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CHAPTER 13

The Cell Surface, Virus Modification, and Virus Transformation

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I. Introduction*

Role of the Cell Surface

The surface plays a crucial role in the biology of a single cell, and also in the interplay of cell with cell, contributing to and molding the organismic whole. Of particular interest in the present context are those phe-

* Abbreviations used: $GlcNH_2$, glucosamine; GlcNAc, *N*-acetylglucosamine; RSV, Rous sarcoma virus; ts, temperature-sensitive; UV, ultraviolet light.

TABLE I

Cell Functions Mediated by the Surface

Function	Modified by neoplastic transformation	Selected references
Enzyme activity		
Various	+	Emmelot and Benedetti, 1967
Adenyl cyclase	+	d'Armiento *et al.*, 1972; Anderson *et al.*, 1973
Proteases	+	cf. Burger, 1973
Glycosyl transferases	+	Roth and White, 1972
Transport systems	+	cf. Holley, 1972; Kalckar *et al.*, 1973
Interaction with viruses	+	cf. Allison, 1971; Križanová *et al.*, 1971; Taylor *et al.*, 1971; Drzeniek, 1972; Sturman and Takemoto, 1972; Weiss, 1973
Interaction with agglutinins		
Lectins	+	Inbar *et al.*, 1972; cf. Burger, 1973
Polymers of basic amino acids	+	cf. Inbar *et al.*, 1972
Antigenicity		
Blood group substances		Pann and Kuhns, 1972; Dimmock *et al.*, 1972; Hakomori *et al.*, 1972
H-2 antigens	+	Klein, 1971, 1972; Lilly, 1971
HL-A antigens	+	Reisfeld *et al.*, 1971; Osoba and Falk, 1974
Tissue-specific antigens	+	Boyse and Old, 1969; Dickinson *et al.*, 1972; Iyje *et al.*, 1972; cf. Tillack, 1972; Snell *et al.*, 1973
Fetal antigens	+	Alexander, 1972
Forssman antigen	+	Hakomori and Kijimoto, 1972
Immunoglobulins	+	Aisenberg and Bloch, 1972
Interaction with immune system		
Immunogenicity	+	cf. Haughton and Nash, 1969; cf. Klein, 1971
Reaction with immune lymphocytes		Wekerle *et al.*, 1972
Reaction with macrophages	+	Hibbs, 1973
Reaction with cytotoxic sera	+	cf. Haughton and Nash, 1969; cf. Klein, 1971; Kurth and Bauer, 1972
Reaction with complement		Müller-Eberhard, 1972

TABLE I (*continued*)

Function	Modified by neoplastic transformation	Selected references
Growth, contact inhibition		
Of movement	+	Abercrombie, 1967
Of mitosis	+	cf. Martz and Steinberg, 1973
Of progression through cell cycle	+	cf. Dulbecco, 1971
Morphogenesis		
Cell recognition	+	cf. Lilien, 1969; Roth *et al.*, 1971; Pessac and Defendi, 1972
Adhesion	+	cf. Weiss, 1973
Intercellular junction Formation	+	cf. Goodenough and Gilula, 1972
Interaction with regulatory molecules		
Hormones	+	Krug *et al.*, 1972; Amir *et al.*, 1973; Lesniak *et al.*, 1973
Acetylcholine		Patrick *et al.*, 1972; Sytkowski *et al.*, 1973

nomena that are greatly modified during neoplastic conversion by oncogenic viruses. The illustrative information set out in Table I indicates the wide range of phenomena involved. It will be the assumption of this discussion that specific molecules at the surface of cells carry out the various functions here tabulated. In some cases, this postulate rests on very few, but intriguing, observations; in others the data have converted hypothesis to established fact.

The working model for the cell surface derives from that proposed by Singer and Nicolson (1972). The plasmalemma is considered to comprise a fluid lipid phase into which are inserted structural and functional proteins and glycoproteins, the distribution of which is subject to continual quantiative and qualitative modification during growth and development. A central problem in the present consideration is the mechanism whereby the cell surface is formed from its constituent building blocks. There is considerable information on the general patterns of synthesis of neutral lipids (Spector, 1972), phospholipids (McMurray and Magee, 1972), sterols (Rothblat, 1972), glycolipids (cf. Roseman, 1970), proteins, and glycoproteins (cf. Schachter and Roden, 1973) by animal tissues. But little is known about the reactions by which individual cells generate these components and mold them into a functioning plasmalemma.

Of specific importance will be the synthesis and incorporation into the cell surface of glycolipids and glycoproteins. It will be assumed that *de novo* synthesis of these molecules by the various fibroblast types discussed proceeds by procedures already established. In the case of the glycolipids, the sugars are added stepwise to a preformed ceramide base through the action of specific glycosyl transferases (cf. Roseman, 1970). The protein of glycoproteins is synthesized on polyribosomes and there, upon completion, accepts the first sugar residue. Once again carbohydrate residues are added sequentially by specific sugar-transferring enzymes, as the glycoprotein is transported through the Golgi apparatus to its site at the cell periphery (cf. Schachter and Roden, 1973). Our own studies on the synthesis of surface glycoprotein by mouse fibroblasts are in accord with the general aspects of the latter model, since treatment of cells with puromycin or cycloheximide brings rapid inhibition of formation of peptide and oligosaccharide moieties (Sheinin and Onodera, 1970).

This chapter will address itself to the following questions: (i) What are the modifications to the metabolism of surface molecules, which are inflicted upon cells by their infecting viruses? (ii) Under what conditions, can these modified pathways become stablized, thereby producing a permanent alteration of the cell surface? (iii) In what way do the oncogenic viruses resemble, or differ from, their nononcogenic counterparts in their effects on cell surface metabolism?

II. Interactions of Viruses with the Cell Surface

A. Introduction

The interactions of viruses with the surface of cells may perhaps be classified under three headings: (i) reactions by which an infecting virus is permitted entry into the cell; (ii) reactions by which newly replicated virus is permitted exit from the cell; (iii) reactions of biogenesis of the integral plasma membrane (Singer and Nicolson, 1972) and its peripheral components which are directly or indirectly modified as a result of virus infection.

This chapter will concern itself primarily with class (iii) phenomena, for these appear to offer the most suitable target for oncogenic conversion. The many processes embraced under classes (i) and (ii) are discussed elsewhere (Allison, 1971; Poste, 1970; Krizănová *et al.*, 1971; Dales, 1973a,b; Medzon, 1973). Of these, the action of viral neuramini-

dases (cf. Drzeniek, 1972) may be relevant, and will be commented upon below.

