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Absence of a Cell Membrane Alteration Function in Non-transforming Mutants of Polyoma Virus*

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Abstract. A surface receptor for an agglutinin, exposed in transformed but not in normal cells, arises in normal mouse cells during lytic infection by polyoma virus. The structural change in the surface membrane characteristic of transformed cells and of cells productively infected by wild type virus fails to occur in normal mouse cells infected by mutants of the virus that are unable to cause transformation. The exposure of the receptor site by wild type virus is reversibly blocked by inhibitors of DNA synthesis.

A change in structure of the cell surface membrane, associated with the loss of contact inhibition of growth, can be detected by a cytoagglutinin present in wheat germ lipase.[1,2] A glycoprotein isolated and purified from wheat germ[3] causes the agglutination of several virus-transformed and other malignant cells, but not of normal cells or of phenotypic revertants of virus transformed cells.[2] The site on the membrane to which the agglutinin binds is present but not exposed in normal cells; mild protease treatment leads to the exposure of the site in normal cells and, concomitantly, to their release from contact inhibition of growth.[4] The exposure of the site parallels the loss of growth regulation of cells both *in vitro* and *in vivo*[5].

The ability of a tumor virus to bring about this cell membrane change during productive infection is investigated in the present study. The surface alteration can be demonstrated with the wheat germ agglutinin in normal mouse cells lytically infected by wild type polyoma virus. Upon infection of the same cells with mutants of the virus that have lost the ability to transform, the membrane change does not occur. Although these polyoma mutants have been selected to be able to carry out productive infection only in polyoma-transformed mouse cells, they nevertheless adsorb to and carry out some intracellular functions in normal mouse cells (ref. 6, and unpublished results). These findings thus implicate a function of polyoma virus which results in the alteration of the cell membrane as an important factor in the transformation process.

Materials and Methods. Wheat germ agglutinin was purified as described.[1] Conconavalin A, obtained from Calbiochem, was further purified by precipitation with ammonium sulfate. Cells were harvested by rinsing the culture dishes twice with saline at 37°C and then suspending the cells with an EDTA solution at 37°C as described.[2] The agglutination tests[3] were standardized to 4 min of mixing on a rocking

platform with a constant temperature plate at 22°C. Scores of 0, (+), +, +(+), ++, ++(+), +++, +++(+) and ++++ agglutination correspond, respectively, to 88–100%, 64–87%, 44–63%, 33–43%, 23–32%, 16–22%, 10–15%, 4–9%, and 0–3% single cells. Half maximal agglutination (++) was determined by interpolation of results at various concentrations of agglutinin; such determinations have been shown to have a standard deviation of not more than 10%.

Cell lines and virus strains were described.[6] Infections were at a multiplicity of 5–10 plaque forming units (pfu)/cell. Unadsorbed virus was removed by rinsing the infected monolayers at the end of a 1-hr adsorption period. Monolayers of 3T3 cells were infected while subconfluent in order to facilitate the resuspension of cells at the time of harvest.

Results and Discussion. In Table 1 are shown the results of agglutination

TABLE 1. *Agglutinabilities of infected 3T3 cells.**

Expt.	Infection by	Degree of agglutination
1	Wild type polyoma virus grown in baby-mouse kidney cultures	+++
2	Polyoma virus mutant NG-18	0
3	Wild type polyoma virus grown in polyoma-transformed 3T3 cells	++(+)
4	Wild type and mutant NG-18†	++(+)
5	Wild type inactivated by ultraviolet light (<0.001% survival)	(+)
6	Wild type; 4 μg/ml puromycin present from 2 to 12 hr after infection	0

* Tests of agglutinability were carried out with 45 μg/ml of agglutinin. Cells were tested 24–30 hr after infection.

† Multiplicity of infection of each virus was 5–10 pfu/cell, as in the single infections.

tests of 3T3 cells infected under a variety of conditions. By 24–30 hr after infection, wild type virus (Expt. 1), but not virus mutant NG-18 (Expt. 2), causes exposure of the site which binds the agglutinin. Since wild type polyoma is routinely grown in baby-mouse kidney cultures, while the mutant had to be grown in cultures of polyoma transformed mouse cells,[6] the possibility of a host-induced modification determining the ability of the virus to expose the site was tested and ruled out by showing that wild type virus, grown under conditions identical to those of the mutant, also exposes the site (Expt. 3). In a double infection by wild type and mutant viruses, the site is exposed (Expt. 4); thus, the effect of the mutant is not to destroy the site or render it nonexposable. Ultraviolet inactivation of the plaque-forming ability of the virus (Expt. 5) prevents the development of agglutinability, showing that gene function by the virus is required. Blocking protein synthesis with puromycin in 3T3 cells infected by wild type virus results in the failure to develop agglutinability (Expt. 6). In a control experiment, puromycin did not prevent agglutinability of transformed cells, and therefore does not interfere with sites already exposed.

