

# Caught Nek-ing: cilia and centrioles

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*Journal of Cell Science* 118, 5161-5169 Published by The Company of Biologists 2005  
doi:10.1242/jcs.02681

## Summary

The Nek family of cell-cycle kinases is widely represented in eukaryotes and includes numerous proteins that were described only recently and remain poorly characterized. Comparing Neks in the context of clades allows us to examine the question of whether microbial eukaryotic Neks, although not strictly orthologs of their vertebrate counterparts, can provide clues to ancestral functions that might be retained in the vertebrate Neks. Relatives of the Nek2/NIMA proteins play important roles at the G2-M transition in nuclear envelope breakdown and centromere separation. Nek6, Nek7 and Nek9 also seem to regulate

mitosis. By contrast, Nek1 and Nek8 have been linked with polycystic kidney disease. Results of statistical analysis indicate that the family coevolved with centrioles that function as both microtubule-organizing centers and the basal bodies of cilia. This evolutionary perspective, taken together with functional studies of microbial Neks, provides new insights into the cellular roles of the proteins and disease with which some of them have been linked.

Key words: Flagella, Cell cycle, NIMA, Kinase, Basal body

## Introduction

The NIMA-related protein kinases (Nrks or Neks) have been called the 'third family of mitotic kinases' (O'Connell et al., 2003). The Nek proteins have a well-conserved N-terminal kinase domain but have divergent C-terminal tails of varying length (see O'Connell et al., 2003). Together with the Polo and Aurora kinase families, some members of the Nek family participate in the regulation of downstream events following Cdc2 activation. Although functional characterization of most of these kinases is in its early stages, studies so far suggest that most Neks have cell-cycle-related functions.

There are eleven orthologous Nek genes in mice and humans (Table 1) (Caenepeel et al., 2004; Forrest et al., 2003). A phylogenetic analysis of 81 Nek sequences from a wide variety of eukaryotes and four kinases from outside the family produces a phylogenetic tree in which most branches are poorly resolved (not shown). Of these, the three well-resolved clades are shown in Fig. 1. Note this analysis does not include the 39 Neks recently found in the genome of the ciliate *Tetrahymena thermophila* (J. Gaertig, personal communication). This large expansion of the Nek family may reflect the numerous and specialized cilia found on this sophisticated unicellular organism, since several of these proteins appear to regulate cilium length (J. Gaertig, personal communication). Nevertheless, lineage-specific expansions, such as those seen in the higher plants and *Tetrahymena*, are less likely to be informative with respect to ancestral functions. Thus, in this Commentary we focus on the members of the family that are more likely to provide such clues.

Below, we review what is known about the Nek proteins mainly in the context of clades because, despite the potential for substantial evolution of function, we wish to explore the possibility that proteins within a clade might share ancestral protein-protein interactions and conserved cellular functions.

We focus on the best-characterized members of the family rather than provide a comprehensive catalog of initial characterizations. We then examine the hypothesis that the Nek family coevolved with centrioles that serve dual functions as basal bodies and foci for mitotic spindles.

## The Nek2/NIMA clade: G2-M regulation and centrosome separation

The proteins in the Nek2/NIMA clade are the best-characterized members of the Nek family and include representatives from diverse organisms including unicellular and filamentous fungi, flies, mammals, nematodes and slime mold (Fig. 1). The founding member of the Nek family, NIMA from *Aspergillus nidulans*, is essential for mitotic entry and efficient progression through mitosis, and its degradation is required for mitotic exit [for a clear and concise review of earlier work on NIMA and the Nek family see (O'Connell et al., 2003)]. Mitosis in *Aspergillus* is closed – instead of nuclear envelope breakdown, there is a reorganization of certain cytoplasmic and nuclear proteins (for example, tubulin is transported into the nucleus, where it can form the mitotic spindle). Recent work (Wu et al., 1998; De Souza et al., 2003) indicates that NIMA regulates entry into mitosis by controlling the localization of cyclin-dependent kinase 1 (Cdk1) by interacting with and partially disassembling nuclear pore complexes (NPCs). Another interesting aspect of mitosis in *Aspergillus* is that, during spore germination, there are several rounds of nuclear division without cytokinesis. This means that nuclear membrane fission must be mediated by mechanisms that are not coupled to cytokinesis. TINC (two-hybrid interactor with NIMA C) physically interacts with NIMA and expression of a  $\Delta$ N-TINC causes destabilization of NIMA and defects in nuclear membrane fission (Davies et al., 2004).

