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Kinetochore and Mitosis

Goal

To understand kinetochore structure and function.

Background

Reliable chromosome segregation depends on the correct attachment of the two sister chromatids to microtubules (MTs) emanating from the opposing spindle poles. The chromosomal structures that mediate this interaction are the kinetochores (KTs). They are composed of several conserved multiprotein complexes that either bind to the centromere DNA, constitute the KT–MT interface or serve as linkers between these two. In addition, proteins that regulate kinetochore function interact with the core components in a mitosis stage specific manner. These include the proteins that regulate the spindle assembly checkpoint (SAC), a signaling mechanism that prevents the initiation of anaphase as long as unattached kinetochores (uaKTs) are present. A second group of proteins that may serve a regulatory function at the KT are proteins that influence the dynamics of MTs. These include the CLASP protein family. CLASP proteins contain two or more TOG-like domains (TOGL). TOG or TOGL domains consist of 6 HEAT repeats that can bind to alpha/beta tubulin dimers via their intra HEAT repeat loops. CLASPs exploit this feature probably to promote MT rescue. A prominent example in this respect is the stabilization of the mitotic spindle. In anaphase, CLASPs promote the stability of interpolar MTs (iMTs) by localizing to the spindle midzone. For metaphase however the principals of CLASP localization and function are not well understood. At the KT, CLASPs are thought to regulate kinetochore microtubule (kMT) dynamics and KT-MT interaction. However, the regulation and dependencies of KT localization as well as the exact function at the KT are unclear.

Research Highlights

We have analyzed how the individual domains (Fig. 1) of the *S.cerevisiae* CLASP, Stu1, contribute to the localization and function of Stu1 in space and time. In prometaphase Stu1 is sequestered (possibly via oligomerization) at uaKTs via the TOGL1 domain and with the assistance of the CL domain. Thus Stu1 is not available for the stabilization of spindle MTs (Fig. 1A, B, E). Sequestering Stu1 at uaKTs therefore serves as a checkpoint that prevents the formation of a stable spindle and keeps the spindle poles in close proximity in prometaphase. This guarantees fast bipolar attachment of newly captured KTs. Stu1 is important for the capturing process. It is however unclear whether this requires the localization of Stu1 at uaKTs. Upon capturing of an uaKT, Stu1 moves with the KT to the pole and subsequently a majority of Stu1 relocates to spindle microtu-

bules. In metaphase Stu1 localizes to the overlap of iMTs (Fig. 1A, C). This depends on a basic, serine rich sequence (ML domain), that confers lateral MT interaction but not on Ase1, the protein that establishes the spindle midzone. We thus assume that Stu1 interacts with the MTs directly in metaphase. Furthermore, dimerization of Stu1 (via the endogenous D4 domain or an ectopic dimerization domain) is important for efficient localization to the overlaps of the iMTs in metaphase. We thus assume that Stu1 crosslinks iMTs in metaphase and thus assists the formation of a stable metaphase spindle. In addition, localizing Stu1 to iMT overlaps allows microtubule rescue. Surprisingly only one of the two TOGL2 domains present in Stu1 (TOGL2) is required for this activity and consistently only TOGL2 binds a tubulin dimer. As observed for uaKTs, TOGL1, enables Stu1 to interact with the KT in metaphase (Fig1A, F). In addition, this localization requires lateral MT interaction. Localization of Stu1 to metaphase KTs is essential to stabilize kMTs (that is kMTs shorten dramatically if KT localization is compromised). Moreover Stu1 at metaphase KTs apparently regulates kMT length in correlation to the tension at the KT-MT interface. One model how this may be achieved is depicted in Fig.1F: TOGL2, located between the KT-interacting TOGL1 and the MT-interacting ML, may be displaced from the MT plus ends in the absence of tension at the KT-MT interface and thus allow MT depolymerization. Upon tension TOGL2 may be placed at the MT plus end to support MT rescue. With the beginning of anaphase Stu1 detaches from the MT lattice and binds to the Ase1 dependent midzone via the D4 domain (Fig. 1A, D). This relieves the iMT crosslinking by Stu1 while guaranteeing the continued localization of the (MT-rescuing) TOGL2 to the iMTs overlap. Thus the strategy of Stu1 localization in meta- and anaphase fits the needs: To stabilize metaphase spindles when tension is applied at the unresolved sister chromatids and to allow MT gliding in anaphase. Stu1 also dissociates from KTs in anaphase (Fig. 1A, G) and consistently the kMTs shorten (anaphase A). The dissociation of Stu1 from KTs at the metaphase to anaphase transition therefore is sufficient to regulate the initiation of anaphase A.

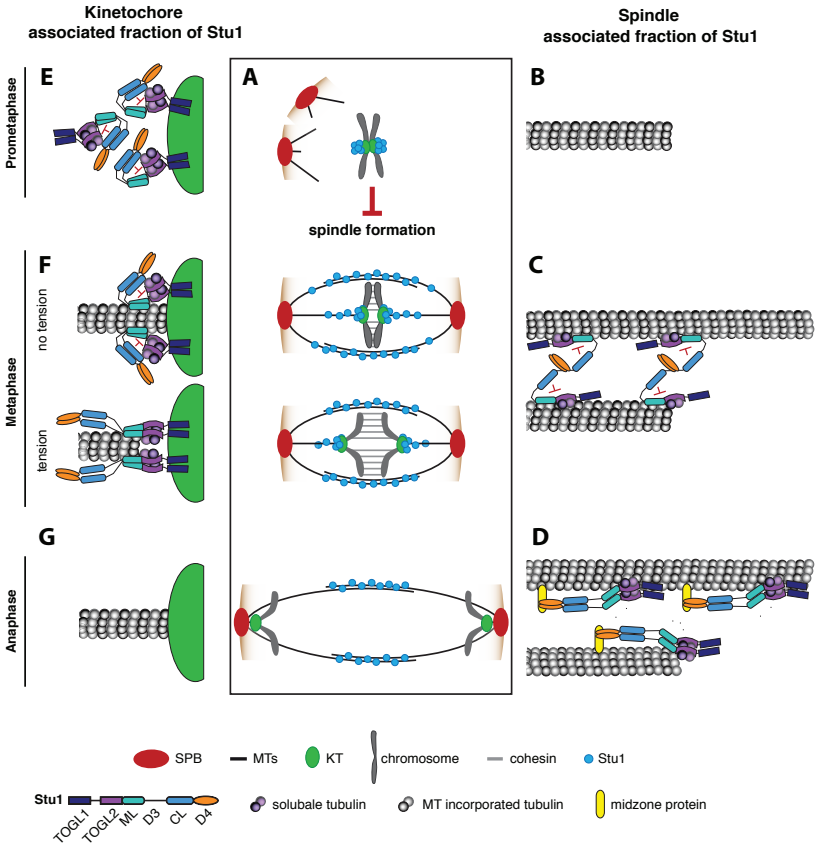

Fig. 1: Model of Stu1 localization and function during mitosis.

Selected Publications

Kemmler, S., Stach, M., Knapp, M., Ortiz, J., Pfannstiel, J., Ruppert, T., and Lechner, J. 2009. Mimicking Ndc80 phosphorylation triggers spindle assembly checkpoint signalling. *EMBO J* 28(8): 1099-1110.

Ortiz, J., Funk, C., Schäfer, A., and Lechner, J. 2009. Stu1 inversely regulates kinetochore capture and spindle stability. *Genes Dev* 23(23): 2778-2791.

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