# Positioning the cleavage furrow: All you need is Rho

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RhoA controls cleavage furrow formation during cell division, but whether RhoA suffices to orchestrate spatiotemporal dynamics of furrow formation is unknown. In this issue, Wagner and Glotzer (2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201603025) show that RhoA activity can induce furrow formation in all cell cortex positions and cell cycle phases.

The completion of the cell cycle necessitates separating the newly replicated chromosomes and cytoplasm into two separate daughter cells. This process is normally initiated during anaphase, in which a contractile cleavage furrow forms at the middle of the cell and ingresses/constricts to ultimately split the cell in two. Though many of the cellular players in cytokinesis have been identified (Chang et al., 1996; Eggert et al., 2004; Kittler et al., 2007), how this process is controlled in space and time is not fully understood.

For proper chromosome partitioning during cell division, the contractile furrow must form at the proper physical position in the cell (a plane between the newly separated chromosomes) as well as at the proper phase of the cell cycle (after chromosome replication and separation). This process is normally controlled by the mitotic spindle (Rappaport, 1985), the same machinery that separates the chromosomes, through its regulation of the small GTPase RhoA (Miller and Bement, 2009). RhoA cycles between a GTP- and a GDP-bound state, and this cycle is regulated by activating guanine nucleotide-exchange factors (GEFs) and inactivating GTPase activating proteins. Active RhoA promotes cytokinesis by stimulating actin nucleation and myosin activation, thus forming the actomyosin ring (Matsumura, 2005; Watanabe et al., 2008) that generates the contractile forces to create the cleavage furrow and eventually separate the cell in two.

Loss-of-function experiments demonstrate the necessity of RhoA for furrow formation. Pharmacological inhibition of RhoA by C3 blocks the initiation of cleavage and induces regression of preexisting cleavage furrows (Drechsel et al., 1997; O'Connell et al., 1999). This shows that RhoA activation is necessary for actomyosin ring assembly and cleavage furrow formation. However, the sufficiency of RhoA in activating furrow formation has not been tested. The major obstacle to this experiment has been the lack of tools to manipulate protein localization and activity with fine spatiotemporal control. The advent of optogenetic tools for light-induced protein interactions (Tischer and Weiner, 2014) now enables several open questions in the cell division field to be tackled. Is the sophisticated regulation of furrow position and timing primarily dictated by when and where Rho activity is generated, in which case artificial activation of Rho should suffice to induce furrowing in any cell position and cell cycle time? Or does Rho have other key collaborators in furrow formation that limit its competence to act in space or time?

In this issue, Wagner and Glotzer demonstrate the sufficiency of RhoA activation in furrow initiation with light-mediated control of RhoA activation through an opto-engineered GEF. The membrane-targeted photosensitive domain LOVpep changes its conformation with 405-nm light illumination and allows binding of the PDZtag (Strickland et al., 2012), which is fused to a RhoA-specific GEF. For ease of manipulation, Wagner and Glotzer (2016) use mammalian tissue culture cells for their experiments. With this setup, focal light illumination suffices for opto-GEF recruitment, RhoA activation, and local F-actin and myosin accumulation. To probe the spatial sufficiency of RhoA in initiating furrow formation, light-inducible RhoA activation can be generated at a specific location of the cell to test whether it can induce local furrow ingression. But first the endogenous pathway of RhoA activation during anaphase must be crippled to give the light-inducible RhoA a clean background on which to operate. The authors used two different approaches to block endogenous RhoA activation: a pharmacological inhibitor that blocks Polo-like kinase 1, which regulates the key Rho activator Ect2 (Yüce et al., 2005; Nishimura and Yonemura, 2006), and siRNA to deplete the Cyk4 GTPase activating proteins that participates in Ect2-mediated Rho activation (Zhang and Glotzer, 2015). Both approaches generated noncontractile anaphase cells that were used as a test bed for light controlled RhoA.

The cleavage furrow is normally generated at the cellular equator during anaphase. To test whether light-induced RhoA activation can replace the endogenous system, Wagner and Glotzer (2016) first investigated the ability of their optogenetic system to direct furrowing at the normal cellular position and cell cycle phase. They found that a band of RhoA activation of the equator suffices to initiate a cleavage furrow. With this important control in hand, the authors next tested the competency of other cellular locations to support furrow formation. If additional key furrow regulators are confined to the equatorial region, then optogenetically-driven RhoA should be spatially limited in its ability to initiate a furrow. In contrast, if RhoA is the sole control point, light-induced activation of RhoA at locations other than the equator will also induce furrow formation. Consistent with the second hypothesis, light-mediated recruitment of RhoGEF to the poles also sufficed to initiate furrow

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formation with a similar extent of ingression and constriction rate as activation at the equator. Surprisingly, there is not even a restriction to forming a single furrow—when light is applied to both the equatorial zone and the poles, both regions initiated furrows that form simultaneously and constrict to a similar degree. Therefore, the anaphase cell cortex is uniformly responsive to RhoA activation and local furrow initiation.

After establishing that the cortex is uniformly competent for Rho-induced furrow formation during anaphase, Wagner and Glotzer (2016) next addressed whether or not Rho can drive furrowing in other phases of the cell cycle. Is Rho sufficient to initiate furrow formation at any cell cycle phase, or can Rho only engage key actomyosin effectors during specific times of the cell cycle? Using the light-mediated recruitment of GEF at both metaphase and anaphase, similar degrees of RhoA-directed furrow ingression were observed. They also found that even interphase cells are competent to initiate a cleavage furrow.