**Table 1. Proposed functions of the mammalian Neks**

Protein	Proposed functions and localizations	Refs
Nek1	hNek1 Interacts with polycystic kidney disease (PKD) proteins	Surpili et al., 2003
	mNek1 Causal mutation in <i>kat</i> mouse model of progressive PKD	Upadhyaya et al., 2000
	Role in DNA damage response pathway	Polci et al., 2004
Nek2	hNek2 Localizes to centrosomes and kinetochores	Fry et al., 1998b
	Phosphorylates C-Nap1 and Nlp At centrosomes	Fry et al., 1998a
	Regulates centrosome splitting at G2-M	Rapley et al., 2005
	Phosphorylates Hec1 at kinetochores; possible role in spindle checkpoint	Chen et al., 2002
Nek3	hNek3 Associates with Vav2; modulates prolactin receptor signaling	Miller et al., 2005
	mNek3 Predominantly localized to cytoplasm; no cell-cycle-dependent changes in Nek3 activity detected	Tanaka and Nigg, 1999
	No known functions	
Nek4	No known functions	
Nek5	No known functions	
Nek6 and Nek7	hNek6 and hNek7 Nek6 and 7 bind to C-terminal tail of Nek9; Nek9 phosphorylates and activates Nek6 during mitosis	Belham et al., 2003
	Inhibition of Nek6 function arrests cells in metaphase and triggers apoptosis	Yin et al., 2003
Nek8	hNek8 Overexpressed in primary human breast tumors	Bowers and Boylan, 2004
	mNek8 Causal mutation in <i>jck</i> mouse model of recessive juvenile cystic kidney disease	Liu et al., 2002
	No known functions	
Nek9	hNek9 Associates with and phosphorylates Bcd2 in vivo	Holland et al., 2002
	Regulates chromosome alignment and segregation in mitosis	Roig et al., 2002
	Mediates centrosomal and chromosomal microtubule organization	Roig et al., 2005
	Activates Nek6 during mitosis	Belham et al., 2003
	Regulates G1 and S progression through interaction with the FACT complex	Tan and Lee, 2004
Nek10	No known functions	
Nek11	hNek11 DNA replication/damage stresses-responsive kinases; may play a role in the S-phase checkpoint	Noguchi et al., 2002
	Colocalized with Nek2A in nucleoli; is activated by Nek2A in cells arrested at G1-S	Noguchi et al., 2004

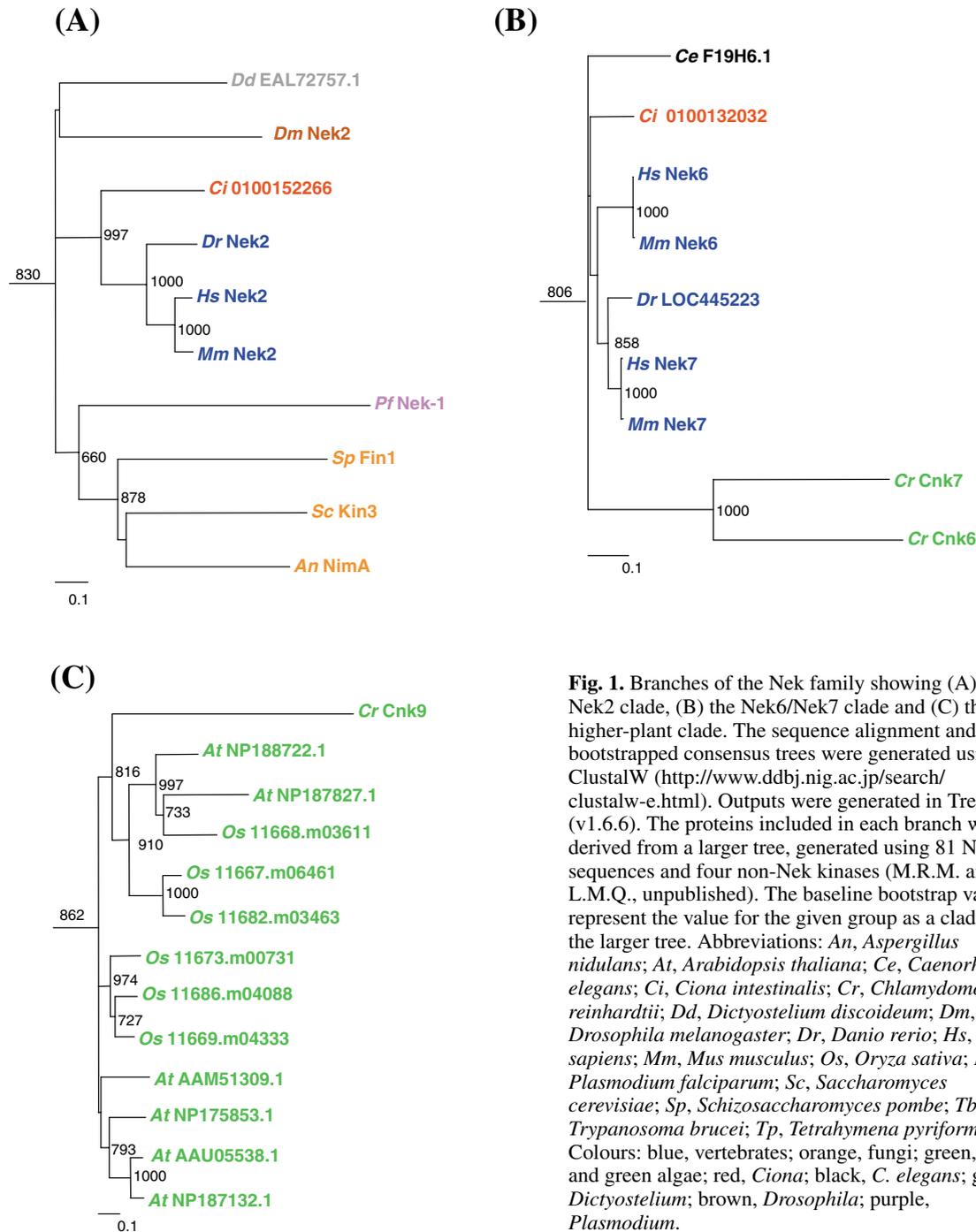
Abbreviations: h, human; m, mouse; FACT, 'facilitates transcription of chromatin templates'.

