kif3, a member of the kinesin-1 family of organelle transporters, appears to be essential for organism viability (51). Individual disruptions of three kinesin genes (*kif1*, *kif4*, and *kif12*) produce distinctive defects in cell growth or organelle transport (30, 42, 46). Analyses of six of the seven other kinesins reveal important phenotypes but only when combined with other motor disruptions or cell stresses. We address here the roles of the remaining two *Dictyostelium* MT-based motors.

*kif6* and *kif9* encode two central motor kinesins in the *Dictyostelium* genome (24). The best-studied isoforms of this motor type are represented by the kinesin-13 family, and they largely function to regulate MT length during cell division (13, 16, 40, 41). In some organisms, kinesin-13 motors also have been shown to operate during interphase and to mediate MT and flagellar length control (3, 4, 15) and perhaps even organelle transport (32, 43, 56). *kif6* encodes the kinesin-13 family member in *Dictyostelium*. We demonstrate that Kif6 activity is essential for viability, and that it plays a primary, conserved role in chromosome segregation during cell division.

The second of the central motor kinesins, Kif9, does not group with an existing family (24, 38). The gene disruption of this motor reveals a completely novel function for a kinesin in maintaining a connection between the MT-organizing center (MTOC) and the nucleus. By electron microscopy (EM), the MTOC of *Dictyostelium* appears as a cytoplasmic cube-shaped structure surrounded by amorphous dense material (39, 44). EM, biochemical analyses, antibody labeling, and live-cell imaging studies have demonstrated that during interphase, the cytoplasmic MTOC is firmly and closely attached to the nucleus (28, 29, 44, 48, 49, 63). Upon entry into mitosis, the

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* Corresponding author. Mailing address: Wadsworth Center, Empire State Plaza, P.O. Box 509, Albany, NY 12201-0509. Phone: (518) 486-1490. Fax: (518) 474-7992. E-mail: Koonce@wadsworth.org.
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MTOC duplicates during prophase and is brought to or into a fenestration of the nuclear envelope, and then it establishes an intranuclear bipolar spindle for division (39, 53, 64). While MTOCs can be purified from Dictyostelium, the methods rely heavily on reagents that actively disrupt the attached nuclei (10, 59). A recent study has identified at least one component of this connection, the nuclear envelope protein Sun-1 (67).

We demonstrate in the current work that the disruption of the kif9 gene (DDB0185204) was obtained from http://dictybase.org/. For homologous recombination, we used a Stock Center (http://dictybase.org/StockCenter/StockCenter.html). The kif9 construct was designed to terminate message coding at H299, a position well upstream of the recombination site (6, 42). The PCR amplification of a 0.9-kb target, with a primer upstream of the recombination site (+3 to +1134) with the 1.6-kb blasticidin cassette (BsrI) was blunt/sticky-cloned into BglII/MluI sites in the MB38 plasmid (2). Southern blotting was performed using chemiluminescence procedures (ECL; Amersham Biosciences), and the blot was probed with the initial 1.2-kb amplified genomic target of kif9 (for kif9, bp +1894 to +2455) was amplified with primers containing 5′ NotI and 3′ MluI restriction sites and was cloned in reverse orientation relative to the actin-15 transmembrane domain.

FIG. 1. Sequence analysis of the two central motor kinesins of Dicyostelium. (A) Cartoon graphic and sequence of Kif6 (kinesin-13). The motor domain is highlighted in blue. Note the extensive repetition of amide residues (N and Q). (B) Conserved domain alignments at the amino-terminal and carboxy-terminal regions of the Kif6 polypeptide. Green boxes highlight identical residues in at least three sequences. Dd, Dictyostelium discoideum (XP_001709959.1); Gi, Giardia lamblia (XP_001026192.1); Lb, Leishmania braziliensis (XP_001563287.1). (C) Cartoon graphic and sequence of the orphan kinesin Kif9. The motor domain is in blue; the sequence highlighted in orange indicates the position of a transmembrane domain.

