Kinesin is essential for cell morphogenesis and polarized secretion in *Neurospora crassa*

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Kinesin is a force-generating molecule that is thought to translocate organelles along microtubules, but its precise cellular function is still unclear. To determine the role of kinesin *in vivo*, we have generated a kinesin-deficient strain in the simple cell system *Neurospora crassa*. Null cells exhibit severe alterations in cell morphogenesis, notably hyphal extension, morphology and branching. Surprisingly, the movement of organelles visualized by video microscopy is hardly affected, but apical hyphae fail to establish a *Spitzenkörper*, an assemblage of secretory vesicles intimately linked to cell elongation and morphogenesis in *Neurospora* and other filamentous fungi. As cell morphogenesis depends on polarized secretion, our findings demonstrate that a step in the secretory pathway leading to cell shape determination and cell elongation cannot tolerate a loss of kinesin function. The defect is suggested to affect the transport of small, secretory vesicles to the site involved in protrusive activity, resulting in the uncoordinated insertion of new cell wall material over much of the cell surface. These observations have implications for the presumptive function of kinesin in more complex cell systems.

Keywords: kinesin/microtubules/*Neurospora crassa*/null mutant/organelle movement

Introduction

Kinesin is a microtubule-dependent mechanochemical enzyme that moves towards the plus-end of microtubules (Brady, 1985; Scholey *et al.*, 1985; Vale *et al.*, 1985; for a review, see Bloom and Endow, 1995). Though conventional kinesin appears to be the most abundant and most readily isolated kinesin isoform in the cytoplasm (Bloom and Endow, 1995), its precise function in microtubule-dependent activities has remained somewhat elusive. Several lines of evidence strongly suggest that kinesin is associated with membranous organelles in the cell and plays an important role in intracellular movements. For example, although kinesin can be isolated from cytoplasmic extracts, a large proportion of the total kinesin is organelle-bound (Hollenbeck, 1989; Elluru *et al.*, 1995). Ligation experiments on axons *in vivo* demonstrate an

association with anterogradely moving organelles that accumulate proximal to the ligation site (Hirokawa *et al.*, 1991). Immunolocalization in a variety of cell types at both the light and electron microscopic level reveals a punctate or vesicular staining pattern that is sensitive to detergent treatment (e.g. Pfister *et al.*, 1989; Brady *et al.*, 1990; Wright *et al.*, 1991; Leopold *et al.*, 1992). Finally, perfusion of anti-kinesin antibodies into squid axoplasm (Brady *et al.*, 1990) or microinjection of antibodies into cultured cells (Hollenbeck and Swanson, 1990; Lippincott-Schwarz *et al.*, 1995) interferes with the traffic of a variety of membrane compartments.

Whereas a role for kinesin in membrane traffic is highly likely, it is still not clear exactly which organelles kinesin moves or which other roles it might play. As a potentially powerful tool to clarify the function of kinesin *in vivo*, mutations of the kinesin heavy chain were generated in *Drosophila* (Saxton *et al.*, 1991) and *Caenorhabditis elegans* (Patel *et al.*, 1993). These mutations resulted in altered embryogenesis, abnormal behavior, impaired action potential propagation and neuromuscular defects (Saxton *et al.*, 1991; Gho *et al.*, 1992; Patel *et al.*, 1993). In both species, kinesin is an essential protein whose deletion leads to larval death. However, the complexity of both the *Drosophila* and *C.elegans* mutants has so far precluded a full understanding of kinesin function in organelle transport in these two model organisms.

Further attempts to generate kinesin 'null' cells in which the role of kinesin can be studied at the cellular level took advantage of antisense oligonucleotides. Suppression of kinesin in cultured hippocampal neurons (Ferreira *et al.*, 1992; Feiguin *et al.*, 1994) or retinal ganglion cells (Amaratunga *et al.*, 1993) interfered with the transport of certain vesicle classes or tubulovesicular structures and, in hippocampal neurons, reduced the length of the neurites extended *in vitro*. In neither system, however, was kinesin expression completely inhibited.

We have begun a study of kinesin function in the simple and experimentally accessible cell system *Neurospora crassa*. Though *Neurospora* mycelia are multicellular, their vegetative phase consists of only one cell type, the multinucleated hypha. We have shown that *N.crassa* kinesin (Nkin) is a distant relative of the 'conventional' kinesins of animals and that it can be purified easily from cytoplasmic extracts (Steinberg and Schliwa, 1995). Its punctate staining pattern by immunofluorescence microscopy suggests an association with membranous organelles. Since the transport of virtually all organelles visible by computer-enhanced video microscopy in *Neurospora* is microtubule dependent (Steinberg and Schliwa, 1993), Nkin is a prime candidate for the motor that drives some or all of these movements. To address the question of kinesin function at the cellular level in more depth, we have generated a kinesin-deficient mutant by the technique

of repeat-induced point mutations (RIP), an efficient method for gene disruption in *Neurospora* (Selker, 1990). Unexpectedly, neither the extent nor the character of organelle movements visible by computer-enhanced video microscopy were changed dramatically in Nkin-deficient cells. However, hyphal morphology and branching were severely altered, indicating that at least one step in cell morphogenesis requires kinesin. Detailed analysis of the mutant strongly suggests a severe defect in the long-range transport and correct delivery of small secretory vesicles required for normal hyphal growth.

Results

Isolation of a *NKIN*-deficient mutant

The RIP phenomenon described previously by Selker (1990) for *N.crassa* is an efficient method for the disruption of any gene of interest, provided a second copy of the gene can be introduced into the genome. During a genetic cross, both copies of the duplicated gene are altered by multiple G:C→A:T transitions, generating mutated genes whose gene products may be non-functional and/or degraded rapidly. We have transformed a wild-type strain with a genomic PCR product spanning nearly the entirely *NKIN* gene (bp 177–3160) and crossed a transformant containing one extra copy with strain NCN235. By screening crude cell extracts of the progeny with an Nkin-specific antibody, we were able to identify *NKIN*-deficient strains. Since we could not detect any difference between these strains by a variety of criteria, all subsequent work was done with one strain, designated NK01. The gene is heavily RIPed (e.g. 11 mutations in 150 3'-terminal base pairs sequenced; data not shown). More importantly, assays used to probe for the presence of Nkin failed to demonstrate a gene product. Neither the Nkin-specific polyclonal antibody (Steinberg and Schliwa, 1995) nor peptide antibodies generated against the highly conserved VDLAGE-motif (Marks *et al.*, 1994), the primary P-loop motif (Marks *et al.*, 1994) or a conserved peptide motif in the C-terminus (G.Steinberg and M.Schliwa, unpublished) detected any signals corresponding to Nkin or fragments thereof in Western blots of whole cells (Figure 1). Moreover, the standard protocol for the isolation of Nkin (Steinberg and Schliwa, 1995) failed to yield motor protein, and the resulting ATP release did not produce any microtubule movement in a gliding assay. Thus the mutant lacks full-length Nkin as well as detectable Nkin fragments or degradation products. This indicates that Nkin is not essential for viability.

