A tumor host range selection procedure identifies p150^{sal2} as a target of polyoma virus large T antigen

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Cancer cells may undergo loss or alterations in functions that certain viruses normally target to promote virus replication. Virus mutants that have lost the targeting function(s) should be able to grow in such cancer cells but not in normal cells. A “tumor host range” (*t-hr*) selection procedure has been devised and applied to polyoma virus based on this rationale. Studies of one *t-hr* mutant have led to the identification of the mSal2 gene product (p150^{sal2}) as a binding partner of the large T antigen. mSal2 encodes a multizinc finger protein and putative transcription factor homologous to the *Drosophila* homeotic gene *Spalt*. The *t-hr* mutant encodes an altered large T protein that fails to interact with p150^{sal2} and is defective in replication and tumor induction in newborn mice.

Transforming genes of DNA tumor viruses act largely as protooncogene activators or tumor suppressor gene inactivators. In these capacities, they perform essential functions for virus growth. A useful approach for identifying these genes and their interactions with the host has been through the isolation of host range virus mutants. The dual function transforming gene of the highly oncogenic mouse polyoma virus was identified through studies of mutants isolated using polyoma transformed 3T3 cells as the permissive host and normal 3T3 cells as the nonpermissive host (1). This approach is based on the idea of complementation between cell-associated wild-type viral genes and an infecting virus mutant. It has also been applied successfully to other oncogenic DNA viruses, e.g., employing 293 cells expressing adenovirus E1A/E1B genes (2, 3) and COS cells expressing the SV40 large T antigen (4). Complementation cell lines have similarly been used for propagating defective virus mutants, for development of viral vectors and vaccines, and for other purposes (5–8). By design, this approach relies on permissive hosts constructed to have specific gains of viral functions. Its applications are directed to mutants in known viral genes.

Alterations in yet unknown cellular targets of viral oncogenes might occur in spontaneous tumors or nonviral transformed cells. This suggests a different rationale for isolating host range mutants, one based on selective growth in cancer cells of nonviral origin. This selection could depend on loss as well as gain of function by the cancer cell; i.e., tumor suppressor gene inactivation or protooncogene activation could create a permissive environment for replication of a mutant virus that is unable to target those functions. Earlier studies with polyoma established a link between virus host range and tumor suppressor gene function in the host. Large T mutants of polyoma that are unable to bind the retinoblastoma gene product pRb are in effect host range mutants, capable of growing in culture only in cells that lack functional pRb (9). Similarly, adenovirus E1B mutants have a host range determined in large part by lack of expression of p53 (10, 11). It is clear from these examples that expression versus lack of expression of a tumor suppressor gene by the host can constitute the essential parameters for isolating host range mutants of oncogenic viruses that are defective in targeting that gene. The possibility of using tumor host range selection as a way to uncover new viral functions and identify cellular targets

including tumor suppressor genes has not been systematically explored.

The mouse polyoma virus possesses several features that help to explain its extraordinary ability to induce tumors, yet important pieces of the puzzle are still missing (12). This virus replicates lytically in over 30 distinct cell types in its natural host, with tumors arising from as many as 15 of these (13). In each of these cells, the virus must be able to override whatever cell-cycle checkpoint controls are in place. Most well characterized DNA tumor viruses target both p53 and pRb tumor suppressors (reviewed in ref. 14). Polyoma, however, seems to have no mechanism for directly inactivating p53 (15). Furthermore, the ability of polyoma large T antigen to bind pRb, although essential for cell-cycle activation and immortalization of cells in culture, is not essential for virus replication or tumor induction in the mouse (9, 16). Other studies have shown that the ability of the virus to induce tumors is not strictly dependent on its transforming functions as defined *in vitro* (17). Together, these findings suggest that polyoma may possess still unrecognized function(s) enabling it to intervene in cell growth regulatory pathways in the intact host. The *t-hr* selection procedure has been devised as a strategy to identify such functions. The procedure is applicable in principle to other viruses.