As noted in Tables II and III, the genome of many viruses carries information that directly, or indirectly, results in modification of the metabolism of molecules at the surface of the host cell. The ingenuity of many investigators has been employed in isolating virus strains, a major expression of which is seen in this function, normal or mutant (Ghendon, 1972; Vogt, 1972; Roizman *et al.*, 1973). Such variants are known to occur in nature (Poste, 1970; Ichihashi and Dales, 1971; Ichihashi *et al.*, 1971; Nagata *et al.*, 1972; Higashi, 1973; Roizman *et al.*, 1973; Dales, 1973b). Many more undoubtedly remain to be discovered, and perhaps even to be produced.

B. Action of Enveloped Viruses

The most profound direct effects on the synthesis and turnover of the plasmalemma is to be observed in vertebrate cells infected by enveloped viruses. These microorganisms contain a core of nucleoprotein surrounded by the capsid proteins, which in turn are encased in a membranous envelope. The genome of enveloped viruses codes directly for the synthesis of proteins and glycoproteins of the viral envelope membrane, which resembles in many ways the membranes of host cells (Guidotti, 1972). So close is the structural analogy that the biogenesis of viral membrane has been taken as a most effective model for the generation of cellular membrane (cf. Dales and Mosbach, 1968; Ben-Porat and Kaplan, 1972; David, 1973).

The same general picture emerges from the study of myxoviruses, paramyxoviruses, rhabdoviruses, herpesviruses, poxviruses, and oncornaviruses (Eiserling and Dickson, 1972; see also references cited in Tables II and III):

(i) The virus genome codes directly for the formation of specific proteins of the viral membrane, while perhaps only indirectly contributing to the composition of the lipid moiety.

(ii) Viral envelope polypeptides and glycoproteins are synthesized within the cytoplasm of the cell and are then transported from the machinery of synthesis to the site of maturation (be it nuclear or cytoplasmic).

(iii) Viral envelope proteins become associated with membrane lipid either before, or in the process of, becoming a part of the membrane site of maturation.

TABLE II

Vertebrate DNA Viruses Affecting Cell Surface

Class	Species (selected examples)	Genome size ($\times 10^6$ daltons)	Natural host		Cells transformed *in vitro*	Envelope	Cell surface modification		Selected references
			Replication	Tumor formation			During replication	In transformed cells	
Poxvirus	Vaccinia	100–200	Cattle	Unknown (U)	U	+	+	U	Dales, 1973; Dales and Mosbach, 1968
	Rabbit fibroma		Rabbit	Rabbit (cottontail, domestic)	U	+	+	+	Shope, 1966
	Yaba		Monkey	Monkey	Primate, human	+	+	+	Tsuchiya and Rouhandeh, 1972
Herpesvirus	Herpes simplex, type 1	100	Human	U	Hamster[a]	+	+	+[a]	Heine *et al.*, 1972; Roizman *et al.*, 1973; Nahmias *et al.*, 1972; Tevethia *et al.*, 1972; Rapp, 1973
	Herpes simplex, type 2		Human	U	Hamster[a]	+	+	+[a]	Heine *et al.*, 1972, Roizman *et al.*, 1973, Nahmias *et al.*, 1972, Rapp, 1973
	Epstein-Barr		Human	Human	Human, Marmoset	+	+	+	Klein, 1972; zur Hausen, 1972
	Lucké		Frog	Frog	U	+		+	Klein, 1972
	Marek's disease		Chickens, turkeys	Chickens	U	+	+	+	Klein, 1972; Nazerian, 1973

Adenovirus	Human	20–25	Human	U	Hamster, human	−	+	+	Schlesinger, 1969; Vasconcelos-Costa *et al.*, 1973
	Canine		Dogs	U	U	−	U		
	Swine		Swine	U	U	−	U		
Papillomavirus	Shope	5	Rabbit	Rabbit	U	−	U	+	Shope, 1966
	Human		Human	Human (benign)	U	−	U	+	Butel, 1972
	Equine		Horse	Horse (benign)	U	−	U	+	Fulton *et al.*, 1970
Papovavirus	Polyoma	2–3	Mouse	U	Rodent	−	+	+	Habel, 1965; Klein, 1971
	SV40		Monkey	U	Rodent, Human	−	+	+	Häyry and Defendi, 1970; cf. Sambrook, 1972
Parvovirus	Minute virus of mice adeno-associated virus	2	Mouse	U	Mouse[b]	−	U	U	cf. Crawford, 1969

[a] Observed with UV-irradiated virus (Rapp, 1973).

[b] One unconfirmed observation.

TABLE III

Enveloped Vertebrate RNA Viruses Affecting Cell Surface

Class	Species (selected examples)	Genome size ($\times 10^6$ daltons)	Natural host		Cells transformed (*in vitro*)	Envelope	Cell surface modified		Selected references
			Replication	Tumor formation			During replication	In transformed cells	
Myxovirus	Influenza	2–5	Primates, birds	Unknown (U)	U	+	+	U	Eiserling and Dickson, 1972; Rott *et al.*, 1972; Compans, 1973
	Fowl plague		Birds	U	U	+	+	U	
Paramyxovirus	Simian virus 5	6–8	Monkeys	U	U	+	+	U	Kingsbury, 1972
	Sendai		Mouse, pigs	U	U	+	+	U	Bachi *et al.*, 1973
	Sindbis		Birds	U	U	+	+	U	Bose and Brundige, 1972
	Measles		Human	U	U	+	+	U	Knight *et al.*, 1972
	Mumps		Human	U	U	+	+	U	Kingsbury, 1972
	Newcastle disease		Birds	U	U	+	+	U	Kingsbury, 1972
Rhabdovirus	Vesicular stomatitis	3–4	Swine, cattle, Rodents, dogs	U	U	+	+	U	Howatson, 1970; Kingsbury, 1972
	Rabies		Rodents, dogs	U	U	+	+	U	Howatson, 1970; Higachi, 1973
Oncornavirus	Mouse mammary	10–12	Mouse	Mouse	U	+	+	+	Bentvelzen, 1972; Dalton, 1972

B type C type	Murine leukemia-sarcoma	10–12	Mouse	Mouse	Rodent, human	+	+	+	Aoki *et al.*, 1973; Mann *et al.*, 1973; Salzber *et al.*, 1973
	Feline leukemia-sarcoma		Canine	Cat	Feline, canine, human	+	+	+	Boone *et al.*, 1973
	Avian myeloblastosis		Avian	Bird	Avian	+	+	+	
	Rous sarcoma		Avian	Chicken	Avian, rodent, human	+	+	+	Rao *et al.*, 1966; Quigley *et al.*, 1972; Robinson and Robinson, 1972
Coronavirus	Human		Human	U	U	+	+	U	Bradburne and Tyrrell, 1971
	Murine		Murine	U	U	+	+	U	Sturman and Takemoto, 1972
Togavirus	Rubella		Human	U	U	+	+	U	Higashi, 1973
	Japanese encephalitis		Avian	U	U	+	+	U	Shapiro *et al.*, 1972

(iv) Viral envelope proteins can be detected (by biochemical and immunological methods) as an integral component of the plasmalemma of infected cells before virion maturation begins.

