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Intercellular Communication and the Control of Growth: XII. Alteration of Junctional Permeability by Simian Virus 40. Roles of the Large and Small *T* Antigens.

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Summary. We studied the action of temperature-sensitive mutant simian virus 40—a transformation-inducing DNA virus—on the junctional permeability to mono-, di- and triglutamate in rat embryo-, pancreas islet (epithelial)-, and 10T½ cell cultures. Junctional permeability was reduced (reversibly) in the transformed state. To dissect the genetics of this alteration, we used two kinds of mutant virus DNA. One kind had a temperature-sensitive mutation on the *A* gene, rendering the large *T* antigen (the gene product) thermolabile ($T^+ \rightleftarrows T^-$). The other had a deletion on the *F* gene, in addition, abolishing (permanently) the expression of the little *t* antigen (t^-). The junctional alteration occurred in the condition $T^+ t^+$, but not in the conditions $T^- t^+$, $T^+ t^-$ or $T^- t^-$. Both antigens, thus, are necessary for this junctional alteration—a genetic requirement identical to that for decontrol of growth (but distinct from that of the cytoskeletal alteration).

Key Words cell junction · cell-to-cell communication · cell-to-cell channel · gap junction · simian virus 40 · DNA virus · tumor antigens · transformation · cancer

Introduction

In this paper we examine the effects of a DNA virus on communicating cell junction, the simian virus 40 (SV40). We chose this virus because its genome is accurately mapped (Lai & Nathans, 1975; Nathans, 1976) and the two transformation-inducing genes, the *A* and the *F* gene, and their respective cellular products, the *T* and *t* antigens, are well studied (*cf.* Tooze, 1981). It thus provided us with the opportunity of gaining further insight into the genetics of the junctional alteration in the transformed state. Besides, there is evidence that the *T* antigen is bound to a phosphorylated protein (Lane & Crawford, 1979) (which, in the case of its functional homologue in polyoma virus, proved to be tyrosine phosphorylating; Courtneidge & Smith, 1983). And so the possibility of a junctional alteration did not seem too remote.

As in the preceding paper dealing with the action of an RNA virus, we resort to temperature-sensitive mutants to analyze the junctional effect of transformation. However, the analysis is more complex because there are at least two viral genes involved here, and temperature-sensitive mutants are available only for one of them. Both, *T* and *t*, are necessary for the induction of abnormal growth. Neither one alone seems sufficient for it, unless the corresponding complementing functional equivalents are supplied in the medium or by the cells (Rubin et al., 1982; Sompayrac & Danna, 1983; *see also* Fluck & Benjamin, 1979; Seif & Martin, 1979; Frisque, Rifkin & Topp, 1980).

We use cells infected with viruses that have a single mutation on the *A* gene, a mutation that renders *T* thermolabile. At 32–34/35°C (the permissive temperature range) both *T* and *t* are expressed ($T^+ t^+$) and the cells are transformed, by the criteria of loss of density-dependent inhibition of growth and of anchorage dependence. At 38.5–41°C only *t* is functionally expressed ($T^- t^+$), and the cells are not transformed (Frisque et al., 1980; M. Anderson & W.A. Scott, *unpublished work*). We will show for a fibroblastic and an epithelial cell type that in the $T^+ t^+$-transformed condition the junctional permeability is reduced.

To analyze the role played by *t* in this junctional alteration, we use cells infected with double mutant viral DNA, that can be switched by a change of temperature from the $T^+ t^-$ to the $T^- t^-$ condition. We make do here with a permanent t^- condition, given by a deletion on the *F* gene, as there are no temperature-sensitive mutants available for that gene. We resort to viral DNAs constructed by Sompayrac and Danna (1983) and by W.C. Topp that combine such an *F*-gene deletion with a temperature-sensitive mutation on the *A* gene. The rationale is straightforward: if *t* is necessary for the junctional alteration, the junctional permeability in the cells

infected by these double mutants should not be temperature-sensitive. We will show for two cell types that this is the case, indeed.

