A Quantitative Assay of Polyoma Virus Mediated Neoplastic Transformation of Cells in vitro

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Introduction:

At the present time there are available two systems for the virus mediated neoplastic transformation of normal cells in vitro. The first of these was worked out by Harry Rubin (1) at the University of California at Berkeley using the Rous Sarcoma virus (an RNA containing particle) which causes fibrosarcomas in chickens in vivo. Rubin's in vitro system employs chicken embryo fibroblasts, and after addition of the Rous Sarcoma virus, transformation of the infected cells becomes visibly apparent in about 10 days.

The second such in vitro system was developed by Dulbecco and Vogt (2) using Polyoma virus on normal hamster embryo cells. The polyoma virus differs in many aspects from the Rous sarcoma virus. In the first place it is a DNA containing particle with an extremely wide host range for neoplastic transformation, including mice, hamsters, rats and rabbits. It also has the remarkable property of being able to induce an immense variety of different types of tumors in each of its hosts. Thus the so-called " virus theory of cancer " was given a tremendous boost by the finding of this polyoma virus, as the requirement for a particular type of virus strain responsible for induction of each of the many hundreds of varieties of tumors was no longer necessary. In one polyoma virus strain

lay the potential of inducing a tremendously wide range of tumor types in several different hosts.

The in vitro transformation system worked out by Dulbecco and Vogt was explored with the intention of learning more about the details of the virus-cell interaction leading to the neoplastic state. Unfortunately this system of transformation was only qualitative in its nature. One could observe only the transformation of entire cultures instead of the more desirable quantitative study of individual cells. The introduction of a quantitative technique into this transformation system was the object of the work described in this paper.

Experimental work:

The first major problem in the quantitization of this system was the selection of the proper type of cell tissue with which to work. Cells infected with polyoma virus have been observed to undergo two different types of reaction with cells in vitro. The first of these is the cytolytic reaction in which the virus enters the cell, converts the cell's metabolic processes for the reproduction of many more virus particles, and then lyses the cell releasing the newly synthesized virus progeny. This reaction is the predominant one in mouse cells, and consequently these are used for the routine harvesting of virus stocks.

The second in vitro pattern of virus-cell interaction is the transformation reaction. Very little is known about this other than the fact that the virus enters the cell and causes some types of changes in it which lead to a dramatic increase in the frequency of mitoses, a loss of contact inhibition, and the ability to causes tumors when injected into immunologically compatible animals. This transformation of normal cells into the "neoplastic" state is the predominant one in rat and hamster tissues.

Thus we see that in order to best study the quantitative aspects of this transformation reaction we must choose a type of tissue in which there is a minimum of cytolytic action and a maximum of transformation. The cytolytic reaction interferes with a quantitative study of the transformation reaction not only because it can destroy cells which are already in the transformation reaction cycle, but also because it leads to synthesis and addition to the culture medium of unknown quantities of extra viruses, thus hopelessly compounding any quantitative aspects of the system.

In the search for a suitable tissue many varieties of cells were tested: mouse, rat and hamster embryo cells; and cells from various organs of mouse, rat and hamster young adults. After extensive work the heart tissue from 15 day old rat or hamster babies was selected as the most desirable.

Work was then begun using this heart tissue in an effort to standardize the transformation of the cells in a manner similar to the Rous sarcoma system. The plan was to infect a culture of cells with virus and observe the amount of transformation in the culture by the counting of discrete colonies of transformed cells (known as foci) made visible by the fact that they have lost their contact inhibition and no longer grow in a monolayer in the culture dish, but instead accumulate in large dark masses which can be seen with the naked eye.

Using a technique developed by Winocour at Caltech, polyoma virus stock titers were obtained at a concentrationof 10^{11} plaque forming units (PFU) per ml. Upon infection of the rat heart tissue, foci were observed to develop in the cultures in 12 days. Each colony, or focus, contained about 10^{5} cells and was actually a clone resulting from a single original infected cell in the culture.

Random foci were picked from the cultures, injected into live rats, and subsequently gave tumors at the site of inoculation, thus confirming the fact that the foci cells were neoplastically transformed. These foci cells were assayed for the presence of virus, but no virus was found.

Linearities were established between the number of plaque forming units and the number of focus

forming units (FFU) in all the stocks used, thus showing clearly that it was the virus which was responsible for the transformation. The following values were obtained:

Physical particles (as determined by the electron microscope)	10^{13}/ml.
Plaque forming units	10^{11}/ml.
Focus forming units	$\sim 10^{5}$/ml.

It was also observed with extreme interest that only certain cells appear to be susceptible to transformation. Only 1 out of 10^{3} cells in a culture could be transformed regardless of the virus titer used. The explanation for this finding is as yet quite speculative.

Discussion:

A brief comparison of the two in vitro cell transformation systems would be worthwhile here. There appear to be two basic mechanisms for viral tumor genesis, one for the RNA viruses and one for the DNA viruses.

As shown by Rubin, in the case of RNA viruses the virus particles themselves are always found to be present inside the infected and transformed cells. This is in direct contrast to the DNA tumor viruses (polyoma & SV40) in which, if the infected cell enters the transformation cycle, all traces of the virus

disappear after two or three days.

In addition, the ratio of physical particles/FFU in RNA viruses is about 10^2 as opposed to the ratio of 10^8 found in the DNA viruses.

These basic differences tend to indicate that the continual presence of the actual information content of the nucleic acid of the RNA viruses is what maintains the neoplastic state in this system. There is no evidence thus far for cell chromosomal damage or other cytologically observable alterations.

The situation with the DNA viruses is as yet quite unclear. All attempts (3) to discover viral DNA in the transformed cells, either as a mature virus, free DNA or as a prophage incorperated into the cell genome have failed. Thus the DNA virus appears not to be continually supplying its own information to the cell as does the RNA virus. Actually it probably causes some cellular alterations in the original infected cell which are passed on genetically to all the daughter cells. Cytological evidence for such alterations has been found with many DNA viruses in the form of chromosomal breakage shortly after infection.

With regard to the extremely large phy. part./FFU ratio in the DNA viruses (which is very near the mutation frequency) two possible explanations are proposed:

1. The particles leading to foci are simply

due to mutations in the original stock.

2. All particles in the original stock are identical. Upon infection a DNAase is created which causes random damage on a chromosomal level, but only in very rare cases (1 out of 10^3) is the breakage in the correct place so as to initiate the neoplastic transformation of the infected cell.

At this point it is completely unclear which, if either, of these hypotheses is correct. However, work on this problem has and should continue to shed light on the general field of possible human cancer viruses. The rapid disappearance of all traces of the DNA viruses from cells undergoing the transformation reaction seems to explain the lack of success thus far in isolating a human cancer virus.

References:

1. Rubin and Temin (1958); Virology: 6: 669-688.
Characteristics of an assay of Rous sarcoma and Rous sarcoma cells in tissue culture.

2. Vogt and Dulbecco (1960); PNAS : 46: 365-370.
Virus-cell interaction with a tumor producing virus.

3. Vogt and Dulbecco (1962); Virology: 16:41-51.
Studies on cells rendered neoplastic by polyoma virus: the problem of the presence of virus-related materials.