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MINIREVIEW

Simian Virus 40 Large T Antigen: the Puzzle, the Pieces, and the Emerging Picture

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INTRODUCTION: THE PUZZLE

In cultured monkey cells infected by simian virus 40 (SV40), the viral regulatory protein large T antigen directs an ordered sequence of events leading from the early phase of infection prior to viral DNA replication into the late phase of infection, in which viral progeny are produced in large numbers (53). The early phase of infection is devoted to subverting cellular control mechanisms to prepare the cell to support the late phase of infection. T antigen accumulates during the early phase of infection in the nucleus; it alters cellular transcription patterns and stimulates quiescent cells to synthesize cellular DNA. Upon transition to the late phase, T antigen autoregulates its own transcription, initiates viral DNA replication, and stimulates late gene expression and virion production. Perhaps as a reflection of its ability to alter cellular transcription and to induce cell DNA synthesis, T antigen can also immortalize primary cells and is responsible for induction and maintenance of cell transformation in cultured cells and for tumor induction in experimental animals. The pleiotropic biological functions of T antigen are accomplished by the multiple biochemical activities identified to date for T antigen and it seems likely that new biochemical activities of this protein remain to be discovered. In view of this remarkable complexity, the question arises of how one polypeptide can fulfill so many different functions and do so in a temporally controlled fashion.

The progress toward a solution for this puzzle has been chronicled in several excellent reviews, to which the reader is referred for detailed background information, specialized aspects of T-antigen function, and more comprehensive citations of the literature (3, 8, 13, 20, 30, 33, 50, 53, 59). This review will briefly describe our current view of the domain structure of T antigen and modification of T-antigen function by protein phosphorylation. In conclusion, a speculative model for the temporal control of viral infection by T antigen will be presented.

DOMAIN STRUCTURE OF T ANTIGEN: THE PIECES

Fundamental to an understanding of T antigen is the realization that the protein is composed of multiple functional domains (Fig. 1). This domain organization is supported by a wealth of evidence derived from detailed biological and biochemical analyses of deletion and point mutations in many laboratories (6, 16, 24, 31). The best-studied region of the protein is the DNA binding domain (1, 6, 14, 26, 51). Lesions in this domain cause defects in specific binding to sites I and II in the control region of the viral genome. The DNA binding domain shown here is sufficient by itself for specific recognition of both sites. Overexpression of large quantities of this peptide in bacteria may soon permit structural analysis of this domain.

The borders of most other functional domains are less well defined, with the exception of the nuclear location signal (NLS) and retinoblastoma suppressor protein (Rb) binding site (Fig. 1). Short synthetic peptides have been shown to substitute functionally for both activities, albeit less efficiently than the intact domains. The host range domain (Fig. 1) is unique in that it is fully functional in the infected cell even when expressed as a separate polypeptide.

The domain organization of T antigen is further supported by analysis of peptides obtained by partial proteolysis of the native protein (40, 43, 45, 46, 62). The coincidence of the protease-resistant peptides with several of the genetically defined functional domains (Fig. 1) argues strongly for the existence of structural folding domains in T antigen. However, this question will remain open until more definitive structural analyses of T antigen are available.

ASSEMBLING THE PIECES: THE WHOLE IS GREATER THAN THE SUM OF THE PARTS

The domain structure of T antigen serves as a basis to begin consideration of its role in control of infection. The interactions between the domains of T antigen are crucial to its function. For example, the interactions between the ATP binding region and the DNA binding domain appear to enable T antigen to temporally order the initial events in the replication of SV40 DNA (reviewed by Borowiec et al. [3]).

The structure and function of T antigen are also modulated by differential phosphorylation (reviewed by Prives [33]). The major sites of phosphorylation have been mapped to two clusters at the N and C termini of the protein, outside the DNA binding and helicase domains of the protein (Fig. 1). Early reports of differential phosphorylation of T antigen from infected monkey cells, different turnover rates of phosphate at different sites, and reduced SV40 DNA binding activity of highly phosphorylated T antigen suggested that phosphorylation of T antigen could regulate its function. Enzymatic dephosphorylation of serine residues in purified T antigen by alkaline phosphatase enhanced specific binding to SV40 DNA and initiation of viral DNA replication in vitro, implying that phosphorylation of at least some serine residues down-regulated these activities, confirming the earlier reports. Helicase and ATPase activities remained unaffected by dephosphorylation.