(v) The surface biology and biochemistry of host cells is profoundly altered when viral envelope proteins are inserted into the plasma membrane. This is reflected in an altered immunopathology and in the reaction of infected cells with lectins.

Thus enveloped viruses can modify the reactivity of cells by coding for the synthesis of specific proteins and glycoproteins which become an integral part of the cell surface. The myxoviruses, and some of the paramyxoviruses, are potentially effective in such functions by virtue of the fact that they carry a neuraminidase as a virion component (cf. Drzeniek, 1972). These enzymes have the ability to remove sialic acid from complex carbohydrate residues at the cell surface, thereby altering the immunological, biochemical, and biophysical properties of cells, their capacity to react with neighboring cells, and their progression through metabolic events of the cell cycle (cf. Weiss, 1973).

C. Action of Nonenveloped Viruses

Unlike the enveloped viruses, those without a lipoprotein–glycoprotein coat appear not to have a direct effect on the synthesis of molecules that become a part of the plasma membrane or other membranous cell constituents. They may, however, indirectly bring about the turnover of membrane components, particularly in association with infection and release of virus from cells (Allison, 1971; Poste, 1970; Dales, 1973a,b; Medzon, 1973) or with transmission of virus from cell to cell.

As noted above, these phenomena will not be discussed further because they do not appear to provide a mechanism for the stable alteration of the surface of surviving cells.

D. General Considerations

When one considers the biological consequences of cell surface modulation by viruses, it is clear that most instances of productive infection are of little direct relevance to the phenomenon of viral oncogenesis because the affected cell dies. There are, however, at least three types of virus–cell interaction in which host cells would survive, but with virus-determined biochemical alteration of the cell surface. These include (i) cells which have established a carrier state and continue to bud off

complete or defective enveloped virions from the cell surface (e.g., Bradburne and Tyrrell, 1971; Howatson, 1970; Lunger and Clark, 1972; Matsumoto, 1972; Higashi, 1973); (ii) abortively infected cells in which are expressed those functions determining synthesis and movement to the surface of viral envelope proteins (cf. Poste, 1970); and (iii) cells infected with virus that is a mutant in some terminal function of the replication cycle, but which does express those functions that modify the cell surface (e.g., Nagata *et al.*, 1972).

These observations are especially important in assessing the potential for oncogenic conversion by viruses. They lead to an appreciation of a major difference between oncogenic and nononcogenic viruses, even within the same class. Tumor viruses can function as donors of genetic information (perhaps even as transducing agents) (cf. Luria, 1959). Cells that survive infection by the oncogenic viruses and undergo stable transformation are known to carry virus DNA as an integral part of the chromosomal DNA (Winocour, 1971; Sambrook, 1972; Temin, 1972; Todaro and Huebner, 1972; zur Hausen, 1972, 1973). This would provide a fourth, and perhaps most efficient, method whereby surface metabolism of host cells could be stably modified.

III. Oncogenic Viruses

A. Introduction

We now know that virus transformation is a two-step process analagous to that illustrated in Fig. 1. Genetic transformation, which results from incorporation of virus genetic material into the chromosomal DNA, is necessary but not sufficient to produce a neoplastically transformed cell. It requires in addition phenotypic expression of a virus function which ultimately modifies the surface of the affected cell. It is the cell surface that embodies a key lesion of neoplastic transformation, for it mediates those phenomena of cell–cell interaction that underlie both normal and cancerous growth and development (see Table I).

To understand the mechanism of oncogenesis by viruses, it is of importance to establish which, if any, virus genes participate in neoplastic conversion and how these interact with genes for cell surface biogenesis. At the biochemical level the problem becomes one of defining the chemical basis of the physiological surface modification to permit identification of the metabolic processes involved.

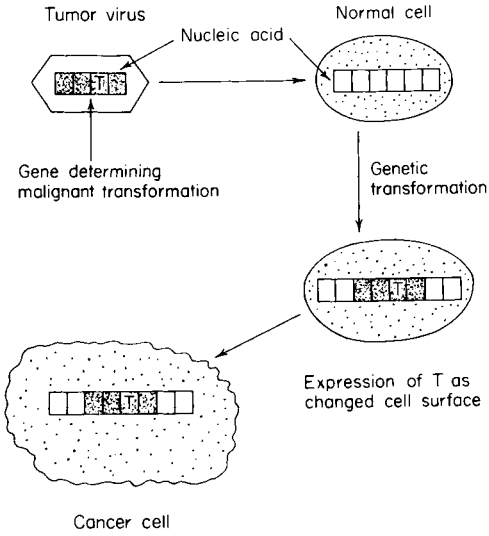

FIG. 1. Model for neoplastic transformation by oncogenic viruses.

B. Genetic Considerations

There seems little doubt that genetic information for modulation of cell surface macromolecules is carried by the genome of oncogenic viruses. Such a conclusion derives from the following observations: (i) Infectious nucleic acid, isolated from highly purified virus, brings about transformation both *in vivo* and *in vitro* (Ito, 1962; cf. Crawford, 1969; cf. Sambrook, 1972; Graham and van der Eb, 1973). (ii) Infection and transformation by these viruses is sensitive to high energy irradiation and mutagenic chemicals (cf. Sachs, 1967; Eddy, 1969; Sambrook, 1972; Vogt, 1972). Because the capacity for viral replication is more sensitive to these damaging agents than is the ability to transform cells, it seems likely that information for the latter function resides only in a segment of viral genome. (iii) Recently a correlation has been established between the nucleic acid composition of various isolates of RSV and their capacity to replicate and/or transform host cells (cf. Duesberg and Vogt, 1973). (iv) Some of the surface alterations characteristic of transformed cells are expressed early in cells infected productively or abortively, by oncogenic virus (Ben-Bassat *et al.*, 1970; Häyry and Defendi, 1970; Girardi and Defendi, 1970; Hakomori *et al.*, 1971, 1972; cf. Eckhart, 1972; cf. Benjamin, 1972). (v) Mutants of these viruses have been isolated [polyoma (cf. Eckhart, 1972; Benjamin,

1972; Sambrook, 1972), Rous sarcoma virus (cf. Vogt, 1972), and murine leukemic-sarcoma virus (cf. Vogt, 1972; McCarter *et al.*, 1974)] which cannot transform cells under nonpermissive conditions and which elicit specific, virus-determined cell surface changes only under permissive conditions.

