

Cilia and the cell cycle?

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A recent convergence of data indicating a relationship between cilia and proliferative diseases, such as polycystic kidney disease, has revived the long-standing enigma of the reciprocal regulatory relationship between cilia and the cell cycle. Multiple signaling pathways are localized to cilia in mammalian cells, and some proteins have been shown to act both in the cilium and in cell cycle regulation. Work from the unicellular alga *Chlamydomonas* is providing novel insights as to how cilia and the cell cycle are coordinately regulated.

Eukaryotic cilia or flagella (the terms are interchangeable) come in a variety of sizes and functional roles but, at the core, they are highly conserved across most phyla. Indeed, possession of centrioles that can act as basal bodies and nucleate microtubule-based axonemes is a primitive condition of eukaryotic cells, that has been lost in some lineages, notably the fungi and higher plants (Stechmann and Cavalier-Smith, 2002; Mitchell, 2004). The best-known role for these organelles is motility, either moving fluid over a surface (e.g., respiratory epithelia) or to propel a cell (e.g., sperm). A second well-known role is in sensory signaling, with examples including the outer segments of vertebrate photoreceptors, the sensory surface of olfactory neurons, and nematode sensory cilia. Primary cilia, which are found on nearly all mammalian cell types (Wheatley et al., 1996) are less well understood, but have long been suggested to play roles in either sensory signaling or in the cell cycle (for review see Pazour and Witman, 2003).

Cilia have been studied most thoroughly in the biflagellate green alga *Chlamydomonas*, whose experimental advantages led to the discovery and characterization of the process by which cilia are assembled, intraflagellar transport (IFT; Kozminski et al., 1993). Cilia are dynamic organelles which undergo growth and turnover at their distal tips; consequently, microtubule motor-based transport of ciliary precursors is necessary for assembly and maintenance of nearly all cilia (Rosenbaum and Witman, 2002; Cole, 2003). Structures associated with basal bodies serve as sites for the organization and targeting of ciliary precursors (Cole, 2003).

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Abbreviations used in this paper: IFT, intraflagellar transport; Nek, NIMA-related expressed kinase; PKD, polycystic kidney disease; SOFA, site of flagellar autotomy.

The primary cilium expressed by most mammalian cells is nucleated from the elder centriole of the centrosome (Sorokin, 1962). The presence of a cilium is associated with the establishment of polarity and differentiation of the cell; tissue culture cells often grow a primary cilium as they approach confluence, and most ciliated cells are probably in the stationary or G₀ phase of the cell cycle (Tucker et al., 1979). In many cells, entry into the cell cycle is preceded by ciliary resorption whereas exit from mitosis is accompanied by ciliary assembly, a relationship which may reflect the use of the basal bodies/centrioles as mitotic spindle poles (Rieder et al., 1979; Tucker et al., 1979; Ehler et al., 1995; Wheatley et al., 1996). There are, however, some cells that do not coordinate ciliary loss and regeneration with the cell cycle. Citing original work by F. Meves from 1900 and J.B. Gatenby from 1917, E.B. Wilson (1928) described the retention of flagella during the final divisions of spermatogenesis in the butterfly; cilia are also retained through division by some unicellular ciliates and flagellates (Kirk, 1998). In these exceptional examples of cells that retain their cilia during division, chromosome segregation occurs with centrioles acting simultaneously as basal bodies and spindle pole organizers (Wilson, 1928; Kirk, 1998). In most cells, a cycle of ciliogenesis is coordinated with progression through the cell cycle and there appears to be an intimate regulatory relationship between the two. Dissecting the bidirectional flow of information between these cellular functionaries is an emerging area of research.

Dysfunctional cilia and pathologies of cell proliferation

If cilia provide information that serves to retain cells in their functioning differentiated G₀ state, then defects in this pathway are predicted to cause proliferative disorders: cancer, cystic diseases, and fibroses of various sorts. To date, the best case for such a role for cilia comes from studies on the polycystic kidney diseases (PKDs). A growing body of evidence indicates that dysfunctional ciliary signaling is the proximal cause of cyst development. Consequently, mutant genes that trigger development of the pathology are candidates for players in the normal pathways of communication between cilia and the cell cycle regulatory machinery.