Phenotype of the kinesin-deficient strain

To identify cellular processes that require functional kinesin, we have examined the phenotype of the *NKIN*-deficient strain. A comparison of cell growth and other morphological characteristics of wild-type and mutant hyphae is summarized in Table I. Whereas the germination rate is not affected by the mutation, mutant conidia exhibit a higher frequency of dipolar and even tripolar extension of germ tubes. The number of branches in hyphae is increased dramatically (Figure 2). In general, the shape of mutant hyphae is knobby and contorted, and their diameter exceeds that of wild-type hyphae by a factor of 2–3. This altered growth produces much shorter cells and results in a greatly reduced rate of mycelial extension

Fig. 1. Coomassie-stained polyacrylamide gel (A) and Western blots (B–E) of wild-type, kinesin mutant and rescued mutant strains of *N.crassa*. (A) Polyacrylamide gel of the polypeptides released from microtubules with ATP (S5 fraction according to the protocol described in Steinberg and Schliwa, 1995) of wild-type, mutant and rescued mutant cells showing the absence of the characteristic polypeptide doublet of Nkin in null cells (arrowheads). The band seen at the level of Nkin in lane 2 is an unrelated polypeptide. (B and C) Western blots of whole cells probed with a polyclonal antibody to Nkin (B) and a peptide antibody directed against the primary P-loop motif (C). (D and E) Western blots of S5 probed with a polyclonal antibody to Nkin (D) and a peptide antibody directed against the primary P-loop motif (E).

(Figure 3). Despite these marked effects on cell elongation and morphogenesis, cell growth *per se* (as determined by an increase in cell mass) is reduced by only ~25%.

Table I. Morphological characteristics of wild-type and mutant *Neurospora*

	Wild-type	Null mutant	Rescued mutant
Conidial germination rate after 5 h ($n = 150$)	80%	81%	82%
Dipolar germination ($n = 100$)	12%	31%	10%
Distance between branchpoints ($n = 30$)	$330 \pm 105 \mu\text{m}$	$76 \pm 57 \mu\text{m}$	$318 \pm 96 \mu\text{m}$
Hyphal diameter ($n = 20$)	$7 \pm 3 \mu\text{m}$	$17 \pm 6 \mu\text{m}$	$6 \pm 3 \mu\text{m}$
Length of the cells ($n = 65$)	$60 \pm 20 \mu\text{m}$	$33 \pm 15 \mu\text{m}$	$62 \pm 18 \mu\text{m}$
Wet weight after 20 h ($n = 5$) (as % of wt)	100%	76%	95%

Rescue of the kinesin-deficient strain

To confirm that the mutant phenotype is caused by disruption of the kinesin gene, we asked whether a copy of the cloned wild-type gene would complement it. The transformation of the mutant with a genomic version of the *NKIN* gene resulted in a complete morphological rescue (Table I). Functional motor could again be isolated from the rescued strain using microtubule affinity purification (Figure 1). When tested in a microtubule gliding assay, the motor from the rescued strain produced microtubule movement with a velocity of $2.63 \pm 0.25 \mu\text{m/s}$ ($n = 20$) which is the same as wild-type Nkin (Steinberg and Schliwa, 1995, 1996). Thus the mutant phenotype is specific for the loss of Nkin and not caused by an unrelated defect that occurred during the RIP procedure.

Organelle movements

Detailed analysis of organelle movements in *N. crassa* by computer-enhanced video microscopy showed that all visible organelle translocations are microtubule-dependent (Steinberg and Schliwa, 1993). Surprisingly, none of these movements were strikingly altered in the mutant. Vacuoles and mitochondria appeared distributed as in control cells, indicating that their movement and positioning was not impaired. Staining with R123, a mitochondria-specific dye (Chen, 1989), revealed brief excursions of these organelles in both directions parallel to the long axis of the hyphae in a manner indistinguishable from wild-type cells. A small difference was found in the behavior of refractile organelles, which easily could be followed in the hyphae. Their frequency of movement appeared slightly reduced (Figure 4), whereas the average velocity of these particles increased from $2.13 \pm 0.55 \mu\text{m/s}$ in wild-type cells to $2.56 \pm 0.67 \mu\text{m/s}$ in Nkin-deficient cells ($n = 90$). However, some of these changes may be a consequence of the altered cell shape.

To test whether the organelles visualized by video microscopy still move in a microtubule-dependent manner in mutant hyphae, cells were mounted in a flow-through chamber and were treated under microscopic control with $10 \mu\text{M}$ nocodazole, a microtubule-depolymerizing drug. As in wild-type cells, all visible transport ceased within 15 min. This block could be reversed by rinsing with medium. Treatment with $20 \mu\text{M}$ cytochalasin D, a compound that affects actin filaments, did not influence organelle movements within a time span of 50 min. Thus, organelle movements in mutant cells still appear to be microtubule-based.

Even though the gelatin embedment procedure used here greatly improved the visualization of particle movements in wild-type and mutant hyphae, complete removal of the cell wall provides still better images of all organelle

Fig. 2. Comparison of wild-type and mutant with respect to colony morphology and cell shape. (A, C and E) wild-type; (B, D and F) kinesin mutant. (A and B) Colony after 1 day of growth on nutrient agar in 10 cm Petri dishes. The white material in the center represents aerial hyphae which are more prominent in the slow-growing mutant. Bar, 1 cm. (C and D) Overview of hyphal morphology, showing extensive branching in the kinesin-deficient strain. Bar, 100 μm . (E and F) High power differential interference contrast micrograph of part of a hypha showing the gnarled appearance of kinesin-deficient cells. Bar, 10 μm .

movements by video microscopy (Steinberg and Schliwa, 1993). In these protoplasts, cell polarity is lost, but organelle movement is still vigorous. These movements are more difficult to quantify due to the variability in protoplast size and morphology. However, extensive video microscopic analysis of wild-type and mutant protoplasts did not reveal any major differences. Nocodazole and cytochalasin D treatment had the same effects as in intact hyphae. Taken together, the analysis of hyphae and protoplasts did not demonstrate a major effect due to the loss of kinesin on the movement of organelles visualized by video microscopy.

