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SPOTLIGHT

Anillin links up with RhoA to break the symmetry of cytokinetic ring closure

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During animal cell cytokinesis, active RhoA assembles actomyosin-based contractile rings that tend to close asymmetrically. Through imaging *C. elegans* zygotes, Lebedev et al. (https://doi.org/10.1083/jcb.202405182) reveal that the scaffold protein, anillin, promotes asymmetric ring closure by locally sequestering RhoA from its canonical effectors and thereby limiting actomyosin contractility.

Cytokinesis is a highly dynamic process driven by spindle-directed RhoA activation at the cell's equatorial plasma membrane. Active RhoA recruits actomyosin effectors, formin and Rho-kinase, to generate a membrane-anchored contractile ring that drives the cleavage furrow inward as it constricts. Active RhoA (RHO-1 in *Caenorhabditis elegans*) also recruits the scaffold protein anillin/ANI-1, a prominent furrow component whose N terminus can bind myosin-2 (NMY-2) and bundle actin filaments and whose C terminus can bind septins and RhoA (1). Our understanding of how ring components self-organize during contractile ring assembly and closure remains limited. One feature of contractile rings, exemplified by the *C. elegans* zygote, is that they tend to close asymmetrically, with different regions constricting at different rates, giving rise to unilateral furrows with faster-closing, leading edges and slower-closing, lagging edges. Previous studies have shown that ANI-1–depleted zygotes close their contractile rings symmetrically and with enhanced levels of NMY-2 (2, 3), but neither the underlying mechanisms nor the significance of asymmetric closure are understood.

Zanin and colleagues (4) report a new series of experiments in the *C. elegans* zygote that further probe the role of ANI-1 in asymmetric ring assembly and closure. They demonstrate that depletion of ANI-1 leads not only to enhanced levels of NMY-2, but also to enhanced levels of RHO-1 effectors, ROK/LET-502 and formin CYK-1, during contractile ring assembly. Strikingly, the enhanced NMY-2 phenotype induced by ANI-1 depletion can be rescued by ANI-1 constructs that retain the ability to bind to RHO-1, but not those that cannot bind RHO-1.

To determine whether ANI-1 impacts RHO-1 levels, the authors generated a new tool, internally tagged RHO-1, which revealed that total levels of RHO-1 at the contractile ring were unaltered by ANI-1 depletion. This suggested that ANI-1's effects might therefore be through binding and sequestering RHO-1 away from its canonical effectors that control actomyosin assembly. Indeed, in vitro experiments using purified RHO-1 and ANI-1 confirmed their interaction through the ANI-1 C-terminal Rho-binding domain. However, these experiments also unexpectedly uncovered a more robust interaction when the central linker region of ANI-1 was included, although the linker does not itself interact directly with RHO-1. This intriguing finding led the authors to posit a hitherto unknown mechanism of ANI-1 self-regulation governed by its linker region.

To explore the functional relevance of the linker region, the authors performed a detailed structure-function analysis in vivo with transgenes that express only specific regions of ANI-1 (N terminus, C terminus, and intervening linker region) and combinations thereof. During ring assembly and closure, the RhoA-binding C terminus (without linker) was sufficient to restore NMY-2 levels to those of control cells, but not the asymmetric distribution of NMY-2, which is ordinarily enriched at the leading edge. However, an ANI-1 construct harboring the C-terminal and linker regions together rescued both the reduced levels and the asymmetric distribution of NMY-2, underscoring the importance of the linker region.

To monitor the asymmetry of furrowing, the authors quantified ring eccentricity (a measure of the closed ring's deviation from its initial centroid) and the time lag between ingression of leading and lagging edges. Again, by these metrics, only the construct expressing both the RhoA-binding C terminus and the linker region rescued the asymmetric, unilateral furrowing.

Finally, cortical flows were quantified at the leading and lagging edges using particle image velocimetry. This confirmed that more pronounced cortical flows at the leading edge precede less pronounced flows at the lagging edge, consistent with prior reports (2, 5). By calculating the divergence of the vector fields generated in the particle image velocimetry analysis, the authors inferred that the leading-edge cortex is under higher compression. To what extent this is a

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cause or a consequence of anillin's symmetry-breaking activity is an important point to resolve in future work.

Taken together, the work of Lebedev et al. shows that anillin promotes unilateral furrowing by binding to RhoA and locally limiting the canonical effectors controlling actomyosin assembly and in a linker-dependent manner. This is important because it demonstrates that anillin is more than a simple actomyosin scaffold, which it has often been labeled as. It also suggests that spatial regulation of RhoA and its effectors can occur independently of the canonical guanine nucleotide exchange factor (GEF)/GTPase activating protein (GAP) cycle, through anillin. Finally, it uncovers an important role for the poorly conserved and structurally disordered linker region of ANI-1.

The authors propose an intriguing model for unilateral furrowing in which anillin binding to RhoA, facilitated by the linker, occurs preferentially at the lagging edge, where cortical flows are reduced, to locally limit actomyosin activation there. Conversely, robust cortical flows at the leading edge impede the linker region's ability to enhance RhoA–anillin interactions, thereby resulting in enhanced actomyosin activity.

It is important to also consider that a direct consequence of RhoA binding to anillin is the recruitment of septins to the plasma membrane to form what we have coined an "anillo-septin" sub-network of the contractile ring (1, 6). Indeed, septins are required for asymmetric closure of the contractile ring of *C. elegans* zygotes (3). The local limiting of actomyosin assembly by ANI-1–mediated sequestration of RHO-1 uncovered by Lebedev et al. ought therefore to coincide with local stimulation of anillo-septin assembly. It would be interesting to determine whether septins are normally enriched at the lagging edge or if septin recruitment at the leading edge might be impaired, together with RhoA, by cortical flows, as one might predict from the author's model. An important unanswered question is why are ANI-1–regulated unilateral furrows able to close at similar rates to ANI-1–depleted, symmetrically closing furrows that have elevated levels of activated actomyosin (2, 3, 4)? Somehow, ANI-1 must act as a kind of brake, as uncovered here, but also as an accelerator. Perhaps ANI-1 may also act to promote positive feedback at the leading edge, in a mechanism analogous to that reported for mammalian anillin, which can dynamically sustain a pool of active RhoA and promote sustained actomyosin contractility (7, 8). Despite the questions that remain and the inherent challenges of understanding such a complex system, this latest work is an important step toward unraveling the mystery of how cytokinetic ring components self-organize to mediate ring closure, whether symmetric or asymmetric.

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