A New Model of Spindle Checkpoint Silencing

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Kinetochores are huge macromolecular machines that connect chromosomes to microtubules of the cell division apparatus, thereby providing daughter cells and gametes with the correct chromosome complement during mitosis and meiosis. This feat is accomplished through chromosome 'bi-orientation,' in which sister chromatids are attached to microtubules extending from opposite ends of the bipolar spindle apparatus. Failures in this process lead to an incorrect chromosome number, or aneuploidy, resulting in certain cancers and genetic disorders such as Down syndrome. A molecular surveillance system known as the spindle checkpoint works to avoid these failures by preventing anaphase until all pairs of chromosomes have made the correct bi-oriented attachments to the spindle apparatus. Across eukaryotes, the highly conserved Mps1, Bub1 and Aurora protein kinases are known to regulate the spindle checkpoint, yet researchers have identified few of the phosphorylation substrates that mediate the checkpoint functions carried out by these enzymes.

In a recent study from the laboratory of Dr. Sue Biggins (Basic Sciences Division), Akiyoshi *et al.* (2010) developed a method for purifying functional kinetochores from budding yeast, and found that Mps1 and Bub1 co-purify with the intact kinetochore particles. This breakthrough in methodology allowed graduate student Nitobe London, principal investigator Dr. Biggins and two of their colleagues to study kinetochore targets of these, and other, candidate checkpoint kinases.

London and co-authors report that Mps1-mediated phosphorylation activity is required for the localization of Bub1 to budding yeast kinetochores, as it is in other organisms. Localization of Bub3, the interaction partner of Bub1, was also found to require the activity of Mps1. By immunoprecipitating candidate kinase substrates and assaying them with radiolabelled ATP, the authors showed that Mps1 phosphorylates the conserved Spc105 protein. London *et al.*then analyzed Mps1-phosphorylated Spc105 in more detail using mass spectrometry, and they discovered that regulation of Bub1 and Bub3 recruitment requires phosphorylation of conserved MELT motifs within Spc105. Bub1 and Bub3 binding to the kinetochores was greatly reduced when these Mps1 phosphorylation sites on Spc105 were knocked out. In this way, the authors experimentally demonstrated that it was phosphorylation by Mps1 that actually causes the recruitment of these key kinases to the kinetochore. The researchers further showed that dephosphorylation of Spc105 by the phosphatase PP1 releases Bub1 and Bub3 from the budding yeast kinetochores. A final line of evidence firmly established the key role of Spc105 and its MELT

motifs in spindle checkpoint regulation: Experimental mutation of the MELT motifs caused defects in spindle checkpoint function, in addition to the specific defects in Bub1 and Bub3 binding that were also detected.

Considering all of their results together, the authors synthesized a new model for spindle checkpoint silencing in budding yeast, which is summarized in the accompanying figure. Given the broad conservation of MELT motifs between fungi (e.g., yeast) and animals, despite great diversity in other parts of the Spc105 protein, it is quite possible that phosphoregulation of Spc105 by Mps1 and PP1 similarly regulates spindle checkpoint function, and therefore maintains genomic stability, in distantly related eukaryotes such as humans.

London N, Ceto S, Ranish JA, Biggins S. 2012. Phosphoregulation of Spc105 by Mps1 and PP1 regulates Bub1 localization to kinetochores. *Current Biology* 22:900-906.

Also see: Akiyoshi B, Sarangapani KK, Powers AF, Nelson CR, Reichow SL, Arellano-Santoyo H, Gonen T, Ranish JA, Asbury CL, Biggins S. 2010. Tension directly stabilizes reconstituted kinetochore-microtubule attachments. *Nature* 468:576-579.

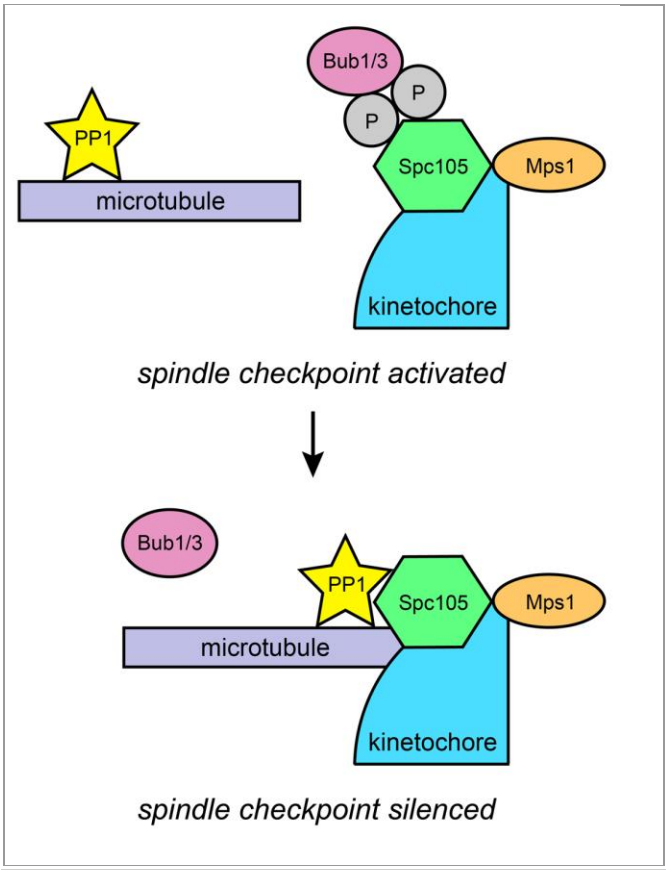

Image modified from London et al. (2012)

The authors' model of how the spindle checkpoint system is silenced. When microtubules are not attached to kinetochores of sister chromatids in the correct bi-orientation, Mps1 phosphorylates Spc105, causing the recruitment of Bub1/Bub3 to the kinetochore. In contrast, correct microtubule attachment promotes PP1-mediated phosphatase activity on Spc105, leading to dephosphorylation of Spc105 and release of Bub1/Bub3. The result is checkpoint silencing and a continuation of mitosis or meiosis.