The *fos* Gene as "Master Switch"

fos gene activity may be central to the cell's ability to convert short-term stimulation to long-term responses—such as growth and memory formation

Few genes would appear to be more central to the cell's regulatory activities than the *fos* gene. In the 5 years since *fos* was isolated, a great deal of evidence has pointed to the conclusion that it serves as a sort of master switch for turning on other genes in response to a wide range of stimuli, including growth factors and agents that trigger nerve cell activity. In effect, *fos* appears to be acting as a sensor that detects incoming signals at the cell membrane and then converts them to long-lasting responses that require gene activity, such as cell division and perhaps even memory formation.

Within the past few months researchers have begun to get a handle on how the *fos* gene accomplishes all this. They have for the first time identified a gene that may be regulated by *fos* and are also beginning to understand how the *fos* gene is itself activated by so many different stimuli.

These findings may help researchers trace how signals are transmitted to the nucleus from the outer cell membrane where agents such as growth factors and neurotransmitters must bind to bring about their effects. This part of signal transmission within cells is still largely a "black box"—it works, but no one knows how. But more than that, a better understanding of *fos* gene activity may provide clues to the larger mysteries of growth control and memory formation.

The *fos* gene first came to light as the oncogene of two closely related mouse viruses that cause osteogenic sarcoma, a type of bone cancer. (The name *fos* refers to its origins in the FBJ and FBR *o*steogenic *s*arcoma viruses.) The *fos* oncogene, like other oncogenes, causes the cancerous transformation of cells and, also like the others, is derived from a normal cellular gene.

Early studies of the cellular *fos* gene focused on its possible role as a regulator of the cell cycle. For example, Michael Greenberg and Edward Ziff of the New York University Medical Center found that expression of the gene is rapidly activated by agents that stimulate cell division, such as platelet-derived growth factor and a tumor-promoting phorbol ester, one of a group of chemicals that cooperates with true carcinogens to cause cancer development. Other growth-stimulatory agents, including epidermal and nerve growth factors, have a similar effect on *fos*, as does blood serum, presumably because it contains growth factors.

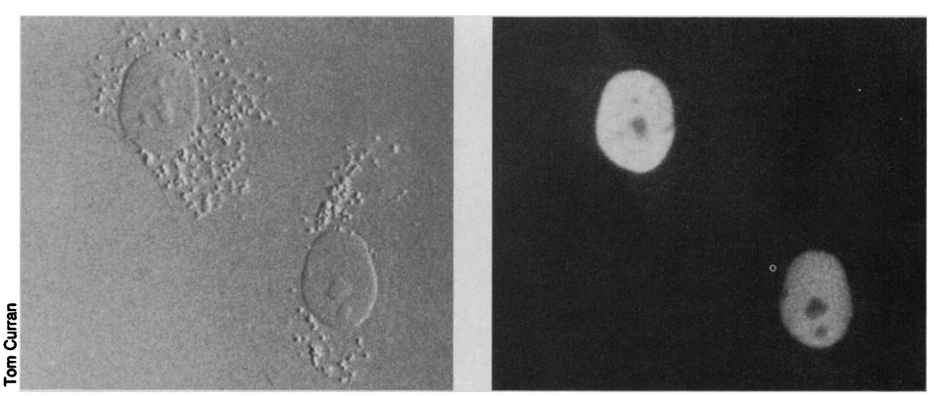
The increased *fos* activity can be detected within 5 minutes of growth factor treatment. "It is a really interesting gene," notes Michael Gilman of Cold Spring Harbor Laboratory. "It makes the earliest known nuclear response to numerous growth factors." These findings led to suggestions that *fos* activity elicits the passage of cells from the resting state into the actively dividing phases of the cell cycle.

Researchers soon learned, however, that the *fos* gene has a much broader scope of action than its postulated role in the cell cycle. The expression of the gene is also stimulated by factors that influence cell differentiation and by several agents that trigger nerve cell activity. Work with cultured cells by Tom Curran and James Morgan of the Roche Institute of Molecular Biology and also by Greenberg, who is now at Harvard Medical School, Ziff, and Lloyd Greene of the New York University Medical Center indicates that the *fos* induction depends on the ability of the neuroactive agents to open channels that allow calcium ions to move into the nerve cells.

