Determination of rules governing spindle pole asymmetry in SIN signalling

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In *Schizosaccaromyces pombe*, cytokinesis initiation and progression is governed by a group of protein kinases called the Septation Initiation Network (SIN). These proteins are recruited and progressively activated on spindle pole bodies (SPB). Interestingly, although in initial mitotic phases some SIN proteins are distributed symmetrically on both SPBs, after sister chromatid separation they become enriched on only one SPB, while fading on the other. This leads to SPB asymmetry at the end of mitosis, which may be preserved after cell division.

It is known that the SIN activity is regulated by nucleotide status of the signaling GTPase, spg1p, whose nucleotide status is controlled by the GAP, cdc16p-byr4p and a putative GEF, etd1p. We are combining automated image analysis and *in silico* modeling to determine the rules governing the transition of SIN proteins from the symmetric to asymmetric state, which correlates with activation of the SIN. We also plan to extend this analysis to mutant cells, to study the consequences of interfering with this control circuitry.

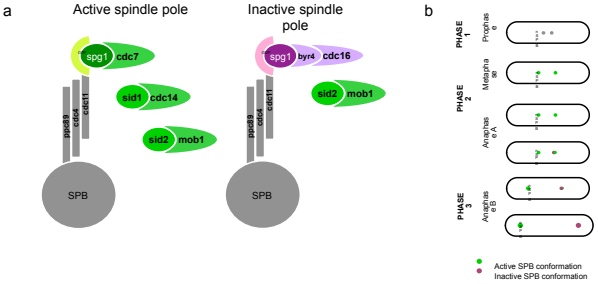

Fig. 1. **Distribution of SIN proteins on active and inactive SPB.**
(**a**) SIN signalling originates from the SPB where it becomes activated in early mitosis. During mitotic progression, active signalling is though to come from then new spindle pole body (nSPB). Signalling from the old SPB is silenced by reassembly of the GAP. The synergy of these two processes leads to tight temporal control of ring assembly, septum formation and cytokinesis. Mutants in which segregation is symmetrical results in deregulated cytokinesis. (**b**) schematic view of SIN activity of SPB during mitosis. After prophase, after SPBs separation, both SPBs recruit cdc7 protein in moderate levels. When the spindle starts to elongate status on both spindles changes. The activating cascade is turned on on new SPB and old SPB recruits inactivating the GAP. Each of these complexes is enriched on their corresponding SPBs during spindle elongation and becomes maximal at the highest length of spindle.

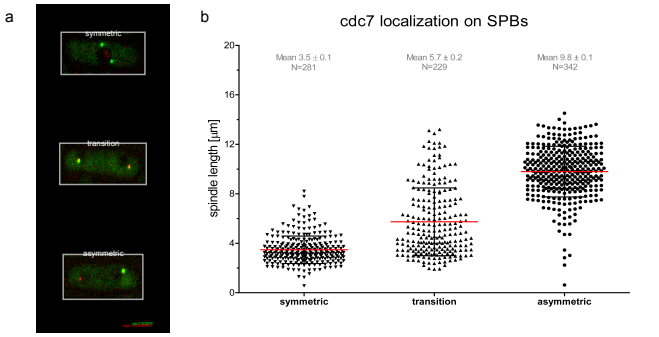

Fig. 2. **cdc7 localizes at SPBs symmetrically until spindle elongation in anaphase B.**
(**a**) The length of spindle was measured in mitotic cells for symmetric (similar intensity on both SPBs), transition (different intensity on both SPBs) and asymmetric (positive only for one SPB) states using ImageJ. (**b**) Using cnp1-mCHERRY protein as a reference for mitotic phase, we established mean spindle lengths for different localizations of cdc7 in mitosis. cdc7 localizes symmetrically on SPBs in phases I and II and gradually fades from oSPB during spindle elongation to become only present on nSPB at the end of phase III.

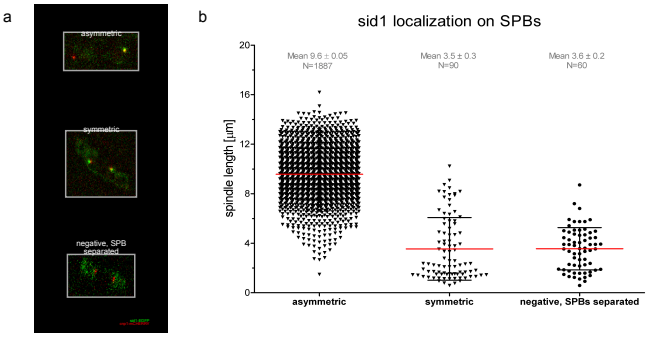

Fig. 3. **sid1 appears on nSPB at initiation of spindle elongation.**
(**a**) The length of spindle was measured in mitotic cells. sid1 appears on nSPB at the onset of spindle elongation, in anaphase B, when kinetochore is attached to SPB. (**b**) Mean spindle lengths for appearance of sid1 on nSPB. A small percentage of cells showed symmetrical staining for sid1 in initial phases of mitosis; the reasons for this are under investigation.

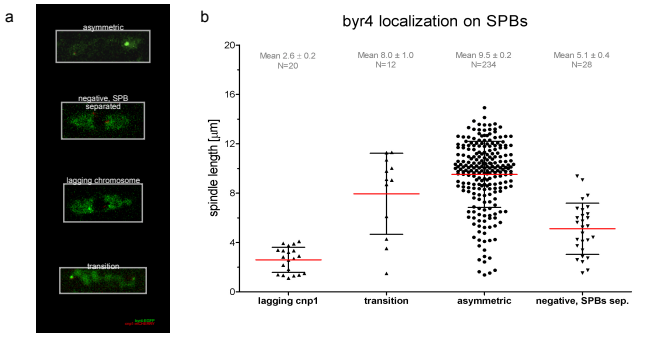

Fig. 4. **byr4 appears on oSPB at initiation of spindle elongation.**
(**a**) The length of spindle was measured in mitotic cells for the different configurations shown on the left. byr4 appears on oSPB at the onset of spindle elongation, in anaphase B. (**b**) mean spindle lengths for appearance of byr4 on oSPB. Small percentage of cells showed staining for both SPBs; the reasons for this is under investigation.

Conclusions:

- cdc7 appears after SPB separation on both SPBs. Symmetric localization correlates with short spindle
- The levels of cdc7 when symmetric on both SPBs are moderate
- After sister chromatid separation, cdc7 becomes gradually enriched on nSPB and fades from oSPB during spindle elongation
- sid1 appears on nSPB at the beginning of anaphase and its levels increase gradually with spindle elongation
- byr4 appears on oSPB and shows similar behavior to that of sid1 on nSPB
- Establishing rules in wild-type cells will help in screen for proteins that affect SIN status and cell cycle control.

Future perspectives:

- Establishment of SIN kinetics in mutant genetic backgrounds
- Development of software enabling semi-automated analysis of SIN kinetics in collaboration with Prof. M. Unser
- Development of quantitative model of SIN behaviour in collaboration with Dr. I. Xenarios