Quality Control Mechanism Preserves Chromosome Stability in Yeast

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The precise partitioning of replicated chromosomes to daughter cells during cell division ensures genomic stability. Kinetochores are the high-molecular-weight protein machines that interact with chromosomes to enable their segregation. Multiple protein complexes assemble the kinetochore in a hierarchical fashion: inner kinetochore proteins directly bind chromosomes while outer (chromosome-distal) proteins interact with microtubules that physically pull chromosomes toward spindle poles. A more recent class of 'middle' kinetochore proteins have been identified that form a key physical link between the inner and outer kinetochore complexes. The Dsn1 protein is one such important middle kinetochore component, and the Biggins Lab has recently discovered a regulatory mechanism that impinges on Dsn1 function to inhibit chromosome segregation in cells with defective kinetochores.

The Biggins Lab recently established a method to purify kinetochores from yeast cell extract that has been instrumental in study kinetochore protein composition and function. Mass spectrometric analysis of the purified kinetochores revealed the presence of the Mub1/Ubr2 E3 ubiquitin ligase complex, previously implicated in the ubiquitination and degradation of a limited number of proteins. Although Mub1/Ubr2 have not been previously implicated in chromosome segregation, their association with kinetochores indicated that ubiquitylation of a kinetochore component might influence the process.

To determine how Mub1/Ubr2 is recruited to kinetochores, the authors used a panel of inner and outer kinetochore mutants to determine whether these mutations disrupt the interaction of Mub1/Ubr2 with kinetochores. Mif2 mutant kinetochores failed to bind Mub1/Ubr2, indicating that Mif2 (CENP-C in higher eukaryotes), a conserved protein known to serve as a link between the inner and outer kinetochore, is required for Mub1/Ubr2 kinetochore association. Although closely associated, Mub1/Ubr2 did not ubiquitylate Mif2. This indicated that another kinetochore protein might be targeted by Mub1/Ubr2 and, with further investigation, Akiyoshi *et. al.* found that Mub1/Ubr2 targets Dsn1 for degradation. Intriguingly, the authors found that Mub1/Ubr2 is particularly important for the degradation of aberrant, likely misfolded or misregulated Dsn1 protein. This is reminiscent of known quality-control mechanisms that target aberrant proteins for degradation, indicating that kinetochore architecture might be under a similar surveillance system.

A final line of investigation sought to determine if Mub1/Ubr2 influence chromosome segregation. Although they found that Mub1/Ubr2 does not influence chromosome segregation in wild-type cells, Akiyoshi *et. al.* reasoned that ubiquitin-mediated regulation of kinetochores might be an important way in which cells inhibit chromosome segregation attempts when malformed kinetochores are present. Indeed, chromosome missegregation of inner and outer kinetochore mutants studied were exacerbated in Mub1/Ubr2 mutants, suggesting a key role for Mub1/Ubr2 in inhibiting aberrant kinetochores from attempting and ultimately failing chromosome segregation (see figure).

Proteolysis is an important mechanism that controls the cellular levels of proteins with a broad range of functions. Akiyoshi *et. al.*now provide additional evidence for ubiquitin-mediated proteolysis in the regulation of kinetochore function by targeting the conserved Dsn1 protein, especially under conditions when kinetochore integrity is compromised. It will be interesting to determine if the Dsn1 homolog in higher eukaryotes is similarly subject to proteolysis to ensure chromosome stability in dividing cells. “In the future, we would like to figure out if this is a conserved quality control system and how it recognizes aberrant kinetochore structures,” says Dr. Biggins.

Akiyoshi B, Nelson CR, Duggan N, Ceto S, Ranish JA, Biggins S. 2013. The Mub1/Ubr2 ubiquitin ligase complex regulates the conserved Dsn1 kinetochore protein. *PLoS Genet.* 9(2):e1003216.

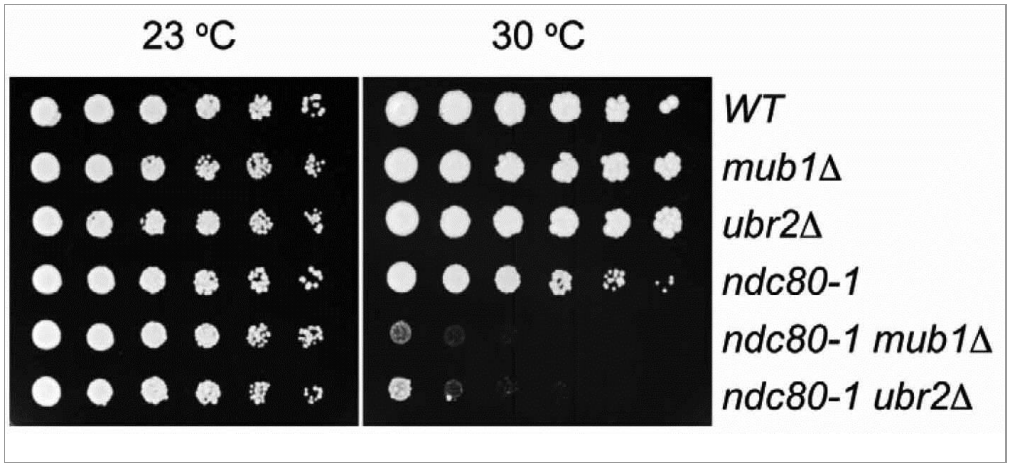

Image adapted from the manuscript

Mub1/Ubr2 deletion exacerbates the slow growth of kinetochore mutants. Yeast of the indicated genotype were serially diluted and incubated at the permissive (left panel) or semi-permissive (right panel) temperature for the ndc80-1 mutant. The deletion of mub1 or ubr2 in wild-type cells has no consequence on cell viability, but greatly enhances the temperature sensitive growth defect of the ndc80-1 mutant. These and other data reveal a key role for the Mub1/Ubr2 complex in kinetochore function.