jun Is Bustin' Out All Over

The product of the jun *oncogene, and other oncogene products as well, act in the signaling pathways that turn on genes when cells are stimulated*

AT THE END OF OCTOBER, molecular biologists gathered in Chatham, Massachusetts, for a symposium devoted to "Gene Regulation and Oncogenes."* According to the symposium chairman, Phillip Sharp of the Massachusetts Institute of Technology, the impetus for the meeting was the recognition that research on the cancer-causing oncogenes is effectively merging with research on the control of gene expression. This is not only providing a better understanding of how oncogenes can make cells become malignant but is also yielding new insights into how cells function normally.

For years, cell biologists had wondered how the incoming signals conveyed by growth factors or hormones are transmitted from the cell membrane to the nucleus where the signals ultimately bring about changes in gene expression. As Sharp points out, "Regulating gene expression is how a cell goes about its business." Researchers are now finding with great regularity that the signaling pathways that culminate in altered gene activity are well paved with oncogene products.

Some of them act very early in the pathways. They are themselves growth factors or receptors for growth factors. However, the Chatham meeting focused mainly on the late-acting oncogenes and in particular on one called *jun*, which featured in nearly a third of the presentations.

The results of several converging lines of evidence now point to the conclusion that the protein encoded by the *jun* gene acts directly to activate gene transcription in response to cell stimulation. Moreover, the product of another oncogene, namely *fos*, which had also been suspected of being involved in regulating gene expression, apparently cooperates with the *jun* product in fostering gene transcription.

The stimuli to which these oncogenes respond include several growth factors but other incoming stimuli, such as nerve impulses, may also work through Jun and Fos (as the protein products of the *jun* and *fos* genes are designated). By activating gene expression the oncogene products may convert short-term signals into long-term responses, such as cell growth and memory formation.

jun is a relative newcomer to the oncogene scene. Peter Vogt and his colleagues at the University of Southern California School of Medicine in Los Angeles identified it about 2 years ago as the oncogene of avian sarcoma virus 17, which causes tumors called fibrosarcomas in chickens. (The name *jun* comes from the Japanese *ju-nana*, meaning the number 17.) Normal avian and mammalian cells also carry a *jun* gene. The virus apparently acquired the gene during the course of a chicken-cell infection.

Many oncogenes were discovered because they were picked up by viruses that acquired the ability to cause cancers as a result. The name *fos*, for example, reflects the identification of this oncogene in the *F*BJ and *F*BR *o*steogenic *s*arcoma viruses. Under normal circumstances the cellular oncogenes do not cause cancer, but researchers usually find that the nucleotide sequences of the viral versions differ somewhat from the cellular gene sequences. These changes can cause the gene products to malfunction and make cells malignant. A malfunctioning transcription factor, for example, might result in the expression of the wrong genes or perhaps the right genes at the wrong time or in excessive amounts, with the result that normal control of cell division is lost.

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In the case of *jun*, Vogt reported at the meeting that the protein encoded by viral gene, is missing a nucleotide sequence encoding 27 amino acids that is present in the cellular protein. The viral *jun* gene also has a few mutations that will result in amino acid substitutions. Vogt and his colleagues have not yet determined, however, whether any of these alterations contribute to the viral gene's ability to induce tumors. The researcher notes that the viral *jun* protein is always highly overexpressed in infected cells. Excess production of an oncogene product can also lead to cancer development.

The first indication that the normal role of Jun might be stimulation of gene transcription came about a year ago. Vogt and his colleagues detected a structural similarity between Jun and another protein called GCN4 that induces the activity of a large set of genes needed for amino acid synthesis in yeast. Kevin Struhl and his colleagues at Harvard Medical School had shown that when GCN4 activates the yeast genes it binds to a specific site on those genes. The Harvard workers localized the DNA-binding domain of GCN4 to the 60 amino acids on the carboxyl end of the protein.