More recently, a stimulatory factor for SV40 DNA replication in vitro was identified as the catalytic subunit of protein phosphatase 2A (PP2A) (56, 57). Dephosphorylation of Ser-120, Ser-123, Ser-677, and Ser-679, all residues with a rapid phosphate turnover in infected cells, was correlated

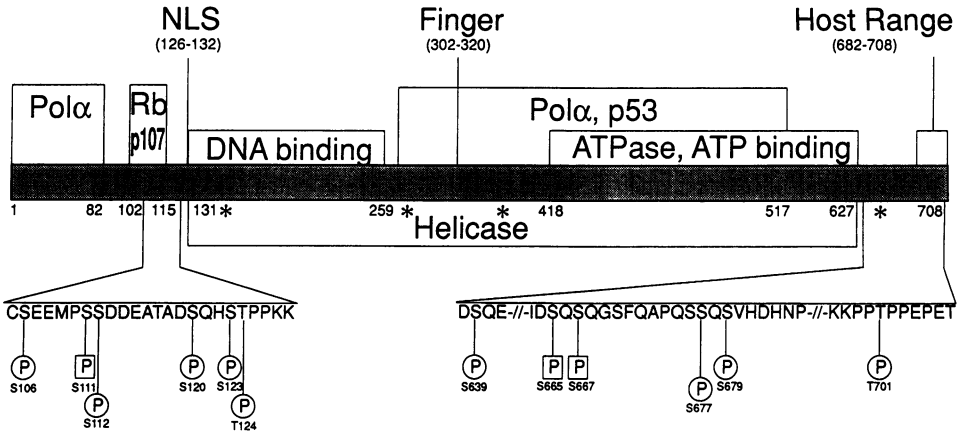

FIG. 1. Functional domains of SV40 T antigen. The DNA binding domain is discussed in the text. The regions of the protein that retain ATPase (6), ATP-binding (4), and helicase (62) activities; binding to DNA polymerase α-primase (Polα) (10 and references therein); the Rb and p107 (7) and p53 tumor suppressor proteins (26, 40); as well as the nuclear location signal (NLS) (18 and references therein), a potential metal binding region (Finger) (22 and references therein), and a host range domain (29, 48, 55 and references therein) are depicted. Asterisks indicate protease-sensitive sites discussed in the text. Phosphorylated residues found in T antigen from mammalian cells are denoted by a circled P, those identified so far only in T antigen overexpressed in insect cells are indicated by a boxed P (5, 36 and references therein). (Modified, with permission, from the Annual Review of Biochemistry, Volume 61, © 1992 by Annual Reviews Inc.)

with enhancement of T antigen's ability to initiate SV40 replication in vitro (39). This enhancement appears to arise through cooperative protein-protein interactions in formation of a double hexamer of T antigen on the origin; highly phosphorylated T antigen fails to bind cooperatively in this reaction and is therefore defective in unwinding origin DNA (12). The enzymatic dephosphorylation studies agree well, for the most part, with a mutational analysis of the individual phosphorylation sites of T antigen (42). For example, a mutant carrying Ala rather than Ser at residue 679 replicated in monkey cells more efficiently than the wild type, consistent with the conclusion that phosphorylation of this site contributes to down-regulation of replication.

Phosphorylation of T antigen may regulate its function positively as well as negatively. A mutant bearing a substitution of Ala for Thr at residue 124 of T antigen was unable to replicate SV40 DNA in monkey cells and bound poorly to binding site II in the SV40 origin (42). Similarly, the mutant protein was defective in SV40 DNA replication in vitro (12, 25, 41). T antigen expressed in bacteria is not modified at Thr-124 and is inactive in the in vitro replication reaction unless preincubated with a mammalian homolog of the fission yeast cell cycle-related protein kinase cdc2 (25), which specifically phosphorylates Thr-124 (15, 25). These results, taken together, support the conclusion that phosphorylation of Thr-124 is required for SV40 DNA replication activity and suggest that this event could be responsible for the S-phase-specific replication of SV40 DNA in monkey cells (25, 33). On the other hand, phosphorylation of Thr-124 on T antigen occurs within minutes after its synthesis in infected cells (36, 37). Moreover, the turnover of phosphate on Thr-124 is quite slow compared with that of Ser-123, Ser-677, and Ser-679 ($t_{1/2}$ 4.5 h versus 1 to 2 h) (35). Baculovirus-produced T antigen is nearly completely modified at the cdc2-targeted site (15). In contrast, when overexpressed in a baculovirus system, Rb and several other proteins known to be modified by cdc2-related kinases in vitro or in vivo in a cell cycle-specific fashion are quantitatively underphosphorylated (2, 21, 32, 52). Finally, a cdc2-related kinase RF-S has been purified from S-phase cell extracts as a stimulatory factor for SV40 DNA replication in vitro (11). However, its stimulatory activity is not thought to be mediated by phosphorylation of T antigen. For those reasons, it is perhaps premature to conclude that phosphorylation by a cdc2-like kinase is responsible for activation of T antigen's DNA replication activity upon entry of the infected cell into S phase.