Materials and Methods

Cells

Single mutants: Rat embryo fibroblasts, NREF, infected with SV40-*ts*A209, designated NREF-*ts*209.4a (Frisque et al., 1980), a gift of Dr. W. Topp, Cold Spring Harbor Laboratory. *Rat pancreas islet cells,* clones RI-D_3, RI-C_3, RI-B_4, RI-C_5. The first two were infected with SV40-*ts*239, and designated RI-D_3-*ts*d_1 and RI-C_3-*ts*d_1; RI-B_4 was infected with SV40-*ts*A58; RI-C_5, with SV40 *dl*54/59(2005).

The temperature-sensitive mutations (Tegtmeyer & Ozer, 1971; Tegtmeyer, 1972) are on the *A* gene Hin-fragment *I* (different loci) (Lai & Nathans, 1974; 1975).

Double mutants: Rat pancreas islet cells, clones RI-B_2, infected with SV40-*dl*54/59(2009)/*ts*A58. These clones, including those above infected with single-mutant virus, were a gift of Dr. W.A. Scott, University of Miami. They were obtained by M. Anderson and W.A. Scott (*unpublished work*) from primary pancreatic islet cells of newborn Wistar-Lewis rats, following a technique described by Niesor, Wollheim, Mintz, Blondel, Renold and Weil (1979); the letters *B*, *C*, *D*, identify the original primary isolate, and the subscripts, the clones. *Mouse C3H 10T½ cells* infected with SV40-*dl*54/59(884)/*ts*A58, designated 10T½-C36, C39, C42, C43, C44, or infected with SV40 wild-type, designated C98 (Sompayrac & Danna, 1983), a gift of Dr. L. Sompayrac, University of Colorado, Boulder.

The *dl*54/59(884)/*ts*A58 DNA was constructed by Sompayrac and Danna (1983) and the *dl*54/59(2009) DNA, by W.C. Topp by ligating a segment containing a deletion between 54 and 59 map units of the *F* gene with a segment containing a temperature-sensitive mutation on *Hin* fragment *I* of the *A* gene.

Medium

All cultures were grown in Dulbecco's MEM supplemented with 10% fetal calf serum in an incubator (6–8% CO_2). The cells infected with wild-type virus were grown at 37°C and those infected with temperature-sensitive virus at 33°C, except where stated differently.

Temperature Changes and Junctional Probing

To shift their temperature to the nonpermissive level, the cultures were moved to another incubator at 41 or 39°C. During the junctional testing the culture dishes were on the microscope stage at room temperature, 30–32°C. The tests were limited to a period of 10–15 min over which the junctional transfer did not change with temperature. (In the SV40 transformed cells, the earliest junctional response set in 4 hr after a shift of temperature.) The devices for temperature control used in the first paper of this series were therefore omitted. The incidence of permeable interfaces was scored within 5–10 min of the microinjection of the probe.

The comparisons of junctional transfer were made between parallel subcultures, as in the first paper of this set (Azarnia & Loewenstein, 1984*a*). For comparisons at matching cell densities, those cells growing more slowly at the nonpermissive temperature were seeded at higher initial density (the time from seeding to junctional testing was constant).

Depending on the cell density, the numbers of first-order neighbors per source cell ranged 3–12 in the rat embryo fibroblast, 2–10 in the pancreas islet cells, and 4–11 in the C3H 10T½ cells.

The methods for microinjection, scoring of incidence of permeable interfaces, statistical treatment, and measurement of fluorescence loss are described in the first paper.

Results

As before, we used those probes, of the list in Table 1 of the preceding paper, which were close to the threshold of junction permeation in the transformed state of the cell. This provided us with a sensitive means for detecting increases in junctional transfer during the transition to the normal state. The probes were: LRB-Glu for the various cells infected with single-mutant virus, and LRB-Glu_2 or LRB-Glu_3 for those infected with the double mutant. In the transformed state, the molecule of the immediately lower size rung in the list was generally still well below the saturation range of our method of measuring transfer. Thus, fluorescein in the first group of cells and LRB-Glu in the latter were useful probes, in addition.

