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# *mlt* Mutants of Polyoma Virus

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New *mlt* deletion mutants of polyoma virus were isolated, and their abilities to produce a lytic response in mouse cells or to transform rat cells were assessed. Their properties were analyzed in terms of the sequences deleted and their effects upon the structure and functions of the viral middle and large T-antigens.

A number of deletion (*dl*) mutants of polyoma virus with lesions in the portion of the genome that codes, in different reading frames, for both the viral middle and large T-antigens (Fig. 1) have been characterized (1, 4, 7, 8, 12). Since the phenotypic properties of these mutants vary considerably, we have chosen to classify them as *mlt* (middle, large T-antigen) mutants based on their genotype (12). In a recent experiment aimed at further probing regions of the genome involved in lytic growth and transformation, linear DNA (produced by the action of the *Hin*fI restriction enzyme on supercoiled polyoma virus DNA in the presence of ethidium bromide and subsequent treatment with the single-strand-specific nuclease S1) was used to transfect whole mouse embryo cells (secondary cultures) by the DEAE-dextran procedure (3). After 24 h, the cells were trypsinized, replated onto 50-mm dishes, and left at 37°C for 2 to 3 weeks. Plaques were then picked and used directly for the analysis of DNA and virus production; that is, the DNAs in 27 plaques were examined and compared with that of wild-type virus strain A2 DNA after restriction with the enzymes *Hpa*II and *Hin*fI, using the simplified protocol described previously (3). Nine deletion mutants were identified by this procedure, of which five were *mlt* mutants; of the rest, one proved to have a lesion near the viral origin of replication, and three were mixtures that were not further characterized. The structures and properties of the *mlt* mutants are described in this note.

All five mutants, designated *dl*-2, *dl*-16, *dl*-17, *dl*-22, and *dl*-27, have lesions which could be shown to reside in either of *Hpa*II fragments 4 or 8, within *Hin*fI fragment 8 (4, see Fig. 1). When the DNA sequences of the mutants were determined, two of them (*dl*-2 and *dl*-16) were found to be identical. Thirty-three base pairs were deleted in *dl*-2 (*dl*-16) and *dl*-17, 57 in *dl*-27, and 99 in *dl*-22. The DNA sequences deleted in the mutants, and the predicted concomitant loss of peptides from the middle and large T-antigens, are shown in Fig. 2. For comparison, the deletions in some previously characterized mutants are included.

Two biological properties of the mutants were investigated, namely, their ability to produce DNA in mouse 3T6 cells and their ability to transform Rat-1 cells. The replication properties of the mutants were compared with those of wild type (strain A2) by using equal titers (PFU) of virus stocks to infect mouse 3T6 cells and assaying the amount of supercoiled DNA produced about 48 h postinfection (4). Transformation was assayed by the ability of the virus to produce dense foci on plastic dishes or colonies in soft agar (see Table 1). Only *dl*-27 grew as well in mouse 3T6 cells as wild-type A2 virus, and its ability to transform Rat-1 cells was not markedly different from that of strain A2. Thus, *dl*-27 has properties reminiscent of those of mutant 45 (1). Mutants *dl*-2 (*dl*-16), *dl*-17, and *dl*-22 all grew slightly less well than wild-type virus. All of them, except *dl*-22, transformed Rat-1 cells by the dense focus assay at least as well as (and in soft agar [data not shown] slightly better than) wild-type virus. Mutant *dl*-22, on the other hand, was only very weakly transforming; three foci were eventually obtained from Rat-1 cells infected at high multiplicity and left for more than 1 month in culture. Cell lines derived from these were found by restriction enzyme-hybridization analysis (10, 14) to contain mutant DNA (data not shown). No colonies in soft agar were ever produced by this mutant. Thus, in its transformation properties, it resembles *dl*-23 (4, 6).

