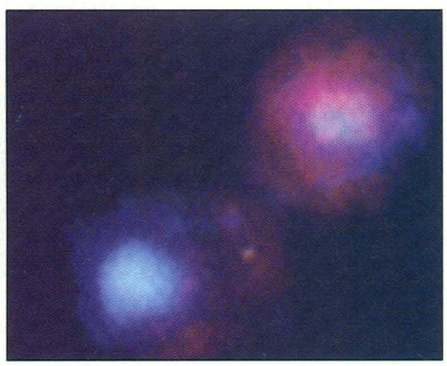
Protein Motors May Drive Cells On Route to Specialization

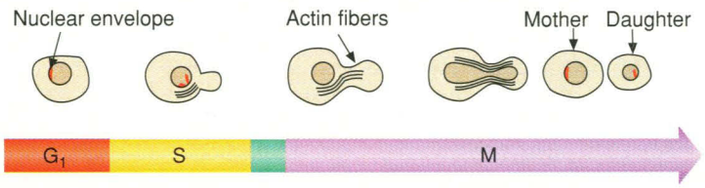
Parents often dream of securing a different future for their children. For parental cells, this isn't a dream—it's often a necessity. When parents divide to create daughter cells with new abilities, even though they have the same genes, it's "a fundamental aspect of development in any organism," says Lucy Shapiro, a developmental biologist at Stanford University. Such specialization, into liver or blood cells, for instance, underlies all complex life, and researchers have long sought to understand how it happens. And they're beginning to get some new answers from an old friend of bakers, brewers, and biologists: the budding yeast *Saccharomyces cerevisiae*.

Yeast mother cells are sexually ambidextrous, able to switch between two mating types known as α and **a**, each one restricted to mating with its opposite type. Their daughters lack this switching ability, however, at least until they've formed young buds of their own. In the 8 March issue of *Cell*, two teams of researchers—working separately at the University of California, San Francisco (UCSF), and at the Research Institute of Molecular Pathology in Vienna, Austria—report new details about how the daughter cells become endowed with abilities different from their mothers'. The answer, it seems, lies in a cellular railway running between mother and daughter. Moving along cytoskeletal "railway tracks," motor proteins shuttle in an unknown regulatory factor that, working through a newly discovered protein called Ash1p, prevents mating-type switching.

As a result of those findings, the yeast pathway is now one of the most carefully mapped journeys toward cell specialization known, and the first one found that's run by a protein motor. "It's a very big step forward," says David Stillman, a molecular geneticist at the University of Utah Medical Center. The findings come on the heels of a report a few months ago of a different pathway in the bacterium *Bacillus subtilis*, which relies on the difference in size between parent and offspring to produce specialized spore cells. In the cramped quarters of each "forespore," a regulatory protein activates certain genes more often than it does in the larger mother, where the regulator can get lost in the shuffle and the vaster spaces (*Science*, 27 October 1995, pp. 578, 637, and 641). So for the first time, developmental biologists say, they are beginning to grasp the myriad ways that nature can create variety. "These papers provide a breakthrough in our understanding," Shapiro says.

For yeast, the road to this understanding began in the early 1980s, when UCSF molecular geneticist Ira Herskowitz, geneticist Kim Nasmyth in Austria, Yasuji Oshima at Osaka University in Japan, and other researchers found that mating-type switching depends on a gene called *HO*, which is activated by a transcription factor known as Swi5p. But that discovery led to a puzzle, because Swi5p is equally abundant in mother and daughter cells, "so it can't be the determinant of switching," says Ralf-Peter Jansen, a geneticist in Nasmyth's lab in Vienna.





Division of labor. In a model of cell specialization, as a daughter yeast buds off a mother, a regulatory protein may be shuttled into the daughter along actin "tracks." This regulator elevates levels of the protein Ash1p (stained pink in photo) in the daughter. Ash1p prevents the daughter from switching mating type; the mother can switch freely.

He, Nasmyth, and their colleagues reasoned that something else must be enhancing Swi5p activity in mother cells—or inhibiting it in daughters. So they embarked on a massive survey of thousands of yeast strains carrying various genetic mutations, looking for errors that affected Swi5p's activation of *HO*. By last year, the scientists had found 46 such mutations in five distinct genes, which they named *SHE* (for *Swi5-dependent HO Expression*) 1 through 5.

