1988 - 1993	Chemistry Studies, University of Athens, Greece
1994 - 1998	Ph.D. - Zentrum für Molekulare Biologie Heidelberg (ZMBH), Germany (Prof. Stefan Jentsch)
2000 - 2005	PostDoc - Institute of Biochemistry, ETH Zürich, Switzerland (Prof. Yves Barral)
2006 - 2013	Junior Group Leader - BZH
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Dimitris Liakopoulos

Spindle positioning in yeast

Goal

To study the mechanisms that bring the spindle to its correct position during asymmetric cell divisions.

Background

Polarized cells have two options when they divide: they can either divide symmetrically, or asymmetrically. Asymmetric divisions are encountered whenever the goal is generation of cellular diversity, for example during embryonic divisions or the divisions of stem cells. Factors that determine cell fate are asymmetrically segregated in one of the two daughters, that consequently differentiates.

In an asymmetric cell division, the cytokinetic machinery must cleave the cell perpendicular to the polarized material, resulting in unequal segregation of the polarized factors. At the same time, the cytokinetic actomyosin ring cleaves the cell midway through the mitotic spindle to ensure equal segregation of chromosomes between daughters. Coordination of cell cleavage with chromosome segregation depends on interactions of astral spindle microtubules (aMTs) with the cortical actin cytoskeleton. A complex network of proteins involving non-motor microtubule plus-end tracking proteins (+TIPs), kinesins, dynein and actin-interacting proteins mediate these interactions (Fig. 1).

Our lab mainly studies the mechanisms and regulation of astral spindle microtubules with the cortical cytoskeleton using one of the simplest asymmetrically dividing organisms, the yeast *S. cerevisiae*.

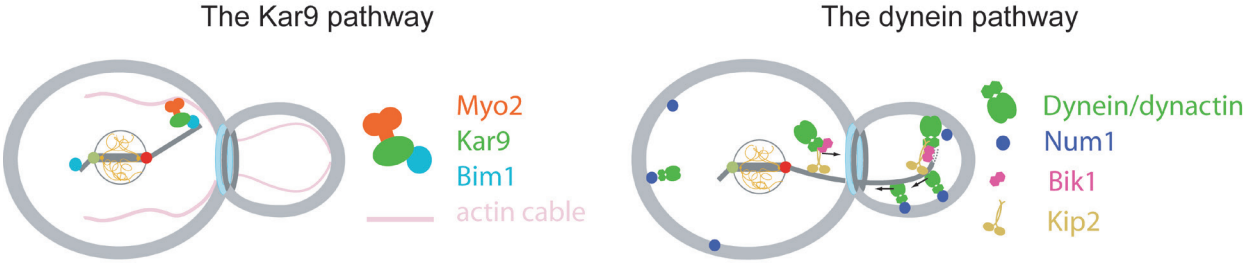


Fig.1: Spindle positioning in yeast. *Thick grey bar: metaphase spindle, red, green spots: old and new microtubule organizing centers (SPBs), thin grey lines: aMTs. Left: The protein Kar9 forms a bridge between aMTs and actin cables through its interaction with Bim1 and Myo2. Myo2 pulls aMTs from the old SPB and the metaphase spindle towards the bud. Right: Dynein is transported by the kinesin Kip2 to the (+)-ends of aMTs and binds to Num1 at the cortex. Num1-immobilized dynein pulls aMTs and orientates the spindle, because its motor activity is directed towards the minus-ends of aMTs that are anchored at the SPB. The blue-gray ring is the actomyosin-based cytokinetic apparatus and the future site of cytokinesis. Only metaphase spindles aligned with the mother-bud axis are able to elongate and partition half of the chromosomes into the bud in anaphase, so that cytokinesis can occur later midway through segregated chromosomes.*