It has been previously noted that the migration of polyoma virus middle T-antigen on sodium dodecyl sulfate (SDS)-polyacrylamide gels is anomalous (6). That is, the wild-type viral protein migrates more slowly (55,000 to 60,000 molecular weight) than expected from its composition as predicted (50,000 molecular weight) from DNA sequence (13), which suggests either

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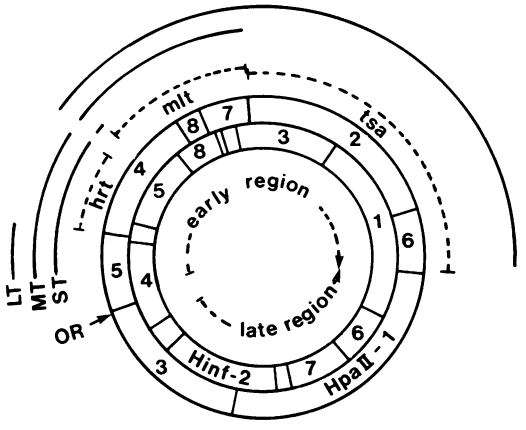


FIG. 1. Physical map of polyoma virus strain A2 DNA showing the location of the *Hpa*II and *Hin*fI restriction sites (4). The *mlt* region, as well as the *hrt* and *tsa* regions, and the location of the viral T-antigens are indicated.

TABLE 1. Comparison of the transformation properties of polyoma virus *mlt* mutants[a]

| Virus | No. of foci per dish at: | |
|---|---|---|
| | 37°C | 32°C |
| A2 (wild type) | 26, 15, 12, 10 (16) | 4, 4, 3, 5, (14) |
| *dl*-8 | 73, 126, 80, 89 (92) | 41, 56, 63, 63 (56) |
| *dl*-23 | 0 | 0 |
| *dl*-16 | 18, 20, 31, 18 (22) | 4, 5, 3, 5 (4) |
| *dl*-17 | 30, 27, 18, 11 (22) | 4, 5, 3, 5 (4) |
| *dl*-22 | 0 | 0 |
| *dl*-27 | 37, 35, 31, 28 (33) | 15, 7, 11, 4 (10) |
| Control | 0 | 0 |

[a] The concentrations of each virus were adjusted so that a dish (90 mm) of subconfluent Rat-1 cells was infected with $10^7$ PFU of virus stock. After 24 h, cells were completely trypsinized and replated onto four 50-mm dishes. Visible foci were scored 3 weeks later; the average numbers per experiment are shown in parentheses.

that its structure is maintained in SDS or that the protein is modified. Moreover, *dl*-8 and *dl*-23, two mutants with deletions of 93 and 102 base pairs, respectively (see Fig. 2), induce middle T-antigens that migrate quite differently; taking into account the deletions, the *dl*-8 antigen has a mobility which resembles that of the wild-type viral protein, and that of *dl*-23 migrates with the mobility predicted from its DNA sequence. Middle T-antigens immunoprecipitated from the mutants described in this note, with the exception of the antigen from *dl*-22, migrated in a manner analogous to the wild-type protein (Fig. 3). The antigen of mutant *dl*-22, on the other hand, was more analogous to that of *dl*-23 in its mobility and migrated ahead of the viral capsid protein VP1 (42,000 to 45,000 molecular weight). Thus, it could be argued that the sequence deleted in *dl*-22 and *dl*-23 (but not in *dl*-27) must be important in determining the mobility of the middle T-antigen. This sequence encodes within the correct reading frame the polypeptide Glu-Pro-Leu-

