Research Roundup

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.