The lack of kinesin affects nuclear distribution and positioning

Previous studies in *Aspergillus* and *Neurospora* have shown that nuclear movement is microtubule dependent (e.g. Oakley and Morris, 1980; Oakley and Rinehart, 1985) and severely affected by mutations in dynein, the

Fig. 3. Mycelial elongation in race tubes prepared as described in Materials and methods. Mycelial growth in the wild-type as well as in the rescued mutant is ~6 times faster than in the mutant. The data shown represent the mean of three independent experiments. The results were so consistent that the error bars are hardly larger than the symbols used for the data points and, therefore, are not shown in the graph.

Fig. 4. Comparison of the frequency of movement of refractile particles within a time window of 10 s in 150 randomly selected wild-type and mutant hyphae. The data are compiled from 30 hyphae each in five independent cultures.

dynactin complex and a protein called ApsA (Plamann *et al.*, 1994; Xiang *et al.*, 1994, 1995; Fischer and Timberlake, 1995; Robb *et al.*, 1995; Tinsley *et al.*, 1996). We have examined whether nuclear distribution is affected in Nkin-deficient cells. 4',6'-Diamidino-2-phenylindole (DAPI) staining of nuclei shows that in wild-type hyphae, nuclei are more or less uniformly distributed along the length of the cell. In the mutant strain, the regular spacing is lost, and nuclei often cluster in small groups separated by larger gaps (Figure 5). The average number of nuclei per macroconidium appears increased (Figure 6). A significant difference was found in the distance between the hyphal tip and the first nucleus, which more than doubled in the mutant cells (Table II). These changes are reminiscent of those observed in dynein/dynactin or ApsA mutations, but the phenotype is less severe in the kinesin-deficient strain.

Growing mutant hyphae lack a *Spitzenkörper*

Elongating *Neurospora* hyphae are characterized by a population of vesicles organized into a visible organelle

Fig. 5. Distribution of nuclei in wild-type (A) and mutant (B) hyphae as seen by DAPI staining. Bar, 50 μ m.

Fig. 6. Number of nuclei per wild-type and mutant conidia, respectively, based on a survey of 100 conidia.

called the *Spitzenkörper* that resides in the apical dome of hyphal tips (Girbardt, 1969; Grove and Bracker, 1970). Video microscopic analysis of growing hyphae revealed a striking difference between wild-type and mutant (Figure 7). Whereas a characteristic *Spitzenkörper* was clearly visible in the majority of wild-type apical cells, none or occasionally only a diffuse structure was seen in mutant hyphae. Elongation proceeded at a much reduced rate (Table III) and was not confined to a distinct apex, but rather occurred in small bulges that frequently shifted location, or in multiple locations simultaneously. Since the presence of a *Spitzenkörper* strictly correlates with hyphal extension in *Neurospora* (Grove and Bracker, 1970; Lopez-Franco *et al.*, 1995), the missing or diffuse

Table II. Distance from the hyphal tip to the first nucleus

Germination time	Wild-type (μm)	Null mutant (μm)
3.5 h ($n = 50$)	3.6 ± 1.4	7.1 ± 1.8
6.5 h ($n = 50$)	3.7 ± 1.8	8.1 ± 3.3

Fig. 7. Phase contrast micrographs of wild-type (A) and mutant (B) apical hyphae. The time interval between the upper and lower micrographs is 90 s. A *Spitzenkörper* is clearly visible in the wild-type but not in the mutant cell. Bar, 10 μm .**Table III.** Presence of a *Spitzenkörper* and apical extension of hyphae growing under a coverslip

	<i>Spitzenkörper</i>			Elongation rate ($\mu\text{m}/\text{min}$)
	Visible	None	Diffuse	
Wild-type	17	3	0	8.5 ± 1.5
Null mutant	0	12	8	2.1 ± 1.6
Rescued mutant	19	1	0	9.0 ± 1.6

Spitzenkörper in mutant cells indicates a defect in the formation of the vesicle supply center.

Electron microscopy reveals a defect in the deployment of apical vesicles

The apical region of extending wild-type hyphae was characterized by a complement of vesicles of ~ 100 nm in diameter that populate the first 0.8–1.5 μm of the tip cell (Figure 8A). Together with a zone within the cluster of apical vesicles that contains smaller vesicles and electron-dense particles (Grove and Bracker, 1970), this region represents the ultrastructural equivalent of the *Spitzenkörper*. In contrast, hyphal tips of mutant cells showed a dramatic reduction in the number of apical vesicles (Figure 8B). The overall shape of the tip was distorted, and numerous bulges were present elsewhere along the length of a hypha. Often small numbers of apical vesicles were associated with these bulges or were found elsewhere along the perimeter of mutant hyphae (not shown). Thus the elimination of kinesin affects the distribution of large apical vesicles.

Lectin staining suggests a difference in cell wall features

To determine whether there are changes in the cell wall of the mutant, cells were stained with a fluorescently labeled lectin, wheat-germ agglutinin (WGA). In fungi, WGA specifically interacts with chitin and its oligomers (Bonfante-Fasolo *et al.*, 1990; Raudaskoski *et al.*, 1994). Most wild-type hyphae were stained brightly at the apex

Fig. 8. (A) Thin section through the apical region of a wild-type hypha showing numerous apical vesicles (arrowheads) in the region of the *Spitzenkörper*. The overall shape of the tip is conical. (B) Corresponding region of a mutant hyphal tip where very few apical vesicles are present (arrowheads). The overall shape is more bulbous and distorted. Bar, 1 μm .

of the distal hypha, whereas the remainder of the mycelium was unlabeled (Figure 9). In contrast, the majority of mutant cells were stained brightly all along their length. Staining was patchy and intensity varied, though a regular pattern could not be identified. It is unclear which physiological status the lectin staining reveals, but the apical staining of wild-type hyphae strongly suggests a correlation with the formation of new cell wall material.

Discussion

Though considerable progress has been made in determining the enzymatic properties of kinesin and its functional interactions with microtubules, the precise cellular roles of kinesin have been more difficult to define. Here we have created a kinesin-deficient mutant in an organism, *N. crassa*, that allows the study of the consequences of the deficiency at the cellular level in a simple model system.