Materials and Methods

Yeast Two-Hybrid Screening. The polyoma PTA large T C-terminal fragment (amino acids 333–781) was cloned into pGBT9 (CLONTECH) to generate pGBT9ITC and used as a “bait” to screen a 10-day whole mouse embryo cDNA library in pVP16 (18) following recommendations from CLONTECH.

Generation of TMD-25 with a Minimum Deletion. Large T C-terminal deletions used in yeast two-hybrid analysis were generated on pGBT9ITC using the Transformer site-directed *in vitro* mutagenesis kit (Promega).

Cloning of Full-Length mSal2 cDNA. A complete cDNA sequence for mSal2 was obtained using the Marathon cDNA amplification kit (CLONTECH) and reverse transcriptase–PCR products from primary baby mouse kidney epithelial cells (BMK). The cDNA was cloned into eukaryotic expression vector pcDNA3 (Invitrogen) as pcDNA-mSal.

In Vitro Glutathione S-Transferase (GST) Pull-Down Assay. Full-length polyoma wild-type large T cDNA and TMD25 large T cDNA were cloned into pcDNA3. mSal2 fragment (amino acids 841–

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Abbreviations: GST, glutathione S-transferase; BMK, primary baby mouse kidney epithelial cells.

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971) was cloned into pGEX4T1 (Amersham Pharmacia) to generate GST-mSal2 fusion protein in *Escherichia coli*. For the association of GST-mSal2 fusion protein with large T antigen, BMK cells infected by the wild-type polyoma strain PTA or 3T3 cells transfected with wild-type or TMD25 large T expression constructs were extracted (19). Five-hundred microliters of cell lysate were incubated with 50 μ l of 50% GST-mSal2 or GST beads for 2 h. After washing four times with PBS, the bound proteins were subjected to Western blot analysis using monoclonal antibody F4 against T antigens (20).

In Vivo GST Pull-Down Assay. Full-length mSal2 was cloned into a eukaryotic GST fusion vector pEBG to generate construct pEBGSAL. NIH 3T3 cells were cotransfected with pEBGSAL and wild-type or TMD25 large T expression constructs in a ratio of 1 to 1 using LipofectAmine2000 (GIBCO/BRL). Lysates were made 48 h posttransfection and incubated with 50–100 μ l of glutathione-Sepharose 4B beads for 2 h. The beads were washed four times with PBS containing 0.01% Nonidet P-40, and the bound proteins were immunoblotted with F4 anti-T and anti-p150^{sal2} monoclonal antibodies.

In Vivo Coimmunoprecipitation of mSal2 and Polyoma Large T. Fifty microliters of 50% protein A beads (Amersham Pharmacia) were incubated with purified rabbit polyclonal anti-N-terminal mSal2 antibody or normal rabbit IgG in 1 ml of Nonidet P-40 lysis buffer at 4°C for 2 h. The beads were washed four times with PBS. BMK cells infected with wild-type virus were extracted 24 h postinfection. Two milligrams of total protein were incubated with either the anti-mSal2 or normal IgG beads in Nonidet P-40 lysis buffer with 1% BSA for 2 h at 4°C. The beads were washed with 0.1% Tween 20 in PBS four times, and the proteins were separated by SDS/PAGE. Polyoma large T and mSal2 were detected using anti-T and anti-mSal2 monoclonal antibodies.

Viral DNA Replication Assays. Plasmid pUCori and polyoma origin replication assay has been described before (21). Cells were grown on 6-well plates; virus infection or DNA transfection has been described earlier. One to 5 μ g of DNA were subjected to restriction digestion. For virus infection, the viral genome was linearized with *EcoRI*. For transfection, pUCori and CMVLT were digested with *DpnI* and *HindIII*. The newly synthesized, but not the input, pUCori DNA is *DpnI* resistant for the lack of methylation in eukaryotic cells. The DNA fragment was resolved on a 1% agarose gel for Southern analysis using origin-specific and LT-specific probes.