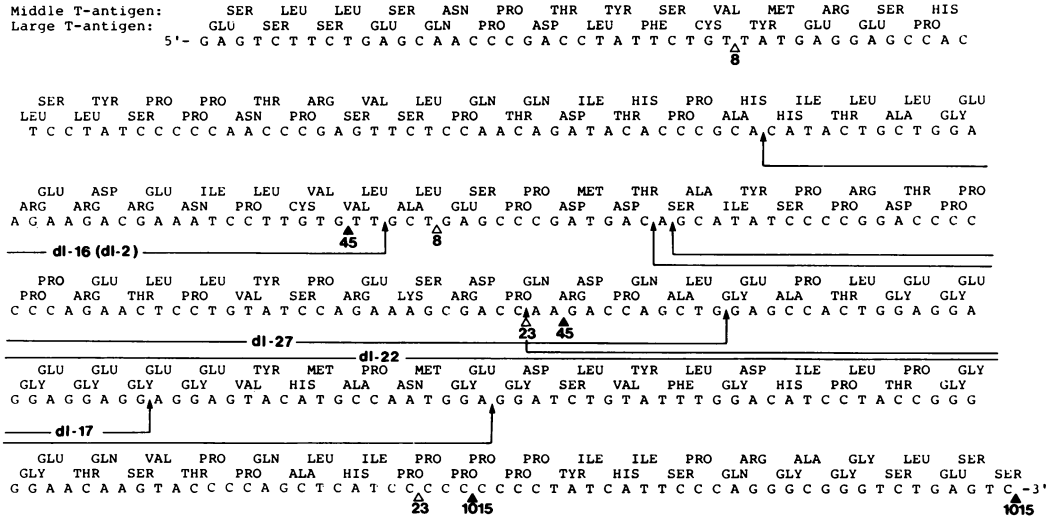


FIG. 2. DNA sequence of the *Hin*fI-8 fragment from polyoma virus strain A2 DNA and the amino acids encoded within it. The sequence extends from nucleotide numbers 960 to 1,273 in this strain (13). Shown are the locations of *mlt* mutants *dl*-16 (*dl*-2), *dl*-27, *dl*-17 and *dl*-22, as well as those of four mutants, *dl*-8 and *dl*-23 (12), mutant 45 (1), and mutant 1015 (8), previously described.

