Protein splicing for immunity

Eukaryotic cells can make several protein variants from one gene via RNA splicing. Now, a chance encounter with an antigen suggests to Ken-ichi Hanada, Jonathan Yewdell, and James Yang (National Institutes of Health, Bethesda, MD) that vertebrate cells might get even more mileage from the genome through protein splicing. Yang's group shows that protein splicing produces a fragment of the FGF-5 protein that is recognized as antigenic by human T cells. T cell recognition was stimulated by a peptide as short as nine residues, as long as it contained two short sequences normally separated within full-length FGF-5 by 40 amino acids. Production of the fusion is post-translational, as untreated cells could process longer synthetic peptides into active antigens, but lightly fixed cells (which are unable to do their own proteolytic processing) could present only an already spliced peptide. Protein splicing probably takes place in the cytoplasm before the fused product is transported to the ER for presentation. The proteasome, which is normally required for antigen presentation and is a highly proteolytic entity, may be responsible for the splicing activity. “Maybe enzymes that cut proteins also ligate them,” says Yang.

The group is putting their findings to use in developing vaccines against cancerous kidney cells, which express a lot of FGF-5. Yang says that other investigators have recently identified additional antigenic epitopes generated by protein splicing, so FGF-5 is not unique. But he is interested in whether spliced proteins have structural or enzymatic functions that differ from the parent proteins. The study of antigen presentation “is the most sensitive way there is of detecting specific peptide sequences,” says Yang. “But it may be a more common phenomenon. Now, we need to know if it has a functional significance.”

Reference: Hanada, K.-I., et al. 2004. Nature. 427:252–256.

Breathe, then breed

New results from Robert Klevecz, Douglas Murray, and colleagues (City of Hope Medical Center, Duarte, CA) reveal that DNA is synthesized when it is least likely to be hit with oxidative damage.

Damaging oxidizing agents are produced by respiration. In yeast, respiration is periodic—it alternates in ~40-min cycles with a stage of non-respiration called the reductive phase. This oscillation was detected decades ago, but it was never thought to be connected to cell cycle progression. Now, Klevecz shows that both S-phase entry and transcription are controlled by the respiratory oscillation.

Microarray analysis revealed that nearly 90% of transcribed yeast genes were maximally transcribed in two peaks during the reductive phase—one early and one late. The transcription of fewer than 700 genes, in contrast, peaked during respiration.

The respiration cycle also gated entry into S-phase. Although only 10% of cells in any given respiration cycle entered S-phase, all those that did entered just as the reductive phase began. Klevecz hypothesizes that the timing strategy evolved when earth’s atmosphere changed from a reducing to an oxidizing environment. “Single-stranded DNA is very susceptible to oxidative damage,” he says. “So the idea would be to avoid damage to DNA, and perhaps to RNA as well. If it’s not broken, you don’t have to fix it.” He is still searching for a synchronized mammalian cell culture system to test whether oscillations are widely conserved.

Klevecz warns that the design of typical treated-versus-control experiments must involve time series sampling and take phase into account, or else significant differences might be due solely to differences in phase. “The cell is in essence an oscillator,” he says. “Normal rules for cause and effect break down in systems that are oscillatory.”

Reference: Klevecz, R.R., et al. 2004. Proc. Natl. Acad. Sci. USA. 10.1073/pnas.0306490101.
Inflexibility in motion

Cell migration depends on polarized actin polymerization at a cell’s front edge. To get the most out of these actin networks, plasma membrane flexibility must be similarly polarized, according to results from Amit Vasanji, Paul Fox (Cleveland Clinic Foundation, Cleveland, OH), and colleagues.

The group shows that the membrane is stiffest at the front of migrating endothelial cells. This oriented flexibility is fine-tuned through cholesterol distribution. Growth factors that induce migration in vascular cells caused cholesterol to concentrate at the leading edge, and this gradient was needed for migration. In liposomes, addition of a modest amount of cholesterol (thus creating a stiffer membrane) promoted the ability of actin to deform the membrane.

One might expect a flexible membrane to be more easily moved by polymerizing actin, but Fox compares actin in a cholesterol-free cell to a finger pressed into a balloon. “It’s so flexible,” he says, “a filament gets completely surrounded. There’s no room for more monomers to come in. With some stiffness, a filament pushes forward a section of membrane that leaves room for more actin.” The effect may be compounded by the exclusion of bundling and cross-linking proteins. This theory is supported by the authors’ findings that growth factors stabilize the forward actin network. The group next hopes to determine how growth factors orient cholesterol trafficking, possibly through transport proteins such as caveolin-1.

Reference: Vasanji, A., et al. 2004. *Dev. Cell.* 6:29–41.

Winding and unwinding ATP synthase

Like a revolving door, bacterial ATP synthase turns two ways, according to Manuel Diez, Michael Börsch (Universität Stuttgart, Germany), and colleagues. One direction makes ATP, whereas the other breaks it down.

ATP synthase is a two-component nanomotor. One part of the enzyme (F₀) lies within the lipid bilayer and translocates protons across the membrane, whereas the other (F₁) makes or breaks ATP. Recent studies have shown that each portion contains a subunit that turns within the rest of the protein framework, thus giving ATP synthase a reputation as a rotor. F₁ rotation had been best shown during ATP hydrolysis, because the microscopy methods used needed soluble protein, but the enzyme requires a proton gradient across a membrane to make ATP.

To solve this problem, the German group used fluorescence resonance energy transfer to study the protein within liposomes, thus allowing them to create a proton gradient. In ATP synthesis mode, the enzyme adopted three sequential positions—similar to the 120° steps seen during hydrolysis with microscopy methods. However, the direction of rotation was opposite to that of hydrolysis. F₀ is thought to rotate smoothly during proton translocation, so researchers next need to determine how that is translated to discrete steps in F₁. Börsch also wonders how cellular conditions toggle the switch from synthesis to hydrolysis and back again.

Reference: Diez, M., et al. 2004. *Nat. Struct. Biol.* 11:135–141.

Nanofibers have the right stuff

Tiny fibers designed by Gabriel Silva, Catherine Czeisler, John Kessler, Samuel Stupp, and colleagues (Northwestern University, Chicago, IL) provide stem cells the environment they need to make clinically desired cells. Although the researchers produced neurons, the design is amenable to many cell types.

The group has created a peptide nanofiber solution that assembles into three-dimensional networks when it contacts biological fluids. On the face of the resulting scaffold sits a laminin-derived epitope that directs neurite growth. In vitro, neural progenitors cells (NPCs) encapsulated by the scaffold differentiated into neurons. On laminin, in contrast, fewer and smaller neurons formed, and some NPCs formed astrocytes.

Astrocytes are thought to be a major obstacle in recovery from paralysis after spinal cord injury, so the nanofibers may speed healing in ways our own physiology cannot. “The [epitope] density [in the scaffold] is a thousand times higher than you would have if you packed [laminin] into a crystal and the epitopes were exposed on the surface,” says Stupp. “Somehow, this epitope presentation causes cell differentiation to change.”

The fibers also assemble when injected in vivo. Although the experiments are still in progress, rats with spinal cord injuries seem to heal faster when treated with the nanofiber solution. With the right epitope, the nanofibers can be modified to support growth of bone, blood vessels, islet cells for diabetic patients, and other cells.

Reference: Silva, G.A., et al. 2004. *Science.* 10.1126/science.1093783.