Jun has a similar sequence on its carboxyl terminal. This result suggested that the protein might bind to DNA. Subsequent work has confirmed that hypothesis. Moreover, Struhl has shown that Jun can replace GCN4 in stimulating gene expression in yeast. "There's not just structural homology in the DNA-binding domain," Vogt remarks, "but there is functional homology between GCN4 and Jun." Among other things, this finding suggests that the gene control machinery is very similar in species as evolutionarily diverse as yeast and birds and mammals.

Another clue to the function of Jun came with the realization that the DNA binding site recognized by GCN4 is very similar to nucleotide binding site for AP-1, a human transcription factor that was identified in Robert Tjian's laboratory at the Howard Hughes Medical Institute at the University of California, Berkeley. A collaborative effort by the Vogt and Tjian groups then provided evidence that AP-1 might actually be the product of the cellular *jun* gene. Jun and AP-1 bind to the same DNA site, for example, and both proteins are recognized by the same antibodies. Moreover, some peptides derived from AP-1 are identical in amino acid sequence to Jun segments.

Meanwhile, Tom Curran and his colleagues at the Roche Institute for Molecular Biology in Nutley, New Jersey, in collaboration with Robert Franza of Cold Spring

*The symposium, which was held from 23 to 27 October, was sponsored by the American Association for Cancer Research.

Harbor Laboratory on Long Island, had been studying the *fos* gene. A great deal of evidence from their laboratories and elsewhere had suggested that Fos alters gene expression in response to cell stimulation.

In the cell, Fos is associated with several other proteins. A major component of these complexes is a protein that had been given the designation "p39" because it has a molecular weight of 39,000. The Curran, Franza, Tjian, and Vogt groups subsequently demonstrated that the p39 protein is in fact Jun. Indications are that Jun and Fos interact in a complex of proteins that regulates gene transcription. "Fos and Jun together give more binding [to DNA] than either alone. This suggests cooperativity," Curran says.

The two proteins may be held together in the complex by a "leucine zipper," a structure originally described by William Landschulz, Peter Johnson, and Steven McKnight from the Carnegie Institution of Washington's Department of Embryology in Baltimore. These researchers noted that several proven or suspected gene regulatory proteins, including Fos and Jun, carry a common structural feature. This is a potential α-helix that has residues of the amino acid leucine projecting out from the same side of the helix at regular intervals.

The Carnegie workers suggested that the leucine side chains from one protein molecule could interdigitate with those of another to hold the two proteins together. According to McKnight, the purpose of the leucine zipper is to somehow juxtapose the DNA-binding domains of two proteins to enable them to attach to specific DNA sites. If two different proteins could be zipped together—and the current work on Jun and Fos indicates that they can—then the repertoire of possible DNA binding sites could be very large. This would help to create the high degree of specificity needed for regulating a large number of genes under different environmental conditions.

There could be the potential for a large repertoire of gene transcription complexes including *fos* and *jun* products. Recent work shows both oncogenes to be members of multi-gene families. With many proteins, many different associations may be possible. "That is sort of what you would want," Curran points out. "There are an awful lot of AP-1 sites around."

Daniel Nathans and his colleagues at Johns Hopkins University School of Medicine in Baltimore have identified two new *jun* relatives, which they are calling *jun-B* and *jun-D*. The two genes were discovered in the course of studies aimed at determining the biochemical consequences of cell stimulation by growth factors. "Our approach, and that of others as well," Nathans says, "is to work on the assumption that the binding of growth factors to their receptors starts a genetic program."

The Nathans group began by looking for genes that were activated within a few minutes of growth factor stimulation. They identified ten such genes, in addition to *myc* and *fos*, which had already been found by other investigators to be turned on shortly after cells are exposed to growth factors. The early-acting gene group included the two new *jun* genes plus the original cellular *jun*.

Moshe Yaniv of the Pasteur Institute in Paris has also identified the *jun-D* gene. He has been calling it *jun-S*, but comparison of the sequence with *jun-D* shows that they are the same, Nathans says.