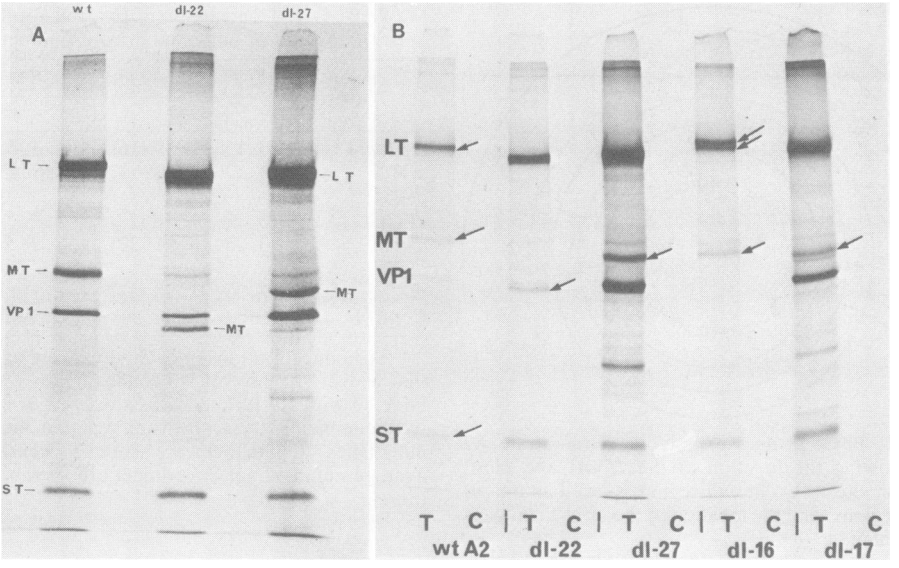


FIG. 3. (A) Autoradiogram of an SDS-polyacrylamide gel showing the location of the large (LT), middle (MT), and small (ST) T-antigens, immunoprecipitated from wild-type (strain A2) and mutant (*dl*-22 and *dl*-27) virus-infected 3T6 cells by using antitumor anti-serum and [$^{35}$S]methionine, according to the procedure of Ito (5). Tracks to the left of each experiment show similar immunoprecipitation results when control serum was used. VP1 is the major viral capsid protein; it immunoprecipitates together with the large T-antigen (2). (B) Autoradiogram of an SDS-polyacrylamide gel showing comparative migrations of wild-type- and mutant-induced, $^{35}$S-labeled T-antigens immunoprecipitated by antitumor antiserum (T) or control (C) serum. The double bands of large T-antigen observed with mutant *dl*-16 (and with *dl*-2, not shown) were also obtained with the monoclonal antibody αPy C1 (2; A. J. Street, personal communication); they were more clearly visible as two species on a more lightly exposed autoradiogram. (The band that comigrated with wild-type middle T-antigen [MT] in all of the experiments with mutant virus is not middle T-antigen [2]; on a darker exposure, it was shown to be present in the tracks where control serum was used.)

Glu-Glu-Glu-Glu-Glu-Glu-Tyr-Met-Pro-Met-Glu, which is particularly acidic and could be predicted to have a marked effect upon the structure of this viral antigen.

Türler and Salomon (15) found that the polyoma large T-antigen induced in lytically infected cells could be resolved into two discrete bands by gel electrophoresis. They interpreted their data as indicating posttranslational modification of the protein. A striking, but as yet unexplained, observation on immunoprecipitates of *dl*-16 antigens is that in spite of the apparent loss of 11 amino acids, the large T-antigen from this mutant migrated on SDS-polyacrylamide gels as two clearly separated bands, both of which appeared slightly larger than their wild-type counterparts (see Fig. 3). The wild-type viral DNA sequence deleted in this mutant is also absent within the larger deletion of *dl*-8. Nevertheless, the latter induced a large T-antigen in 3T6 cells that migrated as a single band with the expected mobility (6).

*mlt* mutants are located at an unusually interesting position on the polyoma virus genome in that their lesions can potentially affect both growth and transformation (4, 13). There are now enough examples of these mutants to allow some tentative conclusions to be reached concerning this region. (i) The area of the genome covered by the restriction fragment *Hin*fI-8 appears to have only a marginal effect upon the lytic cycle of the virus. All the mutants whose deletions are located within *Hin*fI-8 (Fig. 1) grow without the aid of helper virus and lack at least some part of this fragment. (ii) As measured by the ability of the mutants to produce DNA relative to wild-type virus, sequences deleted in mutants *dl*-8, *dl*-2 (*dl*-16), *dl*-22, and *dl*-17 are implicated in viral growth, whereas those deleted in *dl*-23, *dl*-27, and mutant 45 apparently are not (1; see also Fig. 2). Thus, two adjacent but noncontiguous regions of the genome may have subtle effects upon the lytic expression of the virus. (iii) A common portion of the genome (from nucleotide position 1,172 to 1,190; 13) deleted in mutants *dl*-22 and *dl*-23, but present in mutant *dl*-17, appears to be critical for cellular transformation by polyoma virus (4). This sequence encodes a polypeptide, Glu-Tyr-Met-Pro-Met-Glu, whose tyrosine residue has been

suggested to be phosphorylated in the viral middle T-antigen (11). Whether the absence of this residue in *dl*-22 and *dl*-23 can be related to the transformation defect in the mutants remains to be seen. (iv) The biological properties of the mutants give some indications of functional regions in middle T-antigen. The region of the genome covered by *Hin*fI-8 can essentially be divided into three parts. Deletions in the region nearest the N terminus of the protein produce a marked effect upon transformation, as shown by studies with *dl*-8 and to a lesser extent *dl*-16 (*dl*-2) (4, 6, 11; see also Table 1). This is followed by a region of the genome in which deletions have no apparent effect upon transformation, as exemplified by mutant 45 (1) and mutants *dl*-27 and *dl*-17. Deletions, on the other hand, in the region nearest the C terminus have a deleterious effect upon transformation, as seen by the properties of *dl*-22, *dl*-23 (4), and 1015 (7).

Mutants *dl*-22 and *dl*-27 have recently been used to map binding sites of monoclonal antibodies on the T-antigens (2).

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