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Variable Defectiveness for Lytic Growth of the *dl* 54/59 Mutants of Simian Virus 40

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When viral growth in TC-7 cells is compared with that in the simian virus 40 (SV40) transformed CV-1 line C6 some mutants of SV40 deleted between 0.54 and 0.59 on the standard map (*dl* 54/59 mutants) give relative bursts similar to those of wild-type strain 776, whereas others grow markedly poorer in the untransformed cell. In general, viruses which are defective by this criterion have been found to produce neither a fragmentary small-t protein nor a mature small-t mRNA.

Deletion mutants of simian virus 40 (SV40) which map between 0.54 and 0.59 on the standard map are viable in culture (6, 10, 11). Although an early viral protein, small-t (molecular weight, 17,000) (8), is encoded within this region and is substantially deleted as a result of these mutations (3, 11), the effects on viral growth are small and thought to be unrelated to either the size or location of the deletion (10). Early reports also indicated that these small-t deletions had little or no effect on the efficiency of these mutants to transform cells in culture (10). However, more recently a number of studies have shown that the *dl* 54/59 mutants are highly defective in their ability to transform rat cells (2, 11) but not hamster cells (7) when transformation is assayed as the acquired ability to grow suspended in agar or methylcellulose. The reason for the discrepancy is that the *dl* 54/59 mutants induce a phenotype of transformation in rat cells which is partially defective (11). Cells transformed by these viruses form colonies with a transformed morphology but are unable to grow in suspension.

During the course of these transformation studies I noticed that not all the *dl* 54/59 mutants were equally defective; certain viruses consistently gave efficiencies of transformation significantly above both background levels and those of the tighter mutants (2, 11). Similar trends are observed in viral tumorigenicity (W. C. Topp, D. Rifkin, and M. J. Sleigh, manuscript in preparation).

Data support the fact that deleted proteins should be highly defective. Because I wondered how this could be the case, I reexamined the growth of these viruses in an attempt to find some reflection of the trends observed in nonpermissive cells.

Wild-type SV40 strain 776 and the *dl* 54/59 mutants were plaque purified, grown, and titrated on CV-1 cells. Comparison growth curves were carried out on C6 cells (an SV40 transformed CV-1 permissive for viral growth [4]) and on TC-7 cells, an AGMK line which holds a low saturation density.

I knew that all *dl* 54/59 mutants grow well enough in cultured AGMK lines to form plaques. I reasoned that if the viral small-t protein were important for any aspect of lytic growth the *dl* 54/59 viruses would grow better in C6 cells than in TC-7 cells. The endogenous SV40 genome of C6 produces a small-t protein which should complement this defect (9). (The large-T protein in C6 is presumably defective in that it will not complement *tsA* mutants [4]). I chose as our parameter of viability the ratio of viral burst in TC-7 to that in C6. This dimensionless number can be used to compare one virus with another regardless of input multiplicity (viral titer) if normalized to the ratio of TC7:C6 for wild-type virus strain 776. In general we found the viral burst of 776 to be 5- to 10-fold lower in C6, perhaps reflecting interference with the infecting viral large-T by the defective endogenous large-T of C6 (4). In a similar manner we have found that the plaquing efficiency of wild-type SV40 is suppressed up to 10-fold by a lawn of tsA virus at 40°C.

Initial growth curves on several viruses showed a substantial increase in infectious virus 2 to 2.5 days postinfection, so for my comparison, virus was harvested 3 days postinfection. Confluent plates of TC-7 and C6 were infected with aliquots of virus, incubated for 3 days, and frozen and thawed, and the lysates were sonicated immediately before titration. When high input multiplicates were used (2 to 5 plaque-forming units [PFU]/cell) there was no difference between the mutant and wild-type ratios of TC7:C6 (data not shown). However, at lower multiplicities (0.2 to 0.5 PFU/cell) a number of the

mutants consistently grew better in C6 (the helping cell) than in TC7, a defect which could apparently be overcome in high-multiplicity infections. The ratios (viral burst TC7:viral burst C6) for these low-multiplicity infections normalized to a wild-type ratio of 1 are shown in Table 1. Although the differences are small, ranging over only an order of magnitude, they are reproducible. Each point is the average of three independent experiments, with the normalized values differing very little from one experiment to another. (The ratio of burst TC7:burst C6 for 776 ranged from nearly 1 to 10; once normalized to this number the mutant values differed little.) For the purposes of discussion we can arbitrarily assign the viruses into the following three categories (Table 2): defective (e.g., *dl* 884), nondefective (*dl* 891), and virulent (*dl* 2007).

