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SPOTLIGHT

Multifaceted "composite" actin nucleator orchestrates polymerization via dynamic assembly

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In this issue, Magliozzi et al. (https://doi.org/10.1083/jcb.202505039) describe a novel actin nucleation mechanism involving an Aip5–Bud6–Bni1 "composite nucleator," which binds both the formin-mediated barbed end and Aip5-associated pointed end, enabling coordinated filament elongation and maintaining actin cable thickness.

Actin nucleation—the process that kick-starts the assembly of actin filaments—is often the bottleneck in building and reshaping the actin cytoskeleton. This network of filaments is vital for everyday cell functions, such as cell morphogenesis and the movement of organelles, as well as responding to stress. The best-known actin nucleators are the Arp2/3 complex, activated by nucleation-promoting factors (NPFs) for branched F-actin, and the formin family for unbranched F-actin. The Arp2/3 complex not only starts new branches but also caps the pointed ends of filaments to control their growth. Formins, on the other hand, act as processive cappers to nucleate F-actin and enable elongation, which simultaneously protect the end from capping proteins that would otherwise halt growth (1).

A third group of nucleators includes proteins with WH2 domains, which can weakly initiate actin assembly. These WH2 motifs typically play a supporting role, collaborating with other protein domains—such as those that bind filament ends, crosslink filaments, or interact with additional proteins—to facilitate efficient nucleation (2). A recent discovery in yeast by Magliozzi et al. describes a novel "composite" actin nucleator composed of formin Bni1, Bud6, and Aip5 (3, 4, 5, 6, 7, 8). This complex not only assembles actin subunits into a ready-to-grow arrangement but also features pointed-end capping (8) by thioredoxin-fold domain of Aip5 (6), giving it control over both ends of the emerging filament. Remarkably, this setup mirrors the diverse binding behaviors seen in other nucleation complexes with multivalent interactions, creating a stable nucleus for polymerization. For example, growing evidence highlights how molecular condensation of WASP-Arp2/3 complexes or formins can supercharge actin nucleation during specific immune responses in mammals (9) or plants (10). Together, these strategies blend powerful, general-purpose activation with tailored, context-specific boosts through multi-protein interactions. The "composite" nucleator exemplifies this versatility, revealing multicomponent core for nucleation that could inspire insights into similar systems elsewhere.

As the starting point for actin polymerization, these combinations of nucleation strategies offer a spectrum of control. Arp2/3-NPFs and formins rely on conformational changes to switch on robust nucleation, while "composite" or multivalent nucleators form dynamic partnerships locally. This mix allows finely tuned polymerization—strong and reliable in some cases, more adjustable in others—to match a cell's needs, whether in normal physiology or disease states.

In living yeast cells, the composite nucleator achieves precise spatial and temporal control by turning on and off dynamically. At the growing bud tip, Bni1, Bud6, and Aip5 are anchored near the bud tip or neck, likely by direct membrane association and polarisome scaffold protein Spa2 (6). This creates a flexible zone for their multivalent interactions, which recruit free G-actin, align them into a polymerization nucleus, and bind both ends of the initial filament seed right at the tip (8). Notably, a fragment of Bud6 (1–145) directly binds the Aip5 C terminus to recruit G-actin and drive nucleation at physiological concentrations near the bud tip. Since both proteins also partner with formins (6, 7), Aip5 and Bud6 together spark the initial filament seed, then recruit formins to extend the barbed end even in the presence of capping proteins (Fig. 1).

Once elongation kicks off, the growing filament, with elongation forces of a few piconewtons (11), cooperates with differential anchoring; these forces appear to pull apart the Bni1–Bud6–Aip5 tri-component composite nucleator. As a result, Bni1 remains either on the membrane, forming new composite nucleator to generate new filaments, or at the inward-moving barbed end, while Aip5, bound to the pointed end, drifts away from the cell surface. This "catch-to-slip" switching within the composite nucleation complex enables a "nucleate-and-release" mode, where the components dissociate after kick-starting growth. It is intriguing how such modest pulling forces from an elongating F-actin can disrupt seemingly strong interactions between Bni1, Bud6, and Aip5. Regardless, releasing Aip5 from the barbed end at the bud tip may also possibly be crucial by preventing a buildup of too condensed dysfunctional assemblies of "composite