Renal cysts are a devastating feature of a wide range of diseases, many of which have multi-organ pathology. Monoclonal cysts form from epithelial cells in the nephron, which proliferate and detach from their neighbors, eventually leading to end stage renal disease (Wilson, 2004). The idea that defective cilia are the proximal cause for PKD originated with the discovery

that the causative gene of the mouse *orpk* PKD model, Polaris, is homologous to a gene essential for ciliary assembly in *Chlamydomonas*, IFT88 (Pazour et al., 2000). Many proteins associated with PKD and related diseases or syndromes have since been shown to localize to cilia and basal bodies, and/or shown to play roles in ciliogenesis (Badano et al., 2005; Pan et al., 2005). A direct causal connection between cilia and proliferation has been controversial because many of the implicated proteins are not exclusively ciliary. However, the idea that dysfunctional ciliary signaling is the proximal cause of aberrant cell proliferation in the kidney is strengthened by several recent lines of evidence.

The polycystins PC-1 and PC-2 (products of *PKD1* and *PKD2*) are subunits of a mechano-sensitive calcium channel that has been implicated as a flow sensor for the epithelial cells of kidney tubules (Boletta and Germino, 2003). The polycystins have been reported to participate in a confusing plethora of signaling pathways. Postulated mechanisms by which PC-1/PC-2 might retain the differentiated epithelial cells in G₀/G₁ include activation of the cyclin kinase inhibitor p21(waf1) via JAK-STAT (Bhunia et al., 2002) and blockade of cAMP-stimulated cell proliferation via Atk-mediated inhibition of B-Raf (Yamaguchi et al., 2004). Calcium-induced changes in proliferative state require sustained Ca²⁺ changes in order to affect gene transcription; current data implicates both the calcineurin-NFAT pathway (Puri et al., 2004) and AP-1 (Bhunia et al., 2002; Chauvet et al., 2004; Le et al., 2004). It is intriguing to note that PC-1 may mediate some of its effects by regulated cleavage and nuclear translocation (Chauvet et al., 2004). It will be interesting to learn whether the cleaved product of PC-1 travels to the nucleus with KAP: in the sea urchin, the KAP component of the kinesin-2 motor necessary for anterograde IFT moves into the nucleus as cilia resorb before mitosis (Morris et al., 2004). Nuclear KAP may serve as a signal to the cell that the cilia have indeed been resorbed and cell division may now proceed. Another cystogenic protein, inversin, which localizes to centrioles and cilia, binds both calmodulin and Apc2 of the anaphase-promoting complex (Morgan et al., 2002a,b), thus providing another potential link between the sensory function of the primary cilium and the regulation of the cell cycle.

Beyond the kidney, other pathways important for cell growth or differentiation have been found to involve cilia (Pazour and Witman, 2003); for example, in neuronal cells, somatostatin receptor 3 is targeted to primary cilia (Handel et al., 1999). Mouse knockouts of IFT genes essential for ciliary assembly are embryonic lethal, with phenotypes reminiscent of mutations in the hedgehog pathway, suggesting that the hedgehog receptors localize to cilia (Huangfu et al., 2003). In NIH 3T3 cells, the tyrosine kinase receptor PDGFR α , activation of which is sufficient for a quiescent cell to reenter the cell cycle, is expressed specifically on the primary cilium (Christensen et al., 2004). Surprisingly, bovine insulin promotes the growth of low-density cultures of the ciliate *Tetrahymena* (Christensen, 1993). The insulin signal may be received by an insulin receptor-related tyrosine kinase which localizes to the *Tetrahymena*

cilia (Christensen et al., 2003). These studies show that the use of cilia as “antennae” for growth signals may be, like cilia themselves, a primitive condition of the eukaryotes.