Taken together with observations of synthetic phenotypes generated by some NimA alleles, these data suggest that NIMA participates in nuclear membrane fission (Osmani et al., 1991; Davies et al., 2004).

Do these activities reflect those of Neks in other organisms? TINC appears to be a protein specific to filamentous fungi. This mechanism of nuclear membrane fission might therefore be specific to this group (Davies et al., 2004). However, a role for NIMA in nuclear membrane fission might be common to all fungi: the kinase activity of the *Schizosaccharomyces pombe* NIMA ortholog (Fin1p) peaks at the metaphase-anaphase transition, and *fin1*-deletion mutants have extensive elaborations of the nuclear envelope (Krien et al., 2002). Roles in nuclear envelope fission could represent neo-functionalization of the ancestral Nek within the fungi. By contrast, the role of NIMA in mitotic entry might provide more clues to essential early activities. Ectopic expression of the *Aspergillus* NIMA in mammalian cells triggers partial disassembly of the NPC, nuclear envelope breakdown and DNA condensation (Lu and Hunter, 1995a). A mammalian Nek might thus play a role in NPC disassembly (De Souza et al., 2004) and, as the closest relative of NIMA, Nek2 would be the most likely candidate.

Vertebrate Nek2 is an important player in the coordination of centrosome structure and function with mitotic progression, and localizes mainly to centrosomes throughout the cell cycle (Fry et al., 1998b). Most of the Nek2 protein at the centrosome is rapidly turned over ( $t_{1/2} \approx 3$  seconds), and multiple processes regulate its centrosomal abundance (Hames et al., 2005). Nek2 is dimeric and is activated by autophosphorylation (Fry et al., 1999; Fry et al., 1995). There are two splice variants of vertebrate Nek2: Nek2A has an N-terminal kinase domain, a C-terminal domain that includes a leucine zipper, a protein phosphatase 1c (PP1c)-binding domain, and a motif that targets it for destruction by the anaphase-promoting complex [APC/C (Fry et al., 1999; Hames et al., 2005)]; Nek2B, lacks the PP1c-binding domain and the destruction box (Hames et al., 2005). Nek2A is more abundant in adult cells; by contrast, only Nek2B is detected in *Xenopus* eggs and early embryos (Fry et al., 2000). Nek2B is rapidly recruited to sperm basal bodies in the zygotic centrosome and is essential for assembly and maintenance of centrosomes in the early embryo (Fry et al., 2000; Twomey et al., 2004; Uto and Sagata, 2000).

Nek2A is required for centrosome separation (disjunction) at the G2-M cell-cycle transition (Fry, 2002). Among the