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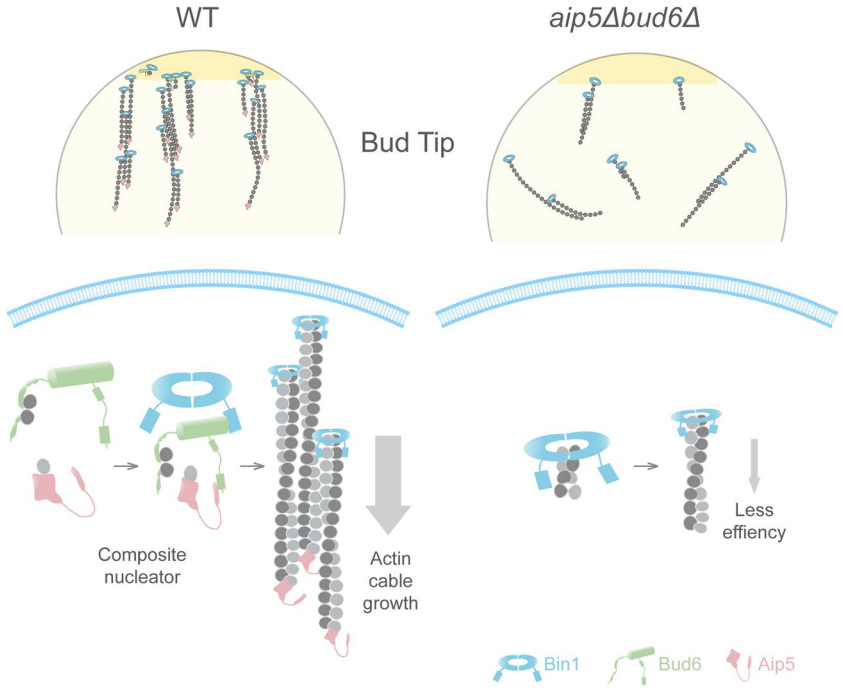

Figure 1. **Composite actin nucleator composed of formin Bni1, Bud6, and Aip5.** In the WT, Aip5 collaborates with Bud6 and Bni1 to form a composite actin nucleator. Bni1 recruits profilin–actin complexes, whereas Aip5 stabilizes the nascent filament and caps its pointed end, thereby enhancing nucleation efficiency and promoting actin cable growth. In the *aip5Δbud6Δ*, disruption of the Aip5–Bud6 interaction compromises actin nucleation, bundling, and thereby cable organization.

nucleator," which could cause a "traffic jam" from imbalanced stoichiometry or excessive multivalent clustering at the bud tip or neck. High-order assemblies might otherwise propagate, stalling elongation by clamping both filament ends too tightly.

In addition, deleting both Bud6 and Aip5 results in not only fewer but also thinner actin cables, which might happen because a subset of these proteins engages in multivalent interactions that bundle filaments for added stability—echoing how a bacterial effector bundles plant actin when its binding sites multiply via flexible self-assembly (12). Furthermore, in Bud6–Aip5 double mutants, actin cables also detach from the bud neck, a region normally rich in formins, Bud6, and Aip5. This hints that the composite nucleator stabilizes not just individual bundles but also their attachment to the cell surface, acting as "anchoring hubs" to guide vesicle transport and support polar growth. Of course, this idea calls for future experiments to explore how just a handful of actin-binding proteins, each with distinct roles, can generate dynamic, combinatorial effects in sequence through varied inter- and intramolecular interactions across time and space—even within a single cell, before factoring in the added complexity of cell type– or isoform-specific rules in multicellular organisms. A key challenge here is building *in vitro* reconstitution systems that mimic *in vivo* spatial and temporally regulated conditions, enabling precise tracking of how these components combine and evolve during polymerization next to the membrane.

This yeast study paints a vivid picture of actin nucleation as a collaborative, adaptable dance, where simple proteins achieve complex feats through teamwork. Unraveling these dynamics could illuminate broader principles of cytoskeletal control across life (Fig. 1).

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