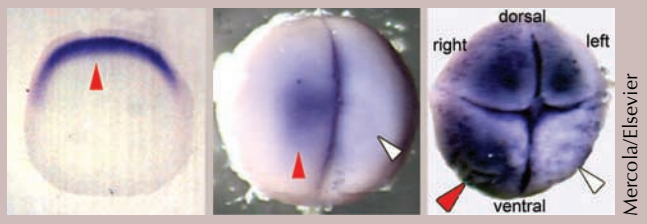
Research Roundup

Choose a side

Dividing an organism into unequal halves just took a giant step backward—in time, that is. Michael Levin, Mark Mercola (Harvard Medical School, Boston, MA), and colleagues have identified what is so far the earliest-acting determinant of left/right (LR) asymmetry. According to their findings, this early determinant establishes voltage differences that distinguish left from right.



Mercola/Elsevier

H^+/K^+ ATPase mRNA (purple) is unevenly localized as early as the two-cell stage (middle) in frogs.

Based on previous evidence that gap junctions are required for LR asymmetry, the group devised a simple model to explain how asymmetry determinants might be driven directionally through gap junctions in a process akin to electrophoresis. Though he did not necessarily believe the model, Mercola says, "the idea that voltage differences and channels or pumps may be important was testable."

And tested it was. In what Mercola likes to call the Sigma catalogue screen, the authors threw hundreds of ion flux inhibitors at developing frog embryos. Many of the chemicals that disrupted LR asymmetry (e.g., caused the normally left-lying heart to be situated on the right) displayed a common attribute: they influenced potassium transport into or out of the cell. More specific compounds revealed that inhibiting the H^+/K^+ ATPase transporter upset asymmetric gene expression patterns known to dictate the hemisphere in which organs form.

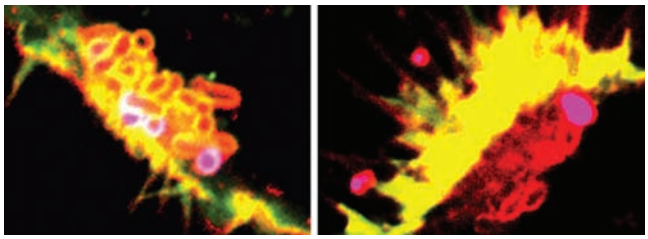
The H^+/K^+ ATPase is the first determinant found upstream of the asymmetric gene expression cascade. In frogs, LR asymmetry was set as early as the two-cell stage, at which point the H^+/K^+ ATPase mRNA was often already asymmetrically localized near the point of cell–cell contact. The H^+/K^+ ATPase mRNA was not asymmetric in chick embryos, but the pump did establish a voltage difference between cells on opposite sides of the primitive streak.

It is still unclear how the pump affects downstream gene expression. Transporter activity on one side of the embryo or streak could make the cell interior more negatively charged, and thus could drive an electrophoresis effect between cells. But, to support their electrophoresis model, Mercola must determine whether the cells affected by the ATPase are the cells linked by the asymmetry-promoting gap junctions. Alternatively, the pump may directly regulate some factor that leads to asymmetry. ■

References: Levin, M., et al. 2002. *Cell.* 111:77–89.

Little PIP, little PIP, let me in

In one simple hydrolysis step, bacteria make the plasma membrane squishy enough to ease invasion, according to new results from two groups.



Grinstein/Macmillan

PIP_2 (green) is eliminated by bacteria (pink) that express SigD (right).

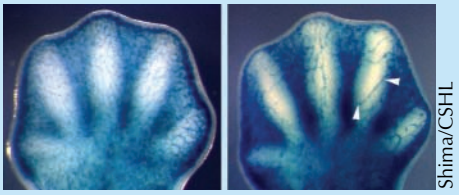
The groups studied different pathogens, but both came to similar conclusions. Both bacteria, *Shigella* and *Salmonella*, inject several invasion-promoting proteins into the host, including a protein with similarity to a mammalian inositol phosphatase. Mauricio Terebiznik, Sergio Grinstein (Hospital for Sick Children, Toronto, ON), and colleagues watched how phosphoinositides were affected during *Salmonella* infection. They found that one PIP_2 species, PtdIns(4,5)P_2, was depleted from the base of membrane ruffles that formed where bacteria were pushing to get in, thanks to the phosphatase activity of the SigD effector protein. Like SigD, the *Shigella* effector IpgD studied by Kirsten Nieburh (Institut Pasteur, Paris, France), Bernard Payrastre (INSERM, Toulouse, France), and colleagues also removed PIP_2 from target cells; in this case, the product was identified as PtdIns(5)P.

The result in both cases was a softer plasma membrane. Expression of either phosphatase in mammalian cells caused membrane blebbing and relaxed attachment between the actin cytoskeleton and the membrane. Vesicles were released from the membrane into the SigD-expressing cells. The loosened membrane sped up bacterial invasion—*Salmonella* mutants lacking SigD were slower to gain entry.

Although PtdIns(5)P may affect membrane-cytoskeleton interactions, both groups imagine that the loss of PIP_2 is at the heart of it all. "Actin cross-linkers are bound by PIP_2," says Grinstein. "Bacteria have taken advantage of this. By chewing away PIP_2, they weaken the interaction." The weakening of the plasma membrane environment may assist the fission of vesicles from the membrane. It remains to be seen whether phosphatase conversion of PIP_2 into PtdIns(5)P is a general mechanism to control membrane properties during other processes, such as motility or endocytosis. ■

References: Niebuhr, K., et al. 2002. *EMBO J.* 21:5069–5078.
Terebiznik, M., et al. 2002. *Nat. Cell Biol.* 4:766–773.