Clearly, there is ample data implicating the virus genome as the repository of information for neoplastic transformation. The problem to be posed concerns the nature of this information. Does it code for specific molecules that are continually elaborated and incorporated into the plasma membrane? Or does it simply provide for alteration of the pattern of regulation of cellular metabolic pathways?

Somatic cell genetics, not yet at a stage that is amenable to sophisticated analysis, has permitted the isolation of cell variants that do shed light on the present problem. Many revertants of virus-transformed cells have been isolated and studied at the genetic, physiological, and biochemical levels (cf. Macpherson, 1971; Wyke, 1971; Nomura *et al.*, 1972; Sambrook, 1972; Stephenson *et al.*, 1973; Yamamato *et al.*, 1973). Some were free of detectable virus genome, indicating the essential contribution of virus. However, other revertants did still carry virus genetic information but were phenotypically normal. These observations suggested a delicate interplay of virus and cellular genome in maintaining the transformed state.

Temperature-sensitive variants of virus-transformed cells have been obtained that do not express the transformed cell phenotype at the nonpermissive temperature (Noonan *et al.*, 1973; Renger and Basilico, 1972). As far as can be ascertained, the virus genome remained associated with these cells and appeared not to be temperature sensitive.

These studies suggested that the virus genome coding for transformation does so through interaction with cell genetic information that determines structure and function of surface molecules. With the early demonstration that virus genome acts in this way, one turned with some confidence to seek out its biochemical expression. As will be noted below, the experimental harvest was bountiful in the extreme, proving something of an embarrassment. It became increasingly difficult to account for the very large number of changes observed in association with virus transformation with the available genetic repository.

This is particularly so in the case of the smaller papilloma and papovaviruses [and even adenoviruses (see Tables II and III)], with genome molecular weights of 2 to 5×10^6 daltons. This relatively small amount of DNA, which must code for some seven (or more) virion and nonvirion proteins (cf. Sambrook, 1972), is unable to accommodate each component change shown in Tables I and IV, if indeed each depends

upon the expression of an unique virus gene sequence. The available genetic analyses suggest that two gene products at most could serve this function (cf. Sachs, 1967; Eckhart, 1972; Benjamin, 1972).

C. Biochemical Studies

The results obtained in studies designed to understand the biochemical basis of the altered cell surface physiology associated with viral oncogenesis, are briefly summarized in Table IV. They embrace a large number of individual biochemical changes, from those involving terminal sialic acid residues at the extreme cell periphery inward to the internal plasma membrane components.

Our own studies compare the surface structure of control 3T3 mouse fibroblasts, and those transformed by polyoma virus and SV40, at the level of the glycoprotein at the extreme periphery of the cell and the proteins, glycoproteins, and glycolipids of the underlying plasma membrane. These two plasmalemma domains are most easily defined in terms of the model shown in Fig. 2. As suggested by Singer and Nicolson (1972) the plasma membrane is considered to be comprised of a lipid bilayer into which are inserted globular proteins and glycoproteins. The extreme periphery, or surface component, of the cells can be defined as that portion of the plasmalemma which is sensitive to treatment with enzymes, under conditions that leave the cells with the underlying plasma membrane functionally intact with respect to the maintenance of cellular integrity and the transport of nutrients for survival.

In our studies, trypsin has been used to define these two plasmalemma domains. Thus, treatment of cells with this enzyme (see Fig. 2) was shown to release surface glycoprotein from the extreme periphery of cells. This left the underlying plasma membrane intact, as indicated by the fact that the plating efficiency of treated cells was unaltered, as was their uptake of nonvital stains (cf. Onodera and Sheinin, 1970; Sheinin and Onodera, 1970).

Figure 3 illustrates the operational definition employed for surface glycoprotein. It relies on the fact that 3T3 mouse fibroblasts (as well as most other animal cells), when grown to confluence, are in a pseudo-G-1 phase of growth. If subcultured with trypsin, and plated at subconfluent concentrations, they will grow synchronously and immediately regenerate their surface glycoprotein. If such cultures are incubated with radioactive $GlcNH_2$ at 12–13 hours post-plating, this precursor is preferentially incorporated into surface glycoprotein. Such specifically labeled surface glycoprotein can be isolated, purified, and characterized (cf.

TABLE IV

Biochemical Changes Observed in Surface of Virus-Transformed Cells

Component affected	Virus	Selected references
Glycoproteins at the periphery of the plasma membrane	Polyoma, SV40	Wu *et al.*, 1969; Meezan *et al.*, 1969; Onodera and Sheinin, 1970; Greenberg and Glick, 1972; cf. Warren *et al.*, 1973
	RSV	cf. Warren *et al.*, 1973; Wickus and Robbins, 1973; Stone *et al.*, 1974; Wickus *et al.*, 1974
Glycoproteins within the plasma membrane	Py, SV40	Sheinin *et al.*, 1971; Sakiyama and Burge, 1972; Sheinin, 1972; Sheinin and Onodera, 1972
	RSV	Wickus and Robbins, 1973; Stone *et al.*, 1974
	EBV	cf. zur Hausen, 1972
Glycolipids	Py, SV40	cf. Hakomori *et al.*, 1972; cf. Sheinin, 1972; Yogeeswaran *et al.*, 1972; cf. Brady *et al.*, 1973; Hammarström and Bjursell, 1973; Schengrund *et al.*, 1973; Murray *et al.*, 1973
	RSV	cf. Hakomori *et al.*, 1971, 1972; Warren *et al.*, 1972; cf. Sakiyama and Robbins, 1973
Enzymes	Polyoma, SV40	cf. Burger *et al.*, 1972; cf. Johnson and Pastan, 1972
	RSV	cf. d'Armiento *et al.*, 1972; Anderson *et al.*, 1973
Sialic acid residues	Polyoma, SV40	cf. Kraemer, 1971; Schengrund *et al.*, 1973; cf. Weiss, 1973

Onodera and Sheinin, 1970; Sheinin and Onodera, 1970).