MATERIALS AND METHODS

**Microbial genetics.** The sequence of the kif9 gene (DDB0185204) was obtained from http://dictybase.org/. For assembly of the disruption construct, we used the following primer combinations to amplify a 1.2-kb genomic fragment from AX-2 cell DNA: forward, 5′ (TGAATCCCTACCATCACACAATTAGGGTCATCAGGTTATGGG) (for Kif6, bp +1894 to +2455) was amplified with primers containing 5′ NotI and 3′ MluI restriction sites and was cloned in reverse orientation relative to the actin-15 promoter in pDL1A15NSN (50). The second segment (+2095 to +2455) was amplified with primers containing 5′ NotI and 3′ MluI restriction sites and was cloned downstream but in a forward orientation adjacent to the initial segment. Expression should yield a single RNA transcript that folds into a hairpin, with a large unpaired loop (for kif6, 165 bases). An identical strategy was followed for the dynein heavy chain and kif3 hairpin constructs. For inducible expression, the entire hairpin loop cassette was excised with SalI and MluI digestion and then blunted/sticky-cloned into BglII/MluI sites in the MB38 plasmid (2). Standard molecular biology procedures were followed for DNA isolation, manipulation, and blotting. The kif9 Southern blotting was performed using chemiluminescence procedures (ECL; Amersham Biosciences), and the blot was probed with the initial 1.2-kb amplified genomic target of kif9.

Quantitative reverse transcription-PCR (RT-PCR) was performed using a LightCycler instrument (Roche Applied Science). RNA-free total RNA was isolated from cells with an RNeasy kit (Qiagen). For each reaction, 1 μg of RNA was transcribed into cDNA (Omniscript RT kit; Qiagen); 1/50 of this product was used for reverse transcription-PCR (RT-PCR) with the SYBR green JumpStart Taq ReadyMix kit (Sigma Chemical Co). Primers and conditions were optimized to produce single bands of predicted sizes, amplifying targets of 300 to 400 bp located outside of the conserved motor domains. Primer sequences and PCR conditions are available upon request.
constitutively active actin-15 promoter would drive their ex-
cloned into the pLD1A15SN plasmid (50), where the robust,
generated fragments by following the strategy depicted in Fig.
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RNA was particularly effective in blocking cell growth. Long
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RNA was particularly effective in blocking cell growth. Long
hairpin RNAi (lhp) constructs were assembled from PCR-
generated fragments by following the strategy depicted in Fig.
S1 in the supplemental material (9, 36). These constructs were
cloned into the pLD1A1SSN plasmid (50), where the robust,
constitutively active actin-15 promoter would drive their ex-
pression. In none of the multiple transformation attempts with
the kif6 lhp construct did we recover any viable colonies of
cells. As controls, we also used lhp constructs for the dynein
heavy chain and the kinesin-5 family member kif13. Dynemin is
an essential protein in Dictyostelium (27, 34), while kif13 can be
deleted without a significant growth penalty (60). The trans-
formation of AX-2 cells with a similarly designed dynein lhp
(see Fig. S1 in the supplemental material) produced the same
lethal result (i.e., no viable colonies) as the kif6 constructs,
whereas transformation with the kif13 lhp routinely produced
dozens of viable colonies per 10-cm dish. These results suggest
that the expression of the kif6 lhp is lethal in Dictyostelium.

Since Dictyostelium transfection is not particularly efficient,
it is not possible to examine individual cells that are trans-
formed with the kif6 lhp and potentially are dying, against a
background of 10^4 to 10^6 nontransformed cells that are sensi-
tive to the G418 antibiotic. As an alternative strategy, we uti-
лизирова the same kif6 and kif13 lhp constructs in the tetracycline-
regulated tetOFF plasmid MB38 (2). By waiting 24 h
posttransfection before removing tetracycline and inducing
kif6 lhp expression, we were able to recover multiple colonies