**Fig. 1.** Branches of the Nek family showing (A) the Nek2 clade, (B) the Nek6/Nek7 clade and (C) the higher-plant clade. The sequence alignment and bootstrapped consensus trees were generated using ClustalW (<http://www.ddbj.nig.ac.jp/search/clustalw-e.html>). Outputs were generated in Treeview (v1.6.6). The proteins included in each branch were derived from a larger tree, generated using 81 Nek sequences and four non-Nek kinases (M.R.M. and L.M.Q., unpublished). The baseline bootstrap values represent the value for the given group as a clade on the larger tree. Abbreviations: *An*, *Aspergillus nidulans*; *At*, *Arabidopsis thaliana*; *Ce*, *Caenorhabditis elegans*; *Ci*, *Ciona intestinalis*; *Cr*, *Chlamydomonas reinhardtii*; *Dd*, *Dictyostelium discoideum*; *Dm*, *Drosophila melanogaster*; *Dr*, *Danio rerio*; *Hs*, *Homo sapiens*; *Mm*, *Mus musculus*; *Os*, *Oryza sativa*; *Pf*, *Plasmodium falciparum*; *Sc*, *Saccharomyces cerevisiae*; *Sp*, *Schizosaccharomyces pombe*; *Tb*, *Trypanosoma brucei*; *Tp*, *Tetrahymena pyriformis*. Colours: blue, vertebrates; orange, fungi; green, plants and green algae; red, *Ciona*; black, *C. elegans*; grey, *Dictyostelium*; brown, *Drosophila*; purple, *Plasmodium*.

centrosomal targets of Nek2 kinase activity are C-Nap1, a core centrosomal protein that helps keep the centrioles together throughout interphase (Fry et al., 1998a; Mayor et al., 2002; Mayor et al., 2000), and ninein-like protein [Nlp (Rapley et al., 2005)]. C-Nap1 and Nlp are displaced from the centrosome at G2-M and phosphorylation of these proteins by Nek2 contributes to this displacement (Fry et al., 1998a; Mayor et al., 2002; Rapley et al., 2005). Although the roles of C-Nap1 and Nlp in centrosome disjunction remain enigmatic, it is clear that Nek2 plays an important role in the process. Transient overexpression of Nek2 causes a splitting of centrosomes (Fry

et al., 1998b), whereas expression of a kinase-dead Nek2A blocks normal centrosome disjunction and, consequently, bipolar spindle formation (Faragher and Fry, 2003). Thus, Nek2A kinase activity is essential for this key mitotic event.

Polo-like kinase 1 (Plk1) is another centrosomal kinase that becomes localized to the centrosome and is active at G2-M (Golsteyn et al., 1995). Recognition of certain substrates by Plk1 is enhanced by prior phosphorylation of the substrates by another kinase (Elia et al., 2003). Studies by Rapley and colleagues showing that Nlp is a substrate for both Nek2 and Plk1, and that the *in vitro* phosphorylation of Nlp by Plk1 is

enhanced by Nek2, suggest that Nek2 might prime Nlp for Plk1 (Rapley et al., 2005). This idea is supported by experiments using the kinase-dead Nek2A, which interferes with Plk1-induced displacement of Nlp from the centrosome (Rapley et al., 2005). Such interactions with polo kinases might be a conserved ancestral activity because Fin1p, the *Schizosaccharomyces pombe* Nek2 ortholog (see Fig. 1A), recruits polo kinase (Plo1) to the spindle pole body (Grallert and Hagan, 2002). However, Fin1p affects the spindle at a later point in the cell cycle, after the metaphase-anaphase transition (Krien et al., 2002). Intriguingly, it binds only to spindle pole bodies that are more than two cell cycles old and appears to regulate the polarity of the septum initiation network (SIN), a conserved mitotic exit network (Grallert et al., 2004; Simanis, 2003).

Nek2 might also regulate the core kinetochore protein Hec1 (for 'highly expressed in cancer 1'; also known as Ncd80), which is essential for the organization of stable microtubule plus end binding sites in the outer plate (DeLuca et al., 2005). Prior to metaphase, Hec1 anchors the spindle checkpoint protein Mad1 to kinetochores that have not yet formed stable microtubule attachments (DeLuca et al., 2003). The *Saccharomyces cerevisiae* Nek2 ortholog Kin3p and human Nek2 can both phosphorylate Hec1 (human and yeast) at Ser165, and this is essential for the integrity of chromosome segregation (Chen et al., 2002). In this context, it is notable that Nek2A has also been identified as a Mad1-binding protein, which has led to speculation about a role for Nek2A in the integration of the spindle checkpoint (Lou et al., 2004).

Nek2 might be a key player in additional checkpoints. It has been identified as a downstream target of the DNA damage response pathway: DNA damage induced by radiation or chemical agents leads to decreased amounts and activity of Nek2 and a corresponding inhibition of centrosome splitting, presumably contributing to the observed G2 delay (Fletcher et al., 2004). Additionally, Nek2A has been reported to translocate to nucleoli and activate Nek11 during G1-S arrest (Noguchi et al., 2004). The cellular role(s) of Nek11, which also has two splice variants, remains to be determined.

