DEVELOPMENTAL BIOLOGY

Defining the First Steps on the Path Toward Cell Specialization

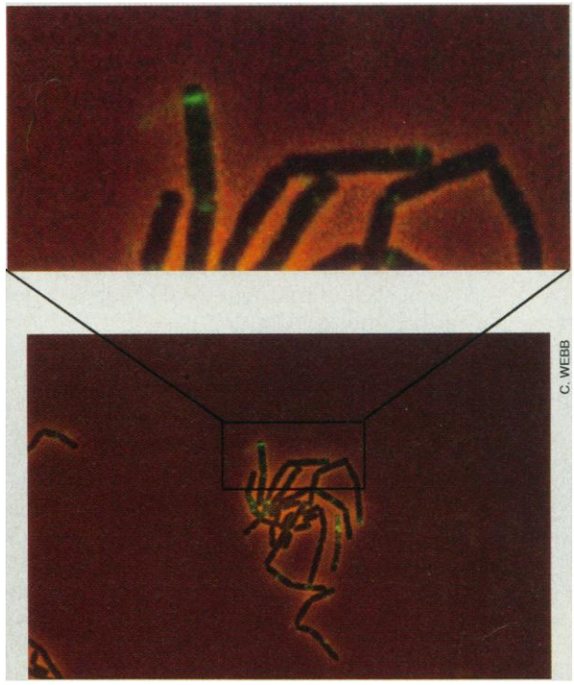
When poet Robert Frost's two roads diverged in a yellow wood, he regretted that he "could not travel both/and be one traveler." But a cell that reaches such a fork in its developmental path isn't forced to choose between one road or the other: It can become two travelers, by dividing into two cells with different fates. This maneuver is the basis for complex life, as a single cell splits into cells with distinct functions, eventually becoming part of specialized tissues and organs. Yet the mechanisms for the initial, asymmetric division—how different genes are activated in each progeny cell—have mystified developmental biologists for decades.

Now a team of researchers in Paris and Cambridge, Massachusetts, has—in a simple model, a bacterium—traced this mechanism back to a single protein. The researchers focused on a phenomenon called sporulation, in which certain bacteria divide to produce two distinct progeny that have identical sets of genes yet take very different paths: a "mother" cell and a "forespore" that is biochemically specialized to become a dormant spore. On pages 637 and 641 of this issue, the investigators report that near the very beginning of this type of cell division in the bacterium *Bacillus subtilis*, a single protein called SpoIIE initiates a cascade of biochemical changes on one side of the burgeoning cell membrane, leading to the activation of scores of genes that convert the forespore into a spore. And this activity may be tied to the size of the nascent cell.

The achievement—by a group led by molecular geneticist Patrick Stragier of the Institut de Biologie Physico-Chimique and developmental biologist Richard Losick of Harvard University—has triggered a chorus of praise from other researchers. "Losick, Stragier, and colleagues have relentlessly and elegantly kept moving the problem of asymmetry back to earlier and earlier stages. They now have a strong suspect for a molecule that may well be what makes the two cells different," says Ira Herskowitz, a developmental geneticist at the University of California, San Francisco (UCSF). Adds David Kirk, a developmental geneticist at Washington University in St. Louis, "It's beautiful work."

Kirk and other researchers have found clues to the relationship between cell size and cell fate in *Volvox carteri*, a species of algae, and to the way signaling proteins are restricted to specific progeny cells in the developing nervous system of the fruit fly *Drosophila melanogaster*. But, says Alan Grossman, a molecular geneticist at the Massachusetts Institute of Technology (MIT), "No other model system has gotten as close as *B. subtilis*." Researchers caution that the Losick-Stragier team still hasn't pinned down the reasons for one crucial initial step, when a membrane called the septum forms near one end of the parent cell. But investigators in their labs, at Oxford University in England, and at other institutions are hot on the trail of this event.

Together, all these projects signal that cell fate studies themselves may be entering a crucial period of discovery. Evolution's inherent economy means that such mechanisms may be conserved in other simple organisms, and perhaps even in higher ones, such as mice and humans. "Mechanisms aren't going to be transferred lock, stock, and barrel across organisms," says Michael Yudkin, a biochemist at Oxford. "But any mechanism discovered in one organism is helpful in inspiring experiments in another."



C. WEBB

Division street. The protein SpoIIE *(green band)* collects at the site of cell division in these bacteria (each cell=2 microns), triggering different patterns of gene activation on either side.

Divide and differ. The findings by the Losick-Stragier team cap decades of study of the ubiquitous *B. subtilis*, which can be readily found in almost any handful of dirt. Sometimes, however, nutrients in the soil are not nearly as abundant as the bacteria. When food is scarce, a *B. subtilis* cell stops reproducing through normal, binary fission, and instead duplicates its chromosomes and partitions them into two contiguous but differently sized cells, the mother cell and the smaller forespore. The mother cell expands and engulfs the forespore, coating it with layers of protective proteins, then dies. With its genetic material bound up in a sturdy, inactive mass, the armored spore can survive starvation in a state of suspended animation for decades.