**RESULTS**

**kif6 and kif9 each encode a central motor kinesin.** The kif6
gene encodes a 1,030-amino-acid polypeptide that contains a
central motor domain (residues 459 to 776) homologous to
members of the kinesin-13 family (Fig. 1A). The motor domain
sequence is most similar to the vertebrate KIF24 subfamiliy, yet
it is positioned in the center of the molecule, as is commonly
found for KIF2 subfamily members (38). Outside of the motor
domain, there are only two short stretches of sequence similar
to other kinesin-13 proteins, an amino-terminal domain
(Dd 8-64) that has homology to a sterile alpha motif (58) and a
short carboxy-terminal region (Dd 919-976) (Fig. 1B). A striking
feature of the Kif6 sequence is the extensive stretches of
repeated amide residues (asparagine and glutamine). Together
these two amino acids account for 29% of the Kif6 polypeptide
(37% total outside of the motor domain), including a 37-
residue stretch of asparagines and a region where 44 of 51
residues are glutamines. Dictyostelium polypeptides are notably
rich in homopolymer tracts of these two amino acids, and thus
the repetitions noted in Kif6 are unlikely to be a product of
sequencing or annotation errors (7). In fact, the Kif6 sequence
outside of the repetitive tracts may be particularly useful in
defining key functional domains of other kinesin-13 motors.
The kif9 kinesis also contains a central motor domain (resi-
dues 356 to 720, out of 1,222) (Fig. 1C). However, unlike the
other 12 Dictyostelium kinesin motors, Kif9 does not group
with previously recognized kinesin families (38). On the car-
boxy-terminal side of the motor domain, there is a region
predicted to form an alpha-helical coiled-coil domain as well as
a transmembrane domain (residues 1182 to 1204) that may be
significant in forming motor-to-cargo linkages.

**kif6 is an essential Dictyostelium gene.** We screened Dictyostelium
AX-2 cells with a collection of kinesin RNA interference
(RNAi) constructs and found that the targeting of the dictyostelium
RNA was particularly effective in blocking cell growth. Long
hairpin RNAi (lhp) constructs were assembled from PCR-
generated fragments by following the strategy depicted in Fig.
S1 in the supplemental material (9, 36). These constructs were
cloned into the pLD1A1SSN plasmid (50), where the robust,
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**FIG. 2.** MT arrays in kif6 lhp cells. (A to C) Representative inter-
phase wild-type (A) and kif6 lhp cells (B and C). There are no obvious
differences in MT or chromatin patterns. (D to L) Mitotic figures in
kif6 lhp cells. Panels D to F show normal-appearance mitotic spindles
in metaphase (D), late anaphase (E), and late telophase (F) (a binu-
cleate cell). (G to L) Aberrant spindle formations due to Kif6 knock-
down. (G and J) Malformed metaphase arrangements; (H and K)
lagging chromosomes and extracrytoplasmic MTs in late anaphase; (I
and L) abnormal binucleate spindle and chromatin figures in late
telophase. MTs are in green, DNA is in blue. Scale bar = 5 μm.
of viable kif6 lhp cells. However, these cells grow poorly. Under the same conditions, there were ~10-fold more colonies formed on kif13 lhp control dishes than on the kif6 lhp dishes. After 1 week in the absence of tetracycline, the kif13 lhp dishes were confluent, while the kif6 transformants covered only 10 to 20% of the dish surface. The two cell strains grew equally well in the continued presence of tetracycline, indicating that kif6 lhp expression negatively affects cell growth. To demonstrate that the kif6 lhp was effective in reducing endogenous levels of kif6 mRNA, we measured message levels in cells using quantitative RT-PCR. Cells were transfected with the kif6 lhp and grown to near confluence in the presence of tetracycline. At this point, cells were washed free of tetracycline to induce hairpin expression. After 19 h of induction, the kif6 lhp cell population showed a 50% reduction in kif6 mRNA relative to wild-type levels in control (MB35) cells (data not shown). This result indicates that the kif6 lhp construct does function to reduce the level of endogenous message and therefore is likely to negatively affect Kif6 protein functioning.

We further examined MT arrays in the kif6 lhp cells. While

| Table 1. Percentages of mono- and multinucleated cells |
|---------------------------------|-------|-------|-------|--------|--------|
| Cell type | Mononucleate | Binucleate | Trinucleate | Other (%) | Total no. of cells |
| Wild type  | 64   | 26    | 6     | 4      | 150    |
| kif9       | 66   | 22    | 6     | 6      | 167    |

FIG. 3. Gene knockout (KO) of kif9. (A) Schematic representation of a recombination construct designed to integrate into the kif9 locus and disrupt expression. (B) Southern analysis of wild-type (WT) and kif9 KO transformant DNA. Fragment mass shift in an EcoRI digest (1.7 to 3.1 kb) and the appearance of a new, 1.5-kb band in a BglII digest are exactly as predicted for marker insertion (see panel A). To reduce congestion, only a partial listing of the Lambda DNA-BstEII mass markers are shown. (C) Growth of kif9 KO cells, demonstrating a substantially reduced rate compared to that of wild-type cells. Error bars indicate standard deviations.