The analysis of the mutation offers novel insights into

Fig. 9. Wild-type (A) and mutant (B) hyphae stained with fluorescently labeled WGA. In wild-type cells, labeling is confined to the cell apex, whereas mutant cells are labeled virtually over their entire surface. Bar, 50 μ m.

the cellular role of these microtubule motors. First, the function of kinesin is not essential in *Neurospora*. Cell growth, as measured by an increase in cell mass, is reduced but not impeded. This contrasts with, for example, *Drosophila*, where kinesin mutations cause growth defects and larval lethality (Saxton *et al.*, 1991). Second, the elimination of kinesin has little or no effect on the movement of organelles visualized by video microscopy. This observation is unexpected in light of findings (discussed below) that at least a proportion of kinesin appears to be associated with mitochondria, lysosomes and other organelles in other cell types. We do observe some changes in particle movements which could be due to an effect on a subpopulation of these organelles in Nkin-deficient cells. However, these minor changes might also be explained by the altered cell geometry which, for example, underestimates the frequency of particle movements in a wide, short cell compared with a narrow, elongated cell. Third, the elimination of kinesin appears to affect nuclear positioning. Whether kinesin is the elusive additional motor postulated in the analysis of the dynein knockout mutation (Xiang *et al.*, 1995), or whether nuclear distribution merely changes in response to an altered cell shape, remains to be determined. Lastly, and most importantly, whereas growth itself is mildly affected, there is a dramatic effect on the way in which growth occurs. An increase in hyphal diameter, a convoluted shape and a high incidence of branching are among the most notable of these growth abnormalities.

Since its discovery, kinesin has been implicated in the movement of membrane-bounded organelles, but studies as to which organelles are being transported have not yet yielded a consistent picture. In neurons, suppression of kinesin by antisense oligonucleotides prevents certain organelle markers from entering the neurites (Ferreira *et al.*, 1992, 1993; Amaratunga *et al.*, 1993), and inhibits the anterograde spreading of tubulovesicular membranes (Feiguin *et al.*, 1994). Kinesin mutations impair the transport of ion channels, but not synaptic vesicles, in larval *Drosophila* neurons (Gho *et al.*, 1992), which is in accordance with the finding that a kinesin-like protein (unc-104), but not kinesin itself, is required for synaptic vesicle transport in *C.elegans* (Hall and Hedgecock, 1991). In rat retinal ganglion cells, on the other hand, two heavy chain variants were observed to co-transport with synaptic vesicles and mitochondria, respectively (Elluru *et al.*, 1995). In a variety of cell types, kinesin is also associated with, and appears to be required for, the transport of lysosomes, mitochondria, Golgi apparatus, endoplasmic reticulum and chromaffin granules (Dabora and Sheetz, 1988; Vale and Hotani, 1988; Hollenbeck and Swanson, 1990; Henson *et al.*, 1992; Leopold *et al.*, 1992; Fath *et al.*, 1994; Jellali *et al.*, 1994; Marks *et al.*, 1994; Schmitz *et al.*, 1994). Recent studies implicate kinesin in transport processes originating in the Golgi apparatus (Steinhardt and Alderton, 1994; Lippincott-Schwartz *et al.*, 1995; Nakata and Hirokawa, 1995), and the radial extension of intermediate filaments (Gyoeva and Gelfand, 1991). While kinesin may be involved in all these processes, in some cases it may be difficult to determine whether the observed effects are direct or affected by other factors of the experimental system or the approach used.

In *Neurospora*, insights into which transport activities fail when kinesin is lost can be gained from a closer examination of the null phenotype. One possibility is that kinesin is responsible for the movement of organelles visualized by video microscopy. If so, then the fact that their motility is virtually unaffected might suggest that the function of kinesin has been taken over by other microtubule-dependent motors. Functional overlap is a well-known phenomenon in other cytoskeleton-related activities (e.g. Schleicher *et al.*, 1995; Jung *et al.*, 1996), and is also observed among certain members of the kinesin superfamily (e.g. Hoyt *et al.*, 1992; Roof *et al.*, 1992). However, no other motor activity could be demonstrated in extracts of cells lacking Nkin, which would seem likely if the fairly abundant Nkin is replaced by another motor. Moreover, if the loss of kinesin were compensated by another motor in null cells, then one probably would not expect such a severe morphological phenotype. Therefore, if a replacement for Nkin exists, it is inadequate. We suggest that the transport of microscopically visible organelles, which is also microtubule dependent, is accomplished by other, as yet unidentified, motor(s) in both wild-type and mutant hyphae. Studies in the mouse have revealed a large family of kinesins (Aizawa *et al.*, 1992), several of which are potential motors for different classes of organelles (for a review, see Hirokawa, 1996). *Neurospora* is bound to have kinesin-like proteins required for cell division; whether it also possesses other kinesins involved in organelle transport processes is presently unknown. If so, they probably represent a minor com-

ponent that cannot be isolated from cell extracts using standard techniques.

An alternative possibility is that kinesin's primary function is the transport of small, less readily visualized organelles. What might be the nature and function of these organelles? The most obvious and most severe alterations observed in mutant cells are a grossly altered hyphal cell shape and defects in cell elongation. The fact that these basic aspects of cell morphogenesis cannot tolerate a loss of kinesin function suggests that Nkin has unique duties in shape determination and cell elongation. Since both are linked intimately to cell wall morphogenesis (Wessels, 1986; Harold, 1990), the simplest explanation for the observed phenotype is a defect in the correct deployment of cell wall precursors, involving the kinesin-dependent transport of small, secretory vesicles.

In support of this hypothesis, perhaps the most significant change observed in the mutant concerns the *Spitzenkörper*, an organelle known to be linked to cell elongation in *Neurospora* and many other fungi (reviewed in Gow and Gadd, 1994). Instead of an apical vesicle supply center, in mutant cells a multitude of growth zones exist that frequently shift position and advance rather slowly, leading to profuse branching and bulging of hyphae. In accordance with these observations on living cells, electron microscopy demonstrates a dramatic reduction in the number of apical vesicles, organelles believed to be involved in cell wall secretion (Wessels, 1986). The apparent defect in the supply of secretory vesicles to the cell apex entails their less focused addition to the cell wall elsewhere. If WGA staining marks sites of cell wall deposition, as suggested by our findings, then it is a vivid demonstration that in the mutant, cell wall is added uncoordinately and unselectively in many places. Cell growth in *Neurospora* has been estimated to require the fusion of tens of thousands of vesicles per tip per minute (Collinge and Trinci, 1974). Their transport must employ a fairly abundant motor, more abundant, in any case, than motors required for the movement of larger, visible organelles. Thus there appears to be a correlation between the abundance of Nkin, which is by far the most prominent microtubule motor species that can be isolated from hyphal cell extracts, and the abundance of its presumptive cargo, the secretory vesicles. However, despite the gel embedment procedure used here to enhance contrast, the movements of small vesicles cannot as yet be visualized in cell hyphae. It will also be important to determine the polarity of microtubules which as yet is not known in any filamentous fungus. In both wild-type and null mutant hyphae, microtubules extend parallel to the cell's long axis (S.Seiler and M.Schliwa, unpublished observations). In our interpretation of the null phenotype, their plus-ends would be expected to be distal, near the hyphal apex.