We injected the probes as single species into the cells, and determined their junctional transfer in the transformed condition—the habitual background state of the cells—and in the untransformed condition, after shifting the temperature (parallel subcul-

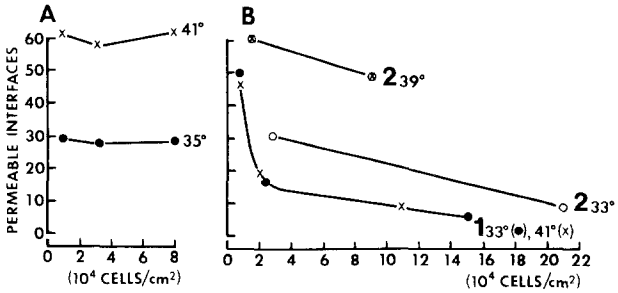

Fig. 1. Density dependence of junctional transfer. (*A*): Rat embryo cells, NREF-*ts*A209. The mean incidence of C-fluorescein permeable interfaces (%) *vs.* cell density at the permissive temperature, 35°C, and the nonpermissive temperature, 41°C (after 4 hr at the last temperature). Data from parallel subcultures. (*See* series 1 of Table 1 for standard errors and confidence levels, *P*, of the differences between the corresponding points of the curves.) (*B*): Rat pancreas islet cells (RI-1D_3-*ts*d_1) probed with LRB-Glu. Two series of experiments (*1, 2*) at the permissive temperature, 33°C, and the nonpermissive temperature, 41 or 39°C; about 4.5 hr at the nonpermissive temperature in series *1*, and 24 hr in series *2*. The data for each series are from parallel subcultures. (The data in *B* are not included in Table 2)

Table 1. Incidence of permeable interfaces at permissive and nonpermissive temperatures. Rat embryo fibroblasts-*ts*A209.4a

Cell system	Experiment series #	Density (10^4 cells/cm^2)			Probe	Time at 41°C (hr)[c]	Permeable interfaces %[a]			
		33°C	35°C	41°C			33°C	35°C	41°C	P[b]
NREF-*ts*A209	1		1.0	1.0	C-fluorescein	4		29 ± 8	62 ± 4	0.0009
	1		3.2	3.2	C-fluorescein	4		26 ± 4	56 ± 5	0.00009
	1		8.0	8.0	C-fluorescein	4		28 ± 4	63 ± 4	0.00002
	2	1.0		1.0	C-fluorescein	5	62 ± 4		81 ± 4	0.005
	3	1.5		1.5	C-fluorescein	5	50 ± 8		76 ± 5	0.005
	4	2.0		2.0	C-fluorescein	5	53 ± 6		87 ± 4	0.0001
	5	5.1		5.0	C-fluorescein	24	25 ± 6		72 ± 8	0.0008
	6	9.0		7.0	C-fluorescein	24	30 ± 6		68 ± 7	0.0018
	7	—		—	C-fluorescein	48	31 ± 18		82 ± 6	0.022
	8	4.0		5.0	LRB-Glu	5	16 ± 6		62 ± 5	<0.0001
	9	7.6		7.1	LRB-Glu	5	20 ± 6		57 ± 4	<0.0001
	10		3.9	2.6	LRB-Glu	24		20 ± 4	65 ± 2	<0.00009

[a] Mean incidence of (first-order) permeable cell interfaces ±SE at the permissive—33, 35°C—and nonpermissive—41°C—temperatures. The total number of cell interfaces examined for each datum ranged 27–211.

[b] Statistical confidence level (*t*-test) of the difference between the means at the permissive and nonpermissive temperatures.

[c] The cultures were habitually kept at the permissive temperature. This column gives the time they had been at the nonpermissive temperature, when the incidence of permeable interfaces was determined.

The data for the permissive and nonpermissive temperatures in each series are from parallel subcultures. The series numbers (and the series letters in Table 2) identify strictly parallel sets of experiments.

