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Biochemistry

Complex of simian virus 40 large tumor antigen and 48,000-dalton host tumor antigen

(virus–host interaction/differential phosphorylation/temperature-sensitive *A* gene effect/chromatin binding)

DANIEL S. GREENSPAN AND ROBERT B. CARROLL

Department of Pathology, New York University School of Medicine, New York, New York 10016

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ABSTRACT **Simian virus 40 large tumor antigen (T Ag) can be separated by sucrose gradient sedimentation into a rapidly sedimenting, maximally phosphorylated fraction and a slowly sedimenting, less phosphorylated fraction. The M_r 48,000 host tumor antigen (48,000 HTA, also called nonviral T Ag) is preferentially complexed with the maximally phosphorylated T Ag. Pulse-labeled T Ag sediments as a 5–6S monomer, whereas T Ag radiolabeled for progressively longer periods slowly increases in sedimentation coefficient to give a broad distribution between 5 S and greater than 28 S. Mutation in the viral *A* locus causes a decrease in T Ag phosphorylation and a marked decrease in 48,000 HTA binding, shifting the sedimentation coefficient of T Ag to the monomer value. The more highly phosphorylated T Ag also has the highest affinity for chromatin.**

Simian virus 40 (SV40) large tumor antigen (T Ag) is encoded by the viral *A* locus (1, 2), which governs initiation of DNA synthesis and the establishment of viral transformation (3, 4). Found in the nuclei of cells infected (5) and transformed (6) by SV40, T Ag is a DNA-binding protein (7–11) that binds DNA with heterogeneous affinities (7, 12). Mutation in the viral *A* locus has been shown to decrease the DNA-binding affinity of T Ag (13, 14). T Ag also sediments heterogeneously with sedimentation coefficients from 5S to greater than 22S (7, 15–20). Mutation of the *A* locus causes T Ag to sediment as a 5–6S species (17, 18), corresponding to the 94,000 dalton monomer (13). Furthermore, T Ag, a phosphoprotein (21), is heterogeneously phosphorylated (22), generating multiple isoelectric focusing species with differing contents of phosphate. Differential phosphorylation may play a physiological role in generating the heterogeneous aggregating and DNA-binding forms of T Ag. Similarly, the addition and removal of the phosphate groups of acidic chromatin-associated proteins of eukaryotic cells has been suggested as a control mechanism for the regulation of their DNA-binding properties (23). In addition, differential phosphorylation states can affect the binding affinities of subunits for each other, as in glycogen phosphorylase (24).

Two recently discovered host gene products, the M_r 48,000 and 55,000 host tumor antigens (HTA), also termed nonviral T Ags, have been shown to complex with T Ag *in vitro* (19, 20, 25, 26). On the basis of results with separate [^{32}P]orthophosphate- and [^{35}S]methionine-labeled proteins analyzed on separate gradients, McCormick and Harlow (20) have proposed that the "53K NVT"-associated form of large T Ag becomes more highly phosphorylated than the free form. The 48,000 and 55,000 HTAs have been shown to be unrelated immunologically and structurally (19). The broad physiological significance of these nuclear phosphoproteins has been established by the fact that they are induced in mouse, rat, hamster, and human cells transformed by SV40 (19). They are expressed, in the absence of SV40, in cells transformed by a variety of carcinogenic agents—including the RNA tumor virus, murine sarcoma virus, and the chemical carcinogen methylcholanthrene (27)—and in spontaneously transformed fibroblasts (27) and embryonal carcinoma cells (26). Because these host proteins are widely expressed in mammalian cells in response to transformation, the elucidation of the mechanism of their interaction with the SV40 transforming gene product, T Ag, will help in understanding the mechanism of viral transformation.

In this study we have determined the ^{32}P/^{3}H ratios and the kinetics of formation of the various physical forms of T Ag. We have shown the physical heterogeneity of T Ag is a result of differential phosphorylation and 48,000 HTA binding, both of which are inhibited by ts*A* (temperature-sensitive) mutation.