If polarized secretion is so important for cell morphogenesis, why is cell elongation not inhibited entirely? The cytoplasm evidently still advances slowly, indicating that the directed delivery of secretory vesicles is not the only factor required for cell elongation. For example, actin-dependent cytoplasmic streaming may support the bulk transport of cytoplasm towards the advancing tip. The elimination of kinesin does not affect these slow protrusive activities, but rather the fast delivery of cell wall precursor vesicles to the cell apex, allowing the vesicles to insert in

a disorderly manner in many locations. Alternatively, another motor may have substituted for Nkin. Neither mechanism, however, is sufficient to cover the defect.

Cell elongation by strictly apical deposition of new plasma membrane involving a *Spitzenkörper* is a special feature of somatic hyphae of higher fungi and, as such, easily may be dismissed as a case of extreme specialization. However, these traits may just highlight a general phenomenon common to many cells. The unusual characteristics of the fungal secretory machinery have, in fact, worked to our advantage since they have facilitated the identification of the defect in mutant cells. Thus the findings reported here may well be generally relevant and may help to pinpoint the basic role of conventional kinesin in other, more complex cell types. In support of this contention, the hypothesis that kinesin is required for a transport step leading to cell elongation is consistent with studies where kinesin expression has been suppressed or eliminated. For example, the length of all neurites of hippocampal neurons treated with antisense oligonucleotides is significantly reduced (Ferreira *et al.*, 1992). Microinjection of kinesin antibodies into fibroblasts results in the loss of long cell processes, an elongated cell shape and suppression of pseudopodial activity (Rodinov *et al.*, 1993). These findings implicate kinesin in the maintenance of cell shape, a process that requires the constitutive delivery of membrane vesicles. Constitutive transport of ion channels and components of the synaptic release machinery may also be defective in larval neurons of *Drosophila* kinesin mutants (Gho *et al.*, 1992). Thus, while kinesin may have additional functions that cause pleiotropic effects upon its suppression, these and our findings argue for a basic role for kinesin in the replenishment of the plasma membrane.

The conclusion that kinesin has a specific role in the rapid elongation of hyphae is supported by the identification of a gene in the Basidiomycete *Ustilago maydis*, Kin2, that displays a high degree of similarity to *Neurospora* kinesin across the entire coding region (see accompanying paper by Lehmler *et al.*, 1997). Deletion mutants of the *Ustilago* kinesin show similarities, but also some differences, to their counterparts in *Neurospora*. In the yeast-like haploid form, cell morphology and growth is hardly affected by the deletion of Kin2. The morphology of dikaryotic hyphae of *Ustilago*, on the other hand, which resembles that of *Neurospora* hyphae, is strongly affected in Kin2 mutants. The cells are shorter, irregular and sometimes branched, and thus resemble the *Neurospora* mutants lacking Nkin. These findings suggest that in *Ustilago*, as in *Neurospora*, the demands placed on organelle transport processes increase with increasing cell length.

In conclusion, the elimination of kinesin function in *Neurospora* leads to striking alterations in cell morphogenesis. The most likely explanation for these alterations is a defect in the transport pathway and deployment of secretory vesicles to sites of cell wall formation. This defect needs to be defined more clearly in future studies.

Materials and methods

Strains and growth conditions

General procedures used in the handling of *N.crassa* have been described by Davis and De Serres (1970).

Neurospora crassa wild-type 74A was grown in Vogel's minimal medium as described (Sebald *et al.*, 1979). As *N.crassa* only grows at the tip of the hyphae, a simple method to measure mycelial elongation is to allow them to grow in long, hollow glass tubes ~30 cm in length and 1 cm in diameter ('race tubes'; David and de Serres, 1970) containing Vogel's minimal agar covering the bottom of the glass tube. Conidia were inoculated at one end and the extent of growth was recorded over time.

General molecular biological techniques

DNA-mediated transformation of *N.crassa* spheroplasts was carried out as described by Schweitzer *et al.* (1981), with modifications introduced by Akins and Lambowitz (1985). DNA sequences were obtained using Sequenase (Pharmacia) according to the supplier's instructions. Genomic DNA was isolated using the method described by Schechtman (1986).

RIP and isolation of the null mutant

A PCR product spanning nearly the entire *NKIN* gene (bp 177–3160; see Steinberg and Schliwa, 1995) was cloned into the vector pGEM-T (Promega). The bacterial *hph* gene which confers resistance to hygromycin under the control of the *trpC* promoter from *Aspergillus nidulans* was introduced into the plasmid to allow selection of transformants in *N.crassa* (Staben *et al.*, 1989). The resulting construct (pSS2) was used to transform *N.crassa* wild-type 74A. Southern blot analysis of hygromycin-resistant isolates probed with the PCR product of *NKIN* (bp 177–3160) revealed strains with a single ectopic integration of the *Nkin* PCR product. These strains were used in a genetic cross with strain NCN235, and single ascospores were selected for further study. Mycelium from liquid cultures was harvested by filtration and ground to a slurry in the presence of 1 g of acid-washed quartz sand per gram of mycelium in 1 ml of API100 buffer supplemented with protease inhibitors (100 mM PIPES, pH 6.9, 2 mM MgCl₂, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 10 µg/ml tosyl-arginine-methyl esters, 10 µg/ml soybean trypsin inhibitor, 1 µg/ml aprotinin, 1 µg/ml pepstatin, 1 µg/ml leupeptin). Following centrifugation at 10 000 g for 30 min, the supernatant was analyzed by SDS-PAGE and Western blotting to probe for the presence of *Nkin*.