Table 1*A*. Data summary. Rat embryo fibroblasts-*ts*A209.4a

Cell system	Experiment series #	Probe	Permeable interfaces %[a]		
			33/35°C	41°C	P
NREF-*ts*A209	1	C-fluorescein	29 ± 4 (33)	60 ± 3 (33)	<0.00009
	2–4	C-fluorescein	54 ± 4 (30)	81 ± 3 (33)	<0.00009
	5–7	C-fluorescein	29 ± 5 (29)	74 ± 4 (15)	<0.00009
	8–10	LRB-Glu	19 ± 3 (31)	61 ± 2 (28)	<0.00009

[a] Mean value ± SE. The comparable data of Table 1 are lumped (*see* text). In parentheses, the number of injected (source) cells (the *n* for all statistics).

tures) to the nonpermissive level for the virus. In response to such upshifts—a switch from the T^+ to the T^- state—we found an increase in the junctional transfer in the cell types transformed by virus DNA with the single mutation, but not in those transformed by virus DNA with the double mutation.

Cells Transformed by Single Mutants

Rat Embryo Fibroblasts-tsA209

These cells gave a temperature response at all densities. With either LRB-Glu or carboxyfluorescein, the incidences of permeable interfaces were markedly increased when the temperature was raised from the permissive range, 33/35°C, to the nonpermissive level, 41°C. The effect was clear by 4 hr and was reversible. It resembled the effect of the Rous sarcoma virus, but in slower tempo (*see* Fig. 3 of the first paper). Table 1 gives the results, series by series; the strictly comparable data, those obtained at equivalent density in parallel runs with parallel subcultures (and identical serum supplement), are aligned horizontally.

As in the cells transformed by the RNA virus, the cell density here did not sensibly influence the junctional transfer over the experimental range, at either temperature (series 1, Table 1; and Fig. 1*A*). Nor did the length of stay at the nonpermissive temperature seem to be a major factor, beyond 4 hr (Table 1). We lumped in Table 1*A*, therefore, the data from the different experimental series in which

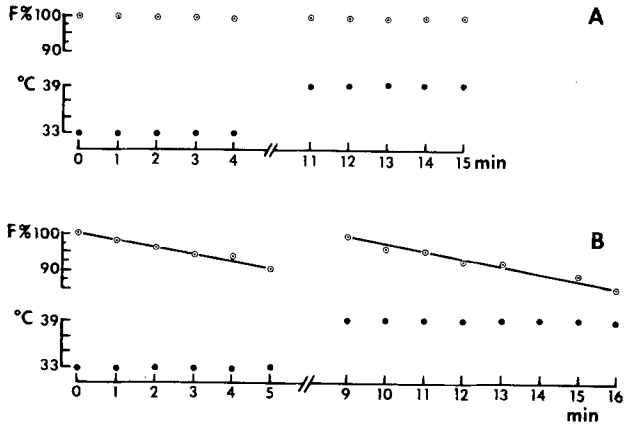

Fig. 2. Rates of fluorescence loss at the permissive and nonpermissive temperatures. Rat embryo fibroblasts-*ts*A209. The LRB-Glu fluorescence intensity (*F*) as a function of time. (*A*): A typical example exhibiting no detectable loss. (*B*): An example of a rare leakier cell; the rate constants are 0.18 and 0.21 min^{-1} at 33 and 39°C, respectively. *F* is the photodiode voltage (logarithmic scale) normalized in respect to the first value in each segment (100%). There is no significant photobleaching with the present excitation parameters (*see* inset of Fig. 5 of the first paper of this set)