Preparations of underlying plasma membrane, greatly depleted of surface glycoprotein, were obtained using cells removed from their solid growing surface by gentle enzyme treatment (cf. Sheinin *et al.*, 1971; Sheinin and Onodera, 1972). Recently we have applied a procedure developed by Mr. Svein Carlsen (Ontario Cancer Institute) to produce plasma membrane preparations carrying almost all of the surface glycoprotein (Sheinin *et al.*, 1973).

We have now studied in detail the surface glycoprotein fraction derived from 3T3 mouse fibroblasts, and derivative cells transformed by

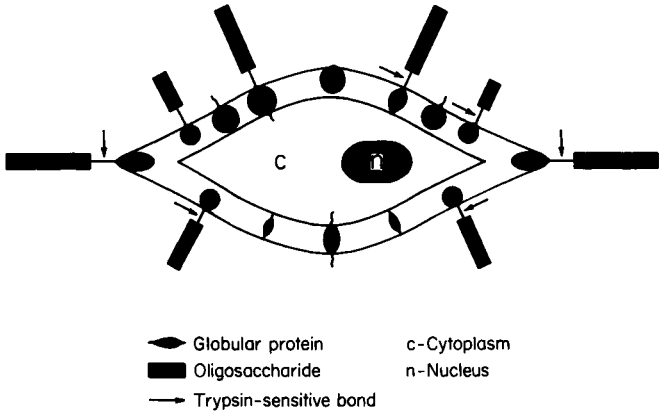

FIG. 2. Model for the structure of the cell and its surface.

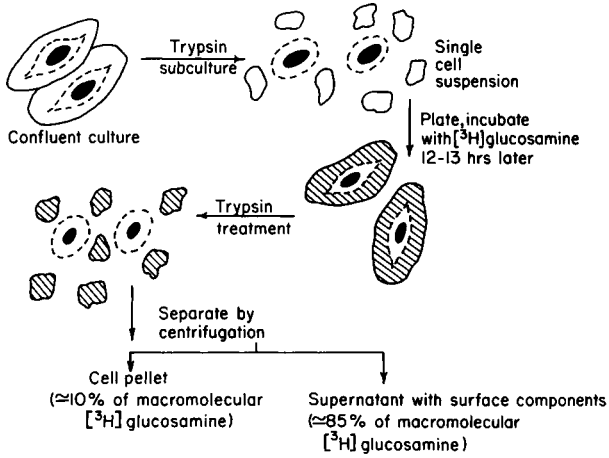

FIG. 3. Operational definition for surface glycoprotein. When confluent cultures of normal and virus-transformed 3T3 mouse fibroblasts are subcultured at $<10^4$ cells/cm^2 of growing surface area, they grow synchronously and proceed to regenerate the surface components released by trypsin treatment. If incubated with [^{3}H]GlcNH_2 at 12–13 hours postplating, this precursor is very largely incorporated into surface macromolecules shown to be glycoprotein. (Onodera and Sheinin, 1970; Sheinin and Onodera, 1970; Sheinin *et al.*, 1973).

polyoma and SV40 viruses. The physiological properties of these cells (3T3-Py6, 3T3-SV479, 3T3-SVCE56, 3T3-SVA26) have been described elsewhere (cf. Yogeeswaran *et al.*, 1972). The surface glycoprotein, as released from cells by sonic vibration and purified, was recovered as a soluble component, which remains in the supernatant after sedimentation at 105,000 *g* for 3 hours. This fraction exhibited heterogeneity when examined by polyacrylamide gel electrophoresis (cf. Sheinin *et al.*, 1973), with a major fraction moving very slowly through the gels in the region of material having molecular weights in excess of 10^5 daltons. When subjected to sedimentation in neutral sucrose density gradients [5–20% (w/w), 16 hours at 23,000 rpm at 2°C) the greatest proportion of the surface glycoprotein exhibited an apparent molecular weight of about 62,000 daltons (Sheinin and Onodera, 1970). The surface glycoprotein, excluded from beads of Sephadex G-200, was barely included after treatment with 8 *M* urea (Sheinin *et al.*, 1973).

The biochemical properties of the surface glycoprotein fraction of control and virus-transformed cells, uncovered to date, are summarized in Table V. Of particular interest is the finding that all of the surface glycoprotein, as defined herein, is homogeneous in the nature of the linkage between peptide and carbohydrate moieties. Its resistance to alkali, and the enrichment for asparagine (cf. Frohlich, 1972) in the protein suggest that this linkage is between an *N*-aspartamido residue and a GlcNAc residue. Such a linkage is characteristic of immunoglobulins and other glycoproteins found at the surface of animal cells (cf. Schachter and Roden, 1973).

Comparative analysis of surface glycoprotein derived from 3T3 cells and from 3T3 cells transformed by polyoma and SV40 viruses, revealed that these were not the same (Onodera and Sheinin, 1970). Preliminary amino acid analyses have uncovered no gross qualitative or quantitative differences; however some quantitative variations in carbohydrate content have been detected (Frohlich, 1972).

The latter findings are in general agreement with those of others (listed in Table IV) in which differences have been observed between glycoproteins at the surface of normal and virus-transformed cells. Although definitive evidence is still to be obtained, it has been suggested that the chain length of the carbohydrate residues may be shorter in the latter situation (Grimes, 1970; Greenberg and Glick, 1972; Sakiyama and Burge, 1972; Warren *et al.*, 1973).

Four kinds of experiments were performed to compare the compositions of surface glycoprotein-depleted plasma membrane preparations obtained from 3T3 cells and virus-transformed 3T3 cells. In the first purified plasma membrane preparations were fully solublized using SDS,

TABLE V

Properties of a Purified Surface Glycoprotein Fraction from Control or Virus-Transformed 3T3 Mouse Fibroblasts

1. Contains peptide and carbohydrate covalently-linked[a]
2. Linkage is alkali-resistant[b]
3. Free of lipid, RNA, DNA[a]
4. Constitutes no more than 0.2% of total cellular protein[a]
5. Carbohydrate makes up approximately 26% of the weight percent (about 14% neutral sugar, 5% amino sugar, and 7% sialic acid)[b]
6. Carbohydrate contains $GlcNH_2$, galactose, mannose, fucose[b], and sialic acid[a], but not glucuronic acid[b]
7. A full complement of amino acids is present with enrichment of asparagine and aspartic acid[b]
8. Insensitive to hyaluronidase,[a] sensitive to neuraminidase[a,b]

[a] Sheinin and Onodera, 1970.
[b] Frohlich, 1972.

mercaptoethanol, and heating. The solublized material was subjected to electrophoresis in polyacrylamide gels to resolve some 30-odd peptides, varying in molecular size from about 15,000–200,000 daltons, (Sheinin *et al.*, 1971; Sheinin, 1972; Sheinin and Onodera, 1972). The summary data shown in Fig. 4 indicated that although the major peptide pattern was similar for various plasma membrane preparations, nevertheless significant differences could be observed. In the second group of studies, plasma membrane harvested from cells generally labeled with radioactive $GlcNH_2$, to tag glycolipids and glycoproteins, were solublized and analyzed as noted above. Quite profound differences were observed between the normal and virus-transformed preparations (Sheinin, 1972; Sheinin and Onodera, 1972). It seems likely that the components here analyzed by their radioactivity were primarily plasma membrane glycoproteins, although the presence of protein-associated glycolipid was not excluded.