What was "rather surprising," Jansen says, was that when they cloned and sequenced four of these genes, none encoded transcription factors or other proteins that might affect Swi5p directly. Instead, one of the genes, *SHE1*, encoded a cytoplasmic "myosin" protein called Myo4. Although Myo4's existence had been known about for some time, its function had not. But the Austrian team now suggests one, noting that Myo4's amino acid sequence closely resembles that of another protein, Myo2, which functions as a tiny molecular motor. It drags protein-laden bags called vesicles from mother cells into daughters along "cables" or "tracks" made from the cytoskeletal protein actin. The sequence similarities of Myo4 imply that it performs a like task. Another *SHE* gene, *SHE5*, was identical to the gene *BNI1*, which encodes a protein that helps to inflate a budding daughter cell with cytoplasm from the mother cell.

The discovery that such motorworks might be connected to cell specialization led the Austrians to a new conjecture. "We had the hypothesis that the She proteins are moving something—that they must move a Swi5p repressor into the daughter cell," Jansen says. To find that repressor, they embarked on yet another search. In cells with defective *SHE* genes, the researchers reasoned, the repressor would accumulate in both mother and daughter cells, preventing *HO* expression—and thus mating-type switching—in both. But after several generations, if any of these *SHE* mutant strains spontaneously regained the ability to express *HO*, the strains would probably be carrying defects in the repressor gene—and those defects could be detected with molecular probes.

The strategy worked: Nasmyth's group found just such a group of defects in a gene named *ASH1* (for *Asymmetric Synthesis of HO*). Without *ASH1*'s protein product, Ash1p, the group showed, there's nothing to prevent daughter cells from switching mating types. That didn't complete the story, however, as there was still no direct evidence that Ash1p is the same factor transported into daughter cells by the She proteins.

At UCSF, meanwhile, Herskowitz and his colleague Anita Sil were arriving at the same answer—but they took a slightly more direct route. Instead of searching for differences in *HO* expression, Sil devised a quick microscopic method for visually distinguishing normal yeast-cell microcolonies (small groups descended from a single cell) from those whose daughter cells were switch-hitters. It was fortunate that the technique was speedy, because Sil needed to examine 86,000 microcolonies before she found one with daughters that consistently switched types. When she cloned the only defective gene in this lone mutant, Sil found that it was the same gene—*ASH1*—just identified by the Austrians. "Interest-

ingly enough," Sil adds, "Nasmyth's group and our group have found that *ASH1*'s protein product, Ash1p, seems to be localized predominantly to the daughter nucleus, which is exactly the right place for a protein that's inhibiting *HO* expression in daughters" (see illustration).

This added up to a neat scenario: More Ash1p ends up in the daughter, hobbling the daughter's *HO* gene, because She proteins drag it in from the mother. At least that's what both labs guessed, until a problem cropped up. After identifying Ash1p, Nasmyth's lab found that it begins to peak in the daughter at the end of the mother's cell cycle, just before the two separate—but after the She proteins that presumably brought it there have already dispersed. So the proteins must have already ferried something into the daughter that helps Ash1p to accumulate. "The model that both Nasmyth's lab and we support is that there is another factor out there controlling the asymmetry of Ash1p synthesis," Sil says. Presumably, this factor is the one transported by the She proteins. "That's the next Holy Grail," says Sil. And both labs are now off in pursuit.

Even though this developmental chain still has a gap, "the number of missing links in yeast is probably smaller now than the number in *Drosophila* embryos" and other examples of asymmetric development, says UCSF cell biologist Andrew Murray. And Shapiro believes that it won't be long before the railwaylike mechanisms for protein localization discerned in yeast turn up in other organisms as well. "The lessons learned from yeast are going to be valuable both up and down—up to the higher mammalian cells and down to the lower bacterial cells," she says. And with those lessons, the many-stranded story of cell divergence may finally start coming together.

