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NoCut: Cytokinesis in Check

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In this issue of *Cell*, Norden et al. (2006) describe a new pathway, NoCut, that links the completion of chromosome segregation to the onset of cytokinesis in budding yeast. In NoCut, Aurora kinase (Ipl1) at the spindle midzone negatively regulates cytokinesis through two proteins previously identified to be involved in cell polarity, Boi1 and Boi2.

The mechanics of chromosome segregation are based on the interaction between kinetochores, specialized structures that form on the centromeres of chromosomes, and microtubules, the cytoskeletal polymer that constitutes the mitotic spindle. The fidelity of segregation depends upon efficient kinetochore-microtubule attachment mechanisms, sister chromatid cohesion, signaling pathways that monitor chromosome bi-orientation, and dissolution of sister chromatid linkages upon satisfying these criteria and progression into anaphase. This is not trivial as chromosomes are extremely long structures; even the smallest yeast chromosome is well over 100 μ in its linear B form. It is equally imperative that chromosome arms segregate completely prior to cytokinesis. In this issue of *Cell*, Norden et al. (2006) have identified a signaling system (NoCut) that relays signals from kinetochores to the spindle midzone to the cell cortex. The authors suggest that this pathway may be critical in delaying cytokinesis until the DNA is completely separated and moved out of the division plane.

Cytokinesis in yeast and other cell-walled fungi requires resolution of the plasma membrane (abscission) and degradation of the primary septum (cell separation or septation). In many cells, the mitotic spindle determines the position of the cytokinesis furrow. In budding yeast, *Saccharomyces cerevisiae*, the site of cell division is predetermined (by the position of the bud) and the spindle migrates toward and through this site during normal anaphase with the elongated anaphase spindle bisecting the cleavage furrow. The anaphase spindle consists of overlapping microtubules, and this zone of overlap, which is equidistant from each pole, is known as the midzone. Chromosome passenger proteins are released from the kinetochore upon anaphase onset and migrate to the midzone. These proteins, collectively referred to as INCENPs (inner centromere proteins), were the first indication that kinetochores may transmit information to the midzone, and this information was proposed to coordinate chromosome segregation and cytokinesis (Earnshaw and Bernat, 1991). Subsequently, Aurora kinase and survivin (an inhibitor of apoptosis protein) were found to relocalize to spindle midzones upon anaphase onset (Wheatley et al., 2001). In budding yeast, the homologous proteins (INCENP, Sli15; Aurora kinase, Ipl1; and survivin, Bir1) form a complex that is required for spindle stability and is required for the proper timing of spindle disassembly (Buvelot et al., 2003) before cytokinesis.

In addition to the INCENP complex, the midzone is populated with microtubule plus-end binding proteins, the structural proteins, Ase1 and Slk19, and several kinetochore components (including Ndc10, a member of the core centromere DNA binding complex, CBF3). These proteins contribute to spindle stability and, in the case of CBF3, may be poised to establish new kinetochore-microtubule interactions for the next cell cycle (Bouck and Bloom, 2005a). The precise nature of which factor does what and how they ensure that the terminal phase of chromosome segregation is complete has remained elusive.

The integrity of the spindle midzone is critical for the NoCut pathway described by Norden et al. (2006). Deletion or overexpression of the gene that encodes Ase1—a microtubule binding protein that localizes to the spindle midzone—delays membrane abscission. In addition, mutations in the genes that encode several kinetochore proteins (such as Ndc10, Cep3) lead to membrane abscission and cell separation defects (Norden et al., 2006; Bouck and Bloom, 2005b). However, as there are reports that membrane abscission but not cell separation occurs in *ndc10* mutants (Bouck and Bloom, 2005a), it is not clear which event requires Ndc10. The class of kinetochore proteins that migrates to the midzone contributes specifically to the NoCut pathway, as mutations of other kinetochore components do not compromise cytokinesis (Norden et al., 2006; Bouck and Bloom, 2005a). Cells lacking the spindle assembly checkpoint divide in the presence of the microtubule poison, benomyl; however, these divisions are abortive, as the cells fail to complete cytokinesis (Norden et al., 2006; Hoyt et al., 1991). This implicates microtubule, not kinetochore, function in cytokinesis. Interestingly, it is spindle microtubules per se, and not the position of the spindle relative to the site of division, that is critical (Norden et al., 2006).

