Analysis of temperature-sensitive mutations in the simian virus 40 gene encoding virion protein 1

(papova viruses/protein stability/virus assembly)

MARLA BEHM, HENRY LOWMAN, SHI-CHUNG NG*, AND MINOU BINA†

Department of Chemistry, Purdue University, West Lafayette, IN 47907

Communicated by Julian M. Sturtevant, September 6, 1988 (received for review April 12, 1988)

ABSTRACT Temperature-sensitive (ts) assembly mutants of the tumorigenic virus simian virus 40 (SV40) fail to follow the normal pathway of virion morphogenesis at 40°C. The mutations were previously mapped to the gene coding for the major virion protein VP1 and fall into three groups: tsB, tsBC, and tsC. We have determined the tsB/C mutations by DNA sequence analysis and deduced the corresponding amino acid substitutions. We find that the mutations are global and span 68% of the VP1 gene. They result predominantly in single amino acid substitutions. The B mutations are localized between nucleotides 1667 and 2091, spanning the VP1 amino acid residues 54-195. With the exception of one mutation in tsC260, the C group mutations occur between the nucleotides 2141 and 2262, spanning VP1 residues 212-252. The tsBC substitutions are not localized within a distinct region. We present a model for the VP1 structure. The model correlates the distribution of ts assembly mutations in the SV40 VP1 gene with the VP1 functional domains, deduced from the phenotypes exhibited by the assembly mutants, and the VP1 structural domains, deduced recently from the cryoelectron microscopic studies of the SV40 virions. We summarize the behavior of the SV40 ts mutants and discuss the possible relationship between the ts phenotype and amino acid substitutions.

VP1, the major virion protein of simian virus 40 (SV40), is a multifunctional protein. The virion capsid is constructed from capsomeres which contain pentamers of VP1 (1). VP1 interacts with SV40 chromatin (2) and DNA (3, 4). VP1 dictates the arrangement of nucleosomes along SV40 DNA *in vivo* (2). The protein has also been implicated in acting as a nucleosome unfolding agent (5). It is thought that such an activity is involved in increasing the rate of transcription of SV40 chromatin *in vitro* (6, 7). Interactions involving VP1 may also play a key role in the topological changes in the SV40 DNA which occur when the SV40 chromatin enters the virion assembly pathway (2, 8-10).

How VP1 interacts with other VP1 molecules is of considerable interest since current evidence indicates that the structure of papova viruses (1, 11, 12) does not conform to the predictions of the quasi-equivalence theory, formulated by Caspar and Klug (13) to explain why icosahedral viruses are icosahedral. The SV40 capsid has a $T = 7$ (dextro) surface lattice (reviewed in ref. 14). The quasi-equivalence theory is most compatible with a capsid constructed from 420 protein subunits: 12 pentameric capsomeres occupying the pentavalent lattice positions and 60 hexameric capsomeres occupying the hexavalent lattice positions (15). However, the results of numerous studies indicate that the capsid of papova viruses is constructed from 360 VP1 molecules and that both the pentavalent and hexavalent morphological units consist of pentamers of VP1 (1, 11, 12). How nonequivalent bonding

occurs in the capsid among apparently identical protein subunits is not known and may be understood from analysis of site-specific mutations in VP1 and of the resulting effects of such mutations on VP1 interactions involved in virion assembly.

When the SV40 chromatin enters the virion assembly pathway, the virion proteins (VP1, VP2, and VP3) associate with it to form initially the 180S previrions and subsequently a mixture of young and mature virions, both of which sediment at 220 S (16-19). Genetic analyses have revealed three classes of SV40 temperature-sensitive (ts) assembly mutants: tsB, tsC, and tsBC (20-22). While all three classes of mutants map to a single cistron, the VP1 gene, the results of complementation analyses between pairs of mutants indicate that, at the nonpermissive temperature (40°C), the B mutants can complement the C mutants and the C mutants can complement the B mutants. The mutants that fail to complement either the B or C mutants have been classified as tsBC (20-22).

The results of biochemical studies have shown that, at 40°C, the tsC and the tsB mutants exhibit distinct phenotypic defects. The VP1 of the C mutants loses its ability to initiate virus assembly (23). The average nucleosome spacing in the tsC chromatin assembled at 40°C is considerably shorter than the average spacing observed for a wild-type (wt) chromatin or for the bulk cellular chromatin (2). Furthermore, nearly all of the tsC minichromosomes that accumulate in cells contain a nuclease-hypersensitive region (9, 10), a structure characteristic of templates that can potentiate replication and transcription in eukaryotic cells (reviewed in ref. 24). Because of its shorter nucleosome spacing, the DNA of the tsC chromatin is less flexible than the DNA of the wt chromatin (25).