Such calcium ion entry is a normal component of neuronal responses to stimulation, and Curran, Morgan, and their colleagues went on to ask whether nerve cell stimulation in the living animal can also result in increased *fos* expression. The answer was yes. The researchers found that a sharp increase in *fos* gene activity occurs in the brains of mice treated with Metrazole, a drug that causes seizures similar to those of human epilepsy. Synthesis of the *fos* protein occurs primarily in the nerve tracts stimulated by Metrazole. The results suggest that *fos* protein synthesis can be used to track the patterns of nerve cell interactions in the brain after stimulation.

The synthesis of the *fos* protein occurs much more slowly than the seizures, however. It is first detected about 15 minutes after Metrazole treatment and persists for up to 3 hours, whereas the seizures begin within a minute or two of drug administration and are over within 30 minutes. "*fos* expression is not involved in the generation of the seizures," Curran explains, "but is a consequence of the seizures."

Curran and Morgan propose that the protein encoded by the *fos* gene mediates the long-term adaptation of nerve cells to Metrazole stimulation, presumably by altering gene expression in the cells. Curran and others have shown that the *fos* protein is located almost exclusively in the nucleus and



Tom Curran

Location of the *fos* protein. *In the micrograph on the right, cells producing a high level of the* fos *protein have been stained with fluorescent antibodies specific for the protein. The fluorescence—and the protein—are concentrated in the cell nuclei. For comparison is a micrograph of the same two cells that was produced under Nomarski optics. In this view, the granulated cytoplasm can be seen.*

is bound to DNA; both findings are consistent with a possible role for the protein as a gene regulator.

One possible target of *fos* regulation is the gene encoding the receptor for the inhibitory neurotransmitter gamma aminobutyric acid (GABA). The neurons on which Metrazole acts produce increased numbers of the GABA receptor, perhaps as an adaptation to counteract the drug's stimulatory effects.

Curran, Morgan, and their colleagues are now looking into the possibility that the *fos* protein alters the expression of the GABA receptor gene. Whether or not that particular gene proves to be regulated by *fos*, the findings about the *fos* gene raise the possibility that its expression may contribute to the changes in gene expression that are widely assumed to be necessary for memory formation.

Meanwhile, Bruce Spiegelman's group at Harvard's Dana-Farber Cancer Institute has made what may be the first identification of a *fos*-controlled gene. The Dana-Farber workers came on the gene more or less accidentally while studying the differentiation of fat cells (adipocytes). "It was basically the result of a hunch and a bit of good luck," Spiegelman says.

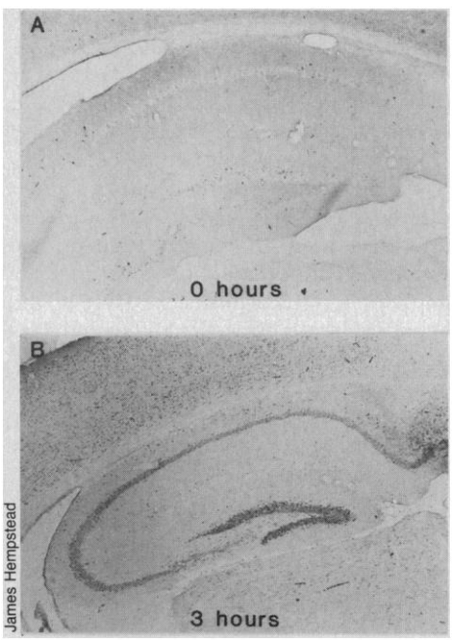
He and his colleagues had identified a set of genes that encode characteristic fat cell proteins and are activated when fat cells differentiate. One of those genes, called the "adipocyte P2" (aP2) gene, proved to contain a regulatory site located about 125 base pairs before the gene start. The regulatory site binds proteins that undergo an as yet undefined change during fat cell maturation. The current supposition is that the change in the binding proteins allows the activation of the aP2 gene.

At this point the hunch came into play because the investigators decided to see whether the *fos* protein is one of those binding to the control sequence. Antibody studies subsequently showed that the binding complex contains the *fos* protein itself, or at least a very close relative. "You cannot distinguish these possibilities using immunological methods," Spiegelman says, "but the connection had not been made previously between a *fos*-like protein and binding to a specific gene."

The control of the *fos* gene is also coming in for intense scrutiny these days, especially with regard to the stimulation of its expression by such a wide variety of agents. As Inder Verma of the Salk Institute asks, "How can this gene be inducible by just about everything we can think of?"

A common way of approaching the question of gene control is to delete specific DNA segments in and around a gene, especially in the several hundred bases just preceding the start site, to see how the deletions affect the gene's responses to various agents. Using this method, Richard Treisman of the Laboratory of Molecular Biology at the Medical Research Centre in Cambridge, England, defined a segment of DNA that is necessary for the cellular *fos* gene to respond to serum with increased expression. The segment, which he calls the "serum response element" (SRE), contains 22 base pairs and is located about 300 base pairs before the start of the human *fos* gene.