Protein of purified plasma membranes was obtained, solubilized, and analyzed by electrophoresis in polyacrylamide gels. Once again the peptide patterns of the normal and virus-transformed cells were generally similar (Sheinin *et al.*, 1971; Sheinin, 1972; Sheinin and Onodera, 1972). However significant differences were observed. It was especially interesting to note (Fig. 5) that the peptide patterns for the plasma membrane preparations derived from the three SV40-transformed cells were remarkably similar, perhaps suggesting a primary expression of virus genome.

A closer examination of the electrophoretic patterns shown in Figs. 3 and 4 reveals that the large molecular weight components (running at the

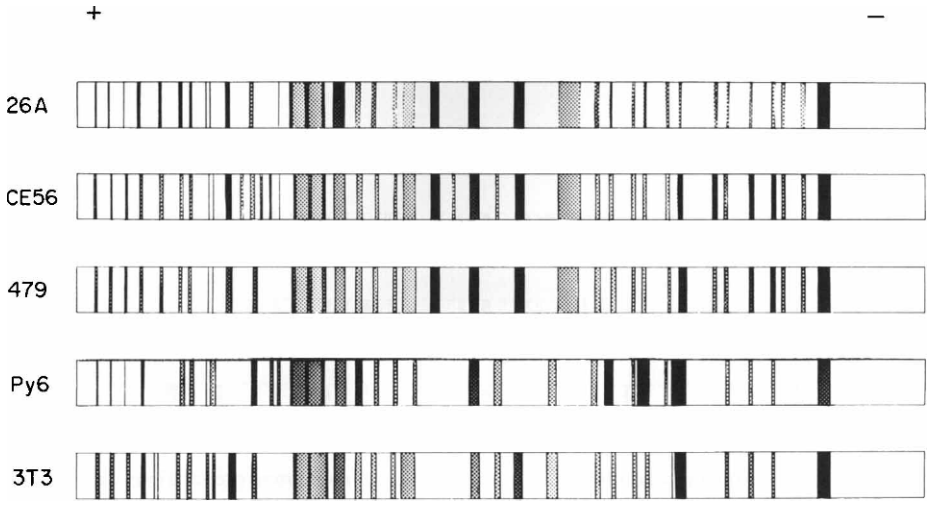

FIG. 4. Electrophoretograms of plasma membrane preparations of various 3T3 mouse cell types, analyzed in sodium dodecyl sulfate-containing polyacrylamide gels. Plasma membrane preparations (depleted of surface glycoprotein) were obtained from 3T3-SV A26, 3T3-SV CE56, 3T3-SV 479, 3T3-Py6, and control 3T3 mouse fibroblasts. These were dissolved in sodium dodecyl sulfate plus mercaptoethanol and analyzed in polyacrylamide gels as described elsewhere (Sheinin and Onodera, 1972). Material in 10 μl volumes containing, respectively, 368, 432, 443, 327 and 250 μg protein was subjected to electrophoresis at 8 mA/gel for 5–6 hours. Intensity of staining with Coomassie blue, is designated as follows: most intense, solid black, decreasing density of stain by decreasing degree of stipling. Reprinted, with permission, from Sheinin and Onodera (1972).

positive end of the gels with apparent molecular weight of $> 10^5$ daltons) are present in virus-transformed cells in lesser amount than in control 3T3 fibroblasts. In addition, the low molecular weight protein components, running at the negative end of the gels, are increased.

These observations obtained with cells transformed by the DNA viruses, polyoma and SV40, are in general agreement with those recently seen in chick embryo cells relatively early after infection with various isolates of RSV (Stone *et al.*, 1974), and under permissive conditions with a ts mutant (Wickus *et al.*, 1974). In addition, the latter infection results in a decrease in the amount of another protein of much lower molecular weight [45,000 daltons (Wickus and Robbins, 1973)].

It seemed likely from our studies with $GlcNH_2$-labeled plasma membrane preparations (Sheinin and Onodera, 1972) that the high molecular weight peptides affected in the course of transformation by polyoma and SV40 viruses are glycoproteins. This conclusion has received strong support from our recent studies (Sheinin *et al.*, 1973), in which we have

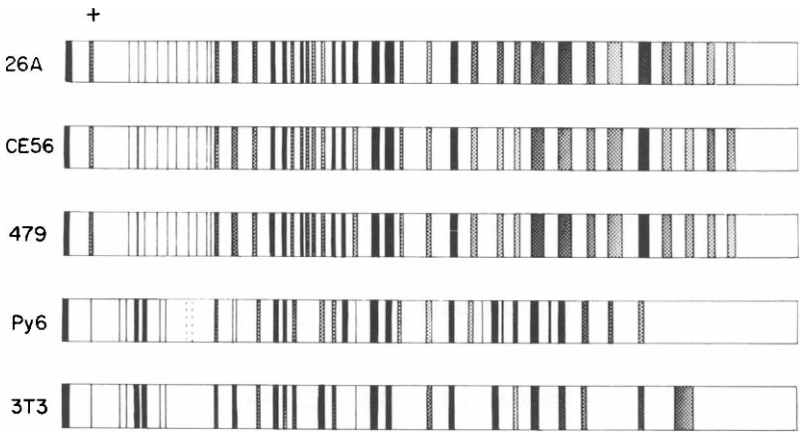

FIG. 5. Electrophoretograms of lipid-extracted plasma membrane preparations of various 3T3 mouse fibroblasts. Plasma membrane preparations (depleted of surface glycoprotein) were freed of lipid, dissolved in phenol–acetic acid and urea, and analyzed on polyacrylamide gels containing acetic acid and urea, as described elsewhere (Sheinin and Onodera, 1972). Preparations derived from 3T3-SV A26, 3T3-SV CE56, 3T3-SV 479, 3T3-Py6, and 3T3 cells contained, respectively, 40.0, 57.6, 56.8, 41.2 and 62.8 μg protein. Reprinted, with permission, from Sheinin and Onodera (1972).

been able to isolate presumably intact plasma membrane by direct processing of cells on the growing surface, without trypsin treatment. Plasma membrane so obtained from cells specifically labeled with $GlcNH_2$ in their surface glycoprotein gave profiles of electrophoresis in which the majority of the label was associated with one or two of the large molecular weight peptides.