To demonstrate that the phenotype of strains lacking detectable *Nkin* could be rescued by the appropriate gene, a genomic version of *NKIN* containing ~0.5 kb of untranslated DNA both 3' and 5' of the coding sequence was used. This fragment was isolated by probing a cosmid library (fungal genetics stock center) with the *NKIN* PCR product. The positive cosmid clone was digested with a panel of restriction enzymes to select a suitable fragment containing the full *NKIN* gene, which was then cloned into a plasmid containing a bleomycin resistance gene for selection in *Neurospora* (Austin *et al.*, 1990).

Biochemical procedures

The biochemical isolation of *Nkin* and the motility assay were as described by Steinberg and Schliwa (1995). SDS-PAGE was performed according to the method of Laemmli (1970) using 7% polyacrylamide gels which were stained with Coomassie brilliant blue. Western blots were prepared according to Towbin *et al.* (1979) and further handled as described by Steinberg and Schliwa (1995). The antibodies used include polyclonals raised against biochemically isolated *Nkin* (Steinberg and Schliwa, 1995), and peptide antibodies against the VDLAGE domain (Marks *et al.*, 1994), the primary P-loop (Marks *et al.*, 1994) and a conserved peptide motif in the C-terminus (G.Steinberg and M.Schliwa, unpublished). Whole cell extracts of *Neurospora* hyphae were prepared in a lysing buffer containing 9 M urea, 10% SDS and 5% mercaptoethanol.

Light microscopy

Light and fluorescence microscopy was done using a ZEISS Axiophot microscope. Images were recorded with a Hamamatsu C2400 camera, fed into a Hamamatsu DVS-1000 image-processing system and stored on videotape using a Panasonic AG6720 video tape recorder. Mitochondria could be distinguished easily from other organelles on the basis of their shape and staining with rhodamine 123, a mitochondria-specific dye (Chen, 1989). The other types of organelle visualized in hyphae are refractile particles with a diameter of 0.5 µm or less showing a complex behavior of stop and go movement in both directions along the long axis of the hyphae (Steinberg and Schliwa, 1993). Only movements longer than 3 µm in one direction were used for analysis. For better visualization of intracellular movements, the hyphae were embedded in medium supplemented with 15% gelatin to minimize the difference in refractive index between cell wall and medium.

For the analysis of *Spitzenkörper* behavior, cells were grown overnight

on coverslips coated with 10% gelatin-containing Vogel's minimal medium. To ensure that the cells were not disturbed during handling and microscopic observation, only hyphae which clearly grew over longer distances (20 µm for the wild-type and 5 µm for the mutant) were used for further analysis.

To visualize the distribution of nuclei, hyphae grown in liquid culture were fixed with 3% formaldehyde and stained with 0.5 µg/ml DAPI for 30 min at room temperature. Staining of conidia was enhanced by two rinses with water after fixation and a 15 min incubation in 100% acetone. After two additional washing steps, nearly 100% of the conidia were brightly stained with DAPI.

For staining with fluorescently labeled WGA, growing mycelia were fixed in 3% formaldehyde for 10 min, rinsed twice with phosphate-buffered saline (PBS) and incubated with 25 µg/ml of fluorescein isothiocyanate (FITC)-labeled WGA (Sigma Chem. Comp.) in PBS for 45 min.

Electron microscopy

For electron microscopy, cultures were grown at room temperature on Vogel's minimal agar overlaid with cellophane (DuPont) until the colony had reached a diameter of ~1 cm. The cultures were quickly flooded with fixative to ensure that growth proceeded up until the time of fixation. Primary fixation was with 2% glutaraldehyde in 70 mM PIPES buffer, pH 6.9, for 1–2 h, followed by 2% osmium tetroxide in the same buffer for 1 h. Incubation in 1% uranyl acetate for 1 h was followed by dehydration in ethanol and embedding in Epon. The hyphal colonies were flat-embedded between two Teflon-coated slides (Euteneuer and McIntosh, 1980). From this thin wafer, appropriate regions in the periphery of the colony were cut out, re-mounted and serial-sectioned on a Reichert Ultracut E microtome (Leica, Munich, Germany). Sections were viewed in a Jeol 1200 CX electron microscope (JEOL, Tokyo, Japan).

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References

- Aizawa,H., Sekine,Y., Tekemura,R., Zhang,Z., Nangaku,M. and Hirokawa,N. (1992) Kinesin family in murine central nervous system. *J. Cell Biol.*, **119**, 1287–1296.
- Akins,R.A. and Lambowitz,A.M. (1985) General method for cloning *Neurospora crassa* nuclear genes by complementation of mutants. *Mol. Cell. Biol.*, **5**, 2272–2278.
- Amaratunga,A., Morin,P.J., Kosik,K.S. and Fine,R.E. (1993) Inhibition of kinesin synthesis and rapid anterograde axonal transport *in vivo* by an antisense oligonucleotide. *J. Biol. Chem.*, **268**, 17427–17430.
- Austin,B., Hall,R.M. and Tyler,B.M. (1990) Optimized vectors and selection for transformation of *Neurospora crassa*. *Gene*, **93**, 157–162.
- Bloom,G.S. and Endow,S.A. (1995) Motor proteins 1: kinesins. *Protein Profile*, **2**, 1105–1171.
- Bonfante-Fasolo,P., Faccio,A., Perotto,S. and Schubert,A. (1990) Correlation between chitin distribution and cell wall morphology in the mycorrhizal fungus *Glomus versiforme*. *Mycol. Res.*, **94**, 157–165.
- Brady,S.T. (1985) A novel brain ATPase with properties expected for the fast axonal transport motor. *Nature*, **317**, 73–75.
- Brady,S.T., Pfister,K.K. and Bloom,G.S. (1990) A monoclonal antibody against kinesin inhibits both anterograde and retrograde fast axonal transport in squid axoplasm. *Proc. Natl Acad. Sci. USA*, **87**, 1061–1065.
- Chen,L.B. (1989) Fluorescent labelling of mitochondria. *Methods Cell Biol.*, **29**, 103–123.
- Collinge,A.J. and Trinci,A.P. (1974) Hyphal tips of wild-type and spreading colonial mutants of *Neurospora crassa*. *Arch. Microbiol.*, **99**, 353–368.
- Dabora,S.L. and Sheetz,M.P. (1988) The microtubule-dependent formation of a tubulovesicular network with characteristics of the ER from cultured cell extracts. *Cell*, **54**, 27–35.