MATERIALS AND METHODS

Preparations. Mouse cells transformed by wild-type SV40 (SVA31E7) were derived from BALB/c 3T3 cells by Y. Ito. This line expresses only the HTA termed "48K" by us (19) and "53K" by others (20, 25). Mouse cells transformed by a ts*A* virus (A21) were isolated by J. Hiscott (28) after transformation of mouse embryo fibroblasts with the ts*A*58 virus of P. Tegtmeyer *et al.* (13). All cells were grown in 100-mm petri dishes in Dulbecco's modified Eagle's medium supplemented with 10% calf serum (GIBCO) (or 10% fetal calf serum where indicated).

Cell extracts for sucrose gradient sedimentation were prepared by freeze-thawing whole cells in 0.01 M Tris·HCl (pH 9.0)/1 mM dithiothreitol/0.14 M NaCl buffer. Detergents were not employed in the extraction. Prior to application to sucrose gradients, extracts were clarified by centrifugation for 20 min at 16,000 × *g* in a Sorvall centrifuge at 4°C. Clarified extracts (0.35 ml) were applied to 5-ml linear 5–20% sucrose gradients (with 0.2-ml 60% sucrose pads). Gradients contained 10 mM Tris·HCl (pH 7.4), 1 mM dithiothreitol, and 0.14 M NaCl and were centrifuged for 12.5 hr at 23,000 rpm in a Beckman SW 50.1 rotor at 4°C. Fractions (approximately 0.27 ml) were collected from the bottoms of the gradients.

Nuclei were isolated from SVA31E7 cells as described by Carroll *et al.* (7). For extraction of nuclei the pH 6.0 buffer contained 10 mM potassium phosphate, 0.1 M NaCl, 20 mM EDTA, phenylmethylsulfonyl fluoride at 0.3 mg/ml, and 1 mM dithiothreitol. The pH 7.0, 8.0, and 9.0 buffers contained 20 mM Tris·HCl, 0.1 M NaCl, 20 mM EDTA, phenylmethylsulfonyl fluoride at 0.3 mg/ml, and 1 mM dithiothreitol.

Assays. Monospecific rabbit antiserum to M_r 94,000 T Ag was prepared and immunoprecipitation, NaDodSO$_4$/polyacrylam-

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Abbreviations: SV40, simian virus 40; HTA, host tumor antigen; T Ag, SV40 large tumor antigen.