FIG. 4. kif9 knockout decouples MTOCs from nuclei. (A to C) Three examples of wild-type interphase cells that show the typical tight association between the MTOC (marked with arrowheads) and nucleus. (A) Mono-nucleate cell with a single MTOC; (B) binucleate cell with two MTOCs; (C) tetranucleate cell with four MTOCs. (D to F) Similar mono-, bi-, and tetranucleate kif9 KO cells. Most MTOCs appear spatially separated from the nucleus. (G to J) Mono- and binucleate kif9 KO cells overexpressing the dynein motor domain. Note the characteristic comet-like appearance of the motile MT array and the enhanced separation between the nuclei and MTOCs. MTs are in green, DNA is in blue. Scale bars = 5 μm.
interphase MT arrays appeared identical to those found in wild-type cells, significant defects were evident in mitotic spindle assemblies in the *kif6* lhp cells (Fig. 2). Examples of spindle malformations include aberrant MT arrangements, extra spindle MTs, and, in particular, lagging chromosomes during anaphase/telophase. In total, obvious defects were found in 38% (26/68) of the mitotic *kif6* lhp cells; 62% of the cells had normal-appearing mitotic figures. This difference likely reflects the known variations in individual cell expression levels for this promoter sequence (2). None of the aberrant spindle morphologies were seen in wild-type AX-2 cells, and they are consistent with the defects described for the kinesin-13 family of motors in vertebrate cells. Thus, these data, taken together, indicate that *Kif6* should be considered essential for viability in vertebrate cells. Thus, these data, taken together, indicate that *Kif6* should be considered essential for viability in vertebrate cells.

**TABLE 2. Number of MTOCs in mono- and multinucleate cells**

| No. of MTOCs | No. of cells |
|--------------|--------------|
|              | Wild type | *kif9* | Wild type | *kif9* | Wild type | *kif9* |
| 1            | 96        | 106    | 1        | 7      | 0        | 0      |
| 2            | 0         | 4      | 38       | 24     | 0        | 0      |
| 3            | 0         | 0      | 0        | 5      | 9        | 6      |
| 4            | 0         | 0      | 0        | 1      | 0        | 4      |

A closer examination of the MT array revealed a striking difference from wild-type cells. In wild-type cells, the MTOC is tightly associated with the nucleus; by our light microscopy analysis, 97% of MTOCs were found within a 1-μm distance of the nucleus (118/122 nuclei; also see reference 39). MTOCs are further maintained at a strict 1:1 MTOC/nucleus ratio, even in multinucleated cells (Fig. 4A to C; Tables 1 and 2). In the absence of *Kif9*, the MTOC could be found spatially separated from the nucleus (Fig. 4D to F). The extent of separation was variable; occasionally the nuclei and MTOCs were found on opposite sides of the cytoplasm. Forty-nine percent of the MTOCs in *kif9*− cells (71/146 nuclei) were greater than 1 μm distant from the nucleus. This separation was further accentuated in *kif9*− cells that contain excess dynein motor (Fig. 4G to J), a dominant-negative effect that stimulates MTOC movement through the cytoplasm (28).

In terms of MT number, length, and arrangement, the interphase MT network in *kif9*− cells otherwise appeared identical to wild-type cells, indicating that MT nucleation is not affected by the loss of *Kif9*. However, the *kif9*− cells showed further variability in the nucleus/MTOC ratio (Tables 1 and 2). While 99% of wild-type cells contained a single MTOC-nuclei pair, >13% of *kif9*− cells contained an abnormal ratio; for example, binucleate cells contained from 1 to 4 distinct organizing centers (Tables 1 and 2). Using a Fisher’s exact test (a two-by-two contingency table), the two-tailed *P* values for both the MTOC-nuclear separation data and MTOC number per nuclei were 0.0001, indicating that these differences between *kif9*-null and wild-type cells are statistically significant. These results suggest that *Kif9* not only functions in maintaining a close physical connection between the nucleus and MTOC in interphase cells but also that this connection is important in the strict control of the number of MTOCs present in the cytoplasm.