- Davis, R.D. and DeSerres, F.J. (1970) Genetic and microbiological research techniques for *Neurospora crassa*. *Methods Enzymol.*, **17**, 79–143.
- Elluru, R.G., Bloom, G.S. and Brady, S.T. (1995) Fast axonal transport of kinesin in the rat visual system: functionality of kinesin heavy chain isoforms. *Mol. Biol. Cell*, **6**, 21–40.
- Euteneuer, U. and McIntosh, J.R. (1980) Polarity of midbody and phragmoplast microtubules. *J. Cell Biol.*, **87**, 509–515.
- Fath, K.R., Trimburt, G.M. and Burgess, D.R. (1994) Molecular motors are differentially distributed on Golgi membranes from polarized epithelial cells. *J. Cell Biol.*, **126**, 661–675.
- Feiguin, F., Ferreira, A., Kosik, K.S. and Cacees, A. (1994) Kinesin-mediated organelle translocation revealed by specific cellular manipulations. *J. Cell Biol.*, **127**, 1021–1039.
- Ferreira, A., Niclas, J., Vale, R.D., Banker, G. and Kosik, K.S. (1992) Suppression of kinesin expression in cultured hippocampal neurons using antisense oligonucleotides. *J. Cell Biol.*, **117**, 595–606.
- Ferreira, A., Caceres, A. and Kosik, K.S. (1993) Intraneuronal compartments of the amyloid precursor protein. *J. Neurosci.*, **13**, 3112–3123.
- Fischer, R. and Timberlake, W.E. (1995) *Aspergillus nidulans* *apsA* (anucleate primary sterigmata) encodes a coiled-coil protein required for nuclear positioning and completion of asexual development. *J. Cell Biol.*, **128**, 485–498.
- Gho, M., McDonald, K., Ganetzky, B. and Saxton, W.M. (1992) Effects of kinesin mutations on neuronal functions. *Science*, **258**, 313–316.
- Girbard, M. (1969) Die Ultrastruktur der Apikalregion von Pilzhyphe. *Protoplasma*, **67**, 413–441.
- Gow, N.A. and Gadd, G.M. (eds) (1994) *The Growing Fungus*. Chapman and Hall, London.
- Grove, S.N. and Braker, C.E. (1970) Protoplasmic organization of hyphal tips among fungi: vesicles and Spitzenkörper. *J. Bacteriol.*, **104**, 989–1009.
- Gyoeva, F.K. and Gelfand, V.I. (1991) Coalignment of vimentin intermediate filaments with microtubules depends on kinesin. *Nature*, **353**, 445–448.
- Hall, D.H. and Hedgecock, E.M. (1991) Kinesin-related gene *unc-104* is required for axonal transport of synaptic vesicles in *C.elegans*. *Cell*, **65**, 837–847.
- Harold, F.M. (1990) To shape a cell: inquiry into the causes of morphogenesis of microorganisms. *Microbiol. Rev.*, **54**, 381–431.
- Henson, J.H., Nesbitt, D., Wright, B.D. and Scholey, J.M. (1992) Immunolocalization of kinesin in sea urchin coelomocytes. Association of kinesin with intracellular organelles. *J. Cell Sci.*, **103**, 309–320.
- Hirokawa, N. (1996) Organelle transport along microtubules. The role of KIFs. *Trends Cell Biol.*, **6**, 135–141.
- Hirokawa, N., Sato-Yoshitake, R., Kobayashi, N., Pfister, K.K., Bloom, G.S. and Brady, S.T. (1991) Kinesin associates with anterogradely transported membranous organelles. *J. Cell Biol.*, **114**, 295–302.
- Hollenbeck, P.J. (1989) The distribution, abundance and subcellular localization of kinesin. *J. Cell Biol.*, **108**, 2335–2342.
- Hollenbeck, P.J. and Swanson, J.A. (1990) Radial extension of macrophage tubular lysosomes supported by kinesin. *Nature*, **346**, 864–866.
- Hoyt, M.A., He, L., Loo, K.K. and Saunders, W.S. (1992) Two *Saccharomyces cerevisiae* kinesin-related gene products required for mitotic spindle assembly. *J. Cell Biol.*, **118**, 109–120.
- Jellali, A., Metz-Boutigue, M.H., Surgucheva, I., Jancsik, V., Filliol, D., Gelfand, V.I. and Rendon, A. (1994) Structural and biochemical properties of kinesin heavy chain associated with rat brain mitochondria. *Cell Motil. Cytoskel.*, **28**, 79–93.
- Jung, G., Wu, X. and Hammer, J.A. (1996) *Dictyostelium* mutants lacking multiple classic myosin I isoforms reveal combinations of shared and distinct functions. *J. Cell Biol.*, **133**, 305–323.
- Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685.
- Lehmler, C., Steinberg, G., Snetselaar, K., Schliwa, M., Kahmann, R. and Böcker, M. (1997) Identification of a motor protein required for filamentous growth in *Ustilago maydis*. *EMBO J.*, **16**, in press.
- Leopold, P.L., McDonald, A.W., Pfister, K.K., Bloom, G.S. and Brady, S.T. (1992) Association of kinesin with characterized membrane-bound organelles. *Cell Motil. Cytoskel.*, **23**, 19–23.
- Lippincott-Schwartz, J., Cole, N.B., Marotta, A., Conrad, P.A. and Bloom, G.S. (1995) Kinesin is the motor for microtubule-mediated Golgi-to-ER membrane traffic. *J. Cell Biol.*, **128**, 293–306.
- Lopez-Franco, R., Howard, R.J. and Braker, C.E. (1995) Satellite Spitzenkörper in growing hyphal tips. *Protoplasma*, **188**, 85–103.
- Marks, D.L., Larkin, J.M. and McNiven, M.A. (1994) Association of kinesin with the Golgi apparatus in rat hepatocytes. *J. Cell Sci.*, **107**, 2417–2426.
- Nakata, T. and Hirokawa, N. (1995) Point mutation of adenosine triphosphate-binding motif generated rigor kinesin that selectively blocks anterograde lysosome membrane transport. *J. Cell Biol.*, **131**, 1039–1053.
- Oakley, B.R. and Morris, N.R. (1980) Nuclear movement is beta-tubulin-dependent in *Aspergillus nidulans*. *Cell*, **19**, 255–262.
- Oakley, B.R. and Rinehart, E. (1985) Mitochondria and nuclei move by different mechanisms in *Aspergillus nidulans*. *J. Cell Biol.*, **101**, 2392–2397.
- Patel, N., Thierry-Mieg, D. and Mancillas, J.R. (1993) Cloning by insertional mutagenesis of a cDNA encoding *Caenorhabditis elegans* kinesin heavy chain. *Proc. Natl Acad. Sci. USA*, **90**, 9181–9185.
- Pfister, K.K., Wagner, M.C., Stenoien, D.L., Brady, S.T. and Bloom, G.S. (1989) Monoclonal antibodies to kinesin heavy and light chains stain vesicle-like structures, but not microtubules, in cultured cells. *J. Cell Biol.*, **108**, 1453–1463.
- Plamann, M., Minke, P.F., Tinsley, J.H. and Bruno, K.S. (1994) Cytoplasmic dynein and actin-related protein Arp 1 are required for normal nuclear distribution in filamentous fungi. *J. Cell Biol.*, **127**, 139–149.
- Raudaskoski, M., Mao, W.Z. and Yli-Mattila, T. (1994) Microtubule cytoskeleton in hyphal growth. Response to nocodazole in a sensitive and a tolerant strain of the homobasidiomycete *Schizophyllum commune*. *Eur. J. Cell Biol.*, **64**, 131–141.
- Robb, M.J., Wilson, M.A. and Vierula, P.J. (1995) A fungal actin-related protein involved in nuclear migration. *Mol. Gen. Genet.*, **247**, 583–590.
- Rodionov, V.I., Gyoeva, F.K., Tanaka, E., Bershadsky, A.D., Vasiliev, J.M. and Gelfand, V.I. (1993) Microtubule-dependent control of cell shape and pseudopodial activity is inhibited by the antibody to kinesin motor domain. *J. Cell Biol.*, **123**, 1811–1820.
- Roof, D.M., Meluh, P.B. and Rose, M.D. (1992) Kinesin-related proteins required for assembly of the mitotic spindle. *J. Cell Biol.*, **118**, 95–108.
- Saxton, W.M., Hicks, J., Goldstein, L.S.B. and Raff, E. (1991) Kinesin heavy chain is essential for viability and neuromuscular functions in *Drosophila*, but mutants show no defects in mitosis. *Cell*, **64**, 1093–1102.
- Schechtman, M. (1986) A moderate-scale DNA prep for *Neurospora*. *Fungal Genet. Newslett.*, **33**, 45–46.
- Schleicher, M., Andre, B., Andreoli, C., Eichinger, L., Haugwitz, M., Hofmann, A., Karakesisoglou, J., Stöckelhuber, M. and Noegel, A.A. (1995) Structure/function studies on cytoskeletal proteins in *Dictyostelium* amoebae as a paradigm. *FEBS Lett.*, **369**, 38–42.
- Schmitz, F., Wallis, K.T., Rho, M., Drenckhahn, D. and Murphy, D.B. (1994) Intracellular distribution of kinesin in chromaffin cells. *Eur. J. Cell Biol.*, **63**, 77–83.
- Scholey, J.M., Porter, M.E., Grissom, P.M. and McIntosh, J.R. (1985) Identification of kinesin in sea urchin eggs, and evidence for its localization in the mitotic spindle. *Nature*, **318**, 483–486.
- Schweitzer, M., Case, M.E., Dykstra, C.C., Giles, N.H. and Kushner, S.R. (1981) Identification and characterization of recombinant plasmids carrying the complete *qa* gene cluster from *Neurospora crassa* including the *qa-1* regulatory gene. *Proc. Natl Acad. Sci. USA*, **78**, 5086–5090.
- Sebald, W., Neupert, W. and Weiss, H. (1979) Preparation of *Neurospora crassa* mitochondria. *Methods Enzymol.*, **55**, 144–148.
- Selker, E.U. (1990) Premiotic instability of repeated sequences in *Neurospora crassa*. *Annu. Rev. Genet.*, **24**, 579–613.
- Staben, C., Jensen, B., Singer, M., Pollock, J. and Schechtman, M. (1989) Use of a bacterial hygromycin B resistance gene as a dominant selectable marker in *Neurospora crassa* transformation. *Fungal Genet. Newslett.*, **36**, 79–81.
- Steinberg, G. and Schliwa, M. (1993) Organelle movements in the wild type and wall-less *fg;sg;os-1* mutants of *Neurospora crassa* are mediated by cytoplasmic microtubules. *J. Cell Sci.*, **106**, 555–564.
- Steinberg, G. and Schliwa, M. (1995) The *Neurospora* organelle motor: a distant relative of conventional kinesin with unconventional properties. *Mol. Biol. Cell*, **6**, 1605–1618.
- Steinberg, G. and Schliwa, M. (1996) Characterization of the biophysical and motility properties of kinesin from the fungus *Neurospora crassa*. *J. Biol. Chem.*, **271**, 7516–7521.
- Steinhardt, R.A., Bi, G. and Alderton, J.M. (1994) Cell membrane resealing by a vesicular mechanism similar to neurotransmitter release. *Science*, **263**, 390–393.

