Animal Viruses: Probes of Cell Function

All molecular biologists know how useful viruses have proved to the study of bacterial cells. Viruses were essential to many of the major discoveries about protein synthesis and the control of gene expression in bacteria. Now many researchers are increasingly turning to animal viruses to study similar cell processes in animal cells. A wide variety of processes lend themselves to this approach. These include cancerous transformation, differentiation, the mechanism of action of hormones, and various aspects of cell metabolism such as protein synthesis and membrane manufacture.

Viruses have proved useful in these studies for several reasons. For example, many genes from tumor viruses are incorporated among cellular genes. The researchers assume that whatever controls the expression of the viral genes also controls the expression of certain cellular genes. Since viruses are easily isolated and purified, they can be obtained in large enough quantities to be useful to the study of gene expression.

These viral probes permit the RNA transcribed from viral genes to be easily identified, which enables investigators to directly determine whether viral genes are being expressed, rather than relying on secondary, and sometimes inaccurate, measures of gene expression. According to Wade Parks of the National Cancer Institute, this means that the use of viruses as probes of cell functions "makes you think physiologically and ensures that what you measure is physiologically relevant."

Most studies that involve viral probes of cell functions make use of tumor viruses. Numerous viruses have been isolated that can transform normal cells of laboratory animals into tumor cells, and many investigators use these viruses to study how transformation occurs. Although viral genes can cause cell transformation, the mere presence of these genes in a cell is not sufficient. In fact, these viral genes are often incorporated among cellular genes and passed from generation to generation for millions of years. For example, cells from monkeys, apes, and humans have viral gene sequences that closely resemble sequences found in baboon RNA tumor viruses, which suggests that these sequences were integrated into primate DNA before the human lineage diverged from the rest of the primates. According to Raoul Benveniste and George Todaro of the National Cancer Institute, most vertebrates and all higher primates have been shown to have viral gene sequences as part of their cellular DNA.

Harold Varmus and J. Michael Bishop of the University of California at San Francisco and their associates are studying the control of the expression of viral genes acquired by infection and subsequently incorporated among cellular genes. Their work was stimulated by the discovery, more than a decade ago, by Ian Macpherson, of the Imperial Cancer Research Fund in England, that baby hamster kidney cells transformed by an avian sarcoma virus sometimes revert to normal appearances. Varmus, Bishop, and their associates then asked what happens to the viral genes when the cells revert.

A few years ago, Varmus and his associate Chun-Tsan Deng, together with Macpherson and his associate David Boettiger (now at the University of Pennsylvania), found that the revertant cells do not lose their viral genes. One copy of a virus-specific DNA sequence is integrated into the genome of each revertant as well as each transformed cell. The investigators were not particularly surprised at this because normal cells do contain viral gene sequences. But they did not anticipate that the viral genes would still be expressed in the revertant cells. However, between 2 and 85 times as many RNA copies of these genes are found in the transformed as in the revertant cells.

It is possible that the viral genes are not effective in the revertant cells because RNA copies of these genes are abnormal. For example, they may not include copies of the genes responsible for transformation or copies of those genes may not make the associations with polyribosomes necessary for translation. Deng, Varmus, Bishop, and their associate Dominique Stehelin recently investigated this hypothesis and reported that they can detect no qualitative differences in the viral RNA made by the revertant cells when compared to transformed cells. The difference between the normal and transformed phenotypes of these cells seems to depend on the amount, rather than merely the existence of, a viral gene product.

In addition to the control of tumor virus genes, many investigators are studying how the product of one or a very few viral genes can cause transformation. Todaro and his associate Joseph De Larco, along with Stanley Cohen of Vanderbilt University, suggest that at least one group of tumor viruses—the mouse and cat sarcoma viruses—may cause transformation by means of a viral gene product similar to epidermal growth factor (EGF). The EGF is a normal growth factor that stimulates cell division when it binds to receptors on the surfaces of these cells and causes them to divide. Cells transformed by mouse and cat sarcoma viruses can no longer bind EGF, although they can bind other growth factors that interact with other membrane receptors. Cells transformed by other kinds of tumor viruses are able to bind EGF.

