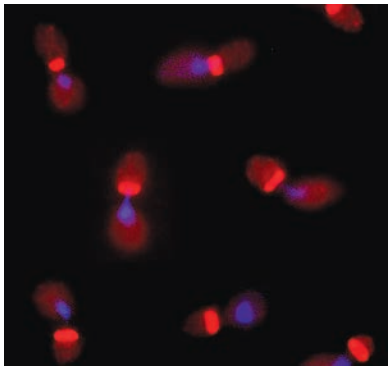
Text By Nicole LeBrasseur
lebrasn@rockefeller.edu

# The kinase knows

Cells monitor the assembly of cytoskeletal structures via direct binding and activation of a checkpoint kinase, as shown by Jessie Hanrahan and Michael Snyder (Yale University, New Haven, CT).



Snyder/Elsevier

**Hsl1 (red), is activated at the bud neck by septin binding.**

Cytoskeletal reorganization is needed during various events in the cell cycle. In budding yeast, for example, a ring of septin filaments separates the mother cell from the forming bud. The ring must be completed before the cell can enter mitosis. This landmark is signaled by the Hsl1 kinase, which is activated upon ring assembly. Snyder wondered, "how do [cells] recognize when a macromolecular complex like the cytoskeleton is assembled? The thought seems daunting at first." But he found a relatively simple answer for Hsl1—it is either bound to the septin ring or is in an inactive conformation.

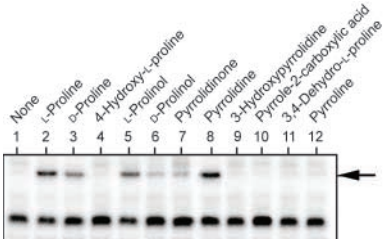
The authors identified a site in the large noncatalytic portion of Hsl1 that binds to and inactivates the kinase domain. This autoinhibitory domain overlaps with three sites that bind to two different septin subunits. In vitro, septins activate the kinase by displacing the inhibitory domain. In vivo, the septins have to be polymerized, as previous results showed that unassembled subunits do not activate Hsl1. This may be due to the spacing of the septin binding sites and the need for all three sites to be filled to displace the inhibitory domain.

This sensing mechanim may extend to other cytoskeletal structures. Microtubules, for instance, are bound by a relative of Hsl1 called MARK. Says Snyder, "[the Hsl1 model] is attractive as a universal mechanism to monitor assembly of cytoskeletons specifically, and maybe macromolecular complexes in general." ■

Reference: Hanrahan, J., et al. 2003. *Mol. Cell.* 12:663–673.

# Transcription keeps it simple

A new report shows that a eukaryotic transcription factor is directly activated by a metabolite of the pathway it controls. Although this idea has been proposed for several regulatory systems, Christopher Sellick and Richard Reece (University of Manchester, UK) are the first to demonstrate direct binding to and activation of a eukaryotic transcription factor by a small molecule metabolite.




Reece/EMBO

**Proline analogues turn on Put3p-activated transcription (arrow).**

The interaction controls the activity of yeast Put3p, an activator of PUT1 and PUT2 transcription. Put1p and Put2p convert proline into glutamate—a high quality source of nitrogen. Put3p binds upstream of the enzymes' transcription start sites whether or not proline is available, but Sellick and Reece show that waste is avoided by limiting activation to proline-rich conditions. In vitro, Put3p alone was inactive, but transcriptional activation required only the addition of proline. The direct binding of proline to Put3p was weak, probably so the pathway is turned on only when proline is abundant.

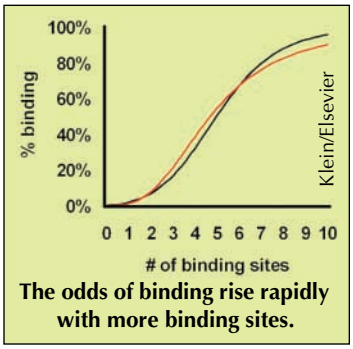
When preferred nitrogen sources such as glutamine are present, Put3p is inactive even in the presence of proline, suggesting that other mechanisms ensures that proline is converted only when alternative sources are not available. ■

Reference: Sellick, C.A., and R.J. Reece. 2003. *EMBO J.* 22:5147–5153.

# Binding takes cooperation

The CDK inhibitor Sic1 gets proteolyzed in late G1, but the regulation of its binding to the Cdc4 ubiquitin ligase subunit has been a conundrum. The proteins do not bind unless Sic1 is phosphorylated on at least six of its nine sites, yet Cdc4 can harbor only one phosphorylated epitope. Traditional thermodynamic arguments cannot explain this puzzle, but a new mathematical model from Peter Klein (Fox Run Management, Greenwich, CT), Tony Pawson, and Mike Tyers (University of Toronto, Toronto, Canada) offers an explanation.

The group imagined that additional binding sites might make Sic1 more likely to rebind at another site than to diffuse away from Cdc4. "It's like seaweed floating in the waves," says Klein. "Pieces get stuck on a rock. A wave might knock it off, but before it floats away, another arm can get stuck." Their model shows that the affinity of Cdc4 for Sic1 grows exponentially with each Sic1 phosphorylation, an effect they call allovalency. As long as diffusion is sufficiently slow and rebinding is rapid enough, the model is possible kinetically when biophysically realistic numbers are considered.




**The odds of binding rise rapidly with more binding sites.**

Extension of this model is possibly limited by the critical assumption that each binding site must be able to move independently, as is the case for the unstructured Sic1. Lectins and their long-chain carbohydrate ligands might also fit this sort of model. ■

Reference: Klein, P., et al. 2003. *Curr. Biol.* 13:1669–1678.