At the nonpermissive temperature, the VP1 of the B mutants retains its ability to form complex structures that contain VP1, the SV40, chromatin, and the minor virion proteins VP2 and VP3, but it appears to be defective in the propagation step of shell assembly. At 40°C, the mutants accumulate in cells as semiassembled virion-like particles (19, 26, 27). In contrast, the mutants of the BC group do not show a distinct phenotype. For example, at 40°C, the 75S chromatin accumulates in the tsBC11-infected cells (28, 29), whereas in cells infected with other mutants of the BC group the capsid proteins can associate with the viral chromatin to yield complexes which sediment at about 120 S (29).

In this report, we present the amino acid substitutions that impart thermosensitivity to VP1.‡ The distribution of ts

Abbreviations: VP, virion protein; SV40, simian virus 40; ts, temperature-sensitive; wt, wild-type; nt, nucleotide; T antigen, tumor antigen.

*Present address: The Howard Hughes Medical Institute, Massachusetts General Hospital, Boston, MA 02114.

†To whom reprint requests should be addressed.

‡The sequence reported in this paper is being deposited in the EMBL/GenBank data base (accession no. J04139).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

mutations in SV40 VP1 gives insight into the relationship between the structural and functional domains of this protein.

MATERIALS AND METHODS

Virus Strains. The mutants (numbered 200–299) were obtained from Robert Martin (National Institutes of Health). These were previously derived (20) from mutagenesis of a wt small plaque SV40 strain named SV-S, which is the same as wt776 (21, 22). A second set of mutants (numbered 1–99) was obtained from Peter Tegtmeyer (State University of New York, Stony Brook). These were derived (30) from mutagenesis of a parental strain named VA45-54.

DNA Isolation and Sequencing. The method described by Hirt (31) was followed for obtaining the viral DNA from BSC40 monkey kidney cells infected with the tsB/C mutants. The DNA was digested with restriction endonucleases (21). The appropriate fragments were isolated from 5% polyacrylamide gels and subsequently cloned in M13-derived vectors (32). The sequence of the single-stranded DNA obtained from isolated clones was determined by the dideoxy sequencing procedure (33). Where possible, the nucleotide changes identified in the mutants were further confirmed by altered restriction enzyme pattern analysis: loss of the *Pvu* II site at nucleotide (nt) 1716 in tsB8, tsB204, tsB211, and tsB265; loss of the *Hae* III site at nt 2259 in tsC260; generation of a new *Hinf*I site at nt 2236 in tsC240; and generation of a new *Hind*II site and a new *Hpa* I site at nt 1999 in tsB201.

RESULTS AND DISCUSSION

Mutations Which Impart Temperature-Sensitivity to VP1. The B/C mutations were induced by mutagenesis with hydrox-

ylamine (20) or nitrosoguanidine (30). Lai and Nathans (21) have localized the mutations on the SV40 physical map by rescuing the ts mutants with specific restriction fragments from wt SV40 DNA. We have isolated the restriction fragment containing the ts mutation from each mutant strain and determined its nucleotide sequence.

Fig. 1 shows the distribution of the mutations along the SV40 VP1 gene and the corresponding amino acid substitutions. VP1 begins with Ala, at nt 1508 (34, 35). The B mutations identified in our study are localized between nt 1667 and 2091. They include the VP1 residues 54–195 and seem to occur in two clusters. The first cluster begins with tsB228, which results from replacement of Gln-54 by Lys. This is followed by tsB218, in which Pro-58 → Arg.

Three independently isolated mutants, tsB204, tsB211, and tsB265, all result from the same amino acid substitution of Ala-71 → Thr. Another type of replacement at residue 71 (Ala-71 → Val) is found in tsB8; however, this mutant also contains a second substitution, Glu-83 → Asp. It is not known whether both replacements are required to cause temperature sensitivity in tsB8. The second B cluster begins at residue 163, where Ala replaces Gly (tsB221), and contains tsB201, which results from the replacement of Ala-166 by Thr. This cluster ends with tsB4, in which Ala-195 is replaced by Val (Fig. 1).

