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Phosphorylation regulates the disassembly of cilia

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Cilia and flagella are conserved organelles protruding from the surface of eukaryotic cells and are composed of a ciliary membrane, axoneme, and basal body. Cilia are widely distributed in protozoans and vertebrates; their main functions are to sense environmental cues and drive cells to move. Defective ciliary structure and function have been implicated in a spectrum of diseases called ciliopathies, including polycystic kidney disease (PKD), retinal degeneration, respiratory disease, and sterility. The prevalence of ciliopathies in man is approximately 1:1000, with no commercially available drugs. Therefore, the study of cilia and the pathogenesis of ciliopathies have attracted great interest.

Cilia are polarized cell organelles, with highly regulated assembly/disassembly mechanisms. When cells exit from the cell cycle and enter into the stationary phase (G0), one of the centrioles moves to the apical plasma membrane and differentiates into a basal body, where it functions as a template for the assembly of the cilia axoneme. Prior to entering into the mitosis phase, cilia disassemble, and the basal body reverts to form the centriole, which functions as the center of spindle microtubule organization. The causal relationship between cilia assembly/disassembly and the cell cycle is still indeterminate. However, there are several ciliary signaling pathways (or proteins) such as Wnt and polycitin-1/polycystin-2 that are involved in cell cycle regulation. Therefore, altering the dynamics of ciliary assembly/disassembly may result in changes in the cell cycle. Although the ciliary assembly and disassembly are equally important, disassembly has received sparse attention to date. Dr. Pan began studying the molecular mechanisms of ciliary disassembly in 2004, at Professor Snell's laboratory at the University of Texas Southwestern Medical Center. He and the member in his group in China found that *Chlamydomonas* aurora-like kinase (CALK) and kinesin-13 (CrKin13) are two important proteins involved in cilia disassembly, while the dissembled products are returned to the cell body via retrograde intraflagellar transport (IFT) [1 3]. Professor Pan's group has recently identified a flagellar shortening mutant *fls1* and proposed a two-phase ciliary disassembly model based on this mutant, thus revolutionizing our view of cilia disassembly [3].

The unicellular green alga, *Chlamydomonas*, is a classical research model for cilia. This model for flagellar disassembly research is advantageous because flagellar resorption occurs prior to mitosis or meiosis and can be induced by adding chemicals such as sodium pyrophosphate (NaPPi). The flagella in wild-type (WT) cells are completely resorbed 3 h after NaPPi induction; however, this interval is extended in the *fls1* mutant, with another 1.5 h needed for complete resorption. The speed of flagellar disassembly in WT is constant at 0.068 μm min^{-1}; however, flagellar disassembly in the *fls1* mutant occurs in two phases: disassembly of the distal part of the flagella at 0.029 μm min^{-1}, lasting for 3 h, and resorption of the proximal part of the flagella at 0.077 μm min^{-1}. In addition, flagellar resorption prior to mitosis also occurs in two phases: the first is a slower phase at 0.08 μm min^{-1}, followed by a faster process at 0.26 μm min^{-1}. As a result, cell division of the synchronized *fls1* mutant is delayed by 2 h as compared to that in WT cells. Based on these results, Hu et al. proposed that flagellar disassembly occurs in two phases, depending on flagellar length, where phase I is FLS1-dependent [3]. Their

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work also indicated that progression of the cell cycle could be adjusted by altering the kinetics of flagellar disassembly.

FLS1 protein has a putative cyclin-binding domain, similar to members of the cyclin-dependent kinase-like (CDKL) subfamily in humans. Immunostaining indicated that FLS1 was enriched at the basal body and is found in both the flagella and cell body when flagella are at steady state. FLS1 is immediately phosphorylated when flagellar resorption is induced, either by incubation with NaPPi or mixing the plus and minus types of gametes for 3 min, without a change in flagellar length. The phosphorylation of FLS1 is an early response of flagellar disassembly. Subsequently, cytosolic CALK is phosphorylated/activated within 10 min in WT cells, but this process is delayed in *fls1* mutant. This phosphorylation occurs after 90 min when flagella are shortened by approximately 2 μm, indicating that FLS1 was upstream of CALK. In addition, kinesin CrKin13 is immediately transported into flagella in WT cells after NaPPi incubation. It may also mediate the depolymerization of axonemal microtubules. Some of CrKin13 is phosphorylated and its microtubule depolymerization activity may be partially inhibited after approximately 1 h when WT flagella were shortened to 6 to 7 μm. However, phosphorylation of CrKin13 in the *fls1* mutant occurred within 10 min after NaPPi induction, thus the depolymerization activity of CrKin13 was inhibited earlier in the mutant as compared to that in WT. The absence of FLS1 may attenuate microtubule depolymerization via both, CALK-histone deacetylase 6 (HDAC6) pathway and CrKin13 pathway. In summary, FLS1 is not only a positive activator of CALK phosphorylation, but also a negative regulator of CrKin13 phosphorylation.

