CELLULAR SIGNALING

Missing Link in Insulin's Path To Protein Production

Over the past several decades, biologists have unraveled some of the ways cells communicate with each other. The long-distance "messages" that the cells exchange are hundreds of different proteins, such as hormones and growth factors. The "receivers" are a multitude of specialized receptors on the surface of the target cells. Beyond these fundamentals, however, the picture of cellular communication gets sketchy. There are very few cases in which scientists understand all the intricate biochemical steps that flow from message received to action inside the cell.

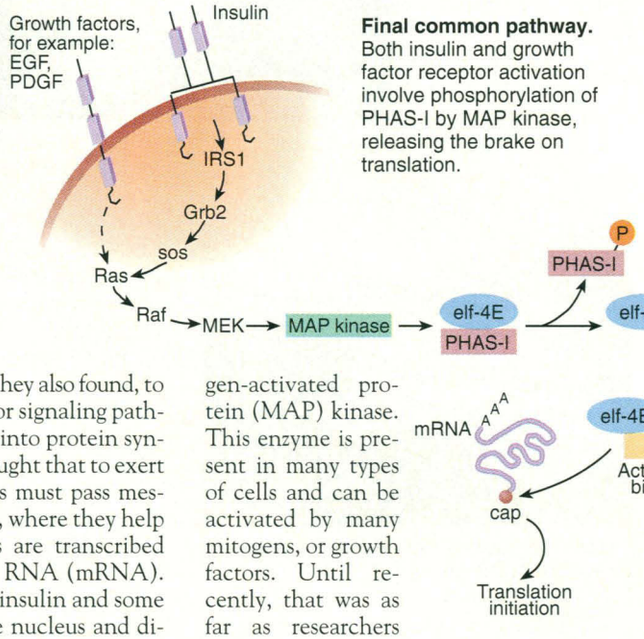
On page 653 of this issue of *Science*, however, a team led by pharmacologist John Lawrence Jr. of Washington University, St. Louis, that includes researchers at the University of Virginia, Charlottesville, and McGill University, Montreal, describes in detail one such chain: The researchers supply a link between the first events inside the cell after the hormone insulin binds to its receptor and the downstream effect of insulin, which is to boost protein synthesis. Insulin plays a key role in regulating the body's accumulation of muscle and fat, and the newly discovered pathway "could be one of the mechanisms by which insulin stimulates [tissue] protein synthesis," says biochemist Philip Cohen of the University of Dundee in the United Kingdom.

The researchers found that this pathway connects with other cell-signaling paths, including those for certain growth factors, which tell cells to divide such as in skin growth. But they also found, to their surprise, that this major signaling pathway is also hooked straight into protein synthesis. It was previously thought that to exert their effects, growth factors must pass messages into the cell's nucleus, where they help to determine which genes are transcribed from DNA into messenger RNA (mRNA). But the new signal used by insulin and some growth factors bypasses the nucleus and directly affects the machinery of translation, the process whereby the information in the mRNA is used to construct proteins.

The novel direct effect on protein synthesis is described in a companion paper, whose authors include several members of the same team, in this week's issue of *Nature*. "It very nicely explains how insulin affects the protein translation machinery," says molecular biologist Nahum Sonenberg of McGill, a co-author on both papers. Biochemist Richard Denton at the University of Bristol Medical School in the United Kingdom adds, "taken together [the two papers] represent a really substantial advance in our understanding of the regulation of translation."

The molecule that provided the context for this advance, insulin, is one of the hormones that control the level of glucose in the blood. Cells in the pancreas, upon detecting a high glucose level, release insulin into the blood, prompting the body to convert glucose into muscle mass and fats. The message insulin carries instructs adipose (fat-storing) cells and skeletal muscle fibers to make more of the enzymes that catalyze synthesis of fats or muscle proteins.

But the pathway by which insulin manages to do all this is far from simple. Once insulin binds to its receptor on the surface of the cells, it triggers an internal reaction path involving at least six intermediate proteins and enzymes; at the end of that chain the signal arrives at an enzyme called mitogen-activated protein (MAP) kinase. This enzyme is present in many types of cells and can be activated by many mitogens, or growth factors. Until recently, that was as far as researchers had gotten in unraveling the route from insulin to protein synthesis, but Lawrence's team has now shown that the next step involves a single protein, also found in many tissues, which they call PHAS-I. When activated by MAP kinase, PHAS-I gains a phosphate group, and it is this reaction that provides the crucial link between insulin and the translation of new proteins, says Lawrence, who is also a co-author on the *Nature* paper. The new protein is able to play its key role because, in its unaltered state, PHAS-I suppresses the start of translation, but if phosphorylated by MAP kinase this action is interrupted, allowing translation to proceed. "There are many steps at which phosphorylation has been implicated [in the cell's response to insulin], and now you've linked it to protein translation," says Lawrence.