Western Blots for Detection of p150^{sal2}. Tissue extracts were prepared from C3H/BiDa mice. One-hundred micrograms of protein from each sample were separated by SDS/PAGE, blotted on nitrocellulose membranes, and probed with anti-mSal2 monoclonal antibody.

Results

The T-HR Mutant Selection Procedure. A general procedure for isolating *t-hr* mutants and identifying cellular targets is outlined in Fig. 1. The initial goal is to screen for virus mutants that replicate better in cancer cells than in normal cells. Virus growth may be followed by plaque assay, by monitoring cytopathic effects, or by a direct assay for viral DNA replication. Differential growth is presumed to arise from an inability of the mutant to interact with some cellular factor expressed in normal cells but which is missing or altered in the particular cancer cell used as the permissive host. Identification of the mutation that underlies the host range allows construction of a suitable bait for identifying the putative cellular factor. The bait is chosen as the region of the wild-type virus corresponding to the site of the mutation. A yeast two-hybrid screen has been used here for target iden-

1. Mutant Virus Selection

2. Target Identification and Validation

Fig. 1. Outline of the tumor host range selection procedure.

tification. Other formats could also be used, e.g., *in vitro* binding using a GST fusion with the wild-type bait and normal cell extracts, or coimmune precipitation followed by mass spectrophotometry to identify interacting proteins. The mutant virus serves as a negative control to verify absence of interaction with candidate target(s). The mutant also provides an important tool to assess the role of specific interactions between virus and host proteins in virus replication, cell transformation, and tumorigenesis.

To isolate *t-hr* mutants of polyoma virus, permissive hosts were first chosen from among mouse cell lines derived from chemically induced, spontaneous, or other non-polyoma-induced tumors or transformed cells. The choice was based solely on the requirement that the cells be susceptible to lytic infection and plaque formation by the wild-type virus. Among many such cell lines tested, two have been used here: TCMK-1, an SV40-transformed baby mouse kidney cell line (22), and A6241, derived from a spontaneous mammary tumor in a C57BR mouse (23, 24). Primary baby mouse kidney (BMK) cells were used as the nonpermissive host. The use of primary cells as nonpermissive host is considered an important departure from earlier studies that used established cells (1) because the latter are known to have undergone loss of tumor suppressor gene function(s) (35). Randomly mutagenized virus was prepared by passage of a plasmid containing wild-type viral DNA through the error-prone MutD strain of *E. coli* (25) followed by excision of the viral genome and transfection into TCMK-1 cells. After several cycles of virus growth, individual plaques were isolated on TCMK-1. Virus from each plaque suspension was inoculated in to BMK cultures. Those that induced little or no cytopathic effect after 10–14 days were amplified on TCMK-1. Mutant DNAs were cloned, reconstituted as virus by transfection of permissive cells, and confirmed to retain the desired host range. The frequency of mutants was roughly one in several thousand plaques tested.

Fig. 2 shows results comparing the growth of wild-type virus with that of a *t-hr* mutant on BMK, TCMK-1, and A6241. Wild-type virus grows well on all three cells, whereas the mutant grows only in the transformed or tumor-derived cells. Extensive cytopathic effect develops in cultures of TCMK-1 and A6241 infected by the mutant. Infectious virus is produced in these cultures, although with slower kinetics and lower final yields

Fig. 2. Growth of a *t-hr* mutant is restricted to transformed or tumor-derived cells. TCMK-1, SV40 transformed baby mouse kidney cells; A6241, derived from a spontaneous mammary tumor. Cultures were either uninfected (Mock) or infected by wild-type or mutant polyoma virus. Photos taken 12 days postinfection show extensive cytopathic effects indicative of virus growth in all infected cultures except for BMK infected by the mutant.

