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Active gels *in vivo*: Patterns and dynamics in cytokinetic rings and their functions in cell division

Summary

Cell division is one of the most basic events for cells. Single cell organisms proliferate by dividing, and multicellular organisms grow and renew tissue by this mechanism. After the chromosomes are separated, a ring of actin, myosin and other proteins forms. Its closure leads to cell separation. This mechanism is conserved in fungi, amoebae and animal cells.

Dynamics of actin and myosin, or more generally filaments and motors are relevant for cell division, but also for cells in general, be it for their internal organization, be it for the sensing of the environment or auto-organization in tissues and embryogenesis. Networks of dynamic filaments and active motors are also termed *active gels*, since they are out-of-equilibrium systems. Therefore they constitute not only important structures in cell, but also a new kind of materials. Numerous theoretical and experimental studies have been carried out on this topic.

It is more challenging to study these gels *in vivo*, since many proteins and signaling are involved in their organization. Due to its circular geometry, the cytokinetic ring is a relatively simple active gel *in vivo*. Its components are well studied. However, it is still not clear how the ring constricts and how stress is generated in this system. By elucidating this question, we hope that we can also contribute to a better understanding of other active gel structures in cells.

Based on an existing setup for fission yeast, invented by Daniel Riveline, we developed a setup and protocol to study cytokinesis in mammalian cells. More specifically, we investigate the cytokinetic ring by using an array of microcavities, which allowed us to orient cells and to see the ring in a single plane of focus. We visualize the ring with fluorescently labeled actin filaments,

myosin and other proteins for two cellular systems: mammalian cells and fission yeast. This setup allowed us to reveal, characterize and compare patterns and dynamics in both cell types.

Interestingly we see a structure within the cytokinetic ring in mammalian cells: myosin and formin form a pattern of regular clusters. This pattern can exhibit global dynamics during closure, like collective rotations or local fusions and separations of single clusters. However, our characterization of the pattern reveals that its properties remain mainly constant throughout closure. This suggests that it is an inherent property of the ring. Interestingly, the pattern is not disturbed after laser cutting the ring. Furrow constriction is even continuing after cutting; this shows that contractility is a local property. We also find an inhomogeneous distribution of anillin and septin in the ring, but no systematic colocalization with myosin clusters. Furthermore we show that cytoskeletal drugs disturb the pattern. In particular they render the pattern less regular. Strikingly this pattern is forming as the ring begins to close. This suggests that the pattern is related to stress generation. Indeed, the model and calculations of Karsten Kruse and Anne Wald support this interpretation. Our model is built on simple assumptions about the ingredients in the ring. It shows that such patterns of regular motor, nucleator and filament clusters form due to a dynamic instability which occurs above a critical motor activity. This pattern leads to an increased stress generation due to the ordering of filaments. Therefore, we propose that the ring components organize in a regular pattern and that this pattern is the key to the stress generation in the cytokinetic ring.

In fission yeast cells, we find also inhomogeneities (*speckles*) of actin, myosin, the wall building machinery (Bgs) and other proteins. But in contrast to our findings in mammalian cells, these speckles are rotating on the ring. This rotation also occurs in the absence of ring closure, which suggests that it is a built-in property of the cytokinetic ring in fission yeast. Inhibition of the myosin speckle rotation leads also to an arrest of cell closure, suggesting a correlation between speckles rotations and ring closure. François Nédélec and Eszter Lakatos proposed that the ring closes because the cytoskeleton components drive the rotation of the wall building machinery. Rotations allow to grow the wall against the turgor pressure. We implemented the ring with a minimal set of ingredients and we can reproduce speckle motion and ring closure *in silico*. Furthermore we propose that another function of rotation might be to render the ring more stable. Indeed our laser ablation experiments show that the ring can repair within seconds.

In both systems we discovered mesoscopic structures in the active gel. We characterized their patterns and dynamics and probed their changes under different conditions. Our observations and measurements led us to two models, explaining the stress generation and ring closure in the respective systems. However, it is surprising that two actomyosin rings exhibit different dynamics. Future experiments will aim at understanding, which system parameters lead to either still or rotating clusters. We suggest that the transition between states of different orders and dynamics might be one way to regulate actomyosin systems *in vivo*, in addition to traditional signaling.