Viruses Yield Clues to Gene Regulation

Studies of viruses may shed light both on normal cellular gene control and on cancerous transformation

The cells of higher organisms have a highly refined ability to discriminate among genes, turning on only those appropriate for their stage of development and mission in life. Within the past few years researchers have finally begun to get a handle on how this discrimination is achieved. One of the emerging themes is *trans*-regulation, in which specific genes are recognized and turned on by factors, presumably proteins, encoded by other genes.

Identification of those regulatory factors and the genes in which they are encoded would go a long way toward solving the mysteries of gene regulation during development and in the everyday life of the cell. But in an entity as complex as the living cell this is a formidable task, and some investigators have turned to simpler surrogates, viruses such as SV40, adenovirus, and herpesviruses, which have just handfuls of genes, not thousands, that must be kept under control.

The viral genes are expressed in infected cells in an orderly, stepwise fashion that resembles the hierarchical pattern of gene activation that is thought to occur, although on a much larger scale, during the development of specialized cells. In particular, the viruses produce early on regulatory proteins that activate later viral genes and may serve as models for cellular *trans*-acting factors. "The viruses are so valuable because they are microcosms of what is happening in the cell," says George Khoury of the National Cancer Institute (NCI).

The viral research may also shed light on the origins of cancer. Adenovirus, SV40, and some herpesviruses, all of which have DNA as their genetic material, can cause the malignant transformation of cells, at least in culture. Transformation by SV40 and adenovirus requires the same early proteins that activate viral genes, and there are indications that the proteins may also turn on cellular genes. These findings have raised suspicions that the viral regulatory proteins transform by activating cellular genes, perhaps those that normally regulate cell division or differentiation. Moreover, this type of transformation mechanism may not be limited to DNA-transforming viruses. Transformation by human T cell leukemia viruses I and II, which have RNA genomes and cause leukemias or lymphomas in humans, has been linked to their production of a *trans*-acting protein (*Science*, 27 July, p. 398).

When viruses such as SV40, adenovirus, or herpesviruses infect cells, the first thing that happens is the switching on of the early wave of viral genes, the ones that make, among other things, the proteins needed for the subsequent expression of later genes in the viral life cycle. These regulatory proteins are the T (for tumor) antigen of SV40, the E1a (early 1a) protein of adenovirus, and the immediate early protein of herpesviruses. Activation of the immediate early gene requires a component brought in by the virus, according to Bernard Roizman and his colleagues at the University of Chicago. But the T antigen and E1a genes are switched on apparently without the intercession of viral products.

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The responses appear to depend on specific regulatory sequences carried by the early genes. For example, SV40 has an "enhancer" element, a DNA segment that strongly stimulates gene transcription and is required for early gene expression. "The SV40 early genes probably respond through the activity of their enhancer to signals ubiquitous or present in most cells," notes Khoury.

The E1a and immediate early genes also carry enhancer-like sequences. For example, the Roizman group made a hybrid herpesvirus gene consisting of the protein-coding region of a gene that would normally be turned on only in the second phase of gene expression plus a DNA segment taken from just upstream of the beginning of the immediate early gene. This hybrid was now controlled like the immediate early gene itself. Investigators, including Joseph Nevins of Rockefeller University, Pierre Chambon of the University of Strasbourg, and Thomas Shenk of Princeton University, have found that the E1a gene region carries sequences that serve as enhancers.

Cellular genes are also being found to have enhancers that permit the genes to be expressed in a tissue-specific manner. The existence of these enhancers constitutes part of the evidence for cellular *trans*-regulation, because their presence implies that the appropriate cells produce proteins or other factors that recognize the enhancers and turn on the genes they regulate.

In any event, once the T antigen and E1a and immediate early proteins are made, they can initiate the next phase of gene expression in their respective viruses. Although there are various ways in which gene expression might be altered, evidence from several laboratories indicates that the viral regulatory proteins act by stimulating the first step, the transcription of the genes into messenger RNA. Moreover, the transcription-stimulating effects of the proteins are not restricted to genes of their corresponding viruses. Investigators have found that, under appropriate conditions, the proteins can also activate the transcription of structural genes for proteins, such as β-globin and insulin, which are normally made only in specific cells.

In these experiments, the structural genes are transferred into cultured cells that also contain one or another of the viral regulatory proteins. Ordinarily, transferred β-globin genes are not expressed efficiently unless they have been attached to a viral enhancer. However, Tom Maniatis and his colleagues at Harvard University have found that the E1a and immediate early proteins activate expression of the transferred β-globin gene in the absence of any such enhancer. They suggest that the proteins may effectively circumvent the need for the regulatory sequence.

The viral regulatory proteins generally do not have the same effect on the corresponding endogenous genes as they have on transferred genes, although there are exceptions to this. Nevins has found that the E1a protein increases the transcription of the cellular gene coding for one of the heat shock proteins, which were given their name because they are turned on by increased temperatures and other

stresses. The results imply that the heat shock gene, which is readily induced by a variety of stimuli, and the transferred genes are somehow more accessible and susceptible to activation by the viral proteins than genes that are normally on only in certain highly specialized cell types.