Final common pathway. Both insulin and growth factor receptor activation involve phosphorylation of PHAS-I by MAP kinase, releasing the brake on translation.

Serendipity played a part in the discovery of how PHAS-I makes the link in this pathway. Protein kinases play a central role in many intracellular signaling pathways, and so researchers often take them as a starting point when seeking to unravel the signaling reactions. Because the kinases catalyze addition of a phosphate group to another protein or enzyme, researchers often look for proteins that are rapidly phosphorylated, in the hope that they will be part of a cell's response to an extracellular message. Denton and his former graduate student, molecular biologist Graham Belsham (now at the United Kingdom's Institute for Animal Health in Woking), were undertaking just such a search in 1980 when they identified a heat-stable, acid-soluble protein they knew became phosphorylated in insulin-stimulated fat cells. However, at the time they did not have the tools to investigate its molecular biology, and earlier this year they were scooped on sequencing the cDNA that codes for PHAS-I by Lawrence's group, who "picked it to clone precisely because it was a major [insulin-] stimulated and phosphorylated protein." Denton recalls, "We were completely gobsmacked. ... There's no doubt that this is the same protein."

But fate, which had been unkind, promptly turned around and dealt Belsham a second chance. Earlier this year he was doing a sabbatical with Sonenberg on an entirely different project when they realized that Arnim Pause, a doctoral student in Sonenberg's lab, had cloned and sequenced the cDNA for the very same protein—but for different reasons. "It was really something of a coincidence that it happened in the lab in which I was working," says Belsham, who adds, "it was useful because I knew how to handle the protein." Sonenberg was looking at events further downstream in cellular function: His interest centered on one of the proteins, known as initiation factors, that are required to get translation up and running. The factor Sonenberg was studying, eIF-4E, binds to the unique "cap" structure at one end of an mRNA molecule and helps attach the mRNA correctly to the ribosome, the cell's protein-synthesizing machine. This "initiation" is generally the rate-limiting step in translation, and anything that interferes

ILLUSTRATION: C. FABER SMITH

with it could be an important regulator of protein synthesis.

One way to interfere with the process would be to bind eIF-4E so that it is no longer free to join up with the mRNA. Sonenberg's group was looking for such 4E-binding proteins, and they had already found two, for which they cloned the genes. When they realized that one of these 4E-binding proteins was PHAS-I, "it was a pleasant surprise," says Sonenberg. "We knew it represses translation, but we couldn't link it to signal transduction."

Along with Lawrence's biochemical experiments, the link to eIF-4E activity established that insulin-activated MAP kinase phosphorylates PHAS-I at a particular site, which causes it to dissociate from eIF-4E, so that the factor can initiate translation. The PHAS-I work "sure is interesting," says Simon Morley of the University of Sussex, U.K., who also studies the regulation of initiation.

The idea that signals from growth factors and hormones can be relayed via MAP kinase directly to the protein translation machinery, rather than traveling via the events in the nucleus, is just beginning to gain acceptance with researchers. But for some, the new results vindicate a long-held belief. Cohen comments, "I'm not surprised—we've been pushing the idea for a long time that MAP kinase has actions [outside the cell's nucleus]." Biochemist George Thomas of the Friedrich Miescher Institute in Basel, Switzerland, was one of the first scientists to realize the potential importance of such signaling pathways 20 years ago. He says he has "argued from the beginning" that mitogens would be involved in the regulation of translation. "It's great to have additional evidence of direct effects of mitogens on ... the translational machinery," he adds.

Although the two papers are based on the single case of fat cells stimulated by insulin, the presence of PHAS-I in other cell types strongly suggests that it will play a key role in other cells and possibly in other pathways. Lawrence points out, "We don't know that MAP kinase is the only regulator [of PHAS-I]—such an important inhibitor is probably the target of other second messenger systems." Sonenberg is therefore busy examining the other phosphorylation sites on PHAS-I. And it's likely that PHAS-I, and the related protein coded by Sonenberg's second cDNA clone, are two members of a family of similar proteins. As Denton says, "This is an incredibly exciting time in protein translation." And, because of the link made in the Lawrence paper, that excitement is not limited to those interested in insulin—it's also spilling over into the fast-growing field of growth factors.