compared with the wild type. In contrast, no discernible cytopathic effect and no virus production is noted in mutant-infected BMK cultures even after extended periods of incubation for up to 3 weeks. Whereas growth of the mutant on TCMK-1 could be viewed as being dependent on complementation with SV40 large T antigen, such cannot be the case with A6241 tumor cells which express no known viral genes (24).

p150^{sal2} Is Identified as a Binding Partner of Wild-Type but Not Mutant Large T. The mutation was localized to the C-terminal half of large T by determining the host range of recombinant viruses bearing complementary fragments of mutant and wild-type viral DNA. Sequencing of this region revealed a 20-bp duplication encompassing the C terminus of large T. The resulting frame shift leads to replacement of the last 12 aa by 11 foreign residues (Fig. 3A). To determine whether this mutation disrupts the ability of large T to interact with some cellular target, a 10-day mouse embryo cDNA library (18) was first screened in a yeast two-hybrid assay using the C-terminal portion of wild-type large T as bait. Twenty-three positive clones were analyzed. Twenty of these were represented by nine independent clones with overlapping sequences. This sequence showed extensive homology with the human gene *hSal2* (26) related to the homeotic transcription factor *Spalt* in *Drosophila* (27). The interacting cDNA clones are centered on amino acids 898–963 encompassing a pair of zinc fingers in the C-terminal region of the protein. A similar bait using the mutant large T failed to interact with these cDNAs (Fig. 3B), supporting the notion that the host range defect of the mutant is related to its inability to bind this protein.

The mutant carrying the 20-bp duplication proved to be

unstable, giving rise to wild-type revertants on further propagation. To obtain a stable mutant and to further pinpoint the region of large T essential for binding this target, a deletion analysis of the wild-type construct was carried out (Fig. 3C). Truncation of the last six amino acids of large T had no perceptible effect on binding to this target in yeast. However, further truncations into the P-L-K sequence at positions 774–776 resulted in loss of interaction. A deletion of these three amino acids in large T was sufficient to prevent interaction and to give rise to the host range phenotype shown in Fig. 2. The large T deletion mutant $\Delta 774-776$ is hereafter referred to as TMD-25 (isolated initially on TCMK cells following mutagenesis of viral DNA in *E. coli* MutD).

A complete cDNA of the target protein was obtained using 5' and 3' rapid amplification of cDNA ends (28). The sequence was found to be identical to that reported recently for mSal2 (29) except that our sequence shows glutamic acid rather than lysine at position 350. The genomic sequence indicates two alternate short 5' exons encoding 24 (exon 1) or 22 (exon 1a) amino acids and one unique 3' exon encoding 980 aa. Our sequence shows the alternate exon 1a (29). Eight zinc fingers are apparent in exon 2, with the C-terminal pair presumed to be an essential part of the large T interaction domain (Fig. 3B). Monoclonal and polyclonal antibodies were made against a GST fusion protein containing 104 aa from the N-terminal region of mSal2 spanning exons 1a and 2. The monoclonal antibody detected a single band corresponding to a 150-kDa protein made as an *in vitro* translation product from mSal2 cDNA. A protein of the same size was also seen in extracts of normal mouse brain using this antibody. A polyclonal rabbit antiserum was also made against a GST

Fig. 3. The *t-hr* mutant has an altered large T C terminus that prevents interaction with host factor mSal2. (A) Sequencing of the original mutant shows a 20-bp duplication of coding sequences at the large T C terminus causing a reading frame shift shown in red. (B) The coding region of mSal2 cDNA is shown schematically in blue with the zinc fingers in orange. Overlapping clones from the C-terminal region of mSal2 interact with the wild-type large T C-terminal fragment (amino acids 333–781) but not with that of the mutant in yeast two-hybrid assays. +, growth on His⁺ plates; His⁺ colonies were also lacZ positive (not shown). (C) Deletion analysis of the wild-type large T C terminus indicating that a minimal deletion of amino acids 774–776 abolishes the binding of mSal2 in yeast.

fusion protein containing 131 aa from the large T interaction domain near the C terminus. This antibody detected a 150-kDa protein in extracts of 3T3 and other mouse cells (not shown). The mSal2 gene product is hereafter referred to as p150^{sal2}.