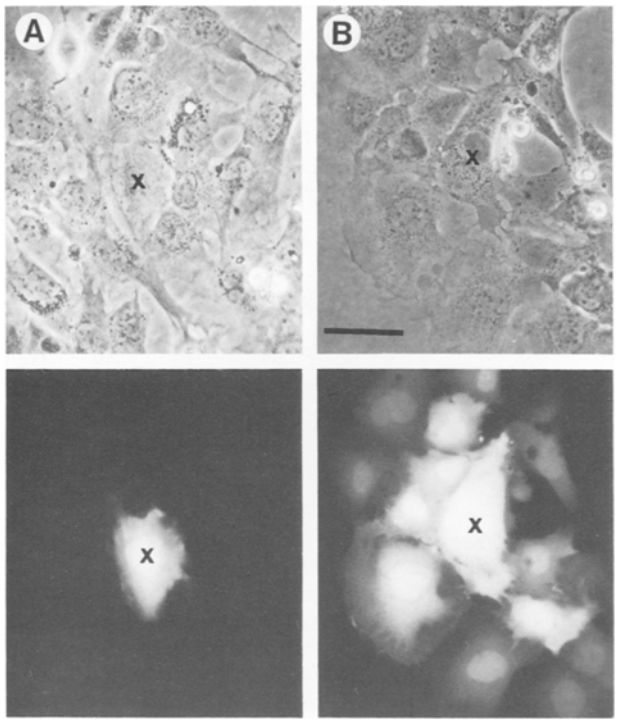

Fig. 3. Cell-to-cell transfer of fluorescent-labelled glutamic acid in pancreas islet cells ($RI\text{-}B_4$) infected with temperature-sensitive SV40 (*ts*A58). (*A*) At the permissive temperature, 33°C, and (*B*) 24 hr after raising the temperature to the nonpermissive level, 41°C. *Top*, phase contrast photomicrograph. *Bottom*, same cells in darkfield. LRB-Glu was injected into cell marked *x*. *A* and *B* are different cell groups. Calibration, 100 μm

the cultures had the identical serum supplement. The virus effect on junctional transfer is as clear there as it is in the more stringent comparisons of Table 1.

Is this effect on junctional transfer due to a change in junctional permeability or to a change in nonjunctional membrane permeability? The latter mechanism was ruled out by measurements of the rates of loss of the LRB-Glu probe from the cells. The rates of fluorescence loss (single cells) were typically virtually zero over 10 min, the maximum period of our scorings of permeable interfaces (Fig. 2*A*). Occasionally, we encountered somewhat leakier cells. But even those had loss rate constants below 0.3%/min, negligible over our times of scoring of junctional transfer (Fig. 2*B*). The situation is analogous to that of the cells transformed by Rous sarcoma virus (Azarnia & Loewenstein, 1984*a*). And the same reasoning as used in that case leads us to conclude that the permeability of the junctional membrane is the determinant of the modifications of junctional transfer: *the junctional permeability is reduced in the SV40-transformed state.*

Rat Pancreas Islet Cells-tsA239 and tsA58

These epithelial cells, unlike all the other transformed cell types we had dealt with so far, were density-sensitive; their junctional transfers diminished with increasing cell density, at least over the range of 0.8–10 (10^4 cells/cm^2) (Fig. 1*B*). Therefore, care had to be taken to closely match the densities of the subcultures used for the comparisons at the permissive- and nonpermissive temperature.

The outcome was the same as in the experiments with the embryo fibroblasts. In the strains of the pancreas islet cells infected with *ts*A239 (the strains $RI\text{-}1D_3$ and $RI\text{-}C_3$), as well as in strains infected with *ts*A58 ($RI\text{-}B_4$), junctional transfer was lower at the permissive temperature than at the nonpermissive one. Figure 3 illustrates an experiment with the last strain, and Tables 2 and 3 summarize the results obtained with all. (The data from one infelicitous match of cell densities—series *d*—are included in Table 2, because the junctional transfer is not very sensitive over that density range; *see* Fig. 1*B*.)

The responsiveness to temperature upshifts seemed to be somewhat slower in these cells than in the embryo fibroblasts. In a series of trials in which the junctional transfer was tested at about 2, 4.5 and 5 hr after the upshift, junctional transfer was found to have increased significantly at the last time point

Table 2. Incidence of permeable interfaces at permissive and nonpermissive temperatures. Rat pancreas islet cells-*ts*A239