Although we initially thought that the endogenous small-t of C6 would complement the viral defect, this is not the case. A T-antigen-negative CV-1 subline which has spontaneously transformed also serves as a helping cell (data not shown). It is likely that the ability of TC7 to reach and hold a low saturation density with very few cells in S phase reveals the small-t defect. Indeed, if exponentially growing TC-7 are infected, *dl* 884 gives a burst only twofold reduced from that of 776, whereas infection of cells 1 day past confluence yields a six- to eightfold difference. These results are in agreement with those of Martin et al. (7) that *dl* 54/59 mutants are defective only in their ability to transform quiescent hamster cells. These trends are similar to those observed in the growth of the *hr-t* mutants of polyoma virus, except they are much less pronounced (1), perhaps because the *hr-t* mutation is actually affecting two viral proteins (5), whereas the *dl* 54/59 mutation alters only one.

TABLE 1. *Ratios of TC7:C6 of dl 54/59 mutants normalized to a wild-type ratio of 1*[a]

Virus	Ratio	(Range)
dl 2000	1.49	(1.4–1.65)
dl 2001	0.85	(0.83–0.90)
dl 2003	0.42	(0.40–0.47)
dl 2004	0.70	(0.57–0.79)
dl 2005	0.68	(0.55–0.80)
dl 2006	1.03	(0.88–1.25)
dl 2007	2.05	(1.6–2.3)
dl 2008	0.96	(0.70–1.35)
dl 883	0.75	(0.60–0.78)
dl 884	0.54	(0.50–0.68)
dl 885	1.41	(1.1–1.7)
dl 886	1.03	(0.95–1.2)
dl 888	0.48	(0.35–0.54)
dl 890	1.40	(1.0–1.78)
dl 891	1.13	(1.0–1.2)

[a] Cells were infected at confluence with a multiplicity of infection of 0.2 PFU/cell. After 3 days, monolayers were frozen, thawed, and sonicated, and the virus was titrated. The numbers are normalized by dividing all values by the ratio of viral titer grown in TC-7 to viral titer grown in C6 for wild-type SV40 strain 776 which was obtained in each experiment. All numbers are the averages of at least three independent experiments. A value of 0.4 would mean that a virus grew relatively more poorly in TC-7 than in C6 as compared with wild-type viral growth and was thus defective for growth on normal cells.

TABLE 2. *Three classes of dl 54/59 mutants*[a]

Defective	Nondefective	Virulent
2003	Wild-type (776)	890
2004	2001	885
2005	2006	2000
883	2008	2007
884	886	
888	891	

[a] Viruses with ratios of TC-7:C6 in Table 1 between 0.4 and 0.75 were assigned to the defective category, those between 0.8 and 1.2 were assigned to the nondefective category, and those 1.4 and greater were assigned to the virulent mutant category. Although there is some natural clustering into these categories, the cut-offs are essentially arbitrary.

It is interesting that those mutants classified as nondefective for lytic growth in Table 2 (e.g., *dl* 2001 or *dl* 890) appear to be leaky for viral transformation and tumorigenicity, whereas those that are defective for lytic growth are tight mutants (e.g., *dl* 884 or *dl* 2003) (10; Topp, Rifkin, and Sleigh, in preparation), although there are exceptions (e.g., *dl* 2004). It is likely that the same viral defect produces both phenotypes.

Khoury et al. (6) have recently reported on the small-t mRNA and protein species produced by the *dl* 54/59 mutants. A comparison of their results with mine immediately shows that five of the six tightest mutants have lost sequences important for message splicing and therefore can not produce a mature small-t message species. All the other *dl* 54/59 mutants should produce an abbreviated small-t message encoding a fragmentary small-t protein. Khoury finds that this is the case, with the fragmentary proteins differing little in stability from those of wild-type small-t (6).

It is striking that the inability to produce a small-t mRNA that would encode a fragmentary small-t should define a class of tight small-t mutants. The viral large-T protein autoregulates its own level, so it is unlikely that the efficiency or inefficiency of large-T production can account for this result. One possibility for this result is that the fragmentary protein produced (6, 9)

when a small-t mRNA matures retains some biological activity.

A second possibility is that the ability to make the small-t splice at 0.54 is important to the production of an RNA species, which serves either as precursor for further splicing events to produce a message (as yet unknown) that is important for both transformation and viral lytic growth or which possesses a biological activity of its own. If the biological activity of a fragmentary protein is involved this suggests that *dl* 2006, a nondefective virus, has deleted virtually all of the sequences unique to the small-t protein (T. Shenk and M. Kelly, personal communication), retaining only nine amino acids unique to small-t, with the remainder being in common with the amino terminus of large-T. From this, one might conclude that sequences held in common with the large-T protein confer biological activity on this protein fragment; therefore, it is plausible that, evolutionarily, small-t might represent a specialization of one aspect of a multifunctional large-T.

I have no explanation of the apparent virulence of viruses such as *dl* 2007, but it is again interesting that *dl* 2000 and *dl* 2007 produce the greatest amounts of small-t protein and mRNA of any of the mutants (6).

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