The connection between spindle midzone and membrane abscission was established upon analysis of

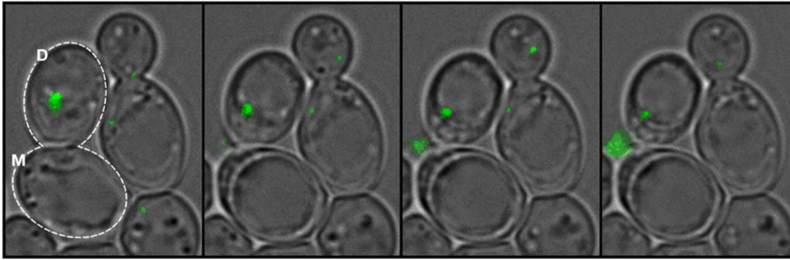

Figure 1. Cytokinesis Gone Awry
The Lac repressor, a DNA binding protein, fused to GFP binds to *lac* operator sequences integrated into the yeast genome. This fluorescently labels a region of the yeast DNA (visualized as a green spot) that can be monitored using timelapse video microscopy. The outlined cell contains a lagging dicentric chromosome that fails to segregate into both mother (M) and daughter (D) cells (panel 1, leftmost panel). Upon cell separation (noted by change in position of the daughter and mother cell between the first and second panel), DNA left in the cytokinesis furrow is extruded from the cell (panel 2) and released from these cells over time (panels 3 and 4). Images courtesy of Doug Thrower and Kerry Bloom.

mutants in the gene that encodes the Aurora kinase (Ipl1). The defect in membrane abscission observed in *ndc10* mutants was rescued in *ipl1 ndc10* double mutants. These results led Norden et al. (2006) to propose that active inhibition of abscission by Ipl1 may delay cytokinesis in response to spindle or midzone defects. However *ipl1 ndc10* double mutant cells remain linked via cell wall constituents. Thus although membrane abscission is restored in the absence of the NoCut pathway, septation is still defective. The lack of cell separation in the absence or presence of Ipl1 remains a common feature of defective kinetochore protein passage to the spindle midzone. Therefore the correct assembly of midzone components is essential for the completion of cell division.

As Ipl1 is nuclear and does not localize to the bud neck, what are the proteins that link this kinase to the spindle midzone/cytokinesis signaling pathway? Norden et al. (2006) reasoned that such proteins may be identified based on changes in localization. Two proteins essential for polarized growth, Boi1 and Boi2 (Bem1 interacting) (Matsui et al., 1996), localize to the bud cortex, are occasionally observed in the nucleus, and could be key intermediates completing the circuitry in NoCut. Boi1 and Boi2 contain PH domains (which mediate interactions with membranes) in their C terminus, as do anillins which are components of the contractile ring and are essential for cytokinesis. In the complete absence of Boi1 and Boi2, *ndc10* mutants form proper septa and were resolved to single-budded cells on treatment with zymolyase (an enzyme that digests the cell wall). Thus, as observed in the absence of Ipl1, *boi1* and *boi2* mutant cells were unable to inhibit abscission in the face of midzone defects. Furthermore, in the absence of Ipl1, Boi1-GFP remained predominantly nuclear. Thus Ipl1 appears to be required for the transit of Boi1 between the nucleus, bud, and bud neck. Although both Boi1 and Boi2 contain Ipl1 consensus phosphorylation sites, mutation of these sites does not alter their localization. This raises the likelihood of additional Ipl1 substrates, alternate Ipl1 sites, or other components in the NoCut circuitry.

Whatever the nature of the signaling cascade, the major question is whether NoCut actually prevents cytokinesis in the presence of lagging chromosomes. In fission yeast, mutations in chromosome segregation lead to cut phenotypes when lagging chromosomes don't "get through" before cytokinesis is completed (Hirano et al., 1986). In extreme cases, the failure to segregate chromosomes before cytokinesis can lead to DNA extrusion from the cell in budding yeast (Figure 1). Norden et al. (2006) monitored the occurrence of DNA breaks with a visual reporter for DNA damage, Ddc1-GFP. Ddc1 is an early responder to damage and accumulates as foci at sites of damage (Melo et al., 2001). In strains with midzone defects and the absence of *boi1* and *boi2*, the frequency of Ddc1 foci is increased. Suppression of cytokinesis via loss of septin (Cdc12) accordingly suppressed Ddc1 foci. Thus if cytokinesis is allowed to proceed in cells with spindle midzone defects, DNA damage ensues.

NoCut is the first pathway that completes the circuit between the spindle midzone and the cleavage furrow. Although several proteins such as INCENPs that shuttle from kinetochores to the spindle midzone and Ipl1, Sli15, Bir1 that regulate spindle disassembly have been reported, the transfer of information between these structures has been elusive. The discovery that Ipl1 is a negative regulator of cytokinesis and functions via the shuttling proteins Boi1 and Boi2 is a significant advance in the field. Like many other processes, the cell takes nothing for granted, and in the case of the final stages of chromosome segregation, Ipl1 sends its Bois to restrain premature cleavage.

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