Proteins in the NIMA/Nek2 clade have thus adopted nuanced roles in the regulation of cell-cycle progression. In general, they appear to be most active during progression through G2 phase and mitosis. Although there is no direct evidence that vertebrate Neks participate in NPC disassembly, the observation that exogenous expression of NIMA in mammalian cells triggers NPC disassembly (Lu and Hunter, 1995b), suggests that regulation of NPC breakdown might be a general role of Nek2-like kinases. Alternatively, it may be an ancestral function lost by Nek2 and retained by another Nek. The well-documented role that vertebrate Nek2 plays in centriole separation might also be an ancestral Nek activity, particularly given that this function is conserved in *Dicytostelium* and *Drosophila* (Graf, 2002; Prigent et al., 2005).

Notably, the *Chlamydomonas* Nek family is not represented in the Nek2/NIMA clade. Mitosis in *Chlamydomonas* is partially closed: nuclear envelope breakdown does not occur and instead the nuclear membrane becomes fenestrated (Johnson and Porter, 1968). It is not known whether partial NPC disassembly occurs during mitosis in *Chlamydomonas*, but we would predict that it does not. Indeed, given the

fenestrations, there would be no need for NPC disassembly. The centrosome-separating activity of Nek2 is also not required in *Chlamydomonas*, because the centrioles/basal bodies are held together by a different mechanism that does not involve C-Nap1 or Nlp.

### The Nek6/Nek7 clade: components of a mitotic kinase cascade

Nek6 and Nek7 are highly similar; for example, murine Nek6 and Nek7 share 87% amino acid identity in their kinase domains (Kandli et al., 2000). Both Nek6 and Nek7 have short C-terminal tails that have no identified motifs or domains and, unlike Nek2, neither dimerizes, which is consistent with evidence indicating that these proteins are not activated by trans-autophosphorylation (Belham et al., 2003). Instead, they appear to be activated through their interaction with another kinase, possibly Nek9 [see below (Belham et al., 2003; Roig et al., 2002)]. Although they were originally reported to be regulators of the p70 ribosomal S6 kinase (Belham et al., 2001), recent evidence indicates that this is not an *in vivo* function of these kinases (Belham et al., 2003; Lizcano et al., 2002). Instead, Nek6, and possibly also Nek7, might participate in a mitotic cascade.

Human Nek6 appears to be required for progression through mitosis: its abundance and kinase activity are increased during mitosis (Belham et al., 2003; Yin et al., 2003) and interfering with Nek6 function by either expression of kinase-dead Nek6 or depletion of Nek6 by short interfering (siRNA) causes mitotic arrest [at metaphase and pro-metaphase, respectively (Yin et al., 2003)]. Whether Nek7 also has a role in mitosis is less clear. *In vitro* studies of recombinant and exogenously expressed Nek6 and Nek7 indicate that the mechanisms of regulation of these two similar kinases might differ dramatically (Minoguchi et al., 2003), but both appear to be activated by Nek9 [see below (Belham et al., 2003)]. The targets of Nek6 (and Nek7) remain to be determined.

Although a great deal of work remains to be done before we understand the cellular roles of the proteins in this clade, it appears that they are part of a regulatory cascade that is important for progression through mitosis (see below). We note that this clade does not include representatives from the fungi or from the higher plants – the two eukaryotic lineages that lack centrioles/basal bodies and cilia. This leads us to speculate that the effects of this regulatory kinase cascade on spindle formation and function are intimately associated with centrioles and possibly cilia. Consistent with this idea, is the finding that the *Chlamydomonas* ortholog of Nek6, Cnk6p, is in the ciliary proteome (Pazour et al., 2005).

### Nek9: roles throughout the cell cycle?

Nek9 (also known as Nercc1) binds tightly to overexpressed Nek6 (Roig et al., 2002), and injection of anti-Nek9 antibodies indicates that the kinase plays a role in chromosome alignment and segregation during mitosis probably by affecting spindle organization (Roig et al., 2002). Nek9 is activated by autophosphorylation of Thr210 in the activation loop, and activated Nek9 is concentrated on centrosomes during mitosis (Roig et al., 2005). Studies with the *Xenopus* ortholog reveal that Nek9 interacts with the  $\gamma$ -tubulin ring complex, which

nucleates microtubules, and localizes to the poles of spindles formed in *Xenopus* egg extracts (Roig et al., 2005). Depletion of Nek9 interferes with formation of bipolar spindles and with the formation of Ran-induced asters, a process that is directed by chromatin rather than by centrosomes. This observation is consistent with the early report that Nek9 binds directly to Ran (Roig et al., 2002). The recent work indicates that Nek9 plays an important role in microtubule organization mediated by either chromosomal or centrosomal pathways. Several lines of evidence support the conclusion that Nek6 (and possibly also Nek7) is an important substrate of Nek9, and phosphorylation of Nek6 on Ser206 by Nek9 serves to activate Nek6 (Roig et al., 2002). However, it is not known whether Nek6 participates in either of the chromosomal or centrosomal pathways of microtubule organization.