In vitro pull-down assays were carried out using a GST fusion protein of the large T interaction domain of p150^{sal2} and extracts of lytically infected or transfected cells (Fig. 4A). Wild-type large

Fig. 4. Interaction of wild-type but not mutant large T with mSal2. (A) *In vitro* binding. GST alone and GST-fusion protein containing the large T binding domain of mSal2 (amino acids 839–969) are used to pull down large T from cell extracts. The filter is blotted with F4 anti-T monoclonal antibody. *, crossreactive cellular band. Lane a, input extract from wild-type infected BMK cells; lane b, wild-type extract from lane a pulled down with GST alone; lane c, wild-type extract from lane a pulled down with GST-mSal2 fusion protein; lane d, input extract from 3T3 transfected with wild-type large T cDNA; lane e, input extract from 3T3 cells transfected with TMD-25 large T cDNA; lane f, wild-type extract from lane d pulled down with GST alone; lane g, wild-type extract from lane d pulled down with GST-mSal2 fusion protein; lane h, mutant extract from lane e pulled down with GST-mSal2 fusion protein. (B) *In vivo* binding. (Left) 3T3 cells were cotransfected with full-length GST-mSal2 fusion construct and either wild-type or TMD 25 large T. Following GST pull-down, blots were developed with anti-T antibody (Upper) and with monoclonal anti-mSal2 (Lower) to show similar amount of GST-mSal2 was present in both pull-down lanes. Input lanes contain 3% of the extract used for pull-down. (Right) A 24-h wild-type virus-infected BMK cell extract was immunoprecipitated with normal rabbit IgG or purified rabbit polyclonal antibody against the N terminus of mSal2. The blot was probed with anti-T monoclonal antibody and reprobed with anti-mSal2 monoclonal antibody.

T synthesized during lytic infection efficiently binds the GST-mSal2 fragment (lanes a–c). When comparing extracts of 3T3 cells transfected with wild-type or TMD-25 mutant large T cDNAs, only the wild-type shows binding (lanes d–h). To confirm the large T-p150^{sal2} interaction *in vivo*, 3T3 cells were cotransfected with a vector expressing a GST fusion with full-length mSal2 and either wild-type or mutant large T cDNAs. Complexes containing wild-type large T were readily recovered, but no evidence of binding was seen with the mutant (Fig. 4B Left). To test for interaction between large T and endogenous p150^{sal2}, an extract of wild-type virus-infected BMK cells was incubated with a polyclonal rabbit antiserum against the amino-terminal mSal2 fragment. The immunoprecipitate was separated by SDS/PAGE and blotted with an anti-T monoclonal antibody. A portion of the large T present in the virus-infected cell extract clearly immunoprecipitated with mSal2 (Fig. 4B Right). Polyoma large T and p150^{sal2} most likely interact directly through their C-terminal regions, although the presence of additional factors mediating the binding cannot be ruled out.

Large T-p150^{sal2} Interaction Is Essential for Viral DNA Replication. The ability of TMD-25 to replicate and spread in the newborn mouse was examined by whole mouse section hybridization. At 10 days postinoculation, the mutant showed no signs of replication and