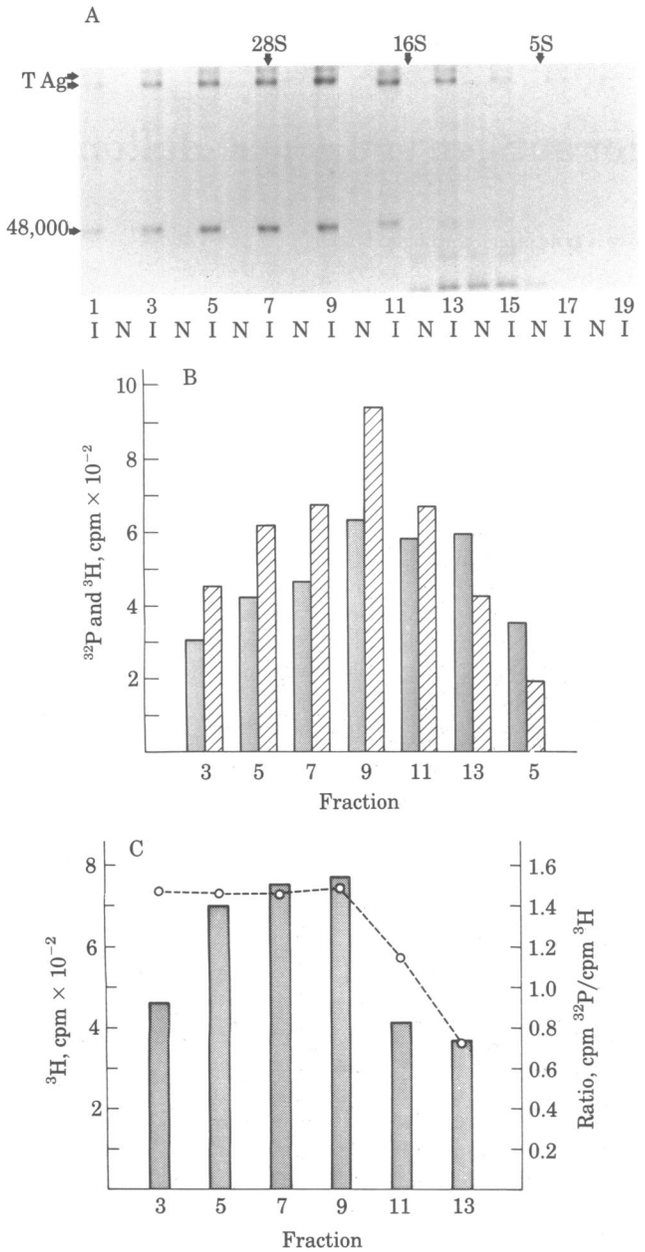

FIG. 1. Cosedimentation of the 48,000 HTA with the highly phosphorylated fraction of T Ag. SVA31E7 cells were labeled for 8 hr in Dulbecco's modified Eagle's medium containing 2% the normal concentrations of phosphate and methionine and 10% dialyzed fetal calf serum with [^{32}P]orthophosphate at 320 μCi/ml and [^{3}H]methionine at 80 μCi/ml (New England Nuclear; 1 Ci = 3.7 × 10^{10} becquerels). Clarified extracts were then centrifuged through 5–20% sucrose gradients and fractions from these gradients were immunoprecipitated with hamster anti-tumor serum. (*A*) Autofluorogram of NaDodSO$_4$/7.5–10% polyacrylamide slab gel electrophoresis of fraction immunoprecipitates. Columns labeled N and I correspond to samples immunoprecipitated with normal and immune hamster sera, respectively. S values were those of rRNA markers in a parallel gradient. (*B*) ^{32}P (hatched bars) and ^{3}H (stippled bars) in bands of 94,000 T Ag excised from the slab gel illustrated in *A*. (*C*) ^{32}P/^{3}H ratios of T Ag bands in *B* (○- - -○) compared to ^{3}H in corresponding bands of 48,000 HTA from gel illustrated in *A*. Bottoms of gradients are to the left.

ide gel electrophoresis, and subsequent fluorography were performed as described (29). Excised gel bands were completely dissolved in 0.5 ml of 30% H_2O_2 (wt/wt) (stabilized with sodium stannate) for 36 hr at 80°C. The ^{32}P and ^{3}H counts in dissolved gel slices were then determined as described by Greenspan and Carroll (22).

RESULTS

The 48,000 HTA Cosediments with Highly Phosphorylated T Ag. In order to determine if the multiple sedimenting forms of T Ag differ in their degree of phosphorylation, an extract of SV40-transformed mouse cells (SVA31E7) radiolabeled with [^{3}H]methionine and [^{32}P]orthophosphate was centrifuged on a 5–20% sucrose gradient. Immunoprecipitates of fractions from the gradient were then analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis and fluorography (Fig. 1*A*). The distribution of T Ag across the gradient is broad, as might be expected from an aggregating protein whose various aggregation states are in equilibrium with one another. It can be seen that the 48,000 HTA is detectable only in complexes with sedimentation coefficients of 16 S or greater.