TEXT BY NICOLE LEBRASSEUR
LEBRASN@ROCKEFELLER.EDU


Shima/CSHL

Blood vessels are missing branches in mice with no ECM-bound VEGF (right).

Branching out requires VEGF

Not all isoforms are created equal. New results from Christiana Ruhrberg, David Shima (Cancer Research UK, London, UK), and colleagues reveal that the stickiness of vascular endothelial growth factor (VEGF) can make all the difference between blood vessels growing larger or finding new territory.

VEGF initiates the formation and expansion of the vascular system. VEGF isoforms differ in their ability to bind to the extracellular matrix (ECM), but, in vitro, endothelial cell proliferation is stimulated by VEGF regardless of its ECM-binding ability.

In the new study, Shima's group examined mice engineered to make only single VEGF isoforms. These mice reveal that VEGF isoforms have opposing effects on growing vessel networks. Although all forms stimulated cell growth in vivo, mice that expressed only soluble VEGF had expanded microvessels with fewer branches. In contrast, microvessels in mice with only ECM-binding VEGF were narrow and branched excessively.

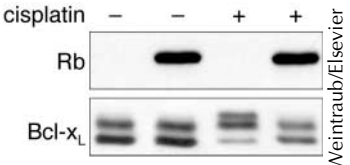
"Our results indicate that growth is integrated with tissues, because the tissue provides localized cues by depositing VEGF in precise spatial patterns," says Ruhrberg. The road map is provided by ECM-bound VEGF, which attracted filopodia extending from the tip of a new vessel branch. Soluble VEGF did not remain where it was secreted, but rather traveled further away, thus stimulating expansion of existing vessels from a distance. The authors hope to raise awareness of isoform-specific effects for those considering the use of VEGF for therapy to increase or block angiogenesis. ■

Reference: Ruhrberg, C., et al. 2002. *Genes Dev.* 16:2684–2698.

An amidation a day keeps apoptosis away

Rapidly dividing cells such as tumor cells are susceptible to DNA damage that then induces apoptosis. As a result, DNA-damaging chemicals such as cisplatin are used as anticancer treatments. How the majority of nontumor cells survive chemotherapy has been mysterious. The trivial explanation is that the cells are growth-arrested and thus less susceptible to DNA-damaging agents. But a more precise explanation is put forth in a new article by Benjamin Deverman, Steven Weintraub (Washington University, St. Louis, MO), and colleagues, who have identified an antiapoptotic activity necessary to keep damaged but nondividing cells alive.

The proapoptotic activity that allows tumor cells to die is an unusual modification of the antiapoptotic protein Bcl-x_L caused by DNA-damaging agents. This modification, deamidation of two asparagine residues, inactivated Bcl-x_L, thereby allowing cell death to proceed. Growth-arrested cells escaped apoptosis by blocking deamidation. To prevent deamidation, cells needed Rb, a tumor suppressor protein that inhibits cell cycle progression. Because tumor cells lack Rb, and cycling cells down-regulate Rb, they are more sensitive to DNA-damaging agents. "Deamidation is like a checkpoint," says Weintraub. "If you undergo DNA damage in the absence of Rb, then cells are susceptible to death." ■



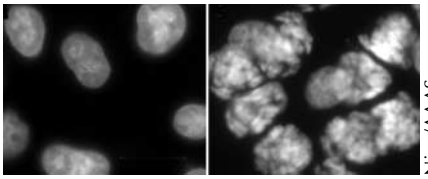
Weintraub/Elsevier

Bcl-x_L modification by cisplatin is blocked by Rb.

Reference: Deverman, B., et al. 2002. *Cell.* 111:51–62.

Arrested cells are Mad as Hec

The spindle checkpoint works even when on the move, according to new results from Silvia Martin-Lluesma, Volker Stucke, and Erich Nigg (Max-Planck Institute of Biochemistry, Martinsried, Germany). Against all that has been sacred in the field, these researchers find that mitosis can be arrested when checkpoint proteins leave the kinetochore.


Nigg/AAAS

Cells without Hec1 (right) stall in mitosis although Mad is not at kinetochores.

The checkpoint is activated by Mad1 and Mad2 proteins, which bind to kinetochores that are not attached to the spindle microtubules. The prevailing theory has been that release of Mad1/Mad2 inactivates the checkpoint and allows mitosis to proceed.

Nigg and colleagues looked for human Mad1-interacting proteins and found Hec1, which recruited Mad1 and Mad2 to kinetochores. Given this function, the group was surprised to find that reducing Hec1 prevented cells from dividing, despite the fact that Mad1/Mad2 were not on the kinetochores. "This is different from all that has been shown so far," says Stucke. "The components are depleted [from kinetochores], but you still get arrest and an active checkpoint." Hec1-depleted cells that also lacked Mad2 continued through mitosis and ended in mitotic catastrophe. Thus, checkpoint components can signal from the cytoplasm.

According to one model of Mad function, the Mad proteins make a brief visit to kinetochores to sample the environment, but then propagate their inhibitory signal in the cytoplasm. Hec1 may regulate this process, but for now the details of Hec1 action remain obscure. ■

Reference: Martin-Lluesma, S., et al. 2002. *Science.* 297:226–270.