Fig. 5. p150^{sal2} imposes a block to viral DNA replication. (A) TMD-25 fails to replicate in the mouse. Newborn mice were inoculated intraperitoneally with TMD25 or PTA (1×10^6 pfu/animal) and killed 10 days later. Whole mouse sections were hybridized with ³⁵S-labeled viral DNA with overnight exposure (32). Wild-type PTA shows extensive replication especially in kidney, skin, and bones. TMD25 mutant shows no signs of viral DNA replication. (B Left) TMD25 fails to replicate in BMK cells 36 h postinfection. BMK cells were infected with TMD25 and wild-type virus (Wt Py). Low molecular weight DNA was isolated at 0, 18, and 36 h postinfection (p.i.) for Southern blot with viral DNA probe. (Right) p150^{sal2} represses viral origin replication. Polyoma origin (Ori) and large T-expressing plasmid (Wt LT cDNA) were cotransfected with increasing amounts of plasmid expressing mSal2. Newly replicated DNA was detected with origin specific probe (Upper). Total amount of plasmids is adjusted to the same level in each transfection using empty vector. The filter was stripped and reprobed with LT and origin-specific probe to show that similar amounts of origin and LT DNA were present in each transfection. (C) Inhibition of replication by mSal2 requires the large T interaction domain and can be overcome by wild-type but not mutant large T. Origin replication assay was carried out similarly to B, with increasing amounts of plasmids expressing either wild-type mSal2 or mSal2 with deleted C-terminal zinc finger (Del Sal2) as shown in the left six lanes. With the amount of mSal2 held constant, increasing the amount of wild-type LT (Wt LT) but not TMD LT abolishes mSal2 inhibition as shown in the right six lanes.

spread, whereas the wild-type virus established a disseminated infection (Fig. 5A). To investigate whether binding of p150^{sal2} by large T antigen is necessary for viral DNA replication, low molecular weight DNA was extracted from infected BMK cells and analyzed by Southern hybridization. The results showed that the mutant is unable to amplify its DNA in the nonpermissive host (Fig. 5B Left).

p150^{sal2} may function in an essential manner in conjunction with large T to promote viral DNA replication. Conversely, p150^{sal2} may act directly or indirectly to inhibit viral DNA replication, thus requiring binding by large T to remove the inhibition. Using a polyoma origin-containing plasmid as a reporter and introducing large T and mSal2 expression vectors in 3T3 cells, p150^{sal2} can be shown to inhibit viral DNA replication in a dose-dependent manner (Fig. 5B Right). A mutant p150^{sal2} lacking the C-terminal zinc finger pair in the large T interaction domain is unable to inhibit viral DNA replication. When inhibition is established using normal mSal2, further addition of wild-type but not mutant TMD-25 large T can

Table 1. Tumor profiles of mutant TMD-25 and wild-type PTA virus

	TMD-25	PTA*
Fraction of mice with tumors	7/7	32/32
Mean age at necropsy	202 d	82 d
Epithelial tumors		
Hair follicle	0/7	32/32
Thymus	0/7	29/32
Mammary gland	0/7	16/32
Salivary gland	0/7	23/32
Mesenchymal tumors		
Fibrosarcomas	7/7†	1/32
Renal medulla	0/0	7/32
Bone	0/0	6/32

*Data on PTA are taken from ref. 13.

†Subcutaneous tumors were found only at the site of inoculation.

reestablish polyoma origin-dependent replication (Fig. 5C). These results show that p150^{sal2} imposes a block to viral DNA replication and that the block can be overcome by wild-type large T.

TCMK-1 and A6241 cells show detectable levels of p150^{sal2} expression in Western blot analysis (data not shown), but the functional status of mSal2 in these cells is not known. These or other permissive hosts could carry mutations in the mSal2 gene itself or in some interacting partner or effector of p150^{sal2}. Consistent with the latter possibility is the fact that permissive hosts for mutants of polyoma or adenovirus defective in binding pRb or p53 may express the tumor suppressors themselves but have defects in INK4A gene products that impinge on pRb and p53 (9, 36–38).

TMD-25 Is Defective in Tumor Induction but Transforms Cells *in Vitro*.

Newborn mice infected with wild-type virus develop a broad spectrum of tumors, whereas mutant-infected mice develop only subcutaneous fibrosarcomas (Table 1). The latter tumors were found only around the site of inoculation, suggesting that direct infection of target cells by the mutant virus can lead to transformation. TMD-25 is expected to retain wild-type transforming ability based on its retention of normal middle and small T antigens. This was tested directly using an established line of rat embryo fibroblasts. Transformation of these cells does not depend on virus replication, and middle T alone suffices (30). Mutant virus-infected or transfected cells gave rise to foci and growth in soft agar in a manner similar to that of wild-type virus (data not shown). The failure of TMD-25 to induce a broad spectrum of tumors is therefore based not on a transformation defect but on a failure to replicate and spread.