With the exception of one mutation in tsC260, the C group mutations occur between nt 2141 and 2262 (Fig. 1). The mutations begin with tsC219, in which, as previously reported, Pro-212 is replaced by Ser (36). The tsC219 is followed by tsC240, in which Leu-244 → Ile. The tsC260 mutant contains two substitutions. One may be defining the end of the C region, where Pro-252 is replaced by Leu, and the other (Gly-40 → Glu) occurs in the amino-terminal region

GCC	CCA	ACA	AAA	AGA	AAA	GGA	AGT	TGT	CCA	GGG	GCA	GCT	CCC	AAA	AAA	CCA	AAG	GAA	CCA	GTG	CAA	GTG	CCA	AAG	1582
ALA	PRO	THR	LYS	ARG	LYS	GLY	SER	CYS	PRO	GLY	ALA	ALA	PRO	LYS	LYS	PRO	LYS	GLU	PRO	VAL	GLN	VAL	PRO	LYS	25
A(C260)																									
CTC	GTC	ATA	AAA	GGA	GGA	ATA	GAA	GTT	CTA	GGA	GTT	AAA	ACT	GGA	GTA	GAC	AGC	TTC	ACT	GAG	GTG	GAG	TGC	TTT	1657
LEU	VAL	ILE	LYS	GLY	GLY	ILE	GLU	VAL	LEU	GLY	VAL	LYS	THR	GLY	VAL	ASP	SER	PHE	THR	GLU	VAL	GLU	CYS	PHE	50
G(LU)																									
T(B8)																									
TTA	AAT	CCT	CAA	ATG	GGC	AAT	CCT	GAT	GAA	CAT	CAA	AAA	GGC	TTA	AGT	AAA	AGC	TTA	GCA	GCT	GAA	AAA	CAG	TTT	1732
LEU	ASN	PRO	GLN	MET	GLY	ASN	PRO	ASP	GLU	HIS	GLN	LYS	GLY	LEU	SER	LYS	SER	LEU	ALA	ALA	GLU	LYS	GLN	PHE	75
LYS																									
G(B218)																									
A(B204, B211, B265)																									
THR																									
VAL																									
ACA	GAT	GAC	TCT	CCA	GAC	AAA	GAA	CAA	CTG	CCT	TGC	TAC	AGT	GTG	GCT	AGA	ATT	CCT	TTG	CCT	AAT	TTA	AAT	GAG	1807
THR	ASP	ASP	SER	PRO	ASP	LYS	GLU	GLN	LEU	PRO	CYS	TYR	SER	VAL	ALA	ARG	ILE	PRO	LEU	PRO	ASN	LEU	ASN	GLU	100
ASP																									
GAC	TTA	ACC	TGT	GGA	AAT	ATT	TTG	ATG	TGG	GAA	GCT	GTT	ACT	GTT	AAA	ACT	GAG	GTT	ATT	GGG	GTA	ACT	GCT	ATG	1882
ASP	LEU	THR	CYS	GLY	ASN	ILE	LEU	MET	TRP	GLU	ALA	VAL	THR	VAL	LYS	THR	GLU	VAL	ILE	GLY	VAL	THR	ALA	MET	125
TTA	AAC	TTG	CAT	TCA	GGG	ACA	CAA	AAA	ACT	CAT	GAA	AAT	GGT	GCT	GGA	AAA	CCC	ATT	CAA	GGG	TCA	AAT	TTT	CAT	1957
LEU	ASN	LEU	HIS	SER	GLY	THR	GLN	LYS	THR	HIS	GLU	ASN	GLY	ALA	GLY	LYS	PRO	ILE	GLN	GLY	SER	ASN	PHE	HIS	150
PHE																									
TTT	TTT	GCT	GTT	GGT	GGG	GAA	CCT	TTG	GAG	CTG	CAG	GGT	GTG	TTA	GCA	AAC	TAC	AGG	ACC	AAA	TAT	CCT	GCT	CAA	2032
PHE	PHE	ALA	VAL	GLY	GLY	GLU	PRO	LEU	GLU	LEU	GLN	GLY	VAL	LEU	ALA	ASN	TYR	ARG	THR	LYS	TYR	PRO	ALA	GLN	175
ALA																									
A(B201)																									
ACT	GTA	ACC	CCA	AAA	AAT	GCT	ACA	GTT	GAC	AGT	CAG	CAG	ATG	AAC	ACT	GAC	CAC	AAG	GCT	GTT	TTG	GAT	AAG	GAT	2107
THR	VAL	THR	PRO	LYS	ASN	ALA	THR	VAL	ASP	SER	GLN	GLN	MET	ASN	THR	ASP	HIS	LYS	ALA	VAL	LEU	ASP	LYS	ASP	200
TYR																									
T(B4)																									
AAT	GCT	TAT	CCA	GTG	GAG	TGC	TGG	GTT	CCT	GAT	CCA	AGT	AAA	AAT	GAA	AAC	ACT	AGA	TAT	TTT	GGA	ACC	TAC	ACA	2182
ASN	ALA	TYR	PRO	VAL	GLU	CYS	TRP	VAL	PRO	ASP	PRO	SER	LYS	ASN	GLU	ASN	THR	ARG	TYR	PHE	GLY	THR	TYR	THR	225
SER																									
GGT	GGG	GAA	AAT	GTG	CCT	CCT	GTT	TTG	CAC	ATT	ACT	AAC	ACA	GCA	ACC	ACA	GTG	CTT	CTT	GAT	GAG	CAG	GGT	GTT	2257
GLY	GLY	GLU	ASN	VAL	PRO	PRO	VAL	LEU	HIS	ILE	THR	ASN	THR	ALA	THR	THR	VAL	LEU	LEU	ASP	GLU	GLN	GLY	VAL	250
ILE																									
GGG	CCC	TTG	TGC	AAA	GCT	GAC	AGC	TTG	TAT	GTT	TCT	GCT	GTT	GAC	ATT	TGT	GGG	CTG	TTT	ACC	AAC	ACT	TCT	GGA	2332
GLY	PRO	LEU	CYS	LYS	ALA	ASP	SER	LEU	TYR	VAL	SER	ALA	VAL	ASP	ILE	CYS	GLY	LEU	PHE	THR	ASN	THR	SER	GLY	275
LEU																									
T(*BC)																									
G(BC11)																									
ACA	CAG	CAG	TGG	AAG	GGA	CTT	CCC	AGA	TAT	TTT	AAA	ATT	ACC	CTT	AGA	AAG	CGG	TCT	GTG	AAA	AAC	CCC	TAC	CCA	2407
THR	GLN	GLN	TRP	LYS	GLY	LEU	PRO	ARG	TYR	PHE	LYS	ILE	THR	LEU	ARG	LYS	ARG	SER	VAL	LYS	ASN	PRO	TYR	PRO	300
SER																									
ATT	TCC	TTT	TTG	TTA	AGT	GAC	CTA	ATT	AAC	AGG	AGG	ACA	CAG	AGG	GTG	GAT	GGG	CAG	CCT	ATG	ATT	GGA	ATG	TCC	2482
ILE	SER	PHE	LEU	LEU	SER	ASP	LEU	ILE	ASN	ARG	ARG	THR	GLN	ARG	VAL	ASP	GLY	GLN	PRO	MET	ILE	GLY	MET	SER	325
TCT	CAA	GTA	GAG	GAG	GTT	AGG	GTT	TAT	GAG	GAC	ACA	GAG	GAG	CTT	CCT	GGG	GAT	CCA	GAC	ATG	ATA	AGA	TAC	ATT	2557
SER	GLN	VAL	GLU	GLU	VAL	ARG	VAL	TYR	GLU	ASP	THR	GLU	GLU	LEU	PRO	GLY	ASP	PRO	ASP	MET	ILE	ARG	TYR	ILE	350
GAT	GAG	TTT	GGA	CAA	ACC	ACA	ACT	AGA	ATG	CAG	TGA														2593
ASP	GLU	PHE	GLY	GLN	THR	THR	THR	ARG	MET	GLN	end														361