The role of Nek9 might not be restricted to mitosis. It has been shown to localize to the nucleus and associate with the FACT (for 'facilitates transcription of chromatin templates') complex, and Nek9-knockdown cells exhibit delayed progression through G1 and S phase, which indicates a role for Nek9 in interphase (Tan and Lee, 2004).

### Nek1 and Nek8: cilia, cell cycle and cystic kidneys

Neither Nek1 nor Nek8 falls into well-resolved clades; thus, phylogenetic analysis does not provide a clear indication of which Neks from non-mammalian species might share conserved functions. Nevertheless, Nek1 and Nek8 are important as products of disease-linked genes, and functional studies of Neks in other organisms could provide clues to their cellular roles.

The first vertebrate Nek to be cloned, mNek1, was identified in a mouse expression library screened with antibodies to phospho-tyrosine (Letwin et al., 1992). Its kinase domain is 42% identical to that of NIMA (indicative of the well-conserved sequences of the kinase domains of Neks) but the two proteins have divergent C-terminal domains. Nek1 is larger than NIMA (1258 amino acids compared with 699) and the C-terminus of Nek1 includes a larger coiled-coil domain than NIMA, although both contain PEST motifs (O'Connell et al., 2003). mNek1 is highly expressed in testes and ovaries (Arama et al., 1998; Letwin et al., 1992), as well as in peripheral and motor neurons (Arama et al., 1998), which suggested it might function in meiosis and/or differentiation (Arama et al., 1998; Letwin et al., 1992). However, Upadhyaya and colleagues (Upadhyaya et al., 2000) subsequently reported that mutations in mNek1 are the causal defects in two allelic mouse models of progressive polycystic kidney disease, *kat* and *kat<sup>2l</sup>* (for 'kidney, anemia, testis').

Renal cysts are characteristic of a wide range of diseases, many of which have multi-organ pathology (Wilson, 2004). The *kat* mice show a progressive increase in the size and number of cysts in the kidney cortex. This is accompanied by male sterility as a result of testicular hypoplasia, dilation of the ventricles of the brain, multiple large cysts in the choroid plexus, very small olfactory bulbs, facial dysmorphism and runting (Janaswami et al., 1997; Vogler et al., 1999). Given the similar pathology in the kidney and the choroid plexus, Nek1 could play similar roles in renal and choroid epithelial cells, which when compromised lead to aberrant cell proliferation and fluid accumulation (Janaswami et al., 1997). But what

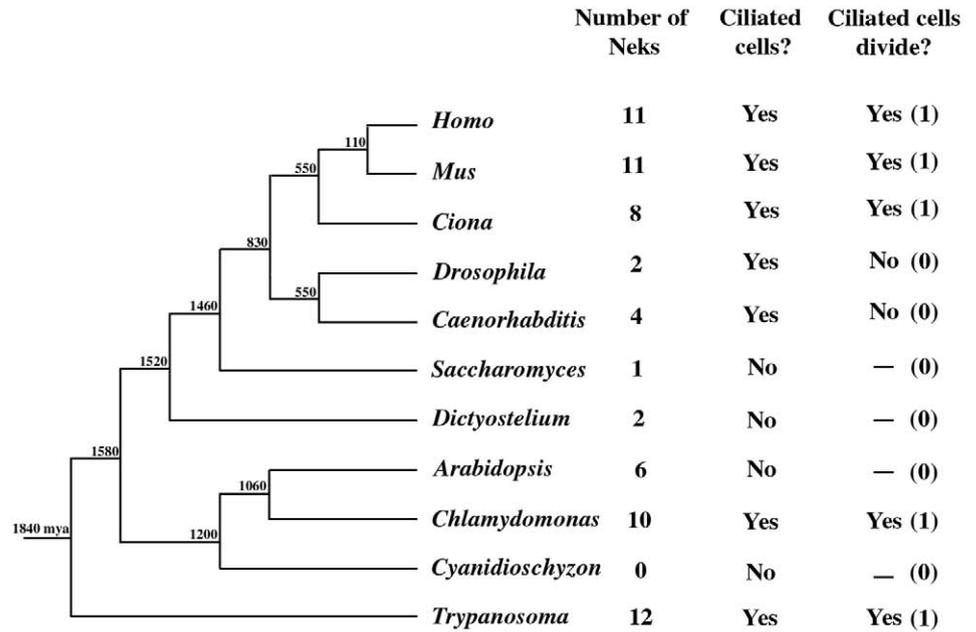
those roles might be and what cellular defects caused by Nek1 mutation might trigger the other aspects of the pleiotropic phenotype, remain to be established.