Treisman and at least three additional groups of investigators, one including Gilman, who was then working with Robert Weinberg at the Massachusetts Institute of Technology, the second from Ziff's laboratory, and the third including Ron Prywes and Robert Roeder of the Rockefeller University, have now isolated a protein, apparently the same protein, that binds specifically to the SRE. Binding of the protein appears to be necessary for the *fos* gene to respond to serum stimulation, although Treisman notes that proving definitively that the protein activates the gene in living cells is difficult. Nevertheless, all the evidence points in that direction. Treisman, for example, has made SRE variants with altered abilities to bind the protein. "The ability to stimulate transcription correlates with the avidity of binding," he says.



***fos* Expression in the mouse brain after Metrazole treatment.** *The dark stain indicates the presence of the* fos *protein in these brain sections. Before Metrazole treatment (A) the brain shows little sign of making the protein. Three hours after administration of the drug (B), increased production of the protein spreads through the brain along the nerve tracts affected by Metrazole stimulation. It becomes evident first in the dendate gyrus (V-shaped structure) and later in the surrounding hippocampus and the cerebral cortex. The time course of increased* fos *gene expression is much slower than that of the seizures evoked by Metrazole treatment, indicating that the gene expression is an effect, rather than a cause, of the seizures.* [Science *237, 192 (1987)*]

Moreover, expression of the gene for actin, a prominent protein of the cell skeleton, is stimulated by most of the same agents that increase *fos* gene expression. Tim Mohun of the University of Cambridge (England) has found that an actin gene from the toad *Xenopus laevis* contains a 20–base pair sequence that closely resembles the SRE of *fos*. It is the only similarity between the two gene sequences. According to Mohun and Treisman, the *Xenopus* sequence also behaves like an SRE, even in mammalian cells. These results indicate that both the SRE sequence and the binding protein have been highly conserved during evolution.

The SRE is apparently not the only sequence involved in *fos* gene regulation, however. "It wasn't clear whether the *fos* gene stimulatory agents all act in the same way," Prywes says. "Now we think there are multiple regulatory elements in the gene."

Even different growth factors may act through distinct sites. Although epidermal growth factor and phorbol esters work through the SRE, Brent Cochran and his colleagues at the Massachusetts Institute of Technology have evidence suggesting that another regulatory site, located about 25 base pairs before the SRE, may be involved in *fos* induction by platelet-derived growth factor.

In addition, some *fos*-stimulating agents use cyclic AMP (cyclic adenosine monophosphate) as a messenger for transmitting their signals to the cell interior. Gilman and Tobe Fisch of the Roeder group have both found that mutations of the SRE do not affect *fos* activation by cyclic AMP. This result indicates that induction of the gene by cyclic AMP requires a different, as yet unidentified, regulatory sequence. Calcium ions, which mediate *fos* stimulation during nerve cell activation, may use still another site, according to Fisch.

Researchers have also identified regulatory sites within the first 100 base pairs of the start of *fos* that appear to be needed for expression of the gene, although not for its ability to respond to inducing agents. "The *fos* gene has a complex promoter," Verma explains. "It has a number of motifs necessary for induction and a number of motifs for basal expression."

Finally, *fos* may be subject to negative regulation as well as positive. Verma and his

Salk colleague Paolo Sassone-Corsi have evidence indicating that cells' contain factors that repress transcription of the gene in addition to factors, such as the SRE-binding protein, that increase transcription.

Previous work in Verma's laboratory showed that conversion of the normal cellular *fos* gene to a transforming gene requires the loss of a noncoding sequence located after the termination site. This apparently increases the stability of the messenger RNA transcribed from the gene, thereby permitting more prolonged production of the *fos* protein.

Cell biologists currently know very little about how signals are transmitted from the cell membrane to the genes in the nucleus. Dissecting *fos* gene regulation may help in this regard by enabling researchers to track backward from the nucleus.

Identifying the *fos* regulatory sites and the proteins that bind there is one step toward accomplishing this goal. Indications are, for example, that the SRE-binding protein is already in place when appropriate stimuli are received, and that the protein undergoes some modification that brings about an increase in gene transcription, which is the first step in protein synthesis.