*BC208, BC214, BC216, BC217, BC248, BC274

FIG. 1. Distribution of ts mutations in the SV40 VP1 gene and the corresponding amino acid substitutions. The numbers at the right indicate the nucleotide and amino acid residues, respectively (34, 35). The initiation codon is not shown. The nucleotide changes identified in the ts mutants are shown above the DNA sequence of the wt parental strain. The corresponding amino acid exchanges responsible for VP1 temperature sensitivity are presented below the VP1 amino acid sequence. The number in parenthesis indicates the ts mutant strain.

of VP1 (Fig. 1). Marker rescue analysis indicates that both substitutions in tsC260 are required to produce a ts VP1; correction of either substitution results in loss of the ts phenotype (21).

Ng *et al.* (37) have previously reported the mutations that define the BC group (20). The tsBC223 mutant results from the replacement of His-193 by Tyr and thus is located in the B region (Fig. 1). Six other mutants of the BC group contain an identical mutation, which causes the exchange of Pro-283 to Ser (37). Another mutant of the BC group (tsBC11) results from the exchange of Lys-287 to Thr (ref. 37; Fig. 1).

Relationship Between the Distribution of ts Mutations in VP1 and the Capsid Structure. Since the amino acid sequence of the SV40 VP1 is very similar to the sequences reported (34, 38) for the major capsid proteins of other members of the papova virus B-genus family—which includes polyoma, BK, and JC viruses (see Fig. 2)—it is likely that the VP1 of papova viruses share a common structure and exhibit similar biological activities. The SV40 VP1 contains 361 amino acid residues (35). The polyoma virus VP1 is somewhat longer and contains several stretches of amino acids that are not present in the SV40 VP1 or in the protein from the human papova viruses, BK and JC (refs. 34 and 38; Fig. 2). The first eight amino acid residues of the SV40 VP1 have been shown to contain a signal which is sufficient for the transport of the protein to the cell nucleus (39). Ludlow and Consigli (40) have recently localized a Ca^{2+} -binding site in the polyoma virus VP1 (Fig. 2).