How many *jun* genes there might be is still not known. According to Nathans, however, "It's conceivable that there are many more yet to be discovered within this general family."

The same may be true for the *fos* gene family. For example, Curran's group has recently cloned a gene that they are calling *fra-1* (for Fos-related antigen 1). The sequence of the protein encoded by this gene is similar to that of Fos, especially across the leucine zipper and DNA-binding domains. Curran's work points to the existence of more Fos-related proteins, although these genes have not yet been cloned.

The work on Jun and Fos provides ample evidence that oncogenes products can participate in protein complexes that directly regulate gene transcription, although the complexes may well contain additional proteins that may or may not be of oncogene origin. And, as already mentioned, several oncogene products have been found to act in and around the cell membrane at the beginning of signal transduction pathways.

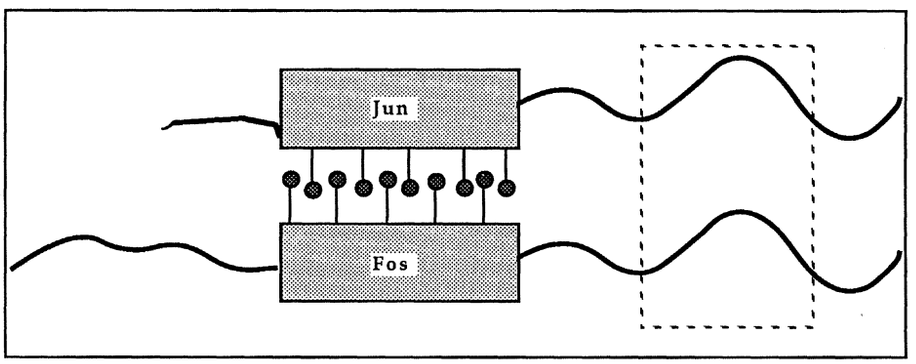
The least understood aspect of signal transmission is the route from the membrane to the nucleus. At the Chatham meeting, however, Tom Roberts of Harvard's Dana-Farber Cancer Institute in Boston reported results suggesting that the product of the *raf* oncogene may carry growth signals through the cytoplasm to the nucleus.

The Raf protein is a type of enzyme known as a serine-threonine kinase, meaning that it attaches phosphate groups to proteins on residues of the amino acids serine and threonine. Agents that stimulate cell division, such as platelet-derived growth factor (the product of the *sis* oncogene) turn on the Raf kinase. "Our data are consistent with the notion that anything that is mitogenic is activating Raf," Roberts says.

This activation may be the result of the Raf protein moving to the cell membrane and becoming phosphorylated by the PDGF receptor, which is a kinase that attaches phosphates to tyrosine residues. The receptor kinase is stimulated when it binds the growth factor.

After Raf is activated, it may move to the nucleus and in turn phosphorylate, and thereby activate, components of transcription factor complexes, such as Jun, that regulate gene expression. Alternatively, it may phosphorylate transcription factors that turn on *jun* or *fos* expression. Roberts cautions that the evidence for much of this scheme is still circumstantial, however. "We have to pin these things down, but, fortunately, they are testable," he remarks.

Researchers would also like to pin down the identities of the genes that Fos-Jun containing complexes activate. These genes are presumably involved in controlling cell growth and differentiation. Oncogenes, once maligned as laboratory artifacts of little interest, are now proving to be reliable guides to some of the cell's most fundamental activities. ■ JEAN L. MARX



Holly Bishop

Zipping proteins together. *The diagram illustrates how the Jun and Fos proteins may be held together by a leucine zipper. The leucine side chains (indicated by the sticks-and-balls) on Fos interdigitate with those on Jun to bring the two proteins together and form a specific DNA binding site (indicated by the dashed lines). Theoretically any two proteins with the leucine motif may associate, thereby generating a large number of DNA-binding domains with different specificities.* [*Adapted from a drawing by Tom Curran*]