The imaging of live *kif9*− cells transformed with a GFP-tubulin expression construct revealed a dynamic association of the MTOC with the nucleus during interphase (Fig. 5). As in wild-type cells, examples could be found with the MTOC sufficiently tethered to the nucleus to produce nuclear distortion upon MTOC movement. Other cells showed no obvious connection between the MTOC and nucleus. In these cases, the MTOC underwent transient back-and-forth motions, but there were no corresponding shape changes or movement of the MTOC-nucleus connection in live cells. (A to F) Sequence of a binucleate *kif9*− cell with two MTOCs, in which the movements of the MTOCs appear independent of the nuclei. (G to L) Control panel showing the close apposition of a single MTOC with the nucleus during identical time frames. Nuclei are outlined in white, and the GFP-labeled MTOCs are highlighted with arrowheads in panels A and G. Time is in seconds. Scale bar = 5 μm.
nuclear envelope to indicate linkage. We could discern a number of intermediate cases where the MTOC appeared to attach and detach from the nucleus or where a single MTOC appeared to associate with more than one nucleus in a multinucleated cell. These examples further support the role of Kif9 in maintaining a connection between the MTOC and nucleus but also demonstrate that Kif9 is not the sole means of attachment.

Mitotic spindle elongation appears normal in \textit{kif9}^{-} cells. We monitored the spindle assembly process in \textit{kif9}^{-}/GFP-tubulin cells. In all cases (12/12) where a bipolar spindle was observed, the spindle continued on through division in a manner indistinguishable from the process in wild-type cells (Fig. 6). Spindle elongation proceeded at a rate of $2.1 \pm 0.5 \ \mu m/min$ and achieved an average length of $11.0 \ \mu m$ before breakage ($n = 6$) (wild-type spindles elongate at $1.6 \pm 0.2 \ \mu m/min$ and attain a length of $10.9 \ \mu m$; these data were taken from reference 60). In no case was there any evidence for lagging chromosomes, a phenomenon observed in knockdowns of the other central motor kinesin, Kif6. This result indicates that Kif9 does not play a significant role in spindle elongation or function.

However, there were a few interesting observations during the earliest stages of mitosis. We viewed a number of cells with supernumerary MTOCs entering mitosis ($n = 10$). In these cells, all of the MTOCs had lost their cytoplasmic MTs, but only those MTOCs that appeared properly associated with the nucleus went on to develop a bipolar spindle (Fig. 7). Second, we also found a couple of double-spindle arrangements. Multiple MTOCs appear to have been tethered to the nucleus upon mitosis; at least two in Fig. 7 appear to have duplicated
and assembled independent spindles. This result suggests that there are multiple MTOC docking sites on the nucleus. Finally, despite the consequent strong growth defect and potential for multipolar divisions, the $kif9$ deletion did not appear to increase the ploidy of cells. The percentages of single-, bi-, and tri-nucleate cells in the $kif9^{-}/H11002$ population remain unchanged from the proportions seen in wild-type cells (Tables 1 and 2).

**DISCUSSION**

We have individually disrupted functions of the two central motor kinesins in *Dictyostelium* and have shown here that they participate in independent MT-nuclear-based processes. Kif6 (of the kinesin-13 family) appears essential for viability, with a role in chromosome segregation during cell division; Kif9 (an orphan) participates in maintaining a connection between the MTOC and nucleus during interphase. On a broader scale, the present work completes a survey of the individual kinesin motor actions in *Dictyostelium*, as summarized in Fig. 8.

Eight *Dictyostelium* kinesins (Kif2, Kif4, Kif6, Kif8, Kif10, Kif11, Kif12, and Kif13) contain sequence homology with families known to participate in at least some aspect of cell division (24). However, only Kif6 appears to be absolutely required for mitosis (18, 30, 42, 60). The defects seen here in kif6 lhp cells (lagging chromosomes, malformed spindles, and extraneous cytoplasmic MTs) are very similar to phenotypes associated with disruptions of kinesin-13 motors in other organisms (40), indicating a likely role in coordinating kinetochore-MT attachments and spindle MT depolymerization. Defects associated with kif12 (encoding the kinesin-6 isoform of the MKLP family) knockout in *Dictyostelium* (such as cytokinesis and spindle assembly defects) (30) also are comparable with this motor’s known biological activities in other systems (see, e.g., reference 37). Thus, kinesin-13 and kinesin-6 isoforms appear to perform similar functions in a wide range of organisms and diverse spindle configurations. However, in contrast to their essential roles in vertebrate cells (19, 45, 47, 65), Kif13 (the kinesin-5 isoform of the BimC/Eg5 family), Kif4 (the kinesin-7 isoform of the CENP-E family), and Kif2 (the kinesin-14 isoform of the NCD/Kar3 family) are not strictly required for division in *Dictyostelium*. In addition, there are no obvious mitotic defects in knockouts of the other three kinesins, Kif8 (the kinesin-4 isoform of the chromokinesin family), Kif10 (the kinesin-8 isoform of the Kip3 family), and Kif11 (a truncated kinesin-7 family member) (42). Thus, the relatively small, compact intranuclear spindle of *Dictyostelium* offers an opportune model to compare and contrast the evolution of essential mitotic motor activities.

