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The first buds of Cdc42

In 1990, John Pringle and colleagues identified the small GTPase and demonstrated its role in yeast cell polarization.

As every fan of *The Hitchhiker's Guide to the Galaxy* knows, the answer to life, the universe, and everything is 42. For cell polarity researchers, however, "Cdc42" might be closer to the truth. By regulating the actin cytoskeleton, the small GTPase controls the polarization of all sorts of cells, from early *C. elegans* embryos to human neurons. But, as for so much of modern cell biology, the story began in *S. cerevisiae,* when a pair of *JCB* papers identified yeast Cdc42 and described its role in bud formation (1, 2).

John Pringle didn't set out to study cell polarity when he started his own lab at the University of Michigan in the mid-1970s. As a postdoc with Leland Hartwell, Pringle had become interested in yeast *cdc24* mutants, which were unable to bud but continued to replicate and divide their chromosomes. This suggested that multiple pathways might operate independently of each other during the cell cycle (3), but, because *CDC24* was the only gene known to regulate bud emergence, it was also possible that the *cdc24* mutants might have lost a control mechanism that normally coordinated budding and nuclear division.

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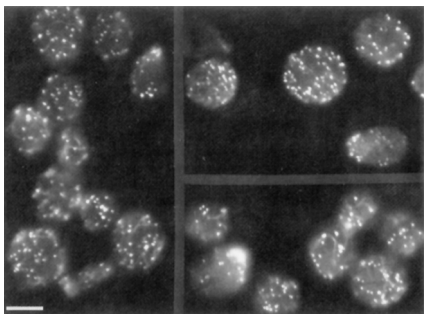
In 1980, therefore, Pringle and his graduate student Alison Adams performed a genetic screen to identify additional mutants defective in bud formation (1). "The first 24 mutants we pulled out had alleles of *CDC24*," Pringle, now at Stanford University, recalls. "An ordinary student would've quit!" The 25th mutant, however, turned out to be in a new gene that the researchers named *CDC42*. Just like cells lacking Cdc24 function, *cdc42* mutants failed to bud, but their nuclear division cycle was also partially impaired, leaving the question of whether budding and nuclear division were independent processes unresolved. (In 1995, Danny Lew and Steven Reed (4) would demonstrate that a morphogenetic checkpoint does, in fact, link bud formation to nuclear division.) This ambiguity, combined with the researchers' inability to clone and sequence *CDC42* using the technologies available to them at the time, meant that Adams et al.'s screen wouldn't be published for another decade.

In the meantime, Pringle and colleagues began to realize that *cdc24* mutants failed to form buds because they were unable to polarize their actin cytoskeleton and specifically target cell wall components to the prospective bud site (5, 6). *cdc42* mutants were similarly impaired, and so, when postdoc Doug Johnson joined the lab in 1985, Pringle was keen for him to use the molecular biology techniques he'd learned as a graduate student to characterize the *CDC42* gene.

Johnson undertook the then arduous task of cloning *CDC42* and proving that defects in this gene inhibited cell polarization and budding (2). The real breakthrough, though, came when Johnson began to assemble the gene's sequence, one tiny fragment at a time, and realized that it encoded a protein that was highly similar to the oncoprotein, and small GTPase, Ras. "Back then, you didn't compare sequences over the Internet. Instead, you had a set of five floppy disks," Johnson, now a professor at the University of Vermont, recalls. "Nothing came up on the first four disks, but—because it starts with the letter R—*ras* was on the fifth disk and so, all of a sudden, the printer started streaming out similar sequences." Cdc42 was even more homologous to a GTPase of unknown function called Rho and showed higher similarity still to several fragments of a protein called G25K, which would turn out to be its human orthologue (7).



ADAMS ET AL., 1990

Instead of localizing asymmetrically to a bud site, actin puncta are distributed randomly in *cdc42* mutant cells grown at a restrictive temperature.

The fact that Cdc42 was a small GTPase suggested that it was probably playing some sort of signaling role to organize the actin cytoskeleton. "For the first time, we could start to think about what Cdc42 might actually be doing," Pringle says. A few years later, Alan Bender and colleagues would demonstrate that Cdc24 was a guanine-nucleotide exchange factor that activated Cdc42 at the bud site (8).

In the years following the publication of Adams's screen and Johnson's sequence analysis, Cdc42's role as a widespread regulator of actin and cell polarity became firmly established. "At first, I was just interested in how a yeast cell made a bud," Pringle reflects. "It was only after we saw that there was a close homologue of Cdc42 in humans that we started to think that budding was like polarizing a mammalian epithelial cell. It's a nice example of the importance of basic, curiosity-driven research."

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3. Hartwell, L.H., et al. 1974. *Science*. 183:46–51.
4. Lew, D.J., and S.I. Reed. 1995. *J. Cell Biol.* 129:739–749.
5. Sloat, B.F., et al. 1981. *J. Cell Biol.* 89:395–405.
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7. Shinjo, K., et al. 1990. *Proc. Natl. Acad. Sci. USA*. 87:9853–9857.
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