The Iliopoulos et al. study shows that a transient oncogenic trigger can lead to cellular transformation. This is mediated through an inflammatory signal and subsequent activation of a positive feedback loop containing IL-6, NF-κB, let-7, and LIN28B. From a therapeutic point of view, this study and others raise the possibility that tumors with this overactive positive feedback loop—LIN28B^{HIGH}/Let-7^{LOW}/IL-6^{HIGH}—may be eradicated efficiently by interference with this loop, which also may inhibit the growth of cancer stem cells.

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Compete Globally, Bud Locally

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How cells generate a single axis of polarity for mating, division, and movement is unknown. In this issue, Howell et al. (2009) use a synthetic biology approach to demonstrate that rapid competition for a soluble signaling component (Bem1) is essential to ensure a unique axis of polarity in budding yeast.

Cell polarity underlies many fundamental cell processes such as directional migration, cell division, and mating. Because of its powerful genetics, the budding yeast *Saccharomyces cerevisiae* is one of the most thoroughly understood examples of cell polarity. Budding yeast undergo isotropic growth (uniform growth in all directions) for most of their lives but polarize their growth under two circumstances: when they divide by forming a bud and when they mate by forming a mating projection called a "shmoo." In both cases, activation and recruitment of the small GTPase Cdc42 in one part of the cell directs trafficking and secretion of cell wall-modifying enzymes to that single location at the cell periphery. By rewiring the yeast signaling cascade that directs Cdc42 polarization, Howell et al. (2009), reporting in this issue, demonstrate that competition for the rapidly diffusing Bem1 protein is essential to ensure that each yeast cell has only a single bud site.

Cdc42 activation in yeast is normally biased by internal landmarks such as the previous bud site in the case of cell division or by external landmarks such as the pheromone gradient in the case of shmoo production. Remarkably, even in the absence of internal and external landmarks, there is spontaneous polarization of Cdc42 activation and recruitment, demonstrating the strongly self-organizing nature of cell polarity in these cells. Genetic and pharmacological analyses of budding yeast have revealed two positive feedback loops that may contribute to polarity (Figure 1A). The first is a signaling-based positive feedback loop in which active Cdc42 locally recruits the scaffolding protein Bem1 from the cytoplasm to the plasma membrane. Bem1 interacts with a guanine nucleotide exchange factor (GEF) for Cdc42, known as Cdc24, which locally activates more Cdc42, thereby leading to autocatalytic activation of Cdc42 (Butty et al., 2002). The second feedback loop is based on the ability of active Cdc42 to generate actin cables, which act as tracks to locally transport vesicular Cdc42 to the plasma membrane, also resulting in autocatalytic recruitment of Cdc42 (Wedlich-Soldner et al., 2003). GTPase-dependent positive feedback loops may also form the core of polarity in other contexts, such as movement and morphogenesis in neutrophils, the slime mold *Dictyostelium*, and neuronal cells (Brandman and Meyer, 2008). Furthermore, evidence in these systems for both soluble signaling and cytoskeletal-based feedback loops suggests a broadly conserved architecture of signaling for directing cell polarization.