Because the mother cell and the forespore are genetically indistinguishable, spore formation must depend on the activation of distinct genes in each cell. Experiments in Losick's lab in the late 1960s led researchers to suspect that differential activation is accomplished by a series of transcription factors (proteins that bind to sites near the beginning of genes to initiate messenger RNA synthesis) called σ factors. By 1991, Losick and colleagues had identified one such factor, σ^F, that is active only in the forespore (*Science*, 25 October 1991, p. 562). Says Losick, "It became clear that if we wanted to understand how cell-specific gene expression begins, we'd have to look at the mechanisms that restrict σ^F activity to the forespore."

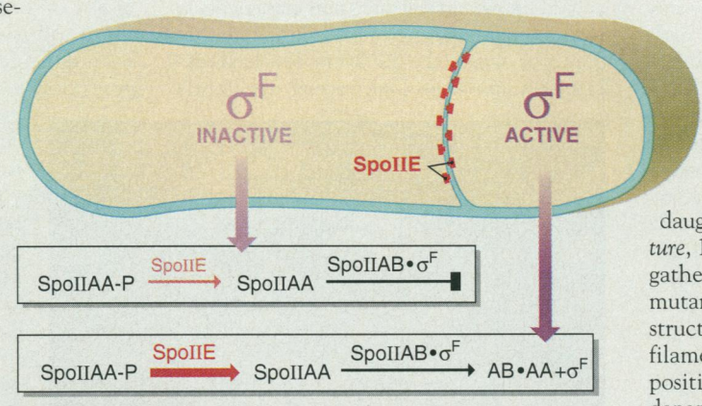
One promising candidate for this mechanism was the protein SpoIIE. Losick's group found that mutant bacteria lacking the protein also lacked σ^F activity. Eager to see where SpoIIE was acting in living bacteria undergoing sporulation, the group starved the bacteria to induce spore formation and labeled the protein with fluorescent antibodies or a green fluorescent protein. In the first of their two papers, Scott Alper, Fabrizio Arigoni, Leonard Duncan, Kit Pogliano, Chris D. Webb, Losick, and Stragier demonstrate that SpoIIE collects exclusively at the developing membrane between mother cell and forespore. Says Losick, "It's just the perfect vantage point to somehow activate the transcription factor on one side of the division but not the other."

Yet that "somehow" was troubling. The group "still didn't know what SpoIIE was doing biochemically," Losick says. Then, last year, they defined the likely functions of two other players in the σ^F regulation game: the "anti-σ" protein SpoIIAB, which binds to σ^F and thus blocks its activity; and the "anti-anti-σ" protein SpoIIAA, which inhibits SpoIIAB's binding action. Alper, Duncan, and Losick showed, in test-tube experiments, that the three substances interact in a partner-switching process. SpoIIAA binds to SpoIIAB, forcing the latter to give

up any bound σ^F, freeing the transcription factor—in theory—to activate genes (*Cell*, vol. 77, p. 195, 1994).

But SpoIIAA can't bind to SpoIIAB—and release σ^F—if it's carrying a phosphate group, and that's where SpoIIE seems to come into play. A group led by Yudkin and molecular geneticist Jeffrey Errington at Oxford had shown this phosphate-driven inhibition of SpoIIAA-SpoIIAB binding—without discerning its exact consequences for σ^F—in 1993 (*Cell*, vol. 74, p. 735, 1993). That meant, Duncan says, that "if a pool of unphosphorylated SpoIIAA is required to generate free σ^F, there had to be a phosphatase" at the beginning of the cascade that was capable of dephosphorylating SpoIIAA. "It occurred to us that SpoIIE could be the phosphatase."

SOURCE: R. LOSICK



Cramped quarters. In one view of bacterial spore formation, SpoIIE contacts SpoIIAA more often in the forespore *(right)*, removing a phosphate group. This allows AA to bind to SpoIIAB, freeing σ^F—which activates genes—from AB's grasp.

Indeed it is. In in vitro studies, phosphorylated SpoIIAA exposed to purified SpoIIE lost its phosphate group. Further experiments showed that when a single amino acid residue, the binding site for the phosphate group, was changed on SpoIIAA, SpoIIE failed to dephosphorylate it—suggesting that, in their nonmutant forms, the two proteins are tailored specifically to interact with each other in a pathway for the release of σ^F. Comments Yudkin, "I think the results are robust. Everybody's views are moving toward convergence" on the importance of phosphorylation reactions in σ^F's release.

