

*SURFACE-SPECIFIC CHARACTERISTICS OF A  
CONTACT-INHIBITED CELL LINE CONTAINING  
THE SV40 VIRAL GENOME\**

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*Communicated by Michael Heidelberger, January 24, 1969*

**Abstract.**—A cytoagglutinin purified from wheat germ lipase agglutinated five related murine fibroblast cell lines in the order of their saturation densities. One cell line, which was transformed by the oncogenic papova virus SV40 but which had a low saturation density, agglutinated poorly. Preincubation of cells with trypsin increased their agglutinability, but trypsin made no cell line more agglutinable than the cell line with the highest saturation density.

**Introduction.**—During transformation with the papova viruses polyoma and SV40, the viral genome is incorporated into the genetic apparatus of the cell.<sup>1</sup> Variant cell lines have recently been isolated from virally transformed cell lines of murine and hamster origin<sup>2</sup> which reverted back to the normal growth pattern insofar as they regained contact inhibition of growth in culture and had a markedly reduced ability to initiate *in vivo* growth as tumors.<sup>2</sup>

Despite their revertant phenotype, variants of a murine cell line transformed by SV40 still contained the SV40 virus genome, and lytic SV40 virus could be recovered by cell fusion with susceptible lines.<sup>2</sup> In addition, at least one gene, the one giving rise to tumor antigen production, was still expressed in this phenotypically reverted cell.<sup>2</sup>

The question arose whether surface characteristics more easily defined in biochemical terms than contact inhibition were restored concomitant with the recovery of contact inhibition.

An agglutinin has been isolated and purified from wheat germ lipase<sup>3</sup> that agglutinated several virally transformed cell lines, among them BHK21 hamster fibroblasts which had been transformed by polyoma virus, while not agglutinating the parent lines.<sup>4, 5</sup> Hapten inhibition studies indicated that the receptor site on the surface of the transformed cells contained *N*-acetylglucosamine or di-*N*-acetylchitobiose.<sup>3</sup>

It is generally assumed that the cell surface is altered during the *in vitro* transformation of cells by a tumor virus, this alteration leading to such phenomena as the loss of contact inhibition of growth in culture and metastasizing and invasive growth *in vivo*.<sup>2, 6, 7</sup> Evidence is presented here for a close correlation between the availability of the agglutinin receptor site and the loss of contact inhibition of growth characteristic of malignant cells.

**Materials and Methods.**—All cultures were maintained at 36.5° in 20-cm<sup>2</sup> plastic Petri dishes in Dulbecco and Vogt's modification of Eagle's medium supplemented with 10% calf serum.<sup>2</sup> The medium was changed twice weekly. The cells used were established murine fibroblast lines. The selection of sublines with increased sensitivity to contact inhibition is described elsewhere.<sup>2</sup>

Wheat germ agglutinin was purified from a preparation of wheat germ lipase by a method described elsewhere<sup>3</sup> and is a glycoprotein of about 26,000 molecular weight.

*Assay of agglutination:* All cell lines were seeded four days before the assay at  $10^4$  cells/plate, so that the assay was carried out only on subconfluent, dividing cultures. Cells were suspended with EDTA, pelleted at 900 rpm for 5 min, and homogeneously resuspended in serum-free medium at a concentration of  $5 \times 10^6$  cells/ml; 0.2 ml of cells and 0.2 ml of agglutinin were mixed, and a hanging drop of this preparation was examined with the microscope. Agglutination of cells was determined 15 min after the addition of agglutinin to the cell suspension. A serological scale of 0 to ++++ was used to estimate the degree of clumping. Relative agglutinability of different cell lines was estimated by assay of agglutination in the presence of decreasing amounts of agglutinin. The concentration of agglutinin needed to clump cells of different lines varied from 3 to 1000  $\mu\text{g/ml}$ .