Together, these pieces of evidence show that local activation of RhoA is sufficient to initiate furrow ingression irrespective of the position of cell cortex and cell cycle phase (Fig. 1). However, light-activated RhoA is not able to sustain furrowing upon light removal. Furthermore, light-induced furrows do not fully constrict to complete cytokinesis for adherent cells. What could account for this failure in furrow ingression? Wagner and Glotzer (2016) ruled out the possibility that RhoA activation by light is lower than endogenous RhoA activation by using a RhoA biosensor (Piekny and Glotzer, 2008). Based on their observation that furrow ingression of nonadherent cells is more complete than that of adherent cells and that mitotic entry and cell rounding increase cortical tension, the authors raised the possibility that cortical tension plays a role in the extent of furrow ingression. To test this hypothesis, they increased cortical tension with a short pulse of light to drive global RhoA activation and found that this decreased the extent of ingression and rate of constriction. Conversely, when cortical tension was decreased by the application of a $Na^+/H^+$ exchanger inhibitor, they observed an increase in the degree of ingression. These data suggest that cortical tension regulates the degree of furrow ingression. But the failure of light-driven furrows to completely ingress cannot be completely explained by cortical tension. When Wagner and Glotzer (2016) superimposed their optogenetic Rho modulation on top of the endogenous RhoA pathway, this interfered with the completion of furrow ingression, suggesting subtleties of spatial or temporal modulation of Rho activity control the completion of furrowing. Investigating this question with more sophisticated monitoring and spatiotemporal modulation of Rho activity will be an exciting future direction.

Where to go from here? Wagner and Glotzer (2016) made powerful use of light-controlled Rho activation to interrogate the role of this GTPase in organizing the timing and placement of the cleavage furrow. But some of the most interesting aspects of furrow formation, such as its ability to self-organize and sustain itself as well as the ability of cells to restrict the number of furrows to one, are not observed in the current implementation of this system. It is important to note that the crippled system designed by the authors not only blocks the endogenous RhoA activation but also inhibits potential Ect2-mediated positive feedback loops that are thought to form the basis of this self-organization

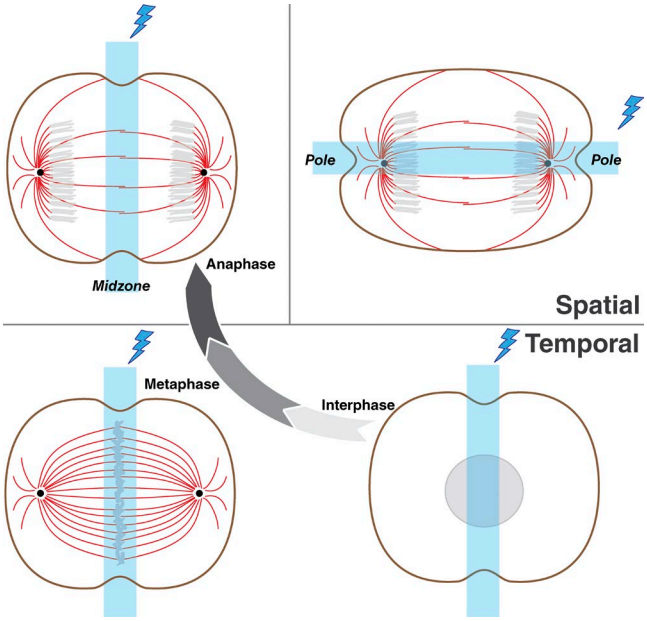


Figure 1. **Light-mediated RhoA activation is sufficient to drive furrow formation irrespective of cell cortex location and cell cycle phase.** In a noncontractile anaphase cell (top left), light activation of RhoA at the midzone induces localized furrow formation. Spatial and temporal regulation of furrow formation is exerted at the level of RhoA. Light activated RhoA in the poles of a noncontractile anaphase cell (top right) suffices to drive furrow formation. Additionally, light-gated RhoA activation at metaphase (bottom left) and interphase (bottom right) also suffices to drive furrow formation. Wagner and Glotzer (2016) demonstrate that RhoA activation is sufficient to initiate the timing and spatial position of cytokinetic furrow ingression.

(Bement et al., 2015; Zhang and Glotzer, 2015). In future experiments, it will be important to extend these optogenetic studies to probe these feedback circuits when intact. It is certainly more challenging to probe a cell when the applied optogenetic inputs are modulated by positive and negative feedback. In these cases, more sophisticated spatial and temporal modulation of the light inputs may be needed to probe network function. For example, imaging-based feedback controllers can be used to clamp optogenetic inputs at defined levels despite cellular feedback (Toettcher et al., 2011), and systematic quantitative experiments can be performed on individual cells to overcome cell-to-cell heterogeneity (Toettcher et al., 2013).

The dissection of RhoA activation in furrow formation with optogenetic tools by Wagner and Glotzer (2016) shows the power of this approach in probing the spatiotemporal logic of signal integration in cells. It is satisfying that the field of optogenetics continues to move beyond proof-of-concept experiments to shed new light on long-standing biological questions regarding cellular organization in space and time. It will be exciting to see how these optogenetic tools continue to complement and extend classical cell biology approaches.

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