A matter of concentration? This release is triggered in the forespore, and not the mother cell, because SpoIIE is more concentrated in the former, the researchers think. "The answer may be that SpoIIE goes to the septum," says Stragier. He points out that because the forespore has a smaller volume, the molecules are crammed into less space, and that means more phosphorylated SpoIIAA comes into contact with SpoIIE. Comparatively more dephosphorylation reactions occur, leading to the release of more σ^F. The researchers are currently testing this theory in vivo.

The next big question, of course, is how parent cells form a septum at one end to produce progeny cells of unequal sizes. During binary fission in *B. subtilis* and several other bacteria, a cytoskeletal protein called FtsZ forms a contracting ring at the dividing cell's midpoint. Errington suggests that the same process probably occurs near one pole during sporulation, but only after utilization of a central "marker" for cell division is somehow blocked. "The problem is, we don't even understand how the cell can choose [to divide at] the central position" during normal cell division, Errington says. Losick's group suggests that sporulation induces a chemical "switch" that frees FtsZ to assemble at other markers closer to the poles. But "there's still no sense of what might be attracting [FtsZ]" to these new markers, Losick says.

B. subtilis isn't the only species in which relative cell size may make a developmental difference. Developing embryos of the green alga *Volvox carteri*, for example, have identical cells through the first five rounds of cleavage. But these cells eventually develop into two distinct kinds: small, nonreproducing somatic cells and much larger "gonidia" or reproductive cells. It's in the sixth round of cleavage that some embryonic cells begin dividing asymmetrically, producing both small and large progeny. All cells under 6 microns in diameter develop as somatic cells, Kirk and his colleagues at Washington University have found, while those over 9 microns develop as gonidia (*Journal of Cell Biology*, vol. 123, p. 191, 1993).

Kirk suggests that the difference in cell fates may be partly dictated by the ratio of chloroplasts to nucleus in each cell. Because the number of chloroplasts in each *Volvox* cell grows with increasing cell size, but each cell has only one nucleus, that ratio is naturally higher in larger cells. The abundance of chloroplast proteins entering the nucleus might therefore be higher as well, Kirk says. And while many of these proteins coordinate the transcription of nuclear genes essential for photosynthesis, he speculates that some could play roles in the transcription or repression of genes that specify somatic and gonidial cell fate.

Tracing the simple and the complex. Asymmetric cell division is also giving up some of its secrets in the fruit fly *Drosophila melanogaster*. The two nerve cells and two support cells that make up an organ called an external sensory bristle, part of the fly's peripheral nervous system, all develop from a single precursor cell. The progeny cell that will later divide to become the nerve cells receives the precursor cell's entire complement of a membrane-associated protein called Numb and a transcription factor called Prospero, both believed to be involved in the determination of cell fate. But how these substances are segregated only to the preneural cell remained unknown—until recently.

UCSF geneticists Jürgen Knoblich, Lily Jan, and Yuh Nung Jan reported in last week's issue of *Nature* that during mitosis Numb and Prospero collect in a crescent-shaped spot overlying one of the two centrosomes, organizers of the mitotic spindle that pulls separated daughter chromatids to the cell poles (*Nature*, 19 October 1995, p. 624). The proteins gather on one side of the dividing cell even in mutant cells with extra centrosomes or when structures such as microtubules and actin filaments are destroyed, suggesting that their positioning is coordinated with—but not dependent on—the cytoskeletal changes characteristic of mitosis. Knoblich thus speculates that a "master organizer" provides positional information for both spindle orientation and the Numb-Prospero crescent. "The key point is, if the cell wants to divide asymmetrically, there is machinery at hand to segregate proteins into one of the two daughter cells," Knoblich says.

As other studies of asymmetric cell division move ahead in the bacterium *Caulobacter crescentus*, the budding yeast *Saccharomyces cerevisiae*, the nematode *Caenorhabditis elegans*, and other organisms, scientists hope that even complex problems such as tissue specialization in mammalian embryos may eventually be better understood. The differential activation of identical genomes segregated to two progeny cells "is an absolutely central mechanism in biology, and one of enormous importance," says Yudkin. And while it's a big leap from bacteria, algae, and flies to mammals, tactics of asymmetric division such as protein phosphorylation and localization are likely to be used over and over, he and others say. Says MIT's Horvitz: "The biochemical data, although it may seem like organism-specific nitty-gritty, really tends not to be. We have to understand the minutiae in order to know the big picture." In other words, the details are what make all the difference.

–Wade Roush

Additional Reading

H. Robert Horvitz and Ira Herskowitz, "Mechanisms of Asymmetric Cell Division: Two Bs or Not Two Bs, That Is the Question," *Cell* **68**, 237 (1992).

Lucille Shapiro, "Protein Localization and Asymmetry in the Bacterial Cell," *Cell* **73**, 841 (1993).