Several proteins that bind to Nek1 have been identified. Yeast two-hybrid assays using an mNek1 kinase domain, including an inactivating G13R mutation as bait, identified a leucine-zipper protein termed Nurit that is only expressed in testis and appears to be involved in late spermiogenesis (Feige et al., 2002). Studies using the central coiled-coil region of human Nek1 as bait identified 11 proteins (Sarpili et al., 2003). These include KIF3A, a ubiquitously expressed kinesin motor that is important for ciliogenesis, neuronal polarity and the establishment of laterality during early embryonic development, and whose kidney-specific knockout results in the proliferation of renal cysts (Lin et al., 2003); tuberlin, a tumor suppressor that plays an important role in the membrane localization of polycystin 1, mutations in which are responsible for the most common form of polycystic kidney disease (Kleymenova et al., 2001); and, finally, several proteins that participate in DNA repair pathways (Sarpili et al., 2003). This is consistent with the report by Polci and colleagues (Polci et al., 2004) showing that mNek1 expression and activity are stimulated following DNA damage. The *in vivo* veracity and the specific cellular roles the various interacting proteins play in the physiological functions of Nek1 remain to be determined. Significantly, mutations in another Nek, Nek8, have since been shown to cause renal cysts in a mouse model of recessive juvenile-onset cystic kidney disease, *jck* [for 'juvenile cystic kidney' (Liu et al., 2002)].

The Nek8 protein (703 residues) is smaller than Nek1 and lacks a coiled-coil domain and PEST motifs, but contains an RCC1 (regulator of chromosome condensation) domain (see O'Connell et al., 2003). RCC1 domains form  $\beta$ -propeller structures similar to, but distinct from, WD40 domains. The role of the RCC1 domain in Nek8 is not known; however, a G448V substitution (within the RCC1 domain) is responsible for the *jck* phenotype (Liu et al., 2002). Overexpression of mutant forms of Nek8 (including G448V) in tissue culture cells leads to the formation of enlarged multinucleate cells and reduced numbers of actin stress fibres, although tubule cells in *jck* mice are not multinucleate. These and other *in vitro* observations led Liu and colleagues (Liu et al., 2002) to speculate that the cellular role of Nek8 is related to regulation of the cytoskeleton. Indeed, overexpression of a kinase-diminished hNek8 mutant in U2-OS cells affects the expression of actin (Bowers and Boylan, 2004). A proteomic study of the renal cysts from *jck* mice revealed overexpression of galectin-1, sorcin and vimentin (Valkova et al., 2005). We are clearly a long way from understanding the cellular role of Nek8.

The cellular basis of renal cyst formation is not understood, but recent evidence indicates that defective signaling from the primary cilium of epithelial cells is an important factor (reviewed by Pazour, 2004). How defective ciliary signaling might cause renal cyst formation is unknown. Cyst formation requires several changes to the epithelium, and the process by which these epithelial cells re-enter the cell cycle could be analogous to transitions that normally occur during development and tissue remodeling. For example, in developmental epithelial-mesenchyme transitions, epithelial cells (many of which are ciliated) partially dedifferentiate

**Fig. 2.** Phylogenetic tree of model organisms indicating the number of Neks and the presence or absence of ciliated cells. NIMA kinase homologs were identified using genome-wide BLAST searches with the *Aspergillus* NIMA protein sequence. Candidate genes were used in reciprocal BLAST searches against the NCBI protein database (<http://www.ncbi.nlm.nih.gov/BLAST/>) to identify true Nek homologs. The data were codified as follows: '1' was assigned to organisms that possess ciliated cells that re-enter the cell cycle, and '0' was assigned to organisms that do not have ciliated cells that undergo cell division (numbers presented in brackets). The tree is adapted and modified from (Hedges, 2002; Pazour, 2004).



(D'Souza-Schorey, 2005). In *jck* mice at 2-3 weeks of age (i.e. prior to the development of kidney cysts), the integrity and structure of the tubular epithelial basal membrane is disrupted and epithelial cells occasionally detach from the basement membrane of collecting ducts (Liu et al., 2002). In this context, it is interesting to note that the mammalian Par complex, required for proper epithelial cell polarization, is required for the assembly of, and localizes to, primary cilia (Fan et al., 2004). We predict that Nek family members participate in regulation of these events.

### Coevolution of Nek kinases and centrioles

Several deflagellation-defective mutant strains of the unicellular biflagellate *Chlamydomonas* carry mutations in *FA2* (for 'flagellar autotomy'), a gene encoding a Nek (Mahjoub et al., 2002). Deflagellation (also known as deciliation) is a calcium-mediated stress response in which the cilia are severed at their base and shed into the environment (reviewed by Quarmby, 2004). Why is a member of a family known for its cell-cycle functions responsible for an important ciliary pathway? The *fa2*-null mutation produces a cell-cycle delay at G2-M (Mahjoub et al., 2002) rather than lethality, which suggests that a compensatory pathway exists, possibly involving another member of the Nek family. Indeed, whereas several microbial eukaryotes, including unicellular fungi, have only one or two members of the family (reviewed by O'Connell et al., 2003), *Chlamydomonas* express 10 Neks (Bradley et al., 2004) (B. Bradley and L.M.Q., unpublished). Since the fungi have neither centrioles nor cilia, we have hypothesized that the Nek family is expanded in ciliated organisms and coordinates cilia with the cell cycle (Bradley et al., 2004; Quarmby and Parker, 2005).