Cell system	Experiment series	Density (10^4 cells/cm^2)			Probe	Time at 41°C (hr)	Permeable interfaces %[a]			
		33°C	39°C	41°C			33°C	39°C	41°C	*P*
RI-1D$_3$-*ts*d$_1$	a	2.8		3.0	LRB-Glu	5	18 ± 5		51 ± 5	0.0001
	b	4.3		4.0	LRB-Glu	5	36 ± 3		56 ± 2	<0.0001
	c	8.7		8.2	LRB-Glu	5	10 ± 3		33 ± 4	0.0001
	d	21.0	9.1		LRB-Glu	24	9 ± 13	48 ± 10		<0.00009
RI-1C$_3$-*ts*d$_1$	e	10.0		9.7	C-fluorescein	5	56 ± 6		80 ± 4	0.003

[a] Mean ± SE. The number of interfaces examined for each datum ranged 40–115.

Table 3. Incidence of permeable interfaces at permissive and nonpermissive temperatures. Rat pancreas islet cells-*ts*A58

Cell system	Density (10^4 cells/cm^2)		Probe	Time at 41°C (hr)	Permeable interfaces %[a]		
	33°C	41°C			33°C	41°C	*P*
RI-B$_4$-*ts*A58	5.2	4.3	C-fluorescein	24	62 ± 4	93 ± 3	<0.00009
	5.2	4.3	LRB-Glu	24	20 ± 3	66 ± 5	<0.00009
Controls							
RI-B$_4$-*wt*	2.5	2.2	C-fluorescein	24	82 ± 4	87 ± 4	0.19
	2.5	2.2	LRB-Glu	24	53 ± 2	55 ± 4	0.37
RI-C$_5$-*dl*54/59(2005)	6.3	5.1	C-fluorescein	24	4 ± 3	1 ± 1	0.22
	6.3	5.1	LRB-Glu	24	0 ± 0	0 ± 0	

[a] Mean ± SE. The numbers of interfaces examined for each datum ranged 51–64.

only. (Fig. 1, series 1, gives an example of a lack of the response at about 4.5 hr, as determined over a range of cell density.)

Controls with cells infected with wild-type virus gave no response to temperature, as demonstrated by probings with LRB-Glu (Table 3, RI-B$_4$-*wt*).

We also included the data from saturation-range probings with carboxyfluorescein in the control section of Table 3 because, like the LRB-Glu data, they bring out a difference between the wild-type- and temperature-sensitive transformants (RI-B$_4$-*ts*A58): the junctional transfers at 33°C are higher in the wild-type transformants (*P*, 0.002 and <0.00009 for carboxyfluorescein and LRB-Glu, respectively). This echoes the situation encountered with Rous sarcoma virus (Azarnia & Loewenstein, 1984*a*).

Another set of controls using cells infected with virus with an *F*-gene deletion (RI-C$_5$-*dl*54/59), which, in a way, also served as control for the experiments with the double mutant, gave no response to temperature, either (Table 3). These data should not be compared with those of the cells infected with the wild-type or the temperature-sensitive mutants (vertically in Table 3), as the experiments lack the needed culture parallelism.

Controls Against Transfer by Protoplasmic Bridges

To control against possible contribution to the cell-to-cell transfer by protoplasmic bridges, we injected the junction-impermeant fibrinopeptide (fluorescein isothiocyanate-labelled) into embryo fibroblasts (NREF-*ts*A209) and pancreas islet cells (RI-1D$_3$-*ts*d$_1$). In both cell types, such contributions proved to be negligible. The frequency of the transfer was 1.0% at 33°C and 2.3% at 41°C in the former cells, and 1.5% at 33°C and 0% at 41°C in the latter.

