Peeling the Onion

Protein by protein, Stanford researchers are working out how a cell differentiates its front from its back.

BY WILLIAM WELLS

A CELL IS NOT JUST AN AMORPHOUS BAG OF CHEMICAL REACTIONS. A fertilized egg immediately defines its front and back so it can develop into a baby with a mouth at one end and diapers at the other. Without a set of coordinates a cell will, at best, grow up to be a puddle of ooze. • The generation of a difference in one part of a cell (asymmetry) leading to the definition of a front and a back (polarity) happens repeatedly in development. Nerve cells define their receiving end from their transmitting end. Cells in the gut define in and out so they excrete digestive fluids only into the stomach and not into surrounding muscles. And cells called neuroblasts split in two, with one end producing another neuroblast and the other end producing a nerve cell.

Something must have been placed in the nerve cell to inform it that it will be a nerve cell and not a neuroblast. But what could that something be? How did it get there and what told it to go in that direction?

These were some of the questions addressed earlier this year at a two-day symposium organized by the students of the Stanford School of Medicine Department of Developmental Biology, led by students Karen Ho and Amy Kiger.

Fourteen speakers from ten universities discussed cell polarity. They explained how yeast cells make their progeny different from themselves, how fruit flies make nerve cells from normal dividing cells, how light from above triggers water plants to grow leaves at one end and roots at the other, how single-celled ciliates make sure they put their mouth and anus at the opposite ends of the cell, and how worm embryos create cells with different fates from the very first cell division.

WHAT MAKES CELLS DIFFERENT?

"Polarity is universal," says developmental biologist Lucy Shapiro, PhD, the first Virginia and D.K. Ludwig Professor of Cancer Research in the School of Medicine. "It is becoming clear that it is important in all kinds of cells," she says. Thanks to Shapiro, even bacteria were represented at the symposium. She described her efforts to understand the asymmetry of *Caulobacter*. Every time this bacterium splits in two it produces two different cells. The stalk cell stays put and immediately gets ready to produce two new cells. But the swarmer cell has a propeller-like tail and can swim off and colonize new lands. Eventually it settles down, becomes a stalk cell and begins producing new stalk and swarmer cells.

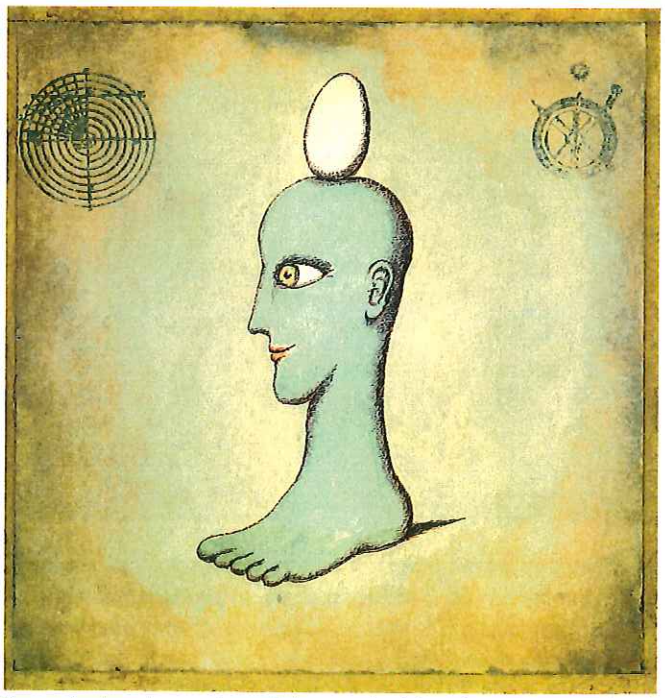
The key to becoming a stalk cell or a swarmer cell seems to be a protein called CtrA. Shapiro's lab found CtrA by looking for a protein that binds to genes that switch on construction of the swarmer's propeller, or flagellum. CtrA also attaches itself to the origin of replication and blocks the swarmer cell from duplicating its DNA. Only when the swarmer cell begins its transformation into a stalk cell is the CtrA destroyed, allowing the stalk cell to reproduce itself.