These observations add weight to a conclusion drawn earlier (Onodera and Sheinin, 1970), that transformation by polyoma and SV40 viruses results in a change in glycoprotein at the extreme periphery of the plasma membrane. In this context it is of interest to note that the analogous large molecular weight peptides of plasma membrane from chick embryo cells, which decreased as a result of transformation by RSV, also appear to be at the external surface of the cells, as indicated by the fact that they can be labeled using iodination with lactoperoxidase (Podluso *et al.*, 1972; Stone *et al.*, 1974; Wickus *et al.*, 1974).

The final series of experiments in our comparison of the plasma membranes of normal and virus-transformed 3T3 cells were concerned with the glycolipids, which appear to be concentrated primarily in the plasma membrane of animal cells (Sheinin *et al.*, 1971; Sheinin, 1972; Yogeeswaran *et al.*, 1972, 1973). Assays of cell extracts, and extracts

of plasma membrane, again revealed great differences between normal cells and those transformed by polyoma and SV40 viruses. The data obtained with one polyoma- and one SV40-transformed cell line were generally in accord with those reported by others (see Table IV) in that marked simplification of the ganglioside pattern had occurred. However, two other SV40-transformed cell lines exhibited a complex pattern similar to that of control cells, but with a quantitative difference.

We can as yet say little with precision about the chemical basis underlying the differences observed between the glycoproteins and proteins of the plasma membranes of normal and virus-transformed cells. Analyses of glycolipids does indicate that at least in some virus-transformed cells the gangliosides have shorter and less complex carbohydrate chains, carrying fewer sialic acid residues. These observations and those of others are in accord with the hypothesis that formation of the more complex, highly sialyl-substituted gangliosides is modified in virus-transformed cells (cf. Hakomori *et al.*, 1972, 1974). Similar conclusions, but with less direct evidence, have been drawn with respect to the glycoproteins of such cells.

IV. Virus Transformation: Its Relationship to Cellular Metabolism of Surface Molecules

The extensiveness of the surface changes that accompany neoplastic transformation by viruses is evident from the data just described, as well as those summarized in Tables I and IV. An accommodation of these myriad biochemical changes, with virus genome available for coding for cell surface modulation with transformation, may come from a consideration of the postulate that integrated virus genome interferes with cellular regulatory processes that normally control the formation and turnover of surface molecules (Wallach, 1969). Such a model, shown in several forms in Fig. 6, would readily embrace the following observations.

(i) Virus transformation is associated with the appearance of species-, tissue-, or even cell-specific surface alterations, which seem not to be mediated by virus genome (cf. Haughton and Nash, 1969; cf. Alexander, 1972; cf. Tillack, 1972).

(ii) Cells that undergo virus transformation suffer a derepression of certain pathways of surface molecule synthesis. These, normally operative in cells during embryonic life, are repressed during subsequent differentiation (cf. Sachs, 1967; Boyse and Old, 1969; Hakomori and

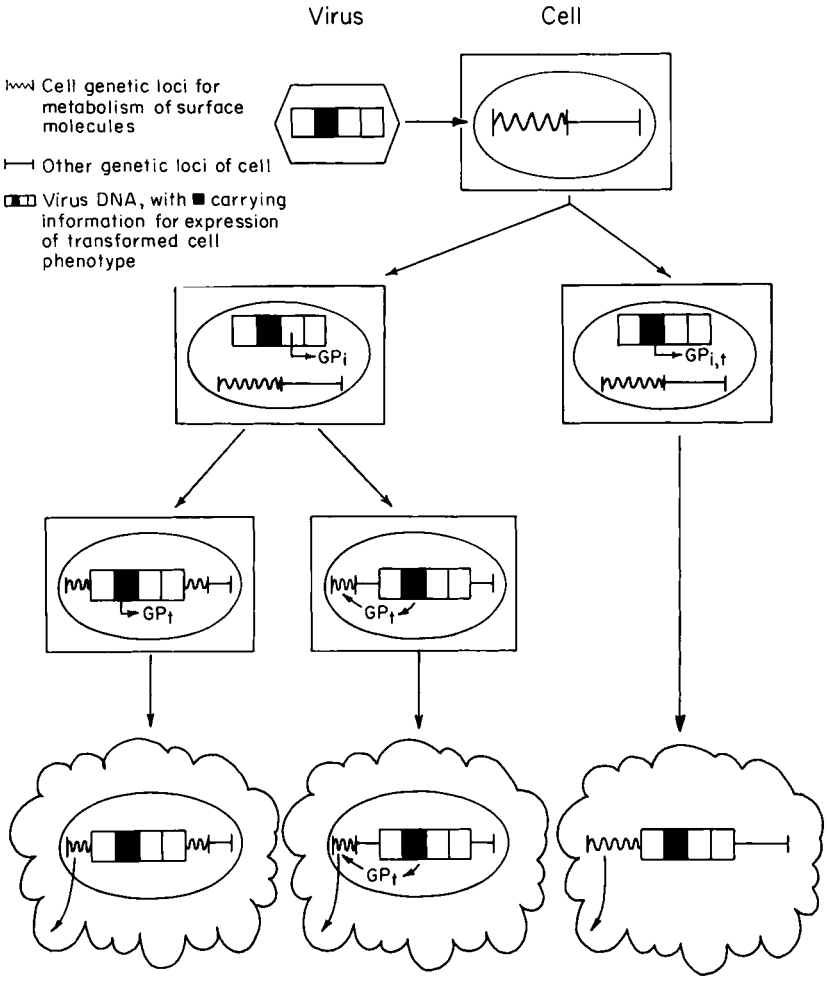

FIG. 6. Models for modulation of metabolism of surface molecules by oncogenic viruses. GP, gene product; i, for integration; t, for transformation.

Kijimoto, 1972). The end products of such metabolic sequences, present on both embryonic and transformed cells, are embraced within the general class of oncofetal antigens (cf. Alexander, 1972).

