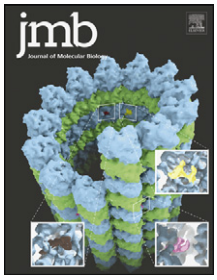

Multimodal and Polymorphic Interactions between Anillin and Actin: Their Implications for Cytokinesis

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Abstract

Cytokinesis of animal cells requires the assembly of a contractile ring, which promotes daughter cell splitting. Anillin is a conserved scaffold protein involved in organizing the structural components of the contractile ring including filamentous actin (F-actin), myosin, and septins and in forming the subsequent midbody ring. Like other metazoan homologs, *Drosophila* anillin contains a conserved domain that can bind and bundle F-actin, but the importance and molecular details of its interaction with F-actin remain unclear. Here, we show that in a depletion-and-rescue assay in *Drosophila* S2 cells, anillin lacking the entire actin-binding domain (ActBD) exhibits defective cortical localization during mitosis and a greatly diminished ability to support cytokinesis. Using *in vitro* binding assays and electron microscopy on recombinant fragments, we determine that the anillin ActBD harbors three distinct actin-binding sites (ABS 1–3). We show that each ABS binds to a distinct place on F-actin. Importantly, ABS1 and ABS3 partially overlap on the surface of actin and, therefore, interact with F-actin in a mutually exclusive fashion. Although ABS2 and ABS3 are sufficient for bundling, ABS1 contributes to the overall F-actin bundling activity of anillin and enables anillin to switch between two actin-bundling morphologies and promote the formation of three-dimensional F-actin bundles. Finally, we show that in live S2 cells, ABS2 and ABS3 are each required and together sufficient for the robust cortical localization of the ActBD during cytokinesis. Collectively, our structural, biochemical, and cell biological data suggest that multiple anillin–actin interaction modes promote the faithful progression of cytokinesis.

Introduction

Cytokinesis of animal cells proceeds via three successive stages: contractile ring (CR) assembly, intercellular bridge assembly, and abscission [1]. It involves dramatic reorganization of the cells' cytoskeletal networks, with microtubules, filamentous actin (F-actin), and septins all collaborating to shape the plasma membrane and ultimately separate it into two cells. The scaffold protein anillin has emerged as a major coordinator of these networks as it can bind to many of the other components [2–4] and is

required for the transition from the CR to the midbody ring (MR) during the formation of the intercellular bridge [5,6].

Anillin was first purified from *Drosophila* embryos by virtue of its interaction with F-actin and was shown to localize to CRs and MRs [7]. A neighboring but distinct domain of *Xenopus* anillin was later found to bind to the phosphorylated, active form of non-muscle myosin II [8]. This myosin binding domain has also been shown to interact with the F-bar protein, Syndapin, in *Drosophila* cells [9]. Through highly conserved C-terminal anillin homology and pleckstrin homology