Recent work using *Chlamydomonas*, *Leishmania*, *Tetrahymena*, and mammalian cells indicate that phosphorylation of CALK, aurora A, kinesin-13 (KIF2A in the mammalian cell), HDAC6, and never-in-mitosis gene A (NIMA) plays pivotal roles in the regulation of ciliary disassembly [2,4,5]. During the FLS1-dependent shortening phase I, signals from the plasma membrane, cilia, or nucleus may induce the phosphorylation of FLS1, followed by the activation of tubulin deacetylase, HDAC6. Phosphorylated HDAC6 moves into the cilia and prevents the acetylation of tubulins, in turn promoting the destabilization of the axoneme. FSL1 might also play a role in dephosphorylating CrKin13 in cilia to increase its depolymerization activity (Figure 1A). In the FLS1-independent phase II, CALK activity is regulated by other factors such as length of shorting flagella, as suggested by Hu et al. [3]. Since CrKin13 is present in the cilia in both, phosphorylated and dephosphorylated forms, with the dephosphorylated form showing stronger microtubule depolymerization activity, it is possible that the speed of ax-

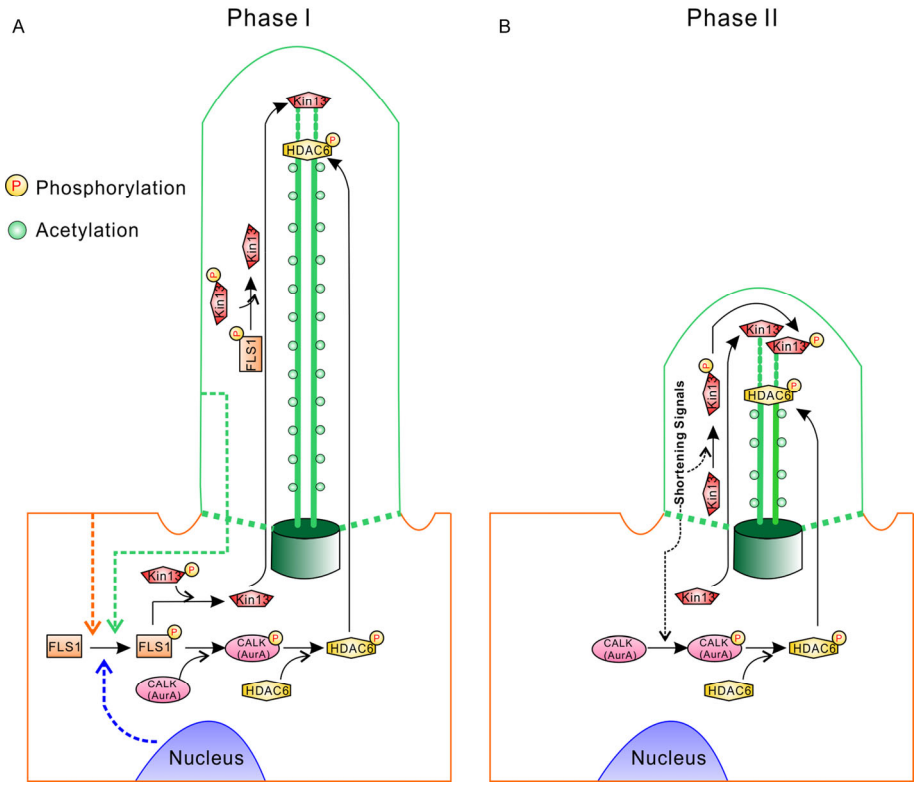

Figure 1 (color online) Two-phase model of flagella disassembly. A. Phase I, signals from the plasma membrane, cilia, or nucleus may induce phosphorylation of FLS1, followed by activation of CALK, subsequently resulting in HDAC6 phosphorylation to deacetylate axonemal tubulin in cilia. FSL1 may inhibit CrKin13 phosphorylation indirectly in the cell body or flagella, to maintain the high depolymerization activity of dephosphorylated CrKin13. B. Phase II, flagella are shortened to half-length and a signal is generated to activate CALK-HDAC6 pathway and also cause the phosphorylation of CrKin13 to keep the balance between phosphorylated and dephosphorylated forms of CrKin13 in cilia.

oneme disassembly could be regulated by adjusting the relative amount of these two forms of CrKin13 (Figure 1B).

Similar to other significant discoveries, the two-phase model of ciliary disassembly has raised several interesting questions. First, what are the physiological functions of the two-phase model? To this, Hu et al. suggested that distal ciliary resorption generates signals that induce cell cycle transition from G1 to S, while signals from the proximal part of cilia help to release the centrioles [3]. Another speculation is that cilia play an important role in sensing environmental signals, where the two-phase model ensures that cells can regenerate full-length cilia immediately from half-length. However, both speculations need experimental confirmation. The second question is if there are any structural differences between distal and proximal cilia to support the two-phase model. The beak-like structures associated with the flagellar microtubule doublet and polyglutamylated tubulins are only found in the proximal flagella, where a length-control kinase, LF5, is also localized. Further study is required to determine if any defect in flagellar disassembly occurs when the localization of these structures or proteins is changed. The third question is if another disassembly pathway in flagella is possible, as flagella could still shorten to half-length, in the absence of FLS1. Other interesting questions include the mechanism of synchronized disassembly of the axonemes and ciliary membrane, if other posttranslational modifications such as ubiquitination are involved, and mechanisms of regulation of FLS1 phosphorylation. Hu's work has built a solid foundation to answer the above questions and will speed up research on the mechanism of ciliary disassembly.

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