The agglutinabilities of 3T3 cells infected with four nontransforming mutants of polyoma virus are shown in Table 2. The cells remain essentially nonagglutinable (similar to uninfected 3T3) for up to 30–34 hr after infection. In the wild type infection of the same duration, the cells become as fully agglutinable as polyoma-transformed 3T3 cells. Agglutinability could not be tested reliably after longer incubations, due to spontaneous agglutination of cells after the de-

TABLE 2. *Agglutinabilities of normal, transformed, and infected 3T3 cells.*

3T3 Cells	Half-maximal agglutination concentration (μg/ml)
Uninfected	300–500
Transformed*	40
Infected by:†	
NG-18	200–300
NG-23	200–300
HA-33	200–300
NG-59	200–300
wild type	20–40

* 3T3-Py-6, essentially identical to 3T3-Py-3[6], was measured.
† Infected cells were tested 30–34 hr after infection.

velopment of the cytopathic effect that begins around 30 hr in both wild type and mutant infected cultures.

The hapten inhibition experiments shown in Table 3 indicate that the reaction of wheat germ agglutinin with the site exposed in lytic infection by polyoma virus is similar to that described previously for the reaction of the agglutinin with transformed cells.[3]

In addition to the *N*-acetylglucosamine-containing site which reacts with the wheat germ agglutinin, lytic infection by wild type polyoma virus also exposes the receptor site for the jack bean agglutinin, concanavalin A,[7] which has α-methylglucose and α-methylmannose specificity. The results given in Table 4 demonstrate that the concanavalin A site is exposed only by wild type virus, not by the mutant.

The kinetics of exposure of the wheat germ agglutinin site by wild type virus is shown in Fig. 1. In several experiments, the time at which the site began to be exposed, though somewhat variable, was around 18–20 hr; agglutinability reached its maximum by about 24–26 hr after infection.

There is a requirement for DNA synthesis in wild type infected cells in order for the membrane change to occur, as shown by the experiments in Table 5. FdU, which acts primarily as an inhibitor of thymidylate synthetase,[8] blocks the

TABLE 3. *Inhibition of the agglutinin reaction.**

Cells	Degree of agglutination
Uninfected	+
Infected	+++
Infected; in 1.8×10^{-3} M *N*-acetylglucosamine	+
Infected; in 8 μg/ml ovomucoid	0

* Cells were resuspended for testing 28 hr after infection; agglutination determined 6 min after mixing inhibitor, agglutinin, and cells, in that order. The agglutinin concentration was 181 μg/ml.

TABLE 4. *Agglutination of infected 3T3 cells by concanavalin A.*

3T3 Cells tested	Concentration of concanavalin A (μg/ml) 0	90	181
Uninfected	0	0	0
Infected by wild type virus (26 hr)	0	+	++(+)
Infected by mutant NG-18 (26 hr)	0	0	0

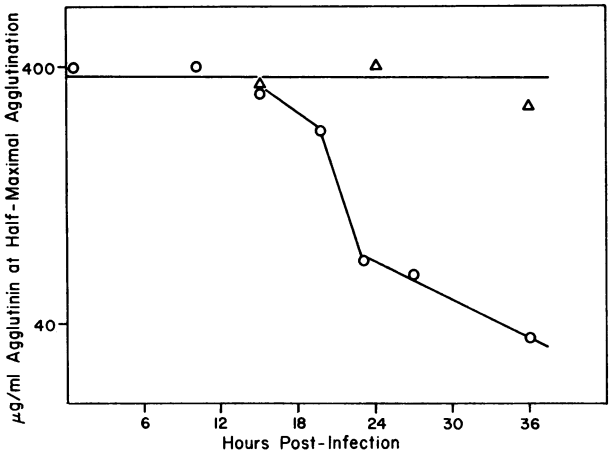

FIG. 1. Kinetics of exposure of the receptor site for wheat germ agglutinin in 3T3 cells.
(O) Infection by wild type polyoma virus.
(Δ) Infection by polyoma virus mutant NG-18.

exposure of the site when present from 0–24 hr after infection. The possibility that this drug interferes with nucleotide-sugar metabolism, thereby affecting synthesis of membrane components, need not be considered, since the same results were obtained with feedback inhibition of dCDP synthesis by thymidine,[9] not known to be a carbohydrate carrier in mammalian cells.

