1985	Ph.D. - University of Regensburg, Germany
1985 - 1987	PostDoc - University of Regensburg
1987 - 1990	PostDoc - University of California, Santa Barbara, USA
1994	Habilitation - University of Regensburg
1994 - 1999	Group Leader - University of Regensburg
since 1999	Group Leader - BZH

Johannes Lechner

Kinetochore and Mitosis

Goal

To understand the function of regulatory proteins at the kinetochore.

Background

Reliable chromosome segregation depends on the correct attachment of kinetochores (KT) to KT-microtubules (kMTs). In budding yeast, chromosomes detach during S-phase. Subsequently the unattached KTs (uaKTs) are captured by nuclear random microtubules (nrMTs) in prometaphase. While they are unattached, KTs activate the spindle assembly checkpoint (SAC) and thus prevent cells from entering anaphase. Crucial for SAC activation is that in the absence of KT-microtubule interaction, the KT protein Spc105 becomes accessible to phosphorylation by the KT-localized protein kinase Mps1. This leads to the assembly of the SAC proteins at the KT and consequential metaphase arrest. Interestingly, uaKTs not only assemble SAC proteins but also sequester the CLASP, Stu1. CLASPs contain TOGL domains that provide microtubule rescue function. Stu1 has two N-terminal TOGL domains (TOGL1 and 2). Only TOGL2, provides direct rescue activity whereas TOGL1 serves as a KT-binding domain. In metaphase, Stu1 has at least two roles. First, it stabilizes interpolar MTs (ipMTs) via a direct interaction that involves the TOGL2 domain and a basic serine-rich unstructured region (ML). Secondly, it localizes to the KTs via TOGL1 and ML and stabilizes kMTs. Consequently, Stu1 is essential for the formation of a metaphase spindle. In the following we were intrigued to reveal the regulatory mechanism of Stu1 sequestering at uaKTs and particularly what benefit it provides for the cell.

Research Highlights

Mechanism and regulation of Stu1 sequestering.
We revealed that besides Stu1, also Slk19, another protein important for spindle stability, is accumulating at uaKTs. Slk19 is required for Stu1 sequestering and vice versa. However, either protein can localize to uaKTs in the absence of the other in basal (low detectable) amounts. Furthermore, we showed that Stu1 sequestering and localization requires Spc105, the activity of Mps1, and the presence of the six Mps1 consensus sites in Spc105 whose phosphorylation initiate SAC protein assembly. In addition, if Mps1 is ec-

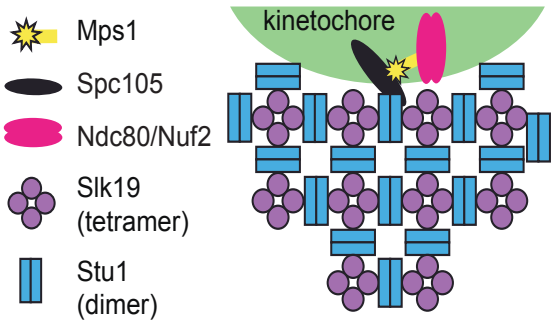

Figure 1. *Model depicting the sequestering of Stu1 at unattached kinetochores*

topically localized close to Spc105, SAC activation (as published by others) and Stu1 sequestering become independent of KT detachment. Thus taken together, the signaling mechanism that translates KT detachment into SAC protein assembly and Stu1 sequestering is similar (if not identical). Our current model (Figure 1) thus assumes that the Mps1-dependent phosphorylation of Spc105 promotes the binding of Stu1 to uaKTs. This may cause a conformational change in Stu1 that initiates the co-polymerization of Stu1 dimers and Slk19 tetramers via propagated conformational changes.