According to the current educated guess, the modification is the addition (or removal) of phosphate groups. The next step in tracing signal transmission back to the cell membrane would therefore be identifying the enzyme that adds the phosphates or accomplishes whatever alteration of the SRE-binding proteins does bring about increased *fos* transcription.

Clearly, a great deal still remains to be learned about *fos*. Control of the gene is turning out to be very complicated. Nevertheless, researchers now have toeholds that may enable them to learn both how *fos* is regulated and what the cellular consequences of its activity are. The central role of the gene in cells' responses to stimuli guarantee it the attention of the research community. ■ JEAN L. MARX

ADDITIONAL READING

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Searching Land and Sea for the Dinosaur Killer

The impact that triggered a mass extinction and possibly the death of the dinosaurs left clues to its location

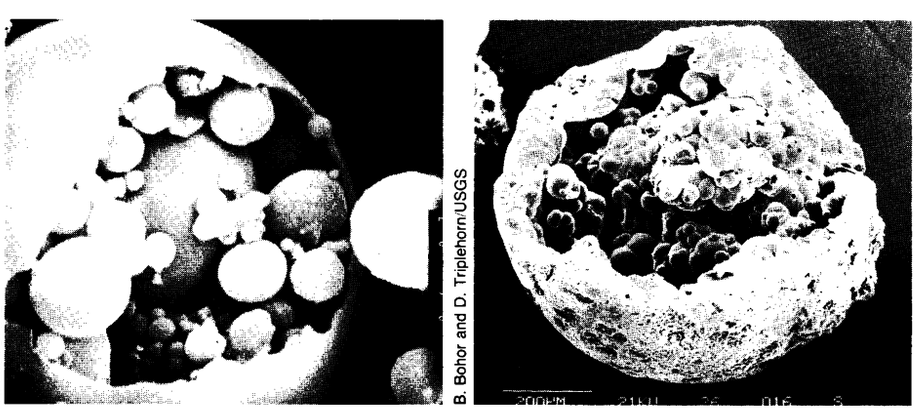
SOMETHING 100 kilometers or more in diameter would not seem that difficult to find, but the scar left by the asteroid or comet that hit Earth 65 million years ago and triggered a mass extinction is proving elusive. The impact did leave subtle clues as to its location that are being extracted from the dust and debris scattered around the globe. So far, opinion tends to favor an impact that threw up both continental and oceanic debris, suggesting that the impactor hit ocean crust loaded with sediments washed from a nearby continent. The continent may have been North America. Alternatively, an impact might have nearly dug through a continent into ocean-like rock, or two or more simultaneous impacts could have created the mixed debris.

An early potential clue to the whereabouts of the crater involved the layer of iridium that first led researchers to conclude that there had been an impact. Rare on the surface of Earth, the iridium carried by the impactor formed a distinctive layer at the boundary between the Cretaceous and Tertiary periods (called the K-T boundary). But a map of the amount of iridium in the boundary layer, rearranged to account for 65 million years of continental drift, showed no pattern that might point to the crater.

If chemistry could not pin down the crater, geochemists reasoned, perhaps isotopes could at least narrow the search. Donald DePaolo and Frank Kyte of the University of California at Los Angeles and their colleagues determined the strontium and neodymium isotopic compositions of the K-T boundary layer at Caravaca, Spain. They concluded that debris from at least 3 kilometers down in the ocean crust dominated the boundary material.

Narrowing the search to the ocean would certainly help, but there was a potential downside. Since 65 million years ago, ocean crust equal to 20% of the surface area of Earth has sunk into the mantle at deep-sea trenches. That subduction has wiped out the crust of the eastern, northern, and western Pacific and much of the Indian Ocean that existed then. If the impact hit any of these parts of the ocean, the crater is gone forever.

In 1984 the discovery in the boundary layer of quartz grains damaged by the shock of an impact seemed to point to a continental, not an oceanic, impact. Bruce Bohor and his colleagues at the U.S. Geological Survey (USGS) in Denver first found K-T shocked quartz in Montana and have now found it in the North Pacific, Europe, and New Zealand (*Science*, 8 May 1987, p. 666). Quartz is abundant in continental rock but scarce in ocean crust. In addition, the largest grains



R. Lauf/ORNL

B. Bohor and D. Triplehorn/USGS

The old and the new ash? *The fly ash on the left came from a modern coal-burning power plant. The object on the right is 65 million years old and was found in association with the debris of the large impact that caused a mass extinction. The spherules may have formed as hot mantle rock exposed by the impact exploded into fine dust. The diameters of the enclosing spherules are about 35 micrometers (left) and 800 micrometers (right).*