When the various bands of T Ag in Fig. 1*A* were excised and the ^{32}P and ^{3}H contents were determined, it was found that T Ag contained in complexes with sedimentation coefficients of 24 S or greater had a higher ^{32}P/^{3}H ratio than any of the more slowly sedimenting forms (Fig. 1*B*). Fraction 13 (15–16S), for example, has half the ^{32}P/^{3}H ratio of the 24S form. Comparison of the ^{32}P/^{3}H ratios of the various sedimenting forms of T Ag with the distribution of the 48,000 HTA on the same gradient (Fig. 1*C*) demonstrates that the 48,000 HTA sediments predominantly with those forms of T Ag having the highest ^{32}P/^{3}H ratios. The relationship of ^{32}P/^{3}H ratios between the species of

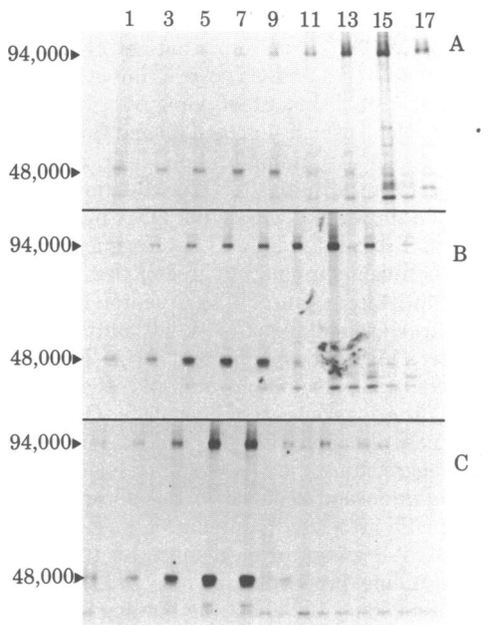

FIG. 2. Sedimentation of T Ag and 48,000 HTA as a function of duration of radiolabeling. Identical cultures of SVA31E7 cells were radiolabeled with [^{35}S]methionine for 30 min (150 μCi/ml in Dulbecco's modified Eagle's medium lacking methionine and containing 10% dialyzed calf serum), 2½ hr (120 μCi/ml in Dulbecco's modified Eagle's medium containing 2% the normal concentration of methionine and 10% dialyzed calf serum), or 19½ hr (20 μCi/ml in the same medium containing 10% the normal concentration of methionine and 10% dialyzed calf serum). The 2½-hr and 19½-hr radiolabeled cultures were then chased with complete Dulbecco's modified Eagle's medium containing 10% calf serum for 30 min and 3 hr, respectively. Cells were extracted and the extracts were sedimented on three parallel 5–20% sucrose gradients. Immunoprecipitation from gradient fractions was with hamster anti-tumor serum (numbered fractions) or normal hamster serum (even fractions). Autofluorograms are shown of the NaDodSO$_4$/7.5–15% polyacrylamide slab gel electrophoresis of fraction immunoprecipitates from gradients of 30-min pulse-labeled cells (*A*); 2½-hr-labeled cells chased for 30 min (*B*); 19½-hr-labeled cells chased for 2½ hr (*C*).