Cells Transformed by Double Mutants

Rat Pancreas Islet Cells—d154-59/tsA58

These cells exhibited no temperature sensitivity in their junctional transfer (Table 4). They were more

Table 4. Incidence of permeable interfaces of rat pancreas islet cells infected with double mutant *dl*54/59(2009)/*ts*A58

Cell system	Experiment series #	Density (10^4 cells/cm^2)		Probe	Time at 41°C (hr)	Permeable interfaces %[a]		
		33°C	41°C			33°C	41°C	*P*
RI-B_2-*dl*54/59(2009)/*ts*A58	12	6.8	7.3	LRB-Glu	24	88 ± 3	86 ± 4	0.3
	12	6.8	7.3	LRB-Glu_2	24	77 ± 2	83 ± 4	0.1
	12	6.8	7.3	LRB-Glu_3	24	48 ± 10	43 ± 4	0.3

[a] Mean ± SE. Each mean value is based on the examination of 52–65 interfaces.

junction-permeable at both temperatures, the permissive and nonpermissive, than any of the pancreas islet cells infected with the single-mutant virus, as shown by the probings with LRB-Glu, LRB-Glu_2 and LRB-Glu_3. Even the large and charged LRB-Glu_3 gave incidences of permeable interfaces of the order of 40%. However, there was no sign of an increase in that incidence, nor in that of the other (less sensitive) probes, when the temperature was raised from the permissive to the nonpermissive level.

This transformed cell strain is the analytical counterpart for the foregoing cells infected with single-mutant virus, in particular for the RI-B_4 strain. Both strains stem from the same primary cell isolate (*B*) and both carry a viral genome with the identical temperature-sensitive *A*-gene mutation (*ts*A58). They differ by the *F*-gene deletion (*dl*54-59) of the double mutant. *Thus, we conclude that the absence of junctional responsiveness to temperature is due to that deletion, that is, to the t^- phenotype of the cells infected with the double-mutant virus.*

10T½ Cells—dl54-59(884)/tsA58

These cells behaved like the double-mutant transformants above. We examined five strains of 10T½ cells (C-36, C-39, C-42, C-43, and C-44) infected with double-mutant DNA. The *F*-gene deletion here was similar to that in the pancreas islet cell system, and the temperature-sensitive mutation was identical. None of these strains showed a change in junctional transfer upon upshift of temperature (Table 5).

Discussion

On the Genetics and Epigenetics of the Junctional Alteration

The present results reveal that SV40 causes reduction of junctional permeability. Both, the *A* and the *F* gene of the virus play a role: the junctional alteration was not produced in conditions where either the large *T* antigen (the *A* gene product), or the little *t* antigen (the *F* gene product) failed to be expressed. Both proteins seem necessary for the junctional effect.

We don't know by what mechanisms these proteins alter the permeable state of the cell-to-cell channel. An attractive possibility is that they act by way of the *cellular src* gene product, $pp60^{c\text{-}src}$. This idea is nursed by the knowledge that *T* is present at the cell membrane and occurs bound to phosphoprotein (Lane & Crawford, 1979; Tevethia, Greenfield, Flyer & Tevethia, 1980) and that the middle tumor antigen of polyoma virus—a functional homologue of SV40 *T*—binds to $pp60^{c\text{-}src}$, enhancing its tyrosine kinase activity (Courtneidge & Smith, 1984; Bolen, Thiele, Israel, Yonemoto, Lipsich & Brugge, 1984; *see also* Eckhart, Hutchinson & Hunter, 1979). Thus, it is not inconceivable that SV40 exerts an action on the cell-to-cell channel by the same final tyrosine phosphorylating mechanism as the Rous sarcoma virus does. The finding that the junctional effect induced by SV40 is slower than that induced by Rous sarcoma virus is in keeping with this idea.

The requirement for both *T* antigens sets the genetics of the junctional alteration apart from those of the cytoskeletal alteration by SV40. For the latter, the small *t* seems sufficient (Frisque et al., 1980). Thus, as in the case of the Rous sarcoma virus, a junctional effect by way of the cytoskeleton appears unlikely.

On the other hand, the requirement for the two antigens is like that for decontrol of growth (Rubin et al., 1982; Sompayrac & Danna, 1983). The same viral genes determine the junctional alteration and the growth-control alteration, and this also holds for the *src* gene, as we have seen in the two preceding papers. This genetic correspondence is consistent with the notion that the junctional alteration plays a role in the decontrol of growth (Loewenstein, 1979). We examine the implication further, below.