Expression Pattern of p150^{sal2} in the Mouse. To determine whether the pattern of mSal2 expression overlaps with sites of virus replication in the animal, normal mouse tissues were extracted and tested for p150^{sal2} expression by Western blot (Fig. 6). Ovary was found to express the highest levels. Kidney, lung, and brain were also positive. Liver, skeletal muscle, spleen, salivary gland, and heart were either negative or very low in expression. These results are generally in line with those from Northern analysis (29). The finding of kidney and lung as sites of strong expression is relevant to the natural route of transmission of polyoma, which is thought to infect newborns via the lung and to amplify primarily in the kidney (31, 32). Successful virus replication in these tissues would require a mechanism for overcoming inhibition by mSal2. High level expression of p150^{sal2} in the ovary is of no apparent significance with respect to virus transmission but may have some relevance to human ovarian cancer (see Discussion).

Fig. 6. Expression of p150^{sal2} in mouse tissues. Tissues were taken from 2-week-old mice except in the first lane, which was from a newborn mouse. Protein (100 μ g) was loaded on each lane. The blot was probed with the monoclonal antibody against the N terminus of mSal2.

Discussion

A tumor host range (*t-hr*) selection procedure has been designed to identify new functions of oncogenic viruses and cellular targets altered in cancer cells. Applying this procedure to the mouse polyoma virus, a function and binding partner of the large T protein has been identified. The target is mSal2, a member of the *Spalt* homeotic gene family. The *t-hr* selection procedure has some advantages over approaches used in the past to investigate molecular aspects of virus–host interaction. Extensive genetic studies of polyoma have failed in the past to uncover the large T function involving interaction with mSal2 despite the fact that this interaction is essential for virus growth both in tissue culture and in the mouse. Coimmune precipitation has been the key tool in discovering interactions between viral oncoproteins and cellular proteins such as p53, pRb, p300, and pp60^{c-src}. Other approaches are needed, however, to detect target proteins with low levels of expression, ones which are not stabilized by viral infection, or ones whose functions are not readily assayed in immune complexes. Isolation of a *t-hr* mutant coupled with the power of the yeast two-hybrid screen has allowed the identifi-

cation of such a target in p150^{sal2}. By using tumor-derived or transformed cells of nonviral origin as permissive hosts, the *t-hr* procedure forces selection on the basis of altered cellular function(s) rather than on direct complementation with integrated viral genes. In addition, using normal primary cells as the nonpermissive host rather than “normal” established cells provides a better opportunity to uncover virus–tumor suppressor gene interactions.

The *Sal* (or *spalt*-related) gene family of putative transcription factors is conserved in evolution from flies to man. Some of these proteins are known to play important roles in embryonic development, although little is known about their molecular function(s). Here, it has been shown that p150^{sal2} acts to inhibit polyoma viral DNA replication and that binding by large T can overcome this inhibition. Synthesis of viral DNA depends on the virus being able to override G₁ checkpoints and induce host cell DNA synthesis (9, 33). This suggests that p150^{sal2} may function in some contexts as a negative regulator of cell growth. The hSal2 gene resides on chromosome 14q12 (26), possibly overlapping a region of LOH in human ovarian cancers (34). These observations, coupled with the finding of high levels of expression of p150^{sal2} in the mouse ovary, raise questions about a possible tumor suppressor function of Sal2. Recent work has shown that p150^{sal2} is highly expressed in normal human ovarian epithelial cells but is missing or altered in a majority of ovarian carcinomas (unpublished results). Extensive functional studies of Sal2 should lead to an understanding of its role(s) in normal development, in virus replication, and possibly in suppressing certain cancers.

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