CELL BIOLOGY

Two Major Signaling Pathways Meet at MAP-Kinase

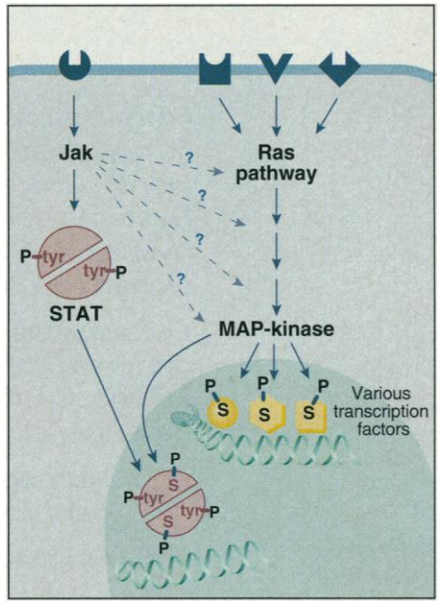
Like explorers mapping trails through uncharted territory, cell biologists labor to map out the cell's internal signaling pathways, the cascades of biochemical reactions that carry signals from hormones, growth factors, and other outside regulators to the cell interior and translate them into physiological responses. Multiple paths co-exist in individual cells, and recent work has made great progress in tracing their meanderings. Each path arrives at its own unique destination, but occasionally the paths are found to cross. Researchers are drawn to those junctions to understand how they influence the traffic on each of the intersecting trails.

In the most recent such example, several reports (including one on p. 1721) provide evidence for a link between two of the cell's major pathways: the Ras pathway, which passes along messages that most often lead to cell growth, and the Jak-STAT pathway, a separate signaling cascade that can produce a variety of cellular responses. The work suggests that the two pathways meet at an enzyme called MAP-kinase, which was previously thought to be part of the Ras pathway only. "What were looked at as separate signaling cascades are now coming together," says Andrew Larner of the Food and Drug Administration Center for Biologics Evaluation and Research in Bethesda, Maryland, senior author on the paper in this issue. If true, that puts MAP kinase in the position to regulate traffic on two of the cell's busiest thoroughfares.

Evidence of a connection between MAP-kinase and the Jak-STAT pathway has been building for about a year. There are more than six different members of the STAT (for signal transducers and activators of transcription) protein family. All are part of a signaling pathway triggered when any one of a group of cell-surface receptors, including those for interferons and other immune-system regulators, are activated. Once switched on, the receptors turn on kinase enzymes called Jaks that attach phosphate groups to the amino acid tyrosine in certain locations on the STAT proteins. This spurs the STATs to head to the nucleus, where they turn on sets of genes that produce the cell's response to the outside signal.

But researchers have had evidence for several years that in some cases, triggering the Jak pathway could also activate MAP-kinase. And they also had reason to suspect that another kinase beside the Jaks was necessary for the STATs to reach their full gene-activating ability.

That general feeling began taking on a more specific form with a report in the 31 March issue of *Science* by Selina Chen-Kiang and her colleagues at the Mount Sinai School of Medicine in New York. They found that a STAT protein called Stat3 gets phosphorylated, not only on tyrosine, but also on the amino acid serine in response to a variety of extracellular signals. That serine phosphorylation, they found, is necessary for Stat3 to bind to the DNA regulatory regions that turn genes on.



Merging traffic. The enzyme MAP-kinase from the Ras pathway may boost STAT activity by phosphorylating it.

That result led Chen-Kiang and her colleagues to focus on MAP-kinase, both because it adds phosphate groups to serine on proteins, and because interleukin-6, an extracellular signal that acts by turning on a Jak, was known to activate MAP-kinase as well. Looking for evidence that MAP-kinase might in turn phosphorylate the STATs, Chen-Kiang scanned the sequences of several STAT genes and found that the proteins they encode have sites that are similar in amino acid sequence to sites on proteins that are phosphorylated by MAP-kinase. That strengthened the notion that MAP-kinase might participate in STAT regulation.

Then, in a paper in the 28 July issue of *Cell*, James Darnell's team at Rockefeller took the story a step further. They showed that, to be fully activated, Stat1, as well as Stat3, must be phosphorylated on serine. They also identified the particular serine that receives the phosphate and showed that it is part of the MAP-kinase recognition sequence mentioned by Chen-Kiang and her colleagues. Moreover, the Darnell group showed serine to be phosphorylated in the test tube by purified MAP-kinase. But their paper stopped short of showing that MAP kinase does the deed in living cells.

That's where the work described by Larner and his colleagues comes in. They show that type-one interferons, which work through the Jak pathway, activate MAP-kinase, and they also found evidence suggesting that, when activated by Jak, MAP-kinase turns up STAT activity. They show that MAP-kinase and STAT associate with each other in activated cells, but their most telling evidence came when they blocked MAP-kinase activity in cells, and that reduced the activation by interferon of a STAT-regulated gene. That suggests phosphorylation by MAP-kinase is necessary for STAT to fully activate the gene.

That result is "wonderfully complementary to the Darnell paper," says Robert Schreiber, who studies signaling pathways at Washington University in St. Louis. By itself, it doesn't prove that MAP-kinase is directly activating STAT, says Schreiber, but when it is taken together with the Darnell paper, he says, "the most logical substrate [for the MAP-kinase] would be the STAT itself."

If so, MAP-kinase may play two different roles in gene transcription. As a member of the Ras pathway, it regulates the activity of several nuclear proteins that turn genes on or off, says MAP-kinase researcher Thomas Sturgill of the Howard Hughes Medical Institute at the University of Virginia School of Medicine. Now, he says, it looks like MAP-kinase, when activated via the Jak-STAT pathway, "has a new way to signal to the nucleus," by augmenting Jak's activation of the STATs, which move from the cytoplasm to the nucleus to regulate genes.

An important unresolved issue concerns where the two signaling pathways intersect. Jak activation might set the whole Ras pathway in motion, says Schreiber, or "you may have activation of MAP-kinase without having activation of other components of the MAP-kinase–Ras pathway." By activating MAP-kinase alone, he suggests, "you may be able to change the whole biologic response," activating STATs, but not the usual targets of the Ras path.

Until more is known about how the pathways intersect, these suggestions are highly speculative. "This paper raises more questions than it answers," says Schreiber. But with a convergence of two such important pathways will undoubtedly come a convergence of the explorers of those pathways, intent on answering those questions.

–Marcia Barinaga