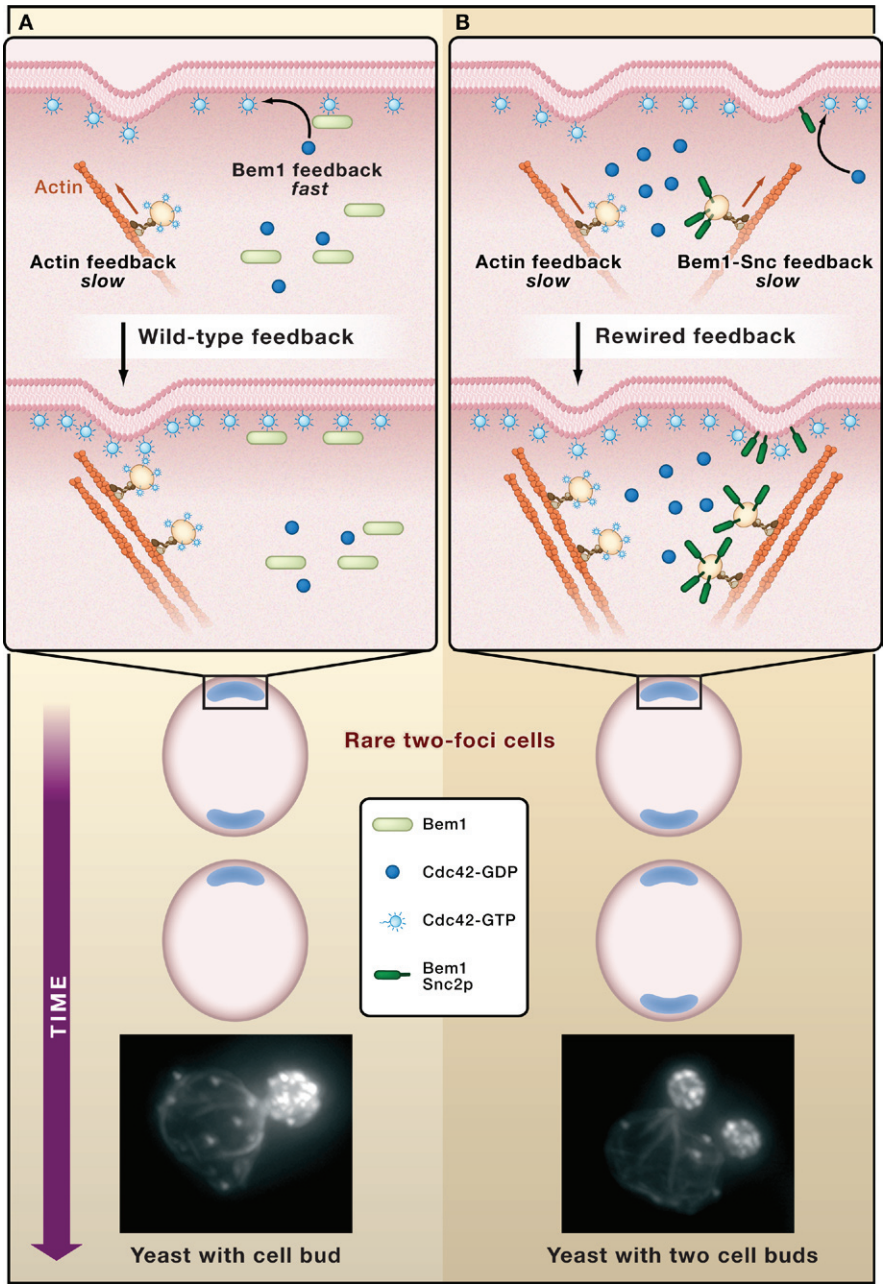

Figure 1. Competition Ensures Singularity in Yeast Budding

(A) Two positive feedback loops are thought to contribute to yeast polarity. In the first, active Cdc42 locally recruits the scaffolding protein Bem1, which recruits a guanine nucleotide exchange factor (GEF) that activates more Cdc42. In the second, active Cdc42 generates actin cables, which enable the transport of more vesicles containing Cdc42 to the plasma membrane. Howell et al. (2009) suggest that rapid redistribution of a limiting pool of Bem1 enables stronger Cdc42 foci to outcompete weaker Cdc42 foci in wild-type cells, ensuring that a single bud is formed.

(B) Budding yeast cells were rewired by decreasing Bem1 mobility through replacement of endogenous cytosolic Bem1 with Bem1 tethered to Snc2, an integral membrane protein (Howell et al., 2009). In these rewired cells, redistribution of the Bem1-Snc2 fusion protein requires actin-based transport, which slows the ability of Cdc42 foci to compete for Bem1. In contrast to wild-type cells, which always form a single bud, a subset of rewired cells generate two buds.

Photos of actin-stained yeast courtesy of A. Howell and D. Lew.

For budding yeast, what keeps these positive feedback loops in check to prevent the entire cell from being overwhelmed with activated Cdc42? In their new work, Howell et al. (2009) address this question by rewiring the yeast signaling cascade that directs Cdc42 polarization. The authors replace the normally cytosolic Bem1 with a Bem1 that is fused to the yeast v-SNARE Snc2, an integral membrane protein (Figure 1B). This Bem1-Snc2 fusion protein is able to rescue from lethality yeast that lack wild-type Bem1 and Bud1/Rsr1 (a budding marker), indicating that even membrane-bound Bem1 can support polarity. The Bem1-Snc2 yeast mutant requires both an intact actin cytoskeleton and endocytosis to support polarity, in contrast to cells with wild-type cytosolic Bem1, which do not. These data suggest that cells with the Bem1-Snc2 fusion protein rely on the actin-based transport feedback loop for instigating and maintaining polarity. Importantly, wild-type cytosolic Bem1 is far more mobile within the cell than the Bem1-Snc2 fusion protein as cytosolic diffusion is faster than endocytosis and actin-based transport.