Low-resolution structures of the polyoma virus empty capsid (11) and the SV40 virions (1) have revealed that the capsid subunits contain at least two structural domains: a shell (S) domain and a protruding (P) domain. Such structural motifs are reminiscent of the structure observed for plant viruses—e.g., tomato bushy stunt virus (41).

We are interested in the correlation between the VP1 structural domains and the clustering of the ts mutations found in the SV40 VP1 gene (Fig. 1). The distributions of the mutations and the phenotypes exhibited by the mutants (2, 10, 19, 23, 26, 29) support the idea (22) that the B and C regions in VP1 correspond to two separate functional domains. We envisage that the shell (S) structural domain includes the B and C functional domains (Fig. 3).

The region which contains the tsB exchanges represents a functional domain, since the mutants of the tsB group exhibit a similar phenotypic defect at the nonpermissive temperature (19, 23, 27, 42). At 40°C, the tsB VP1 associates with the minor virion proteins VP2 and VP3, and with the SV40 chromatin *in vivo* (19). However, a complete capsid is not formed around the chromatin, indicating that the B mutations perturb the VP1 structure in regions involved in contact among the VP1 subunits (42).

The B mutations span a major portion of the SV40 VP1 gene (Fig. 1). Many of the residues found in the B domain are conserved among the VP1 of papova viruses (Fig. 2). These conserved residues may function in VP1-VP1 interactions involved in shell polymerization. The insertions and deletions

FIG. 2. Comparison of the amino acid sequences (refs. 34 and 38; standard one-letter code) of the major capsid proteins from the papova viruses SV40, polyoma (Py), BK, and JC. Dashes indicate gaps introduced in the sequence to maximize sequence similarity. Amino acids conserved in all four proteins are underlined. The amino acid residue numbers are shown at the right. The amino acid exchanges identified in ts VP1 mutants are shown above the SV40 sequence. The complementation class (i.e., tsB, tsC, and tsBC) of each exchange is indicated in parenthesis. N.T. denotes the sequences implicated in transport of VP1 to the cell nucleus (39). The vertical arrows denote the borders of a polyoma VP1 fragment implicated in Ca^{2+} binding; the bracketed region represents a possible Ca^{2+} -binding domain (40).

FIG. 3. Proposed structure of VP1 deduced from biochemical and genetic analysis. The amino-terminal region of VP1 is shown as an unordered chain. The shell (S) domain includes the B and C functional domains. The B domain includes residues 54–195. It is followed by the C domain, which contains residues 212–252. The protruding (P) domain corresponds to the residues that include the VP1 carboxyl terminus. The displayed areas of the B, C, and carboxyl regions are proportional to the number of residues in the respective parts of the VP1 sequence. The division of VP1 into two structural domains has been deduced from cryoelectron microscopic studies of SV40 virions (1).

observed in this region of the sequence (Fig. 2) can be easily accommodated on the capsid outer surface without destroying the quaternary structure shared by the papova virus family.

The tsC region corresponds to a functional domain, since all tsC mutants exhibit the same phenotypic defect at the nonpermissive temperature (2, 10, 23). Except for one of the tsC260 mutations found in the VP1 amino-terminal region, all of the C mutations occur in the middle of the VP1 gene (Fig. 1). At 40°C, the tsC mutants accumulate the 75S viral chromatin and thus are defective in the initiation step of shell assembly (2, 10, 19, 23). This suggests that the tsC region may be involved in formation of the VP1 pentamers and/or interactions of VP1 with VP2, VP3, and DNA. The involvement of the C region in pentamer formation is suggested because the association of VP1 into pentamers precedes shell assembly (12) and capsomere assembly may be one of the factors which influence the initiation step of virion assembly. The involvement of the C region in VP1 interactions with VP2, VP3, and chromatin is supported by the finding that the C mutations alter the VP1 region implicated in changing the average nucleosome spacing of the SV40 chromatin (2). The model shown in Fig. 3 provides a means for these interactions through placement of the C functional domain in the S structural domain. With the tsC region facing toward both the capsid interior and the other subunits, this functional domain is free to interact both with viral chromatin and with other capsid components. The proposed location of the tsC domain in the capsid inner surface is further supported by the finding that this region in general, and the VP1 residues involved in tsC mutations in particular, represent core residues that are highly conserved in VP1 subunits of the papova virus family (Fig. 2). In the model (Fig. 3), we have included the amino-terminal region of VP1 near the C functional domain since both a middle-region substitution (residue 252) and an amino-terminus substitution (residue 40) are required to produce the tsC phenotype in tsC260 (21).

