Fluorescent Imaging of Cell Division

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Declaration

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CD containing supplemental materials is included with the print copy held in the University of Adelaide Library.

List of Publications

Cell division requires a direct link between microtubule-bound RacGAP and Anillin in the contractile ring.

Research Paper

Stephen L Gregory, **Saman Ebrahimi**, Joanne Milverton, Whitney M. Jones, Amy Bejsovec, and Robert Saint

Current Biology 18 (2008) 25-29

Polo kinase interacts with RacGAP50C and is required for the localization of the Cytokinesis Initiation Complex.

Research Paper

Saman Ebrahimi, Hamilton Fraval, Michael Murray, Robert Saint and Stephen L. Gregory

Submitted Manuscript

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Abstract:

Cytokinesis is the final stage of cell division that divides a cell into two. It requires the coordinated assembly and constriction of an Actin and Myosin based contractile ring in anaphase and telophase. The Rho signalling pathway is known to be a key player in regulating the events that lead to the localisation of components of the contractile ring and activation of its constriction. The immediate upstream regulators of RhoA are: the RhoA activator Pebble/ECT2, the Plus-end directed microtubule associated motor protein Pavarotti Kinesin like Protein (Pav-KLP)/MKLP1 and RacGAP50C/MgcRacGAP. At the onset of Cytokinesis, RacGAP50C and Pav-KLP form a complex, termed the centralspindlin complex, on the microtubules. Pebble binds the centralspindlin complex via RacGAP50C forming a complex, here termed the cytokinesis initiation complex. The complex then travels to the cell equator leading to RhoA activation and the subsequent assembly and constriction of the contractile ring.

Two major aims have been the focus of my PhD studies:

1) To investigate the mechanism behind continuous and stable contractile ring constriction after initiation of cytokinesis:

A stable and continuously constricting contractile ring requires the continuous presence of active RhoA. Both microtubules and the centralspindlin complex have been shown to be essential for this process. However, the mechanism by which they ensure this continual activation of RhoA is yet to be elucidated. In a search for potential candidates that would play a role in this process, Fluorescence Resonance Energy Transfer was adapted to the *Drosophila* system and used to detect direct spatio-temporal interactions between components of the contractile ring and the centralspindlin complex. Subsequently confirmed by Yeast Two-hybrid analysis, a direct interaction was identified between

RacGAP50C and Anillin, a RhoA effector that is attached to the Actin-based cell cortex via direct interactions with Actin and Myosin. Live imaging of neuroblast cells lacking Anillin revealed that the contractile ring destabilizes and falls apart after briefly constricting during anaphase. These results suggest that, during constriction, the centralspindlin complex on the microtubules is continuously linked to the Actin-based ring via an interaction with Anillin leading to the continual presence of Pebble and active RhoA. This explains the long known phenomenon that microtubules are required at the cell equator for a continual and stable contractile ring constriction.

2) To investigate the mechanism behind centralspindlin localisation and the subsequent initiation of cytokinesis:

RhoA activation in anaphase is achieved by the localisation of the cytokinesis initiation complex to the equatorial cortex. More recently, Polo Kinase has been found to play an essential role in this process. FRET and Yeast Two-hybrid Analysis revealed a direct interaction between RacGAP50C and Polo Kinase in anaphase leading to the hypothesis that Polo Kinase may be involved in the localisation of RacGAP50C and initiation of contractile ring assembly. To test this hypothesis, anaphase in *Drosophila polo* mutant cells was examined. Immuno-fluorescence of *polo* mutant cells in anaphase revealed that in the absence of Polo Kinase activity, both RacGAP50C and Pav-KLP stall on the microtubules and do not get to the equator where the contractile ring should assemble. This study revealed an essential role for Polo Kinase in the initial localisation of the RhoA activating complex to the cell equator.