Todara and De Larco have isolated a substance from sarcoma virus–transformed cells that binds to EGF receptors, although it is not identical to EGF. They propose that this substance causes the transformed cells to divide and lose control of their growth and that their EGF receptors become saturated with this viral gene product in such a way that no added EGF can bind. They believe that many kinds of cancer may be produced, in a similar way, by gene products that act like growth factors.

When viruses transform cells, they not only cause the cells to lose control of their growth, but they also cause cells to dedifferentiate—that is, cells, such as muscle or retinal cells, lose their characteristic appearances and revert to appearances like those of undifferentiated cells. Investigators hope that an understanding of how viral genes cause this to occur may lead to an understanding of how differentiation is controlled.

Boettiger, Howard Holtzer of the University of Pennsylvania, and their associates are studying dedifferentiation of three kinds of chick cells: muscle cells, chondroblasts, and retinal cells. They infect each kind of cell with temperature-sensitive sarcoma viruses that cause transformation at 35°C but not at 41°C. When infected cells are shifted from the higher to the lower temperature, they immediately cease to make "luxury products" that are characteristic of their differentiated state. For example, the muscle cells stop making myosin, the chondroblasts stop making proteoglycans, and the retinal cells cease to make the black pigment melanin. In fact the retinal cells even destroy the organelles—called melanosomes—in which melanin is synthesized. The transformed cells then morphologically and biochemically resemble embryo cells. Boettiger points out that clues to the control of differentiation may be found in the suppressed differentiation that occurs as soon as viral transformation is evident.

Naomi Rosenberg and David Baltimore of the Massachusetts Institute of Technology are using a leukemia virus to study the differentiation of mouse bone marrow cells (B cells). The virus infects these cells or related cells from the spleen or fetal liver of mice but not other kinds of cells. For example, it does not infect thymus cells, which are also part of the immune system. These investigators find that susceptible mouse cells can be infected and grown in vitro. (Uninfected cells do not grow in cell culture.) The infected cells appear to be immature and lack detectable differentiated functions. Rosenberg and Baltimore hope to use these immature cells to discover markers that distinguish the cells as being related to B cells. Such markers would allow them to detect very early stages of differentiation. They are also looking for agents that will cause the infected cells to differentiate further in vitro.

A somewhat different tack to the study of differentiation is being taken by Arnold Levine of Princeton University, William Topp of Cold Spring Harbor Laboratories, and their associates. They wanted to study the differentiation of cells that are initially undetermined. Their aim is to discover how particular paths of differentiation are originated. They chose to use mouse teratoma cells, tumor cells that differentiate into many different kinds of mouse cells when grown in tissue culture. These differentiated cells, however, have finite life-spans when grown in vitro. Levine, Topp, and their associates infected them with the tumor virus SV40, which makes the cells "immortal." They use certain biochemical markers to see whether particular cells are differentiating and, if so, what sorts of cells they are becoming.

Another use of viral probes is to study the mechanism of action of steroid hormones including the glucocorticoids, estrogens, androgens, and progestins, which apparently act in similar ways. Each hormone is thought to enter a cell, bind to specific target receptor in the cytoplasm, migrate to the nucleus, and cause certain genes to be expressed at greater rates. Investigators would like to determine what happens when the steroid-receptor complex enters the cell nucleus but have had difficulty in finding a biochemical event that represents the initial effect of the complex. For example, estrogen causes ovalbumin messenger RNA (mRNA) to be synthesized by chick oviduct cells, but this is prevented by some inhibitors of protein synthesis. Thus it may not be the initial effect of the hormone-receptor complex because, apparently, proteins must be made before the ovalbumin mRNA is synthesized.