- Tinsley,J.H., Minke,P.F., Bruno,K.S. and Plamann,M. (1996) P150 (Glued), the largest subunit of the dynactin complex, is nonessential in *Neurospora* but required for nuclear distribution. *Mol. Biol. Cell*, **7**, 731–742.
- Towbin,H., Staehlin,T. and Gordon,J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl Acad. Sci. USA*, **76**, 4350–4354.
- Vale,R.D. and Hotani,H. (1988) Formation of membrane networks *in vitro* by kinesin-driven microtubule movement. *J. Cell Biol.*, **107**, 2233–2241.
- Vale,R.D., Reese,T.S. and Sheetz,M.P. (1985) Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. *Cell*, **42**, 39–50.
- Wessels,J.G.H. (1986) Cell wall synthesis in apical hyphal growth. *Int. Rev. Cytol.*, **104**, 37–79.
- Wright,B.D.W., Henson,J.H., Wedaman,K.P., Willy,P.J., Morand,J.N. and Scholey,J.M. (1991) Subcellular localization and sequence of sea urchin kinesin heavy chain: evidence for its association with membranes in the mitotic apparatus and interphase cytoplasm. *J. Cell Biol.*, **113**, 817–833.
- Xiang,X., Beckwith,S.M. and Morris,N.R. (1994) Cytoplasmic dynein is involved in nuclear migration in *Aspergillus nidulans*. *Proc. Natl Acad. Sci. USA*, **91**, 2100–2104.
- Xiang,X., Roghi,C. and Morris,N.R. (1995) Characterization and localization of the cytoplasmic dynein heavy chain in *Aspergillus nidulans*. *Proc. Natl Acad. Sci. USA*, **92**, 9890–9894.

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