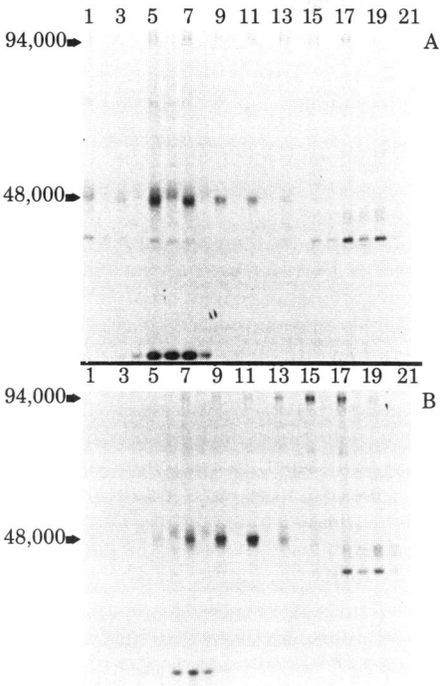

FIG. 3. Effect of tsA mutation upon the T Ag·48,000 HTA complex. The tsA 58-transformed mouse embryo fibroblasts were maintained at 33°C and radiolabeled at either 33°C or 39.5°C (12 hr after shift-up) with [^{35}S]methionine at 60 μCi/ml in Dulbecco's modified Eagle's medium containing 2% the normal concentration of methionine and 10% dialyzed calf serum. Labelings were for 5 and 6 hr at 39.5°C and 33°C, respectively. Extracts were centrifuged through parallel 5–20% sucrose gradients and fractions from these gradients were immunoprecipitated with either monospecific rabbit antiserum to 94,000 T Ag (odd-numbered fractions) or normal rabbit serum (even-numbered fractions). Autofluorograms are shown of $NaDodSO_4$/7.5–15% polyacrylamide slab gel electrophoresis of fraction immunoprecipitates from sucrose gradients of cells labeled at 33°C (*A*) or at 39.5°C. The bottoms of the gradients are to the left.

T Ag remains the same whether short (2 hr) or long (24 hr) periods of labeling are employed. The ^{32}P/^{3}H ratio of the 24S species, for example, is always about twice that of the 15–16S form, which in turn is more than twice the ^{32}P/^{3}H ratio of T Ag sedimenting at 5–6 S. This indicates that differences in ^{32}P/^{3}H ratios reflect differences in the phosphate content of protein species rather than differences in their rates of ^{32}P turnover. The ratio of [^{3}H]methionine in the 48,000 HTA to that in T Ag in each of the fractions (3, 5, 7, 9) is about the same (i.e., 1.5:1), indicating T Ag and the 48,000 HTA occur in a constant ratio in the ≥24S aggregates. The ^{32}P/^{3}H ratios of the 48,000 HTA in each of the fractions 3, 5, 7, and 9 were approximately equal and half that of the ^{32}P/^{3}H ratio of T Ag in the same fractions. ^{32}P/^{3}H ratios of 48,000 HTA declined slightly through fractions 11 and 13.

Sedimentation Rate of T Ag Increases with Duration of Radiolabeling. In order to determine the kinetics of T Ag·48,000 HTA complex formation, a time course study was performed: three identical cultures of SVA31E7 cells were labeled with [^{35}S]methionine: (*i*) from 22 to 3 hr prior to harvest (long label); (*ii*) from 2.5 to 0.5 hr prior to harvest (intermediate label); and (*iii*) for 30 min prior to harvest (pulse). Thus, each of the three cultures had the same content of T Ag and concentration effects on the association were avoided. The extracts were then centrifuged on three parallel gradients and the distributions of antigens on the three gradients were compared (Fig. 2). It can be seen that with increasing time of labeling there is a progressive increase in the S value of T Ag, with the major distribution of T Ag occurring in fractions equivalent to 5–6 S with a 30-min pulse (Fig. 2*A*), 15–16 S after 3 hr (Fig. 2*B*), and 24 S after 22 hr. (Fig. 2*C*). This is consistent with the idea that complexing of T Ag and 48,000 HTA occurs in conjunction with increasing phosphorylation of T Ag. Unlike T Ag, however, newly synthesized 48,000 HTA is immediately incorporated into high molecular weight complexes (Fig. 2*A*).

Quantitation of Phosphoserine and Phosphothreonine in the Sedimenting Forms of T Ag. Walter and Flory (30) have shown T Ag to contain both phosphoserine and phosphothreonine. We have confirmed the existence of both phosphoserine and phosphothreonine in T Ag and, in addition, have that found that T Ag does not contain phosphotyrosine. However, in an experiment in which ^{32}P-labeled T Ag from the bottom (fractions 6–9), middle (fractions 11–14), and top (fractions 16–19) of a sucrose gradient were hydrolyzed in acid and then subjected to two-dimensional thin-layer plate electrophoresis, the three different size classes of T Ag were found to have approximately equivalent ratios of phosphoserine to phosphothreonine: bottom, *P*-serine 2015 cpm (80.3%) and *P*-threonine, 494 cpm (19.7%); middle, *P*-serine 1878 cpm (76.1%) and *P*-threonine 590 cpm (23.9%); and top, *P*-serine 1708 cpm (80.3%) and *P*-threonine 418 cpm (19.7%). It therefore does not appear that aggregation of T Ag correlates with the phosphorylation of a particular amino acid residue, but it does correlate with an increase in both phosphoserine and phosphothreonine content.