A number of researchers believe that studies of mouse mammary tumor virus (MMTV) may enable them to overcome this difficulty. The MMTV is produced in low concentrations by infected cells grown in tissue culture. If these cells are exposed to glucocorticoids, however, the production of the virus is increased 10- to 20-fold. Gordon Ringold, Keith Yamamoto, and their associates found, about 2 years ago, that the accumulation of MMTV mRNA is the initial biological effect of glucocorticoids on the cell nucleus. More recently, Ringold, Yamamoto, Bishop, and Varmus have shown that glucocorticoid hormones stimulate the transcription of MMTV genes rather than alter the processing or degradation of viral RNA—two possibilities that had not previously been eliminated. Similar results were simultaneously reported by Parks and his associates Howard Young, Thomas Shih, and Edward Scolnik. According to Varmus, these results open the way for an investigation of whether the hormone-receptor complex interacts with DNA, and if so, how this affects the expression of MMTV and other genes under hormonal control.

George Fareed and his associates at the University of California at Los Angeles are taking advantage of the fact that cells transcribe and translate viral genes to clone specific DNA segments in mammalian cells. They add DNA segments to the tumor virus SV40 and then infect rat embryo cells or monkey kidney cells with the virus. So far, they have shown that a gene from the bacterium *Escherichia coli* can be transcribed in these mammalian cells. They hope to obtain similar results with mammalian genes. Fareed points out that most of the recombinant DNA research has focused on attempts to add genes from higher organisms to bacteria. But, with the exception of certain genes from yeast, these added genes do not seem to be expressed in bacteria. Fareed believes his approach to recombinant DNA research may overcome this obstacle.

Viral probes used in most studies are tumor viruses, but other viruses are also becoming tools. For example, many viruses consist of RNA and act like mRNA when they enter cells. Because they must use the host cell's enzymes and ribosomes to be translated, these viral RNA's can provide insight into protein synthesis.

A few years ago, Aaron Shatkin of the Roche Institute of Molecular Biology in Nutley, New Jersey, and his associates used reovirus RNA to study protein synthesis and discovered that the reovirus RNA is "capped" on the end at which translation begins with a methylated guanosine linked to nucleotide bases by a triphosphate bridge. This cap was subsequently found on most mRNA's of eukaryotic cells.

Shatkin and his associates believe that the cap on the end of these mRNA's enhances ribosome binding. They found that if they removed the cap, ribosomes bound less well to reovirus mRNA in vitro. Recently, Yasuhiro Furuichi, Alba LaFiandra, and Shatkin obtained evidence that the caps may protect mRNA's from degradation in vivo. They injected reovirus mRNA's into oocytes of the toad *Xenopus* and found that the capped mRNA's were more stable than those whose caps had been removed. This stability seemed to result from protection from cellular degradative enzymes.

Purnell Choppin, of Rockefeller University, and his associates have developed a very different use for viruses. They use influenza and parainfluenza viruses to study cell membranes. When these viruses exit from host cells, they bud—that is, they wrap themselves in a piece of the host's membrane. The viruses are then enclosed by a membrane whose proteins are of viral origin and whose lipids come from the host. Because the proteins are coded for by the viral DNA, they can be genetically manipulated and their effects on the membrane structure followed. This procedure greatly simplifies the problem of studying cell membranes, because the viral proteins are simpler to identify and alter than host cell proteins are. For example, influenza and parainfluenza viruses have only three membrane-associated proteins, whereas their host cells have hundreds. Moreover, Choppin points out that it is relatively easy to isolate a viral membrane but nearly impossible to isolate a membrane from a host cell. This system, he believes, provides a natural way to correlate membrane structure with function.

Most of the studies that use viruses to study cell functions have only recently been initiated and have not yet yielded results of major importance. But they indicate the feasibility and wide applicability of this approach. Since viruses are easy to manipulate genetically and easy to obtain in large enough quantities that their products can de detected in host cells, most investigators agree that viruses have only begun to exhibit their potential as tools in cell biology research.

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