Once DNA duplication is under way in the stalk cell, a new batch of CtrA is made. Initially it spreads throughout the cell. But as the cell builds the wall that will split it in two, the CtrA is destroyed in only the prospective stalk cell. At the end of cell division all the CtrA is in the new swarmer cell, which makes a flagellum and delays copying its DNA. The new stalk cell, however, lacks CtrA and so can immediately enter another division cycle.

Shapiro now knows that CtrA can switch a cell from stalk to swarmer and that the cells differ because CtrA is selectively destroyed in the stalk cell. But why does the destruction happen only in the stalk cell? Is there a CtrA-destroying protein that is only in the prospective stalk cell? If so, how is that protein restricted to the stalk cell? Clearly, Shapiro's work is not finished yet.

"It's like peeling an onion," says Shapiro. "But unless you have an entrée to the system, you will never get anywhere. Now we need to follow all the way back through from CtrA to the source of the asymmetry."

W. James Nelson, PhD, thinks that the source of asymmetry in his favorite cell is a cellular glue called E-cadherin. Nelson, professor and chair of molecular and cellular physiology at Stanford, works on cells originally derived from a dog kidney. In the dog, as in all mammals, the cells fold to form a tube called a nephron. The cells absorb anything useful from the filtered blood passing



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through the tube and send these valuable salts and sugars back to the bloodstream. This process continues until the final fluid — the urine — contains lots of waste products but few nutrients. Nelson wants to find out how the cells know to always send the various molecules in one direction and how the cells first define that direction.

Nelson started in on this problem by looking at what carries out the transport of salt and sugars. One of the key proteins for transporting salt in the kidney is called the sodium/potassium

ATPase. In the kidney nephron the ATPase is found only on the side of the cell facing the outside of the tube, which is the side facing the blood vessels. The ATPase therefore pumps sodium only to the surrounding blood vessels. There is now less sodium in the cell than in the blood filtrate, so sodium flows into the cell through tiny holes in the cell's outer coat. This strategy reduces the amount of sodium (salt) lost in the urine.

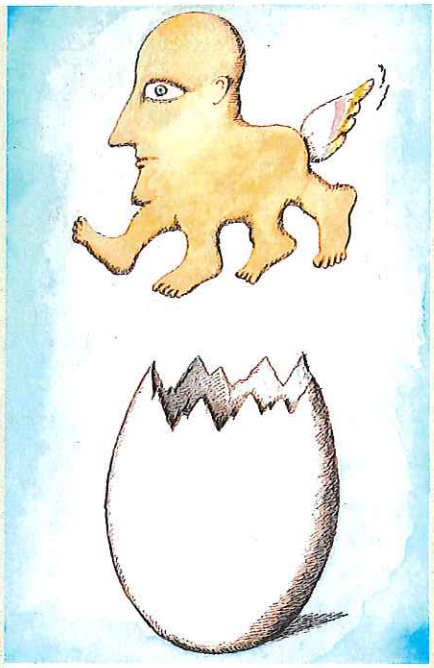
The problem of directional salt transport is therefore reduced; the new problem is how the ATPase gets sent to one side of the cell and not to the other. The answer is of interest to clinicians as much as to basic researchers. Working with Bryan Myers, MD, professor of medicine (nephrology), Nelson found that nephron cells can lose their polarity. In kidneys that are without oxygen for prolonged periods before transplantation, the ATPase disappears from the entire cell surface. The disorganized pumping that follows can cause sodium loss in the urine sufficient to kill the transplant recipient. Meanwhile, patients with polycystic kidney disease localize the ATPase to all surfaces of the cell. Salt and water movement fail, cysts form and swollen, painful and malfunctioning kidneys result.

Patients, or even just a kidney, are hard to work with. So Nelson's "workhorse" for understanding polarity is the batch of cells derived from the dog kidney. Forty-four years after the original kidney dissection the cells — now called MDCK cells — are still going strong in the culture dish. MDCK cells can't concentrate urine. But they spontaneously group together to form a hollow ball that, like the kidney nephron, can shunt chemicals from the outside to the inside of the ball, and vice versa.