![FIG. 8. Summary of the kinesin motor family in *Dictyostelium*. For each kinesin, the motor domains are shown in blue, and the green represents flanking neck and tail domains (drawn to scale). KO, knockout. Numbers in parentheses reference the following texts: 1, reference 51; 2, 17; 3, 5; 4, 46; 5, 60; 6, 30; 7, 42; 8, data presented in this paper; 9, 18. This figure was inspired from reference 24 and adapted with permission from reference 42.](image)
The second central motor kinesin in Dictyostelium, Kif9 (orphan), functions in a nuclear role completely separate from that of Kif6, to form at least part of the linkage between the interphase nucleus and the MTOC. Previous structural studies have described 4- to 5-nm-diameter fibers connecting the centrosome and nuclear envelope, and the tight linkage between these two objects is maintained even in the presence of MT poisons (29, 44). Thus, it is unlikely that the MT system comprises the sole physical connection between the nucleus and MTOC. However, several interesting observations can be made here. Live-cell imaging indicates that in the absence of Kif9, the nucleus-MTOC connection is dynamic: MTOCs are seen either closely tethered to or spatially separated from the nucleus, and they appear to attach and detach from this organelle. Thus, at one level, the Kif9 activity appears to reinforce a tether already formed through another mechanism.

Sun-1 recently has been identified in Dictyostelium (67) as an inner nuclear membrane component of the KASH/SUN pathway of transnuclear linkers (21, 57, 62). The reduced activity of Sun-1 through dominant-negative fragment expression or RNAi produces defects similar to the deletion of Kif9, namely centrosome-nuclear separation and centrosome hyperamplification. Efforts currently are under way to determine whether Kif9 operates in the same pathway as Sun-1 or whether it defines a completely independent mechanism of anchoring the MTOC to the nucleus. A plausible mechanism of action for Kif9 involves a central motor MT depolymerase activity. If Kif9 functions similarly to MCAK-type kinesins (41), then it should bind MTs, effect plus-end depolymerization, and thus progressively move toward the MTOC. If Kif9 were connected to the nucleus, the MT depolymerization activity would provide a constant traction force to keep the nucleus closely apposed to the MTOC. We are working to test this idea to address whether Kif9 moves along or depolymerizes MTs, as well as to determine the function of the protein’s carboxy-terminal transmembrane domain.

In maintaining a nucleus-MTOC connection during interphase, Kif9 has a function thus far undescribed for kinesin motors. It could be argued that because of Kif9’s orphan status, this protein’s function is important only in organisms with similar close linkages between the nucleus and MTOC (11, 12, 52). However, even in vertebrate cells, the centrosome/MTOC is not completely independent of the nucleus, and motors such as dynein have been implicated in nuclear motility and envelope disruption in a number of different organisms (1, 31, 35, 55, 61, 66). Our work could further point toward a general requirement of multiple motor interactions that link nuclei and MTOCs. We suggest that Kif9 plays a role in the transition from interphase to mitosis. Upon entry into mitosis, the MTOC must be positioned into the nucleus for spindle assembly. Movement driven by Brownian motion and guided by fibrous tethers may indirectly dock the centrosome into the nucleus, a mechanism that would account for some cell growth in the absence of Kif9. However, a dedicated motor activity that pulls the MTOC into the nucleus would significantly increase the fidelity of this process, ensuring centrosome duplication and spindle assembly in the proper nuclear environment. We are currently testing this hypothesis.

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