The site is exposed 6 hr after conditions are restored which allow the resumption of DNA synthesis (see Table 5). This rapid appearance of agglutinability suggests that some viral function(s) expressed in the absence of DNA synthesis is involved in the subsequent membrane change. The kinetics of the membrane change in a normal infection (see Fig. 1) would be consistent with the function being "early," occurring before or at the time expected for the onset of viral DNA synthesis. In addition, the membrane change detected by concanavalin A occurs in 3T3 cells infected by simian virus 40[10] where there is little if any viral DNA synthesis (ref 11; Smith, B., Imperial Cancer Research Fund, London, personal communication; Levine, A. J., Princeton University, personal communication). Thus, the requirement of DNA synthesis for the membrane change seems to pertain to cellular DNA, the synthesis of which is stimulated during productive infection by polyoma virus.[12]

Further support for the involvement of cellular DNA synthesis in the membrane change comes from experiments with African Green Monkey kidney cells

TABLE 5. *Membrane change during and after inhibition of DNA synthesis in infected cells.*

Treatment of infected cultures*	Half-maximal agglutinin concentration (μg/ml)
(*a*) Infected	55
(*b*) FdU	>200
(*c*) FdU, reversal	65
(*d*) Thymidine	>200
(*e*) Thymidine, reversal	65

* Agglutinabilities of the infected cells determined (*a*) after 24 hr, (*b*) after 24 hr in the presence of 0.5 μg/ml FdU + 5 μg/ml uridine, (*c*) same as in (*b*) with removal of FdU and addition of 20 μg/ml thymidine from 24 to 30 hr, (*d*) after 24 hr in the presence of 2.5 mM thymidine, and (*e*) same as in (*d*) with removal of thymidine and addition of 0.02 mM deoxycytidine from 24 to 30 hr.

lytically infected with SV40 virus: In CV-1, a monkey line in which host DNA synthesis is stimulated by the virus, the site for wheat germ agglutinin is exposed, whereas in BSC-1, another monkey line in which the stimulation does not occur,[13,14] the site is not exposed. The site can be exposed in BSC-1 cells upon infection with adenovirus 5, which also stimulates some cellular DNA synthesis early in infection[15].

Although the stimulation of host cell DNA synthesis by the virus appears to be necessary for the membrane change, it seems not to be sufficient since polyoma mutant NG-18 is capable of inducing cell DNA synthesis in 3T3 cells (unpublished results). The uncoupling of the membrane change from cell DNA synthesis by the mutant suggests either that some aspect of the DNA stimulation by the mutant is faulty or that two distinct viral functions are involved.

The ability of polyoma mutant NG-18 to give rise to abortive transformation[16] has been tested and found to be lacking (unpublished results). This latter finding implicates the membrane change in the transient establishment of the transformed phenotype.

A single complementation group, defined by temperature sensitive mutants of polyoma virus, has been found to be defective in the ability to initiate transformation;[17-19] the gene function impaired in this class of mutant, however, appears to be different from that of NG-18 since the temperature sensitive mutant retains the ability to cause the abortive transformation response[20].

Further evidence for a viral function causing exposure of the site for wheat germ agglutinin has recently been obtained with a temperature sensitive mutant of polyoma virus (unpublished results).

Summary. The agglutinable state of the cell surface detected by wheat germ agglutinin, previously shown to be characteristic of cells transformed by oncogenic viruses or chemical carcinogens, has here been shown to be acquired by normal cells lytically infected with polyoma virus. A similar change in the normal cell membrane cannot be detected after infection with polyoma mutants which are defective in their ability to transform. These results thus strengthen the previous findings indicating the importance of the agglutinable state in the loss of regulation of cell growth. The 3T3-polyoma system, employing wild type and mutant strains of the virus, should enable further studies of how the membrane change is brought about and on the nature of the coupling between this change and cell division.

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