Text by Mitch Leslie
mitchleslie@comcast.net



# Knl1 shows another face

Kinetochore protein might not recruit key parts of spindle assembly checkpoint and instead might help silence the checkpoint.

Researchers have credited the kinetochore protein Knl1 with functions that range from binding microtubules to docking enzymes. Two new studies help to clarify Knl1's role (1, 2), adding new possible tasks and indicating that it doesn't perform some of the jobs already ascribed to it.

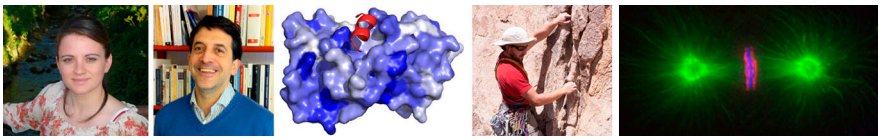
Krenn et al. (1) took on a controversy about how the kinetochore attracts two proteins that help form the spindle assembly checkpoint, which prevents cells from completing mitosis until the chromosomes are correctly connected to the mitotic spindle. Researchers have proposed that two key checkpoint proteins, Bub1 and BubR1, latch onto a kinetochore protein called Bub3 (3). However, near their N termini both proteins also carry three tetratricopeptide repeats (TPRs), which could connect to the KI motifs of Knl1 (4).

To weigh these alternatives, the team determined the crystal structure of a Bub1 fragment bound to a segment of Knl1 carrying the KI motifs. Knl1 adhered to a ridge in the section of Bub1 that contained the TPRs. Mutations in this portion of the Bub1 fragment prevented it from latching on to Knl1.

Krenn et al. discovered that Bub1 and BubR1 variants with mutations in their KI-binding domains were still able to attach to kinetochores. In addition, fragments consisting only of the N terminus of Bub1 (which includes the TPRs) didn't stick to the kinetochores, but longer fragments that also carried the Bub3-binding site did.

The results support the initial hypothesis that the Bub3-binding domain, rather than the TPRs, recruits Bub1 and BubR1 to kinetochores, strongly suggesting that Bub3 is crucial for their recruitment. "The repeats are not sufficient for kinetochore recruitment, and quite possibly they are not necessary," says senior author Andrea Musacchio.

## Focal Point



PHOTOS COURTESY OF CLAUDIO RIGAMONTI (KRENN), ROBERTA PALUMBO (MUSACCHIO), AND JULIEN ESPEUT (ESPEUT); IMAGE (FAR RIGHT) COURTESY OF JULIEN ESPEUT

**Veronica Krenn (far left), Andrea Musacchio (second from left), and colleagues (not pictured) were interested in whether Knl1 recruits the checkpoint proteins Bub1 and BubR1 to the kinetochore. A crystal structure (center) shows a segment of Knl1 (red coil) bound to a portion of Bub1. Julien Espeut (second from right) and colleagues (not pictured) found that Knl1 helps shut down the spindle assembly checkpoint when it detects microtubules. In the far right image, Knl1 (red) adheres to a chromosome (blue) on the mitotic spindle (green) in a metaphase *C. elegans* cell.**

The interaction between the TPRs and Knl1 may instead help the cell dictate where Bub1 and BubR1 are active, Musacchio says. Binding to Knl1 might reshape Bub1 and BubR1, turning on the proteins only at kinetochores.

Meanwhile, Espeut et al. (2) suggest that Knl1 can switch off the spindle assembly checkpoint. How mitotic cells perform this step, which is necessary for their entry into anaphase, has been a mystery. On kinetochores, Knl1 interacts with other proteins, including the Ndc80 complex. Ndc80 forms a load-bearing attachment to microtubules, helping ensure that the filaments attach to chromosomes and can pull them apart (5). Knl1 also hooks onto microtubules, but researchers don't know why.

> "[Knl1 is] probably acting as a sensor controlling checkpoint signaling."

Espeut et al. found that Knl1 doesn't form load-bearing links to microtubules. Chromosomes separated normally in *C. elegans* embryos expressing mutant versions of Knl1 that were unable to bind microtubules. But anaphase began later in these mutants, suggesting that cells had difficulty shutting down the spindle assembly checkpoint.

To test this possibility, the researchers examined embryos with monopolar spindles, which show long delays in mitosis. Knl1 mutants unable to bind microtubules extended mitosis even further, whereas removing the checkpoint protein Mad2 restored normal mitotic timing, bolstering the case that Knl1 helps shut down the checkpoint upon binding to microtubules.

Researchers have previously hypothesized that Knl1 may silence the spindle checkpoint by hooking up with the enzyme protein phosphatase 1 (PP1) (6). Espeut et al. showed that Knl1's microtubule-binding activity is separate from its interaction with PP1.

The work identifies a new function for Knl1. "It's probably acting as a sensor controlling checkpoint signaling," says senior author Arshad Desai. Once Knl1 "feels" microtubules, it somehow helps suppress the spindle assembly checkpoint. The mechanism remains obscure, however, because researchers don't know how the checkpoint generates a "wait" signal at kinetochores. In some organisms, the motor protein dynein helps silence the spindle checkpoint by hauling off some of its components, but Espeut et al. think that Knl1 works independently of this protein.

1. Krenn, V., et al. 2012. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201110013.
2. Espeut, J., et al. 2012. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201111107.
3. Taylor, S.S., et al. 1998. *J. Cell Biol.* 142:1–11.
4. Kiyomitsu, T., et al. 2007. *Dev. Cell.* 13:663–676.
5. Joglekar, A.P., et al. 2010. *Curr. Opin. Cell Biol.* 22:57–67.
6. Meadows, J.C., et al. 2011. *Dev. Cell.* 20:739–750.