MDCK cells naturally stick together because of the E-cadherin glue on their outsides. Nelson discovered that, soon after two cells stick together, the ATPase finds its way to the site where one cell contacts another. A scaffold-like mesh of proteins links the ATPase to any E-cadherin that is attached to E-cadherin on another cell.

The cell is now asymmetric, but the asymmetry is not oriented in a useful direction. The directionality comes from a web of material called extracellular matrix (ECM). "The ECM tells the cell what is down and what is up," says Nelson.

Clumped cells begin shooting ECM proteins out of the cell in all directions. Most of the ECM floats away, but some gets trapped where cells contact each other. The accumulated ECM defines the ATPase side of the cell. Salt transport begins, water transport follows and soon the fluid-filled ball is complete. (In the kidney,



cells surrounding the nephron make the most ECM and so define 'out' as the ATPase side. In the culture dish, this supply of ECM does not exist. The MDCK cells therefore rely on their own ECM and define 'in' as the ATPase side. But the principles of both systems are the same.)

Nelson tested his E-cadherin hypothesis by inducing fibroblast cells — which normally put the ATPase all over their surface — to produce E-cadherin. The new cells clumped and put the ATPase only where one cell contacted another. "This is the ultimate experiment," he says. "You turn a non-polarized cell into a polarized cell; you turn base metal into gold."

"All the cells have to do is to take a surface that is symmetrical and tap it," says Nelson. In this case, that tap is the random collision and gluing of one cell to another. "As soon as the cell responds to that mark, the cell will be polarized. It's a simple idea — not very controversial — and it was probably published a couple of hundred years ago."

Many proteins are found on only one side of the cell, but it seems unlikely that all are linked to E-cadherin. There is just not enough E-cadherin to hold onto them all. Perhaps, thought Nelson, there is a more general system for sorting proteins once E-cadherin and the ATPase have defined which way is up.

At the symposium, and in the May 29, 1998, issue of the journal *Cell*, Nelson described just such a system. The key is a group of proteins called the Sec6/8 complex, first discovered in yeast. As a new yeast cell grows, the complex defines the site on the cell lining where new building materials are delivered.

The mammalian version of the Sec6/8 complex was found by Richard Scheller, PhD, a Stanford professor of molecular and cellular physiology and Howard Hughes Medical Institute investigator. Nelson found that Sec6/8, like the ATPase, moves to the site of cell contact soon after MDCK cells clump. And antibodies that interfere with Sec6/8 stop several proteins from being sent to this side of the cell, although other proteins are still sent correctly to the other side of the cell. His conclusion is that Sec6/8, as in yeast, is the cell's landing pad for protein delivery.

Nelson's story is one of the most complete in the field of cellular asymmetry. But he has still to discover what proteins anchor Sec6/8 to one side of the cell and how proteins that need to be sorted recognize Sec6/8.

As with Shapiro's *Caulobacter*, there are plenty more layers to peel off this onion. **SM**

SHORT TAKE

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"She is a particularly insightful and present human being," Winograd says. "She is able to know what is up with somebody. She is extraordinarily gifted in that respect."

She recalled one day in October 1996 when Traber asked her what medium she'd like to use for the occasion. "How about plates to throw?" responded Winograd, who was full of anger that day. "How about not doing that?" Traber says. "How about drawing your anger?" So she did.

Using colored pencils, Winograd drew her right forearm vibrating with angry red and orange cells. The prose that accompanied the work of art began, "My cells are in overdrive, adrenalized, firing constantly, fighting, fleeing, probably frightened of my own anger... ." Though the visual image is raw and unnerving, Winograd says she felt much calmer when it was completed.

As a result of those sessions, Winograd has produced dozens of works of art, including four 4-by-8-foot self-portraits that reflect her coming to terms with her changing body shape and size.

She was one of 11 artists featured in a permanent, rotating exhibit inaugurated in June by the Art for Healing program. The display is located in a hallway on the first floor of the hospital just outside the endoscopy unit.

In a brochure accompanying the show, Winograd wrote, "As my world collapsed and my physical body became less functional, art has become a place of growth and substance. I feel alive when I am doing art." **SM**