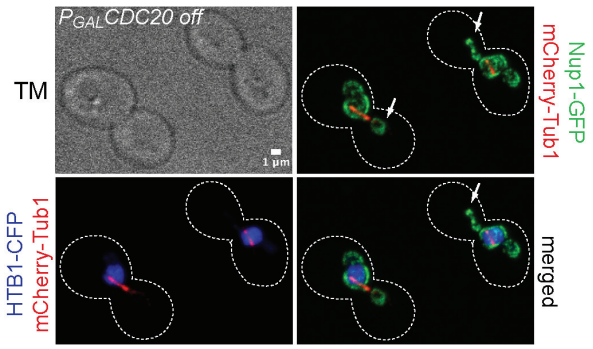
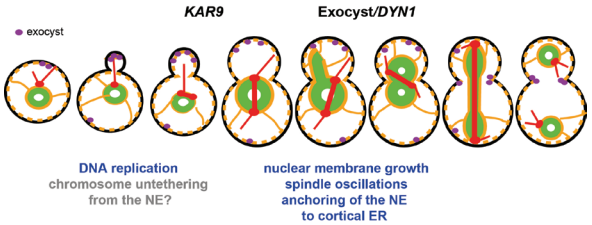


Fig. 2: *Images of living cells showing the relative positions of the nuclear envelope (marked with the nucleoporin fusion Nup1-GFP), the mitotic spindle and the DNA (visualized with the histone H2B fusion HTB1-CFP). Two cells with nucleopodia (arrows). In all cases, the mass of the DNA is associated with the spindle. In dumbbell nuclei, spindle and DNA are inserted in the bud neck, whereas NP enter the bud while the spindle and the bulk of the DNA are still in the mother cell.*

Research Highlights

Our project concerning the mechanics of nuclear migration during closed mitosis led to the discovery of a novel, auxiliary mechanism of nuclear migration in budding yeast (Kirchenbauer et al., 2013). We investigated changes in nuclear morphology during nuclear migration and showed that in pre-anaphase cells, nuclear protrusions (nucleopodia, see Fig. 2) extend into the bud, preceding insertion of chromosomes into the bud neck. Interestingly, formation of nucleopodia did not depend on the Kar9 or the dynein pathway, but instead required nuclear membrane expansion, an intact actin cytoskeleton and the exocyst complex. In addition, DNA replication was also required for generation of nucleopodia. We thus proposed that nuclear membrane expansion, DNA replication and exocyst-dependent anchoring of the nuclear envelope to the bud affect nuclear morphology and facilitate correct positioning of nucleus and chromosomes relative to the cleavage apparatus (Fig. 3). We are now following this project investigating the mechanism that connects DNA replication with nuclear migration.



We had recently found that the protein Kar9, the yeast functional equivalent of the Adenomatous Polyposis Coli (APC) tumor suppressor that links astral microtubules with actin, is regulated by phosphorylation, sumoylation and ubiquitylation (Kammerer at al. 2010). We were now able to identify the enzymatic machinery that mediates Kar9 ubiquitylation and we have elucidated the pathway that connects the three posttranslational modifications which control Kar9 function.

In addition, we initiated the reconstitution and study of Kar9 complexes *in vitro*, in order to generate quantitative information on the function and properties of these complexes.

Finally, we have made additional progress on the regulation of the kinesin Kip2 by the GSK-3 kinase. Yeast GSK-3 phosphorylates Kip2 and reduces its affinity for microtubules and consequently, transport of dynein and Kar9 on aMT ends. Interestingly, Kip2 is able to stabilize microtubules. We collaborated with the lab of J. Howard (Dresden) which has made significant progress on understanding the mechanism through which Kip2 increases microtubule stability.

Since October 2013, the lab has moved and will continue its projects to the CRBM (Centre de Recherche de Biochimie Macromoléculaire)-CNRS in Montpellier, France. We are thankful for all the exciting time in the BZH!

Selected Publications 2011 - 2013

Kirchenbauer, M. & Liakopoulos, D. An auxiliary, membrane-based mechanism for nuclear migration in budding yeast. *Mol Biol Cell* 24, 1434-1443 (2013).

Stevermann,L. and Liakopoulos,D. (2012). Spindle positioning: structures and new concepts. *Curr. Opin. Cell Biol.* 24, 816-24 (2012).

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