–Wade Roush

NEUROBIOLOGY

Rat Study Sheds Light on Cocaine Craving

To a cocaine addict, a small dose of the drug is bad news: It "primes" the brain's reward system, intensifying the craving for more cocaine. And the same effect seems to be triggered by environmental cues that an addict associates with obtaining or taking the drug. Researchers have been trying to figure out the neurobiology behind this phenomenon, because it appears to be a crucial part of the addiction process itself—and if they can understand this reward-priming mechanism, they may be able to find a way to block it with drugs and so prevent relapse of cocaine use in cocaine-dependent people. In a paper on page 1586 of this issue, a team at Yale University School of Medicine, led by Eric Nestler and David Self, reports an important step toward both goals.

The work builds on a growing understanding of how addictive drugs interact with an important neural pathway that uses the neurotransmitter dopamine to send signals between nerve cells. In research reported last month in *Nature*, for example, a team at Duke University verified that cocaine and amphetamines block the transporter molecule that normally mops up dopamine from the synapse, where it activates cell-surface receptors. Now the Yale group has shed some surprising new light on the complex role that this receptor activation plays in the priming mechanism and the processes of addiction. In fact, it appears to play two separate roles.

Dopamine interacts with two types of neural receptors, called D_1-like and D_2-like receptors. When the Yale team gave cocaine-dependent rats a shot of compounds known to stimulate only the D_2-like receptors, the rats' craving for cocaine appeared to increase. But when they administered a shot of compounds that stimulate only D_1-like receptors, they found the opposite effect: The rats no longer sought cocaine, even after they received a priming dose.

Self and his colleagues don't have a complete explanation for this dual effect—in part because it's hard to tell precisely what their experimental rats are experiencing—but it suggests that within the dopamine pathways, different biochemical routes contribute to drug-seeking behavior and other aspects of cocaine addiction. "This is the first demonstration that [different types of dopamine receptors] have qualitatively different effects on cocaine-induced behaviors," says Self, a behavioral neuroscientist. "It's provocative. ... This is extremely difficult research to do," says behavioral pharmacologist Bill Woolverton of the University of Mississippi School of Medicine.

The Yale team used a system in which rats could self-administer cocaine by pressing a lever. The animals kept up a regular routine of lever-pressing over a 2-hour period. When saline was substituted for cocaine for 2 hours, the rats began to press the lever much less frequently—presumably because their brains detected an absence of the drug. Then a "priming" injection containing either a D_1 agonist—a compound that binds and activates D_1-like receptors—or a D_2 agonist was given. Like a priming dose of cocaine, the D_2 agonist triggered a return to regular lever-pressing, but the D_1 agonist had no effect. If the rats were then given a small dose of cocaine, those that had the D_1 agonist still rarely pressed the lever, while the D_2 group pressed even more frequently. "The D_1-like receptor agonist actually blocks cocaine priming," says Self.

It is "as if the D_1 agonist makes [the rats] feel like they've had cocaine," but without making them go back for more, says neuropharmacologist Ken Johnson of the University of Texas Medical Branch at Galveston, adding "this is pretty exciting." Behavioral pharmacologist Ian Stolerman of the Institute of Psychiatry in London agrees that the work is "very interesting." Activation of the D_1-like receptors may produce a sense of gratification, he surmises, while activation of the D_2-like receptors appears to trigger the craving for more drugs.

If so, blocking the D2-like receptors should block drug-seeking behavior. But Self and his colleagues note in their paper that compounds that block dopamine receptors have been found to exacerbate symptoms of cocaine withdrawal. A better approach, they suggest, would be to work on D1-like receptor agonists in the hope that they would provide gratification in cocaine-dependent people without triggering craving for more cocaine. Woolverton says that although the work is preliminary, the results support a move to human tests of D1-like receptor agonists to see if they dampen the craving for the drug. He cautions, however, that the rat model might not correctly predict what would happen in humans. It would also be important to determine whether the agonists themselves are addictive in people.

Bad medicine.
Once touted as a tonic, small doses of cocaine are now known to intensify craving in dependent people.

If the results do hold up, Woolverton and Johnson suggest another interesting possibility: Because some other drugs, such as the opiates, also activate the "reward" pathway involving dopamine, D_1 agonists might eventually be useful in treating addiction to drugs other than cocaine.

–Claire O'Brien

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