While the tsB and tsC regions correspond to different functional domains in VP1, it seems unlikely that the tsBC substitutions are located in a separate, distinct domain since (i) the tsBC223 amino acid substitution of His-193 → Tyr

precedes the tsB4 amino acid substitution at residue 195 (Fig. 1); (ii) the remaining two tsBC mutations occur within a very small region from Pro-283 to Lys-287 (Fig. 1); and (iii) the tsBC mutants do not exhibit a distinct phenotypic defect (29).

The function of the carboxyl terminus of the SV40 VP1 remains to be elucidated. Garcea *et al.* (43) have introduced a relatively large deletion in the DNA region which corresponds to the carboxyl terminus of the polyoma VP1. When the truncated gene is expressed in *Escherichia coli*, it yields VP1 molecules that are 57 amino acid residues shorter than the wt VP1 but contain 6 out-of-frame residues at the carboxyl terminus of the truncated protein (43). This protein can form capsomeres but does not form capsids *in vitro*. This result has suggested that a region near or preceding the carboxyl terminus of VP1 may be involved in the specific bonding for the nonequivalent association of capsomeres (43). It has been shown that the final 18 residues of the SV40 VP1 can be replaced with the carboxyl-terminal region of SV40 large tumor (T) antigen without affecting SV40 viability *in vivo* (44). This indicates that the carboxyl terminus of SV40 VP1 is dispensable and thus not required in virion assembly. This result and the results of our studies are most compatible with a model in which the VP1 carboxyl terminus is located in the P structural domain (Fig. 3). As a part of the VP1 subunit that protrudes from the capsid surface, this domain could accommodate insertions and deletions without a significant alteration in capsid structure (Fig. 3).

Relationship Between the ts Phenotype and Amino Acid Substitutions. The relationship of ts mutations to the general questions of protein stability, structure, and mechanism of folding has been a problem of wide interest. A ts protein has been broadly defined as a protein that can function at a low (permissive) temperature but not at a higher (restrictive) temperature. It appears that the behavior of the SV40 ts mutants that have been examined is rather complex and does not strictly conform to the way the ts proteins have been classified or with the concept that ts mutations simply decrease the overall protein stability, which may in turn be responsible for the inability of the protein to function at the nonpermissive temperature (for examples see ref. 45; reviewed in ref. 46). It appears that in at least two independent examples (42, 47) the ts protein at the nonpermissive temperature represents a stable state which retains structural features similar to those of the wt protein. Prelich and Stillman (47) have found that both the wt and ts mutants of the adenovirus DNA-binding protein show identical cleavage patterns with thermolysin at various temperatures, even when the mutant proteins lose their ability to bind DNA. We have shown that, *in vivo*, the SV40 VP1 with altered biochemical properties can be readily detected in the nucleoprotein complexes isolated from cells incubated at the nonpermissive temperature (19, 29, 42).

We propose that many ts proteins undergo a conformation change in different parts of the molecule to produce the altered structure detected at the nonpermissive temperature rather than the large-scale order-disorder transitions detected in thermal melting studies. For instance, a ts phenotype may arise when a residue found in a secondary structural element is replaced by Pro. As an example, a ts bacteriophage λ repressor results when Leu-12 → Pro in the α -helix 1 or when Ser-35 → Pro in the α -helix 2 (48). Such a substitution would be expected to destabilize the helix, especially at elevated temperatures (46), perhaps without disturbing the overall packing of the molecule.

Alternatively, a ts phenotype may result if a residue found in loops or turns is replaced by another amino acid. Such substitutions may affect the overall loop or turn structure, loop interactions, and/or loop flexibility. Consistent with this is the fact that many amino acid exchanges found to impart temperature sensitivity to proteins involve residues that are

favored in turns and loops. For example, many substitutions involve the exchange of Pro for another amino acid. Pro → Ser exchanges have been identified in ts proteins, including enzymes and protein products coded by viruses: the SV40 VP1 Pro-212 → Ser in tsC219 and Pro-283 → Ser in six independent isolates of SV40 tsBC mutants; the Sindbis virus capsid Pro-218 → Ser (49); the adenovirus DNA-binding protein Pro-413 → Ser (50); the tobacco mosaic virus (TMV) coat protein Pro-63 → Ser (51); and *E. coli* adenylate kinase Pro-87 → Ser (52). Substitution of Leu for Pro, which is strongly disfavored in loops (53), has also been shown to impart temperature sensitivity to several proteins: Pro-427 → Leu in the SV40 large T antigen (54); Pro-252 → Leu in kanamycin nucleotidyltransferase (55); Pro-250 → Leu in the *CDC28* gene product from *Saccharomyces cerevisiae* (56); Pro-32 → Leu in yeast actin (57); and Pro-20 → Leu in the TMV coat protein (51).