SPECULATIVE MODEL FOR TEMPORAL CONTROL OF VIRAL INFECTION

An alternative model for temporal control of viral infection is depicted in Fig. 2. The model starts with the well-established observation that T antigen accumulates to rather high concentrations in the early phase of SV40 infection (53). At about the time maximal levels of T antigen are reached, the late phase of infection commences with the initiation of viral DNA replication. On the basis of phosphopeptide maps of T antigen from infected cells, the bulk of the accumulated protein exists in highly phosphorylated forms, modified at most of the serines, as well as nearly completely at Thr-124 and Thr-701 (36, 37). A minor amount of T antigen, including newly synthesized protein, is nearly completely phosphorylated at Thr-124 and Thr-701 but is relatively underphosphorylated at several Ser residues, most clearly at Ser-120 and Ser-123 (36, 37). Recalling that initiation of new rounds of SV40 DNA replication is dependent on continued production of newly synthesized T antigen (64, 65), I wish to suggest that temporal control is based primarily on a dynamic balance between the phosphorylation rate of total T antigen on key Ser residues (residues 120, 123, 677, and 679) and the production of T antigen unmodified at these residues, either through enzymatic dephosphorylation at these sites or through new synthesis of T antigen. In a newly infected cell, the T-antigen synthesis rate will be maximal and the rate of phosphorylation will ensure that most of the protein accumulates in the highly phosphorylated form, which is proposed to be inactive in viral DNA replication and only partially active in autoregulation of early transcription. However, as the concentration of the highly phosphor-

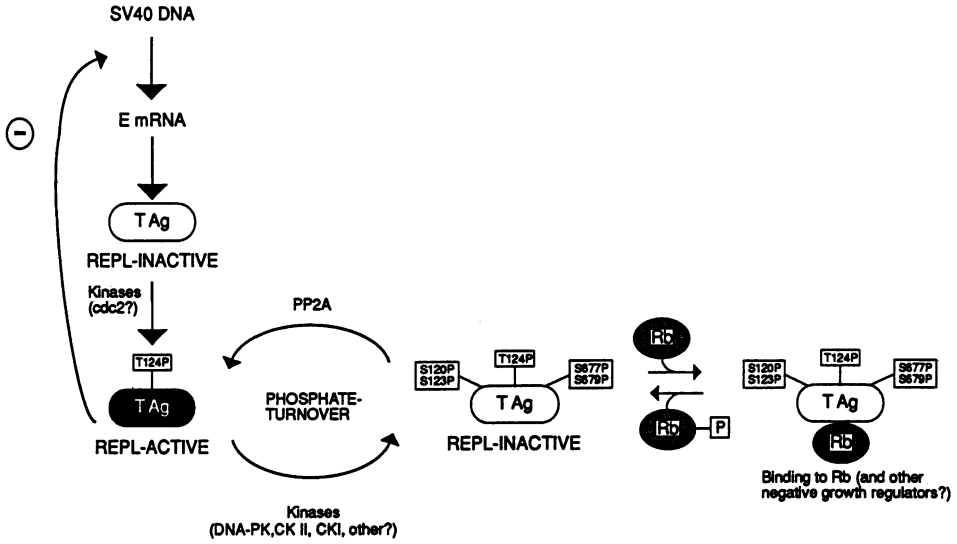

FIG. 2. Speculative model for temporal control of SV40 infection. See text for details.

