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The budding yeast CLASP ortholog Stu1 inversely regulates kinetochore capture and spindle stability

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Faithful chromosome segregation during mitosis is a prerequisite for the maintenance of genomic stability and related defects are associated with a variety of diseases such as cancer. An early and crucial step for faithful chromosome segregation is the capture of chromosomes by spindle microtubules, a mechanism that is widely conserved from yeast to humans. In a first step, chromosomes make contact to the lateral surface of a microtubule and in a second step, the unstable lateral attachment is converted to a stable end-on attachment to the microtubule plus-end. The interaction between chromosomes and microtubules is mediated by multi-protein complexes called kinetochores. CLASPs are components of the kinetochore and their essential function for faithful chromosome segregation has been demonstrated from yeast to humans. As its human orthologs, the budding yeast member of the CLASP family Stu1 localizes to kinetochores and spindles. While its association with the spindle in prometaphase and metaphase has been shown to be important for spindle assembly and integrity, little is known so far about the role of Stu1 midzone localization in anaphase and Stu1 interaction with kinetochores. The phenotype of a kinetochore mutant (okp1-52) that displays a severe spindle defect while Stu1 is sequestered at the defective kinetochores prompted further investigation. Using different approaches, the significance of Stu1 kinetochore and spindle localization was further investigated, revealing new insights into the essential functions of Stu1 for faithful chromosome segregation during mitosis:

1. Stu1 specifically associates with unattached kinetochores
Stu1 specifically interacts with kinetochores that are not attached to spindle microtubules and thus have yet to be captured. This interaction is dependent on the Ndc80 complex, an outer kinetochore component, and the Stu1 C-terminus. The binding of Stu1 to kinetochores triggers Stu1 oligomerization. By this means, a detached kinetochore sequesters most nuclear Stu1 and interferes with Stu1 spindle association.

2. Stu1 is essential for kinetochore capture
Stu1 facilitates kinetochore capture and is in particular essential for the recapture of those kinetochores that have moved further away from the spindle pole bodies. This function is dependent on the Stu1 domain required for oligomerization and microtubule binding. By increasing the surface of a detached kinetochore several fold, Stu1 oligomers could provide a direct and extensive microtubule interaction lattice.

3. Stu1 relocalizes to spindle microtubules when a captured kinetochore interacts with a functional Dam1 complex
Stu1 stays associated with a captured kinetochore during the initial transport along the lateral surface of a microtubule. Only after a stable end-on attachment to the microtubule plus-end through an association with the outer kinetochore complex Dam1 has been achieved, Stu1 dissociates from the captured kinetochore. Thereby, biorientation is no prerequisite for Stu1 release, but syntelic attachment is sufficient. Stu1 that is no longer sequestered at kinetochores relocalizes to spindle microtubules.

4. Stu1 coordinates kinetochore capture and spindle stability
As Stu1 is essential for spindle assembly and integrity, the sequestration of Stu1 at unattached kinetochores interferes with spindle assembly. Only after all kinetochores are captured, Stu1 stays associated with spindle microtubules and promotes spindle assembly. The maintenance of a short spindle until all kinetochores are captured is thought to facilitate the establishment of biorientation.

5. Stu1 association with the spindle midzone in anaphase is dependent on the Stu1 C-terminus and is essential for anaphase integrity
Stu1 is known to localize to spindles in metaphase when all kinetochores are attached to spindle microtubules. This association is mainly independent of C-terminal sequences of Stu1. Contrastingly, the Stu1 C-terminus is required for Stu1 association with the spindle midzone in anaphase and C-terminal deletion mutants even interfere with wild-type Stu1 midzone localization. A failure of Stu1 to associate with the midzone during anaphase results in spindle breakage, revealing the essential function of Stu1 for spindle integrity at this time.

6. Stu1 gets phosphorylated during mitosis
Phosphorylation is a common mechanism for the regulation of protein functions. Stu1 is phosphorylated within 10 min after bud appearance, which is about the time of S-Phase or prometaphase. Interestingly, the phosphorylation of Stu1 is dependent on the Stu1 C-terminus.