Several ts exchanges involve Gly, another residue favored in loops and turns. Substitutions for Gly conferring a ts phenotype to proteins have been observed in the SV40 VP1 mutant tsB221 and in several other proteins, including bacteriophage T4 lysozyme (46).

The role played by Cys residues in producing ts proteins has not been thoroughly examined. While we have not found mutations in the SV40 VP1 that involve Cys residues, there are instances where the replacement of another amino acid by Cys in several DNA-binding proteins results in thermosensitive proteins. For example, ts mutant of the polyoma large T antigen contains a Gly-778 → Cys substitution (58) and two independent mutants of the SV40 large T antigen result when Trp-393 or Trp-422 is replaced by Cys (54). Tyr-22 → Cys substitution in the bacteriophage λ repressor yields a ts protein (48). Residue 22 is buried in the α -helix 2 of the DNA-binding domain of the repressor (59). Similarly, a ts protein results when another buried residue (Gly-53) in the λ repressor is replaced by Cys (48). But there are also examples in which exchanging Cys for another residue yields thermosensitive proteins: Cys-107 → Trp in the *lac* repressor (60) and Cys-121 → Tyr in the *CDC28* gene product from *S. cerevisiae* (56). It is plausible that substitutions involving Cys residues could produce ts proteins either through the loss of a disulfide bridge when Cys is replaced by another residue or through formation of incorrect disulfide bonds, which could occur when Cys replaces other amino acid residues.

The VP1 protein is particularly suited for exploring the effect of Cys residues on the stability of SV40 VP1, since it can provide insight into the structure-function relations of a protein involved both in supramolecular assembly and in assembly of complexes formed between DNA and proteins.

We thank Janet Mertz and Peter Good for communication of their unpublished results. The work was supported by research grants from the National Science Foundation and the American Cancer Society.