Table 5. Incidence of permeable interfaces in rat 10T½ cells infected with double mutant *dl*54-59/*ts*A58

Cell system	Experiment series #	Density (10^4 cells/cm^2)		Probe	Time at 41°C (hr)	Permeable interfaces %[a]		
		33°C	41°C			33°C	41°C	*P*
10T½-C-36	1	5.5	5.9	LRB-Glu	5	20 ± 8	18 ± 7	0.4
10T½-C-36	2	5.5	8.0	LRB-Glu$_2$	24	14 ± 3	15 ± 3	0.4
10T½-C-39	3	8.7	10.0	LRB-Glu	5	69 ± 6	59 ± 3	0.08
	4	3.1	3.7	LRB-Glu	24	64 ± 4	52 ± 4	0.03
10T½-C-42	5	2.5	3.5	LRB-Glu	5	20 ± 8	21 ± 7	0.4
	6	2.6	2.3	LRB-Glu	24	22 ± 6	27 ± 6	0.3
10T½-C-43	7	4.9	6.6	LRB-Glu	5	11 ± 5	13 ± 5	0.4
	8	5.8	5.2	LRB-Glu	24	26 ± 6	30 ± 6	0.3
10T½-C-43	9	17.0	13.0	LRB-Glu$_2$	24	16 ± 3	21 ± 4	0.2
10T½-C-44	10	3.5	3.5	LRB-Glu	5	19 ± 6	17 ± 6	0.4
10T½-C-98 *wt*[b]	11	6.0	6.6	LRB-Glu	24	43 ± 4	46 ± 7	0.35

[a] Mean ± SE. The number of interfaces examined per datum ranged 53–100 interfaces.
[b] Wild-type transformant.

Implications in Growth Control

For the purpose of this discussion, we will distinguish between radical defects, where the junctional deficiency is so extreme that communication is virtually shut off to all molecular species, and subtler deficiencies, such as the permeability reductions by the two viruses here. The two kinds of defect and their relations to growth control have different heuristics. The radical defect, in terms of our hypothesis, would be invariably associated with growth decontrol (but not growth decontrol invariably with the radical defect, of course). Indeed, the experimental evidence has borne this out (Loewenstein, 1979). Moreover, studies on somatic hybrids between such defective cells and normal ones showed, for all hybrids and their segregants, an association between the traits of normal communication and normal growth, on the one hand, and the traits of defective communication and defective growth control, on the other (Azarnia, Larsen & Loewenstein, 1974; Azarnia & Loewenstein, 1977).

The expectations for the subtler defect are not as clear cut. These would depend on the magnitude of the permeability change (specifically) for the growth-controlling molecule (signal), the concentration threshold for control by that molecule, and the volume parameters determining its concentration in the cellular compartments—in final effect, on how much that concentration is shifted with respect to the control threshold—all of which are unknown. Only if the permeability deficiency is severe enough for a critical shift of the signal concentration, would a junctional alteration become a *cause* of growth decontrol (*see* Loewenstein, 1979, for models). So, the question of whether the junctional deficiency by the DNA and RNA viruses plays a role in cancer etiology stays open. The deficiency, no doubt, is a strong candidate for such a role, but a definitive answer may have to wait until the growth-controlling molecule is identified. Meanwhile, there is something to be learned from a genetic analysis of the (subtle) junction-deficient trait. One would like to know how general this deficiency is among virus-transformed cell types and, more to the point, whether the association between this trait and that of growth decontrol will stand the test of genetic reversion and segregation. A set of 3T3 cells revertant from SV40 transformation, currently under study, should tell us just that.

Electrically Coupled SV40-Transformed Cells

Mouse 3T3 and hamster fibrosarcoma cells transformed by SV40 have been found to be electrically coupled to a degree not distinguishable from their normal counterparts (Furshpan & Potter, 1968; Borek, Higashino & Loewenstein, 1969). There is no conflict between these earlier results and the present ones. The electrical measurements were not sensitive enough for detection of the subtle difference in junctional permeability, for the same reasons already discussed regarding the electrical coupling found in cells transformed by Rous sarcoma virus (Azarnia & Loewenstein, 1984*a*).

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