(iii) During the *in vitro* growth of normal cells, the formation of plasma membrane constituents is carefully regulated such that the formation of membrane phospholipids (cf. McMurray and Magee, 1972), neutral lipids (Bosmann and Winton, 1970; cf. Spector, 1972), glycolipids (cf. Bosmann and Winton, 1970; Hakomori *et al.*, 1972; Sakiyama

and Robbins, 1973), and glycoproteins (Bosmann and Winton, 1970; Onodera and Sheinin, 1970; cf. Kraemer, 1971; Nowakowski *et al.*, 1972; cf. Warren, 1972) assumes different patterns throughout the growth cycle. Of importance is the finding that such a shift in metabolic activity is not exhibited by virus-transformed cells.

(iv) Regulation of cell surface formation may be seen in the fact that the core plasma membrane is synthesized primarily at one stage of the cell cycle (Sheinin and Onodera, 1973; Pasternak *et al*, 1974, whereas specific glycolipid (cf. Hakomori *et al.*, 1972; Warren *et al.*, 1972; Sakiyama and Robbins, 1973), glycoprotein (Sheinin and Onodera, 1973), and protein (Kiehn and Holland, 1970; Sheinin and Onodera, 1970, 1973; cf. Warren, 1972; Pasternak *et al*, 1974) reactive groups appear to be inserted or mobilized at other, but equally specific, periods. Some properties of virus-transformed cells mimic those of cells which have not completed their full cycle of duplication (Shoham and Sachs, 1972; cf. Burger, 1973).

(v) Normal cells exhibit many of the properties of virus-transformed cells, if they are first subjected to very gentle treatment with proteolytic enzymes (cf. Burger, 1973). Recently evidence has been obtained suggesting that in virus-transformed cells, plasma membrane-associated proteases may be more active than in normal cells (cf. Burger, 1973; Schnebli, 1974).

(vi) A number of agents are known which, in normal cells, evoke many of the pleiotypic surface alterations exhibited by virus-transformed cells (cf. Kram *et al.*, 1973). In addition, virus-transformed cells selected for reversion with respect to one altered surface function can be at least partially reverted with respect to others (Wollman and Sachs, 1972; Wright, 1973).

V. Conclusions and Comments

The model that invokes modification of cellular reactions of formation of cell surface, as a primary mechanism of phenotypic expression of integrated genome of oncogenic viruses, is an agreeable one. It permits one to consolidate what has often appeared to be an enormous amount of unrelated data showing great changes in surface physiology and biochemistry as a consequence of virus transformation. It is no longer necessary to try to force agreement from different cell types (at various stages of differentiation) transformed by the same, or even different, viruses. Clearly, if the virus genes of transformation code for a product that interferes at the level of regulation of cellular pathways, then one

might expect to observe great variability depending upon the cell under study. Since major structural and functional cell surface molecules are glycolipids and glycoproteins (cf. Winzler, 1970), it is not surprising to find that the metabolism of these molecules is severely affected by transformation.

However, the key problems still remain unresolved. What is the virus gene product that so severely affects the metabolism of surface molecules in transformed cells? And what is its primary biochemical target? The second question may prove to be the more difficult to answer, since the target may vary from cell to cell. Already a number of postulates have been proposed, none of which has proved entirely satisfactory. The metabolism of cAMP, with its extremely far-reaching consequences for growth and development, has received a great deal of attention (Burger *et al.*, 1972; Johnson and Pastan, 1972; Otten *et al.*, 1972), as have transport of nutrients and their regulation of metabolic events (cf. Holley, 1972). Recently the focus has shifted to regulation of function of surface-associated enzymes, the normal action of which may be to modulate surface structure and function (Roseman, 1970; Roth *et al.*, 1971; Roth and White, 1972; Burger, 1973).

Perhaps the evidence may yet enforce the still very attractive suggestion (cf. Hakomori *et al.*, 1972) that the synthesis of the complex heteroglycopolymers (glycolipids and glycoproteins) is curtailed or modified by the virus gene product for transformation. This hypothesis rests directly on chemical evidence described above. In addition it leans on the physiological data concerning altered lectin-binding sites, receptors, antigen reactivity, cell–cell interactions of contact inhibition, adhesion and junction formation, many of which are known to be mediated by glycoproteins and glycolipids of the cell surface (cf. Winzler, 1970; and references cited in Table I). In apparent support of this hypothesis have come studies on specific glycopeptidyl and glycolipid glycosyltransferases (Grimes, 1970; Den *et al.*, 1971; Kijimoto and Hakomori, 1971; cf. Brady *et al.*, 1973; Warren *et al.*, 1972; Schengrund *et al.*, 1973) which show that the level of activity of these enzymes is lower (to the extent of 2- to 11-fold) in virus-transformed cells as compared with normal cells. However whether the decreased enzyme activity is a primary effect of virus transformation, or results from feedback control or repression, is not at all clear. The variations in enzyme activity observed are relatively small, considering the fact that the activity of glycopeptidyl-GlcNAc and sialyl transferases have been observed to vary by as much as 20-fold in 3T3 cells and 3T3-SV479 cells as they move through the cell cycle (R. Sheinin and H. Schachter, unpublished, 1972).

Perhaps there is a common target in all cell types for the action of the genes of transformation of the oncogenic viruses. It is to be hoped that this will emerge from studies with normal and virus-transformed cell variants in which surface expression can be regulated by moving from permissive to nonpermissive conditions. Similar expectations lie with revertants and re-revertants of virus-transformed cells (cf. Macpherson, 1971; McNutt *et al.*, 1973; Yamamoto *et al.*, 1973).

Turning to the question of the nature of the gene product for transformation, here too the possibilities may be several, depending upon the virus in question. For example, the simplest explanation for the action of genome of enveloped viruses is that a portion of the information for viral envelope is transmitted continuously in transformed cells, giving rise to the biogenesis of virion envelope as an integral part of the plasmalemma. Although this possibility cannot be ruled out as an essential component of the mechanism for cell surface modulation by the particular microorganisms involved, it is made less likely by the following observations.

(i) In the case of every tumor virus, neoplastic cells have been derived in which no trace of virus gene product can be detected.

(ii) Virus mutants have been isolated [from RSV (Martin, 1970; Kawai and Hanafusa, 1971) and from polyoma virus (cf. Eckhart, 1972; Benjamin, 1972)] in which the mutated gene does not define a virion peptide, but does determine a protein that is required for maintenance of the transformed cell phenotype.

Our knowledge of the reactions of plasma membrane biogenesis are still rudimentary. New avenues of biochemical and biophysical analysis of the cell surface are now opening up. These, coupled with the powerful tools of virus mutants and somatic cell variants, should permit the development of a clear picture of the synthesis, structural relationships, and structural-functional relationships of surface molecules. From such studies will undoubtedly issue a sharper comprehension of the mechanism of viral oncogenesis as it is expressed in modulation of the surface metabolism of host cells.

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