Clues from *Chlamydomonas*

All cells lose their cilia by one of two mechanisms: resorption or deflagellation/deciliation. Resorption is the process by which the cilium is gradually retracted into the cell, and usually occurs in advance of cell division. Deflagellation is the shedding of flagella that occurs in response to a wide range of stimuli. It involves the precise severing of the nine outer doublet microtubules at the base of the flagellum, but distal to the transition zone between the basal body and the flagellum-proper, at a site known as the site of flagellar autotomy (SOFA; Mahjoub et al., 2004). Deflagellation is a common cellular response: sea urchin embryos, scallop gills, rabbit oviduct, porcine respiratory tissue, and rat cerebral ependymal cells all deciliate in response to stress; cells regenerate their cilia when the stressful stimulus is removed (Quarmby, 2004).

The *FA2* gene was uncovered in a genetic screen for *Chlamydomonas* mutants defective in deflagellation (Finst et al., 1998). Fa2p is essential for calcium-activated axonemal microtubule severing during deflagellation, and, as it turns out, it also plays a role during cell cycle progression (Mahjoub et al., 2002). Cells carrying a complete deletion of the *FA2* gene delay at the G₂/M transition. Fa2p is a member of the NIMA-related kinase family, which is represented by at least eleven genes in humans (the NIMA-related expressed kinases; Neks). Nek family members are known as cell cycle kinases (O’Connell et al., 2003), thus the discovery of a Nek having a ciliary function was provocative.

Fa2p localizes specifically to the SOFA region of the cilium of interphase cells (Fig. 1 A; Mahjoub et al., 2004). Although Fa2p’s ciliary function is directly related to axonemal severing, localization of the protein shows interesting changes during premitotic flagellar resorption and during ciliogenesis. During resorption, Fa2p relocates from the SOFA to the proximal end of the basal bodies; during mitosis, it is associated with the polar region of the mitotic spindle. As the cells exit mitosis, Fa2p accumulates at the proximal end of

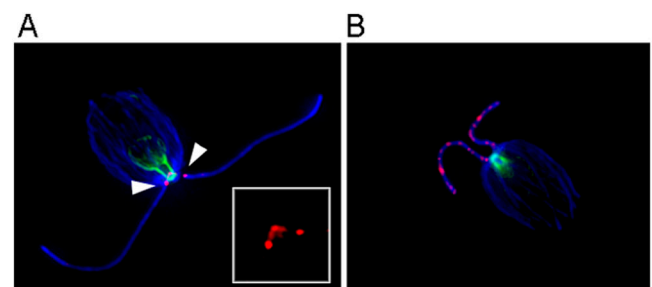


Figure 1. Fa2p and Cnk2p, cell cycle kinases which localize to distinct regions of cilia. (A) Fa2p-HA localizes to the SOFA region of the proximal axoneme (arrowheads) and also associates with basal bodies. The inset shows a magnified view of Fa2p-HA staining. Image by Moe Mahjoub. (B) Cnk2p-HA localizes along the axoneme in a punctate pattern. Image by Brian Bradley. Indirect immunofluorescence images visualized by anti-HA (red), anti- α -tubulin (blue), and anti-centrin (green).

the basal bodies and moves out to the SOFA as soon as ciliogenesis is initiated (Mahjoub et al., 2004). When expressed in IMCD-3 cells (derived from murine kidney), GFP-tagged *Chlamydomonas* Fa2p shows an intriguing pattern of localization. In a confluent culture, Fa2p is observed lying on the presumptive ciliary SOFA and at the base of both centrioles, one of which is serving as the basal body (Mahjoub et al., 2004). During mitosis, Fa2p is associated with the duplicated centrioles and then with the polar region of the mitotic spindle in the mouse cells, as it is in *Chlamydomonas*. Strikingly, it also accumulates at the midbody during cytokinesis. Like the axoneme, the midbody contains acetylated microtubules. Perhaps a functional homologue of Fa2p facilitates the timing of the final break between daughter cells via triggering severing of the acetylated microtubules.