Examination of the genomes of several organisms reveals a correlation between the number of Neks in a particular organism and whether or not it has ciliated cells (Fig. 2). *Drosophila* and *Caenorhabditis elegans* have ciliated cells and

thus might be expected to have more Neks than the higher plants, represented by *Arabidopsis*, which do not have ciliated cells. However, we posit that, because the only ciliated cells in *Drosophila* and *C. elegans* are terminally differentiated, these organisms do not have to coordinate cilia and the cell cycle (i.e. they do not have centrioles that serve as both basal bodies and microtubule-organizing centers). Indeed, employing a binary approach in which '1' is assigned to organisms that possess ciliated cells that re-enter the cell cycle and '0' is assigned to organisms that do not, we have tested this hypothesis by using the evolutionary software Continuous v1.0d13 PPC (Pagel, 1994). The result of comparing a model in which the number of Nek genes and the ability of ciliated cells to divide evolve independently with an alternative model in which the two traits evolve in a correlated fashion strongly supports our hypothesis ( $P=0.01$ ). We propose that the large number of Neks in the higher plants is a consequence of an independent expansion involving substantial sub- and neo-functionalization. In other words, in higher plants, this family of kinases may have been coopted and expanded in the service of cellular activities unrelated to their ancestral functions. Consistent with this is the finding that higher-plant Neks fall into a clade distinct from the Neks of other organisms (Fig. 1C).

### Conclusion/Perspectives

The Neks are a highly conserved family of protein kinases that play roles in the regulation of the cell cycle. Many questions remain about the detailed cellular functions of even the most extensively studied members of this family and, for most Neks, there is little functional information available. Proteins in the NIMA/Nek2 clade are the best-characterized members of the Nek family. The fungal NIMA orthologs play important roles at the G2-M transition of the cell cycle, modulating nuclear pore function and facilitating translocation of Cdk1 into the nucleus. Although no vertebrate Nek has yet been ascribed this function, we predict that a vertebrate Nek regulates this

activity. The vertebrate Nek2 proteins regulate the separation of centrosomes at G2-M, which is essential for the formation of bipolar spindles and high-fidelity chromosome separation. This activity of Nek2 involves the phosphorylation of centrosomal substrates C-Nap1 and Nlp. The phosphorylation of Nlp by Nek2 might facilitate recruitment and activation of Plk1 at the centrosome, a function that might be conserved in yeast, in which Fin1 recruits Plo1 to the spindle pole body. Nek1 and Nek8 are products of disease genes. Their cellular functions remain to be determined, but probably involve signaling between cilia and the cell cycle. Similarly, it will not be surprising to find associations between Nek proteins and other proliferative diseases. Nek8 is overexpressed in primary human breast tumors (Bowers and Boylan, 2004) and human Nek3 appears to play an important role in prolactin receptor signaling, specifically in a pathway that contributes to the progression and motility of breast cancer (Miller et al., 2005).

Here, we have proposed that expansion of the Nek family accompanied the evolution of a robust and complex system for the coordination of progression through the cell cycle with cilia, basal bodies and centrioles. Such a connection is consistent with the role of Neks in the development of polycystic kidneys in mice; however, so far, the only link between all three parameters is provided by *Chlamydomonas*. The Neks Fa2p and Cnk2p are ciliary proteins that have ciliary functions, and both proteins also affect cell-cycle progression (Mahjoub et al., 2004; Bradley and Quarmby, 2005). In addition, there is increasing evidence from both *Chlamydomonas* and *Tetrahymena* that the Nek family play important roles in the regulation of ciliary length (Bradley and Quarmby, 2005) (J. Gaertig, personal communication; J. D. Parker and L.M.Q., unpublished). These data lead us to predict that some of the mammalian Neks localize to cilia and that, in addition to affecting cell-cycle progression, mammalian Neks, like their microbial brethren, regulate ciliary function.

We are grateful to A. Mooers for statistical analysis of coevolution; J. Parker, M. Trapp and B. Bradley for insightful discussions and contributions to the clarity of the manuscript; J. Glover for careful reading of the manuscript and inspired comments; J. Gaertig for generously sharing unpublished data from his lab; and the CIHR, NSERC and MSFHR for financial support of the work in our lab.

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