- Baker, T. S., Drak, J. & Bina, M. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 422–426.
- Blasquez, V., Stein, A., Ambrose, C. & Bina, M. (1986) *J. Mol. Biol.* **191**, 97–106.
- Blasquez, V. & Bina, M. (1985) *FEBS Lett.* **181**, 64–68.
- Soussi, T. (1986) *J. Virol.* **59**, 740–742.
- Moyne, G., Harper, F., Saragosti, S. & Yaniv, M. (1982) *Cell* **30**, 123–130.
- Brady, J. N., Lavialle, C. A. & Salzman, N. P. (1980) *J. Virol.* **35**, 371–381.
- Brady, J. N., Lavialle, C. A., Radonovich, M. F. & Salzman, N. P. (1981) *J. Virol.* **39**, 432–437.
- Chen, S. S. & Hsu, M.-T. (1984) *J. Virol.* **51**, 14–19.
- Blasquez, V., Ambrose, C., Lowman, H. & Bina, M. (1987) in *Molecular Aspects of Papovaviruses*, ed. Aloni, Y. (Nijhoff, Boston), pp. 219–237.
- Ambrose, C., Blasquez, V. & Bina, M. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3287–3291.
- Rayment, I., Baker, T. S., Caspar, D. L. D. & Murakami, W. T. (1982) *Nature (London)* **295**, 110–115.
- Salunke, D. M., Caspar, D. L. D. & Garcea, R. L. (1986) *Cell* **46**, 895–904.
- Caspar, D. L. D. & Klug, A. (1962) *Cold Spring Harbor Symp. Quant. Biol.* **27**, 1–24.
- Baker, T. & Rayment, I. (1987) in *Animal Virus Structure*, eds. Nermt, M. V. & Steven, A. C. (Elsevier, Amsterdam), pp. 335–348.
- Finch, J. T. (1974) *J. Gen. Virol.* **24**, 359–364.
- Garber, E. A., Seidman, M. M. & Levine, A. J. (1978) *Virology* **90**, 305–316.
- Coca-Prados, M. & Hsu, M.-T. (1979) *J. Virol.* **31**, 199–208.
- Baumgartner, I., Kuhn, C. & Fanning, E. (1979) *Virology* **96**, 54–63.
- Blasquez, V., Beecher, S. & Bina, M. (1983) *J. Biol. Chem.* **258**, 8477–8484.
- Chou, J. Y., & Martin, R. G. (1974) *J. Virol.* **13**, 1101–1109.
- Lai, C.-J. & Nathans, D. (1975) *Virology* **66**, 70–81.
- Lai, C.-J. & Nathans, D. (1975) *Virology* **75**, 335–345.
- Bina, M., Blasquez, V., Ng, S.-C. & Beecher, S. (1982) *Cold Spring Harbor Symp. Quant. Biol.* **47**, 565–569.
- Yaniv, M. & Cereghini, S. (1986) *CRC Crit. Rev. Biochem.* **21**, 1–26.
- Ambrose, C., McLaughlin, R. & Bina, M. (1987) *Nucleic Acids Res.* **15**, 3703–3721.
- Bina, M., Ng, S.-C. & Blasquez, V. (1983) *J. Biomol. Struct. Dyn.* **1**, 689–704.
- Blasquez, V. (1985) Ph.D. Thesis (Purdue University, Lafayette, IN).
- Garber, E. A., Seidman, M. M. & Levine, A. J. (1980) *Virology* **107**, 389–401.
- Ng, S.-C. & Bina, M. (1984) *J. Virol.* **50**, 471–477.
- Tegtmeyer, P. & Ozer, H. L. (1971) *J. Virol.* **8**, 516–524.
- Hirt, B. (1967) *J. Mol. Biol.* **26**, 365–369.
- Messing, J. (1983) *Methods Enzymol.* **101**, 20–78.
- Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–4367.
- Tooze, J. (1981) *DNA Tumor Viruses* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
- Kempe, T. D., Beattie, W. G., Weissman, S. & Konigsberg, W. (1979) *J. Biol. Chem.* **254**, 7561–7569.
- Mertz, J. E. (1984) *Virology* **138**, 183.
- Ng, S.-C., Behm, M. & Bina, M. (1985) *J. Virol.* **54**, 646–649.
- Frisque, R. J., Bream, G. L. & Cannella, M. T. (1984) *J. Virol.* **51**, 458–469.
- Wychowski, C., Benichou, D. & Girard, M. (1986) *EMBO J.* **5**, 2569–2570.
- Ludlow, J. W. & Consigli, R. A. (1987) *J. Virol.* **61**, 2934–2937.
- Harrison, S. C., Olson, A. J., Schutt, C. E., Winker, F. K. & Bricogne, G. (1978) *Nature (London)* **276**, 368–373.
- Bina, M., Blasquez, V. & Ambrose, C. (1986) *Biophys. J.* **49**, 38–40.
- Garcea, R. L., Salunke, D. M. & Caspar, D. L. D. (1987) *Nature (London)* **329**, 86–87.
- Tornow, J., Polvino-Bodnar, M., Santangelo, G. & Cole, C. N. (1985) *J. Virol.* **53**, 415–424.
- Hawkes, R., Grutter, M. G. & Schellman, J. (1984) *J. Mol. Biol.* **175**, 195–212.
- Alber, T., Grutter, M. G., Gray, T. M., Wozniak, J. A., Weaver, L. H., Chen, B.-L., Baker, E. N. & Matthews, B. W. (1986) in *Protein Structure, Folding and Design*, UCLA Symposia on Molecular and Cellular Biology, New Series, ed. Oxender, D. L. (Liss, New York), Vol. 39, pp. 307–318.
- Prelich, G. & Stillman, B. W. (1986) *J. Virol.* **57**, 883–892.
- Hecht, M. H., Sturtevant, J. M. & Sauer, R. T. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5685–5689.
- Hahn, C. S., Strauss, E. G. & Strauss, J. H. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4648–4652.
- Kruijer, W., Van Schaik, F. M. A. & Sussenbach, J. S. (1982) *Nucleic Acids Res.* **10**, 4493–4500.
- Wittman-Leibold, B., Jauregui-Adell, J. & Wittmann, H. G. (1965) *Z. Naturforsch. B* **20**, 1235–1249.
- Gilles, A.-M., Saint-Girons, I., Monnot, M., Femandjian, S., Michelson, S. & Barzu, O. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5798–5802.
- Leszczynski, J. F. & Rose, G. D. (1986) *Science* **234**, 849–855.
- Seif, I., Khoury, G. & Dhar, R. (1980) *Nucleic Acids Res.* **8**, 2225–2240.
- Matsumura, M., Yasumura, S. & Aiba, S. (1986) *Nature (London)* **323**, 356–358.
- LoRincz, A. T. & Reed, S. I. (1986) *Mol. Cell. Biol.* **6**, 4099–4103.
- Shortle, D., Novick, P. & Botstein, D. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4889–4893.
- Deininger, P. L., LaPorte, P. & Friedmann, T. (1981) *J. Virol.* **37**, 871–875.
- Pabo, C. O. & Lewis, M. (1982) *Nature (London)* **298**, 443–447.
- Miller, J. H. (1979) *J. Mol. Biol.* **131**, 191–222.