Mutation of the SV40 A Locus Inhibits T Ag·48,000 HTA Complex Formation and T Ag Phosphorylation. tsA 58-transformed mouse cells (A21 cells) were radiolabeled with [^{35}S]methionine at either the permissive or the nonpermissive temperature and extracts of the cells were sedimented on parallel gradients (Fig. 3). Immunoprecipitation was performed with antiserum to 94,000 T Ag. Therefore, any 48,000 HTA immunoprecipitated was necessarily bound to T Ag. T Ag from A21 cells radiolabeled at the permissive temperature sedimented in a broad distribution similar to that of T Ag from wild-type-transformed cells (Fig. 3*A*). The 48,000 HTA was complexed to the most rapidly sedimenting forms of T Ag. However, T Ag from A21 cells radiolabeled at the nonpermissive temperature sedimented predominantly as 5–6S monomeric T

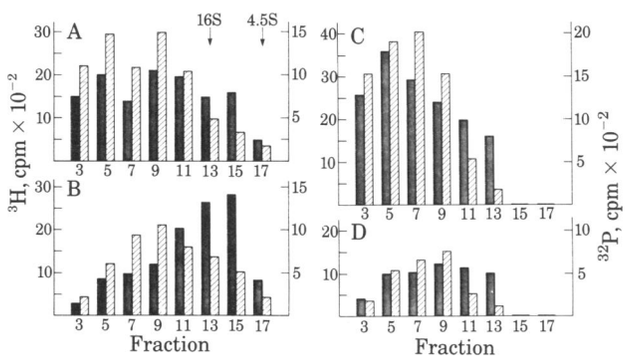

FIG. 4. Effect of tsA mutation upon the ^{32}P/^{3}H-labeled complexes. tsA 58-transformed mouse embryo fibroblasts (*i*) maintained at 33°C or (*ii*) shifted to 39.5°C were labeled with both [^{3}H]methionine (107 μCi/ml) and [^{32}P]orthophosphate (428 μCi/ml) at either 33°C or 39.5°C (15 hr after shift-up). After centrifugation of extracts through parallel 5–20% sucrose gradients and immunoprecipitation of fractions with monospecific rabbit antiserum to 94,000 T Ag and subsequent $NaDodSO_4$/polyacrylamide gel electrophoresis, the T Ag and 48,000 HTA were excised from the slab gels, and ^{32}P (hatched bars) and ^{3}H (stippled bars) contents were determined. (*A*) T Ag radiolabeled at 33°C; (*B*) T Ag radiolabeled at 39.5°C; (*C*) 48,000 HTA radiolabeled at 33°C; (*D* 48,000 HTA radiolabeled at 39.5°C. S values were those of bovine serum albumin (4.5S) and β-galactosidase (16S). The bottoms of the gradients are to the left.