Although yeast cells with membrane-anchored Bem1 exhibit polarity, these cells show some interesting differences from wild-type cells. Most surprisingly, whereas wild-type cells only transiently (<90 s) exhibit more than one Cdc42 focus and never form two buds at the same time, the Bem1-Snc2 cells take longer to resolve multiple foci (6 min), and a significant portion (up to 5%) of these cells go on to become multibudded. These data suggest that Cdc42 foci normally compete for a limiting pool of Bem1 and that slowing the mobility of Bem1 reduces the efficiency of competition. Consistent with this idea, overexpression of Bem1 also slows competition between foci and occasionally (<1%) leads to cells with two buds.

Taken together, these data suggest that yeast use a several-tiered mechanism to ensure singularity of budding. The amplification of Cdc42 activity through recruitment of Bem1 and actin-based transport of Cdc42 generally leads to a single focus of Cdc42 activity. Even in Bem1-Snc2 cells, 80% of cells initiate polarity from a single focus. In the rare cases of two Cdc42 foci being generated at the same time, rapid competition for Bem1 enables stronger Cdc42 foci to outcompete weaker Cdc42 foci for a limiting pool of Bem1. Decreasing the rate

of diffusion of Bem1 or overexpressing this limiting component slows competition sufficiently that singularity cannot be ensured before the onset of budding.

The authors present a mathematical model of yeast polarization that is broadly consistent with their Bem1 data. However, several interesting discrepancies with the experimental data suggest that additional regulatory mechanisms are likely to be at play. First, experiments reveal that bud competition is resolved on a tightly controlled timescale, an observation not predicted by the model. Second, the model predicts that a more intense focus of Bem1 should always defeat a less intense focus, whereas this is not always the case. These data suggest that the concentration of Bem1 at a focus is not the only factor that controls competition and polarity. Likely candidates for additional points of control include the regulation of Bem1's association with essential signaling proteins such as Cdc24 and the kinase Cla4. Furthermore, phosphorylation of Cdc24 appears to modulate when and where this GEF is active, so Bem1 localization is not the only determinant of where Cdc42 activation takes place (Gulli et al., 2000). Finally, actin not only participates in positive feedback loops involved in focus formation, but can also destabilize foci through negative feedback (Ozbudak et al., 2005). It will be interesting to see how these additional layers of regulation combine to robustly generate a unique axis of polarity.

Beyond providing new insight into the mechanism of singularity in budding yeast, the Howell et al. study showcases the power of the nascent field of synthetic biology. Rewiring signaling cascades can uncover the design principles that underlie complex behavior in a manner that is difficult to accomplish with traditional genetic and pharmacological approaches. Relatively simple perturbations such as changing the diffusion constants or subcellular location of proteins have provided insights into not only yeast polarity but also the mechanism of tension sensing during cell division in mammalian cells (Liu et al., 2009) and the control of cell size in fission yeast (Moseley et al., 2009). Adding new positive and negative feedback loops to endogenous signaling cascades has been used to uncover the core module controlling differentiation in bacteria (Suel et al., 2007) and the shape of the MAPK response in yeast (Bashor et al., 2008). These synthetic tools are likely to bring us ever closer to a true mechanistic understanding of how biological circuits generate complex behaviors.

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Finale: The Last Minutes of Smads

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TGF-β ligands induce phosphorylation of receptor-activated Smads at both the C-terminal tail and the linker region. Two papers from Massagué and colleagues (Alarcón et al., 2009; Gao et al., 2009) reveal a dual role for this linker phosphorylation, which is required for activation of Smads and for their degradation.

Members of the transforming growth factor β (TGF-β) family, including TGF-β, bone morphogenetic protein (BMP), activin, and others, are multifunctional growth factors that regulate a variety of physiological events. Deregulation of signal transduction after receptor activation by these ligands is associated with a variety of diseases (Feng and Derynck, 2005; Massagué, 1998). TGF-β/BMP signaling is initiated by binding of ligand to cell surface type I or type II receptor kinases, leading to the formation of a receptor heterocomplex (Massagué, 1998; Zhang et al., 2009). The activated type I receptor, in turn, acti-