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An enzyme with self-control

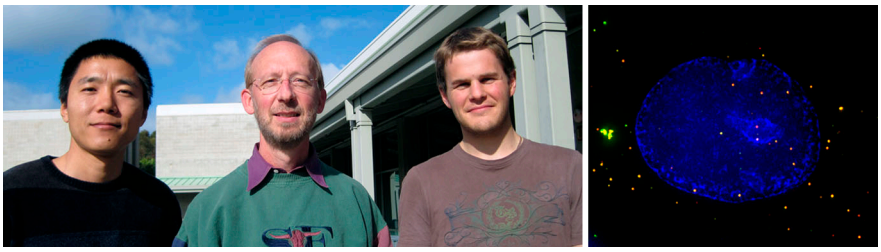
To keep itself in check, Plk4 encourages its own removal.

Unlike enzymes that depend on other proteins to rein in their activity, polo-like kinase 4 (Plk4) does the job itself. Holland et al. reveal that Plk4, which helps regulate centrosome duplication, reduces its activity by initiating its own destruction (1). By sacrificing itself, the protein prevents cells from building too many centrosomes.

Cells prepare to divide by duplicating the centrosome, the organelle that serves as a hub for the cytoskeleton and an anchor for the mitotic spindle. The problem is how to make only one copy before each round of cell division. Extra centrosomes can disrupt chromosome separation during mitosis and might trigger cancer (2), although the evidence for that effect remains controversial. Managing centrosome duplication is the job of Plk4, which prompts the pair of centrioles in the centrosome to replicate. Cells must carefully calibrate Plk4 levels because too much or too little of the enzyme promotes tumors (3, 4). In fruit flies, a protein complex called SCF^{Slimb} helps regulate Plk4's abundance by ubiquitinating phosphorylated copies of the enzyme, spurring their demolition by the proteasome (5). Mammals carry an equivalent to SCF^{Slimb} known as $SCF^{\beta\text{-}TrCP}$, but other aspects of the mechanism that sets Plk4 levels in mammalian cells remain murky.

What little Plk4 cells harbor usually disappears rapidly. The enzyme adds phosphates to targets including other Plk4 molecules, suggesting that self-phosphorylation might spur its speedy demise. To test this idea, Holland et al. engineered human cells to produce normal Plk4 or a nonfunctional variant. The amount of Plk4 was 10 times higher in cells that made the defective enzyme. The researchers also tested a Plk4 version whose phosphorylation ability could be shut down with a drug called 1NM-PP1. Adding the inhibitor to cells caused the amount of Plk4 to shoot up.

Focal Point



Weijie Lan (left), Don Cleveland (middle), Andrew Holland (right), and colleagues determined how the kinase Plk4 curbs its own levels and thus limits duplication of centrosomes. A cell with faulty Plk4 (right) produces multiple centrioles, indicated by the glowing dots strewn around the cytoplasm. The original centrioles are the bright green dots at the left edge.

The picture that emerges from these experiments, the researchers say, is that in mammalian cells Plk4 molecules stimulate their own breakdown by phosphorylating each other. Whether an individual Plk4 molecule can affix phosphates to itself isn't clear, says first author Andrew Holland, but he thinks it's highly likely that Plk4 is autophosphorylating.

The team also nailed down where the phosphates attach to Plk4. Previous studies identified two phosphate-ready amino acids within a region of the protein that attracts the ubiquitinating complex $SCF^{\beta\text{-}TrCP}$. Holland et al. replaced these two amino acids with alanines, which can't be phosphorylated and thus prevent $SCF^{\beta\text{-}TrCP}$ from latching on. The change only modestly boosted Plk4's stability, suggesting that additional phosphorylation sites help regulate Plk4 levels. The team located 13 such sites, spread across a 24–amino acid span in the protein's midsection. Snipping out this segment halted Plk4's destruction and dramatically increased the number of centrioles that cells manufacture, the scientists found.

"This kinase regulates its own stability to help prevent centrosome overduplication and genome instability," says Holland. The finding puts Plk4 in rare company. Scientists have identified only one other kinase, the tyrosine kinase Src, that controls its own levels in a similar way. However, researchers haven't worked out how Src's activity undercuts its stability.

Several questions remain about Plk4 as well, such as whether other enzymes in addition to $SCF^{\beta\text{-}TrCP}$ ubiquitinate it and whether other kinases help diminish its stability. Another mystery is why Plk4 carries so many phosphorylation sites. Holland suggests that the large number of sites results in a degradation delay while the enzyme acquires a threshold number of phosphates. "We think that [the delay] might give the protein the opportunity to perform its function before it kills itself," he says.

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4. Basto, R., et al. 2008. *Cell.* 133: 1032-42.
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