–Claire O'Brien

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NANOENGINEERING

AFM Fabricates a Tiny Transistor

DAVOS, SWITZERLAND—Nanoengineering—the creation of structures smaller than 100 nanometers, or 0.1 micrometer—holds particular allure for the semiconductor industry, where smaller is always better. Until now, however, the promise has remained largely theoretical, because conventional chip-making techniques have trouble with anything smaller than 0.2 micrometer, and newer methods have been confined to the laboratory. But at a meeting here last month, Stanford University applied physicist Calvin Quate reported creating working transistors using a tool created for nanoscale observation, the atomic force microscope (AFM).

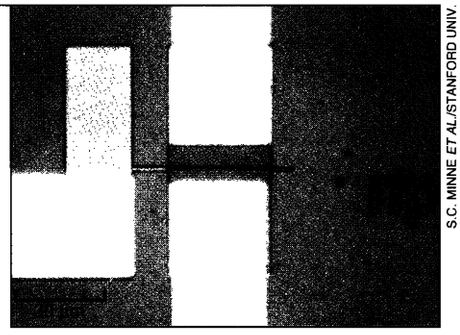
Many delegates believed this is the first example of a working device made with an AFM and, while just on the border of the nanoscale range, the fact that AFMs have the potential to make devices much smaller is getting people excited. "This is really new," says Jürgen Mohr of the Institute of Microstructure Technology at the Karlsruhe Nuclear Research Center in Germany. "We now see that it's possible to use the AFM for fabrication, not just observation."

Quate's report was a last-minute addition to the opening session of the Micro and Nano-Engineering '94 conference. Traditionally geared towards conventional chip-making techniques, the meeting broadened its scope for the first time this year to include local probe techniques such as the AFM. The result? A 70% hike in attendance and a jump in submitted papers from 100 to 172—nano-fever was in the air.

The AFM and its sister instrument, the scanning tunneling microscope (STM), were originally designed for imaging surfaces with atomic-level detail. Both microscopes rely on an extremely fine tip; in the AFM the tip, mounted on the end of a cantilever, rests on a surface. As the tip is moved horizontally, it traces the irregularities of the surface, and a sensor measures the up-and-down movements of the cantilever to generate an image. But in the past several years researchers have also been using them to modify surfaces: punching holes, carving grooves, depositing material, and causing chemical reactions.

Quate and his team used an AFM to draw an ultrafine line that forms the heart of a transistor. They started with a sapphire substrate and coated it with a layer of amorphous silicon and an overlayer of hydrogen. They then moved an AFM tip over the surface, sending a mild electric current from tip to surface. The current cleared away the protective hydrogen layer beneath the tip while oxidizing the surface of the silicon by activating water molecules in the surrounding atmosphere. The resulting silicon dioxide line protected the silicon beneath it while the rest of the silicon was chemically removed from the substrate. The team succeeded in drawing lines less than 0.1 micrometer wide.

Using this AFM line-drawing technique, the team made a number of devices called metal-oxide-silicon field effect transistors (MOSFETs). In such devices, a current passing through a piece of semiconductor is controlled by an electrode above it called the gate. The shorter the gate the faster the transistor. The Stanford team made the bulk of each transistor using conventional lithography, where successive layers are built up and then parts etched away using a series of masks. The AFM was used only for making the gate. (The line width corresponds to the gate length.) Twelve of the transistors they made, with gate lengths ranging from 0.7 to 0.2 micrometer, behaved like conventionally made MOSFETs in performance tests.



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Starting line. A probe microscope was used to make an electrode 0.1 μm wide on this transistor.

Older techniques can create transistor gates just as small as those made by Quate's team, but while conventional lithography is pushing up against the physical limits of miniaturization, scanning probe techniques are still in their infancy and have the potential to go much smaller. This prospect is now drawing interest from industry, but the process is at the moment far too slow for production: Local probes can draw a line on only one device at a time, while conventional lithography imprints millions of transistors in one process. To speed things up, Quate envisions an array of up to 10,000 AFM tips working simultaneously. His team has been experimenting with a prototype five-tip array. Another advantage is cost: Local probes are cheaper than conventional equipment.

–Elizabeth Gardner

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