*Results.*—Five murine fibroblast lines were assayed for agglutinability. The lines differ greatly in their saturation densities when cultured under identical conditions.<sup>2</sup> Due to contact inhibition of cell division, the cell line 3T3 has a very low saturation density<sup>8</sup> (Table 1). The SV40-transformed 3T3 line, SV101, is poorly contact-inhibited and has a saturation density approximately 11 times that of 3T3 (Table 1). F1<sup>2</sup>-SV101 was isolated from SV101 by selection with 5-fluoro-2-deoxyuridine.<sup>2</sup> Its saturation density is less than twice that of 3T3. 3T12 is a murine line of intermediate saturation density.<sup>9</sup> 3T3E is a derivative of 3T3 which lacks the enzyme thymidine kinase.<sup>9</sup> It has a saturation density approximately four times that of 3T3. Relative agglutinability of the five cell lines was in the order of their saturation densities (Table 1).

Despite the presence and partial function of the SV40 genome in both SV101 and F1<sup>2</sup>-SV101, F1<sup>2</sup>-SV101 responded poorly to the agglutinin, as predicted from its low saturation density. These data indicate that contact inhibition of cell division is a property of a cell line accompanied by the relative unavailability of a specific site on the cell surface. This site is unavailable even though the cells of the contact-inhibited lines are all dividing.

Cells that do not agglutinate under the conditions described (rat liver cells, chick embryo fibroblasts, baby hamster kidney cells) are rendered agglutinable by mild digestion of the cell surface with any of a number of proteases, includ-

TABLE 1. *Agglutinability and saturation densities of murine fibroblast lines.*

Line	Experiment	Agglutinability of Wheat Germ Agglutinin ( $\mu\text{g/ml}$ )					Saturation density (Cells/cm <sup>2</sup> $\times$ 10 <sup>-4</sup> )
		666	333	33	17	3	
3T3	(1)	+	0	0			4.0
F1 <sup>2</sup> -SV101	(1)	0	0	0			
	(2)	++	+	0			7.0
3T3-E	(1)	++++	++	+	(+)	0	18
3T12	(1)	++++	++++	+			
	(2)		++++	(+)		(+)	35
	(3)		++++	+		+	
SV101	(1)			++++	++(+)	+	46
	(2)				++(+)	(+)	

Log-phase cells were suspended with  $5.4 \times 10^{-4}$  M EDTA, and agglutination was initiated at  $2.5 \times 10^6$  cells/ml. Each agglutination was scored by more than one person. For each cell type and each experiment a control without agglutinin was observed to detect possible nonspecific clumping of the cells. No such aggregation occurred.

TABLE 2. *Agglutinability of lines following mild trypsinization.*

Line, trypsinized	Agglutinability Wheat Germ Agglutinin ( $\mu\text{g/ml}$ )				
	666	333	33	17	3
3T3		++++	+++	+++	++
Fl <sup>2</sup> -SV101			++++	+++	+
SV101		++++	++++	(+)	+

3T3 and SV101 were incubated with 0.01% trypsin for 10 min, pelleted, and resuspended for assay. Fl<sup>2</sup>-SV101 was incubated with 0.1% trypsin for 10 min, pelleted, and resuspended for assay.

ing trypsin. These enzymes expose on the normal cell surface a receptor site for agglutinin that otherwise is present but not available to the agglutinin.<sup>10</sup>

When either 3T3 or Fl<sup>2</sup>-SV101 cells were exposed to trypsin, the cells became as agglutinable as SV101 (Table 2). Exposure of the highly agglutinable SV101 to trypsin did not further increase its agglutinability.

*Discussion.*—All cells of not only the SV101 but also the Fl<sup>2</sup>-SV101 line contain SV40-specific tumor antigen, evidence that in both lines the viral genome is at least partially functional. Nevertheless, the saturation density of a cell line, rather than the presence or absence of an integrated viral genome, is correlated with the availability on the surface of the cell of specific receptor sites for agglutinin. The response of trypsinized cells indicates further that while the availability of existing surface sites varies with the saturation density of the cell line, all lines have about the same ultimate agglutinability after trypsinization, possibly reflecting the same number of sites per cell.

While normal cells from parenchymal tissues (thymocytes, hepatocytes) do not agglutinate with the wheat germ agglutinin, erythrocytes and polymorphonuclear leucocytes do so.<sup>11-13</sup> The mechanism by which the exposed surface receptor assists in the maintenance of a transformed state is under investigation.

\* Supported by USPHS grants 5T0-5-GM 01-668-05, 2 F2-AI-33,222 (R. P.) and American Cancer Society grant P-450 (M. B.).

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