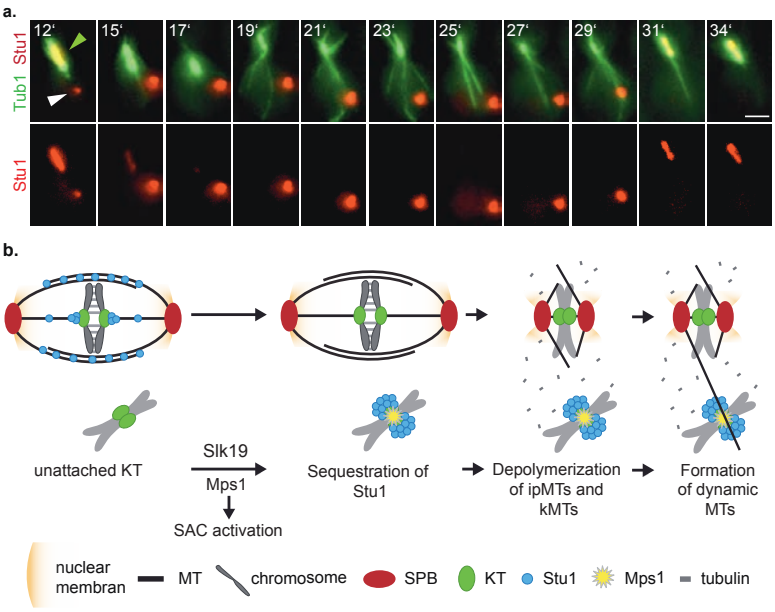

Figure 2. (a) *Time lapse micrographs showing the sequestering of Stu1 at an uaKT (white arrowhead) while Stu1 is withdrawn from the spindle (green arrowhead). With time the spindle collapses and random MTs appear. After capturing of the uaKT (29 s), the effects are reversed. Bar, 2µm.* ***(b)*** *Proposed model how sequestering of Stu1 enhances the formation of nrMTs and thus promotes capturing of uaKTs.*

We found that a putatively unstructured region of Stu1, the C-terminal loop (CL) is essential for sequestering but (in contrast to the KT-binding domain TOGL1) not for the basal localization of Stu1 to uaKTs. Therefore, we suggest that the CL domain plays a major role in Stu1-Slk19 interaction and/or in allowing the necessary conformational changes.

The role of Stu1 sequestering for KT capturing.

In order to observe Stu1 sequestering with time in the presence of an intact metaphase spindle, uaKTs were produced by KT inactivation and reactivation. This revealed that while Stu1 is accumulating at an uaKT it is withdrawn from the spindle MTs and attached KTs (Figure 2a) resulting in the collapse of the spindle. In correlation with this, random dynamic MTs appeared that were revealed to be predominately nuclear. Upon capturing of the KT, the sequestered Stu1 was transported back to the spindle region and the metaphase spindle was re-established. Quantification (in respect to MT number and length) showed that cells exhibited about 20 times more nrMTs occurrence if uaKTs were present than if they were not. This was primarily a consequence of spindle destabilization since the depletion of Stu1 had a similar effect on the formation of nrMTs as the sequestering of Stu1 at uaKTs. On the other hand, *stu1ΔCL* cells that are defective in sequestering and leave the spindle uncompromised showed no enhanced nrMT formation. The occurrence of dynamic MTs that span the nucleus randomly appears ideal for capturing uaKTs. Indeed, we found that the capturing efficiency of *stu1ΔCL* cells was severely compromised in comparison to wildtype cells or Stu1-depleted cells. We thus propose the model shown in Figure 2b. uaKTs withdraw Stu1 from the mitotic spindle and thus destabilize ipMT and kMTs. The freed tubulin is then used to form dynamic MTs that considerably increase the chance for capturing. uaKTs thus drive their own capturing and to do so, they use the same signaling strategy that they use to prolong the time for it (via SAC activation).

Selected Publications

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

Funk, C., Schmeiser, V., Ortiz, J., and Lechner, J. (2014). A TOGL domain specifically targets yeast CLASP to kinetochores to stabilize kinetochore microtubules. J Cell Biol 205, 555-571.

Johannes Lechner
Phone +49 (0)6221-54 4371
E-mail: johannes.lechner@bzh.uni-heidelberg.de