ylated T antigen increases, it will act increasingly as a substrate for cellular phosphatases such as PP2A. Thus, a cycle of phosphate turnover on the key Ser residues will gradually build up, in agreement with the experimental data from infected cells (see above). Assuming a constant rate of production of newly synthesized T antigen in the potentially replication-active form, a slight increase in the rate of dephosphorylation or a slight decrease in the rate of phosphorylation of total T antigen would lead to an increased concentration of the replication-active protein. Upon exceeding a proposed threshold concentration, this minor form of T antigen would activate viral DNA replication. Autoregulation of early transcription and virion assembly would, at some point, reduce the rate of production of newly synthesized T antigen, its concentration would gradually fall below the postulated threshold level, and replication would cease. The model would predict that the rate of conversion of newly synthesized T antigen to the highly phosphorylated forms should be slower during viral DNA replication than earlier in infection, in accordance with experimental findings (58). The model also predicts that little replication-competent T antigen with site II-specific SV40 DNA binding activity should be detectable early in infection before the proposed threshold level is reached, again in accordance with experimental results (49).

Can this model explain the S-phase specificity of SV40 DNA replication and if so, how? Several observations suggest that it can and that a number of mechanisms may contribute. First, T-antigen binding to the negative growth regulator Rb plays a role in stimulation of growth-arrested cells to reenter the cell cycle and progress to S phase (9, 17, 28, 61 and references therein). It seems likely that T-antigen binding to the growth-modulating protein p53 may serve a similar function (20), and interactions with other potential negative growth-regulating proteins may be predicted (9, 61, 63). In complex formation with Rb, it is the highly phosphorylated form of T antigen that interacts most efficiently (23). If this were also true for interactions with the other negative growth regulators (23 and references therein), the accumulating replication-inactive T antigen would essentially titrate the growth suppressors, thereby promoting progression toward S phase. This mechanism could ensure that by the time a threshold concentration of replication-active T antigen is attained, the cell will be in S phase. Release of T antigen upon phosphorylation of Rb in the transition from G_1 to S phase (23) could also lead to a further increase in replication-active T antigen (Fig. 2).

Second, at least two lines of evidence point to a central function for PP2A in temporal control of viral infection. G_1-phase cell extracts support SV40 DNA replication in vitro rather poorly but can be restored to a level comparable to that of S-phase extracts by addition of the purified catalytic subunit of PP2A (56). This result suggests that S-phase cell extracts may have a higher PP2A activity than G_1 extracts and could thus explain how, in the context of the model in Fig. 2, the balance of the phosphate turnover cycle could tip to favor an increased concentration of replication-active T antigen in S phase. However, another recent study found no change in PP2A activity with the cell cycle, suggesting that the nature of the PP2A substrate may be crucial (34). A second line of evidence implicating PP2A in control of viral infection is the association of SV40 small t antigen with PP2A (27, 60). The association of small t antigen with PP2A inhibits its ability to dephosphorylate several substrates, including large T antigen (38, 66). At first glance, one might predict that small t antigen would therefore interfere with viral DNA replication. However, early observations showed that although small t antigen is not required for productive infection of cultured monkey cells, it actually enhanced virion production (44, 53, 54). This apparent contradiction can be resolved in the context of the model in Fig. 2. The presence of small t antigen would presumably allow a larger quantity of the highly phosphorylated replication-inactive large T antigen to accumulate prior to the onset of PP2A-catalyzed dephosphorylation. Two probable consequences can be envisioned. (i) The titration of Rb and possibly other negative cellular growth regulators by a higher concentration of highly phosphorylated T antigen would be more effective, thereby promoting cell cycle progression, and (ii) a larger reservoir of T antigen in the highly phosphorylated state might, upon dephosphorylation, generate more replication-active T-antigen molecules, resulting in the enhanced virus production observed previously.

Although the model presented here is clearly speculative

and does not deal specifically with some important issues such as the mechanism of the early-late viral transcription switch, it does account for much of the considerable experimental data available. The model provides a mechanism for transforming an analog process, the accumulation of T antigen, via a measuring device, phosphate turnover, to a digital signal, entry into the late phase of infection. In this regard, it bears some resemblance to a model for activation of maturation-promoting factor (MPF) just prior to mitosis (47). Remarkably, PP2A also serves as a measuring device to control activation of MPF (19). However, in contrast to the activating role proposed for PP2A in the early-late transition in SV40 infection, PP2A appears to exert a negative control on MPF activation. Thus, it is not difficult to imagine that the apparently reciprocal roles of PP2A in timing control of mitosis and activation of SV40 DNA replication may be a clue that T antigen has borrowed a mechanism that controls timing of cellular DNA replication.

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