We do not yet know how Fa2p mediates its effects on microtubule severing during deflagellation, or on the G₂/M transition. One possibility for the delay in entry into mitosis is that it is a consequence of inefficient flagellar resorption, relating to the ciliary function of Fa2p. Although deflagellation has been considered a distinct pathway from premitotic flagellar resorption, recent evidence indicates that these two mechanisms of flagellar loss share important signaling components (Parker and Quarmby, 2003; Pan et al., 2004). It is also possible that Fa2p independently affects deflagellation and progression through the cell cycle: A presumptive kinase-dead Fa2p localizes correctly to the SOFA, but does not rescue the deflagellation defect of *fa2* cells; this same mutant protein provides full rescue of the G₂/M delay (Mahjoub et al., 2004).

Cnk2p is another member of the *Chlamydomonas* Nek family, and it is the second Nek protein shown to be axonemal (Fig. 1 B; Bradley and Quarmby, 2005). Although Fa2p is specifically localized to the SOFA, Cnk2p is found along the entire length of the axoneme. The roles of Cnk2p in the cilia and in cell cycle regulation are also distinct from Fa2p. Cnk2p affects regulation of both flagellar length and cell size. When wild-type *Chlamydomonas* cells pass the commitment point of the cell cycle, two decisions are made: whether to divide and if so, how many times. Cells then undergo a rapid succession of alternating M/S phases, dividing one, two, or three times, depending on the size of the cell when it passed the commitment point (Pickett-Heaps, 1975). Cells with altered Cnk2p expression are defective at assessing their size at the commitment point: an increase in the amount of Cnk2p results in small cells, while a decrease in Cnk2p results in large cells. These same manipulations of Cnk2p levels produce cells with short and long flagella, respectively. Flagellar length is normally tightly controlled, at least in part by a balance in the rates of assembly and disassembly (Marshall, 2004). Our data indicate that Cnk2p may play a role in flagellar disassembly; whether this role is independent of cell size assessment is unknown. In *Tetrahymena*, at least four Neks localize to cilia and affect ciliary length (J. Gaertig, personal communication). The relationship between the disassembly that contributes to length control, premitotic flagellar resorption and the assessment of cell size are all important connections that remain to be established.

The NIMA-related kinases may be an important link

We speculate that the Nek family provides an important general connection between cilia and the regulation of cell cycle progression. In all organisms where they have been studied, NIMA and its brethren, the Neks, are known as cell cycle kinases (O'Connell et al., 2003). We have noted that this family is expanded in lineages with ciliated cells (Bradley et al., 2004), but the only direct ciliary connection so far in vertebrates comes via studies of PKD models: the causative genes of two mouse PKD models are Neks. Study of *kat* mutant mice established that Nek1 carries the causative mutation in this model of autosomal recessive PKD (Upadhyaya et al., 2000). hNek1 has been found to bind to other cystogenic proteins including kinesin-2, which is also required for ciliogenesis (Surpili et al., 2003). Nek8 carries the causative mutation in the juvenile cystic kidney mouse model, and morpholinos to zebrafish Nek8 cause kidney cysts in that organism (Liu et al., 2002). A kinase domain mutation of the human orthologue of Nek8 affects cell cycle progression, probably at the G₂/M checkpoint. It is currently unknown whether Nek1, Nek8, or any other mammalian Neks, localize to cilia, but the work in *Chlamydomonas* suggests that mammalian Neks will be found in cilia.

Concluding remarks

It is interesting to consider that if cilia are indeed sending signals that regulate cell cycle progression, by physical necessity these signals must pass near or through the centrosome, which is a gathering place for all manner of cell cycle-regulating proteins (Doxsey, 2001; Badano et al., 2005). We are intrigued by the possibility that signals from the cilium are mediated by calcium. Calcium is implicated in both exit from G₀ and at G₁/S (Means et al., 1999), as well as ciliary disassembly (Quarmby, 2004).

It is clear that there is a relationship between cilia, cell size, and cell cycle progression. The extent to which cilia send signals that regulate cell cycle progression (and vice versa) is not yet known, but we predict that this area of research will provide fertile ground for the discovery of new players in the regulation of cell proliferation and differentiation. It is likely that some of these will be disease genes. Available data indicates that most vertebrate cells are ciliated and that the genesis and disassembly of these cilia is coordinated with progression through the cell cycle. We are only beginning to understand how this coordination is achieved, but its significance may be profound.

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