Much of the current research on *trans*-regulation is aimed at understanding the mechanisms by which the viral proteins activate transcription. So far not very much is known. "The link between the proteins and how they turn on other genes is unclear," Khoury points out. "When we better understand how they turn on their own genes we will better understand how they turn on cellular genes."

Nevertheless, there are some leads. Khoury and John Brady, also of NCI, and, independently, James Alwine and his colleagues at the University of Pennsylvania School of Medicine have found that the ability of the T antigen to stimulate late viral gene transcription correlates in part with its ability to bind to SV40 DNA. This suggests that a direct binding of the protein to the viral genome contributes to the transcription effect, although an indirect effect through cellular transcription factors may also be involved. So far there are no clues as to whether the binding might act to remove a repressor of the genes' activity or work in a more positive fashion to facilitate the binding of the transcribing enzyme.

In contrast to the T antigen situation, the E1a protein may not need to bind directly to the DNA to activate gene expression. Although the protein works with different genes, investigators, including those from the laboratories of Nicholas Jones at Purdue University and Phillip Sharp at the Massachusetts Institute of Technology, have not been able to identify any common control sequences to which it might be binding. In addition, Nevins and his colleagues find that the immediate early protein from the pseudorabies virus, a herpesvirus, is even better at turning on an adenovirus gene than is E1a itself. Because the pseudorabies virus and adenovirus are not related, Nevins says, the result makes it unlikely that the E1a and immediate early proteins recognize a specific gene sequence.

Nevins suggests that the proteins act indirectly by increasing the availability or activity of cellular transcription factors that would otherwise not be present in sufficient amounts. "If this is the case, if they are basically mimicking a cellular function, we would expect to see E1a-like activity in cells at particular times," he explains. Certain cell lines, particularly those that are rapidly growing, have high expression of the heat shock gene in the absence of inducing treatments. In these cell lines, Nevins and his colleagues have found, normally E1a-dependent adenovirus genes are active even without E1a. "This suggests that the viral system is quite analogous to cellular transcriptional control," Nevins concludes.

Observations that the viral regulatory proteins can activate transcription of cellular genes have suggested a possible mechanism by which they might transform cells. "Most of these proteins are involved in viral transformation," Alwine observes. "It is not too much a leap of faith to suppose that the *trans*-activation is involved in transformation." This possibility is perhaps more likely for SV40 and adenovirus than for herpesvirus. Herpes simplex virus II transforms cells, but in this case the transformation has not been conclusively linked to any particular viral protein as it has for the T antigen and E1a.

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The E1a protein in particular is interesting because of its resemblances to the product of the *myc* gene, a well-studied transforming gene that was originally identified in an RNA-containing cancer virus of chickens. Normal cells, including those of humans, have their own counterparts of the viral *myc* gene and activation of this gene has been linked to the development of a human cancer called Burkitt's lymphoma.

The amino acid sequences of the *myc* product and E1a protein are somewhat similar, according to Robert Ralston and J. Michael Bishop of the University of California School of Medicine in San Francisco. In addition, the two proteins appear to be functionally equivalent in transformation.

Within the past year or two, researchers have shown that transforming genes, whether from RNA or DNA viruses or of cellular origin, can be subdivided into a minimum of two categories. The products of one category immortalize cells, making them capable of dividing continuously, whereas the products of the second category confer on cells the additional characteristics of transformation such as the ability to form malignant tumors in animals. Both the E1a and the *myc* products fall into the first category of immortalizing genes. "It is a reasonable suggestion," Nevins says, "that at least part of E1a's ability to immortalize is related to its ability to increase transcription." The T antigen is something of a hybrid as it has both immortalizing and transforming capabilities.

Immortalization by *myc* may also be related to increased cellular gene transcription, although more work will be needed to confirm this. Recently, Sharp and his colleagues have shown that the product of an activated *myc* gene from a mouse plasmacytoma, a tumor analogous to Burkitt's lymphoma, can stimulate the expression of genes that have been attached to heat shock regulatory sequences. The increased expression may be due to stimulation of transcription although this has not been directly shown yet. The E1a and *myc* products and the T antigen are all primarily located in the nucleus, which means that they are in the right place to influence gene transcription.

Finally, Philip Leder and his colleagues at Harvard Medical School have shown that the cellular *myc* gene is turned on by agents that stimulate cell growth. Nevins' observation of an E1a-like transcription-stimulating activity in rapidly growing cells presents an intriguing parallel to the *myc* results.

There is a potential problem with suggestions that the transforming activity of E1a is linked to its ability to increase transcription, however. The E1a gene actually produces two protein products, one of which is 46 amino acids longer than the other. Arnold Berk and his colleagues at the University of California at Los Angeles have prepared adenovirus mutants that can produce either the longer or the shorter protein, but not both.

They find that both proteins are necessary for complete transformation of cells, but that only the larger stimulates transcription of adenovirus genes or transferred genes from other sources. "We did not see a correlation between transcription and transformation," Berk says. The experiments do not rule out the possibility that the smaller protein induces the transcription of one or more specific cellular genes, but do introduce a note of caution into attempts to link transformation to *trans*-activation of cellular gene transcription.—**JEAN L. MARX**