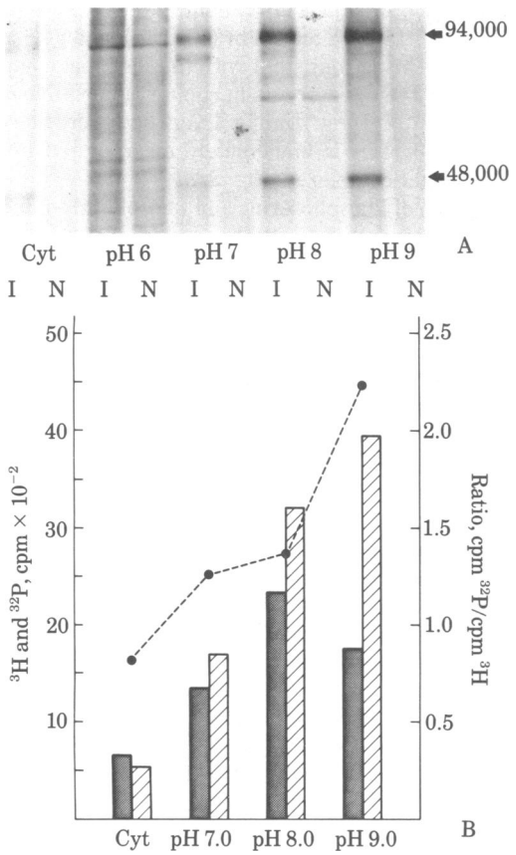

FIG. 5. ^{32}P/^{3}H ratios of different chromatin-binding forms of T Ag. SVA31E7 cells starved for 1 hr in Dulbecco's modified Eagle's medium minus methionine and phosphate and containing 10% dialyzed fetal calf serum were labeled for 3½ hr with 200 μCi of [^{32}P]orthophosphate per ml and 50 μCi of [^{3}H]methionine per ml. Cell nuclei were then isolated and serially extracted with pH 6.0, 7.0, 8.0, and 9.0 buffers and then immunoprecipitated. (*A*) Autofluorogram of NaDodSO$_4$/7.5–15% polyacrylamide slab gel electrophoresis. Immunoprecipitations were with hamster anti-tumor serum (I) or normal hamster serum (N). (*B*) Samples larger than those applied to the slab gel in *A* were electrophoresed in cylindrical NaDodSO$_4$/8.5% polyacrylamide gels. These gels were then sliced and the ^{32}P (hatched bars) and ^{3}H (stippled bars) in the various peaks of T Ag were determined. The background from parallel gels run with normal precipitates has been subtracted. (●---●) ^{32}P/^{3}H ratios. Cyt, cytoplasmic fraction.

Ag not bound to 48,000 HTA (Fig. 3*B*). The persistence, at the nonpermissive temperature, of rapidly sedimenting T Ag·48,000 HTA complexes reflects either leakiness of the mutant or binding of 48,000 HTA to unlabeled T Ag synthesized prior to the shift to the nonpermissive temperature. However, even these complexes (fractions 9 and 11 in Fig. 3*B*) have lower sedimentation coefficients than similar complexes radiolabeled at the permissive temperature (fractions 5 and 7 in Fig. 3*A*). No temperature effects upon the sedimentation behavior of either T Ag or 48,000 HTA were observed in mouse cells transformed by wild-type SV40 (data not shown).

In order to determine if the predominantly 5–6S species of mononomeric T Ag generated in tsA-transformed cells at the nonpermissive temperature is also minimally phosphorylated, A21 cells were radiolabeled with both [^{3}H]methionine and [^{32}P]orthophosphate at either the permissive or nonpermissive temperature. Immunoprecipitation from gradients was performed with antiserum to 94,000 T Ag. Bands of T Ag and 48,000 HTA, located in subsequent NaDodSO$_4$/polyacrylamide slab gels by fluorography, were then excised and ^{3}H and ^{32}P contents were measured. In Fig. 4*A* it can be seen that T Ag radiolabeled at the permissive temperature is found predominantly as rapidly sedimenting highly phosphorylated species. T Ag radiolabeled at the nonpermissive temperature (Fig. 4*B*), however, sediments predominantly as a 5–6S monomeric form (fraction 15). In addition, this species of T Ag was minimally phosphorylated, while the small percentage of T Ag remaining complexed to 48,000 HTA at the nonpermissive temperature was phosphorylated to the same extent as the T Ag in complexes of similar S values at the permissive temperature. This implies a close and perhaps causal relationship between the phosphorylation state of T Ag and its ability to complex HTA.

It can be seen that there is much less of the 48,000 HTA complexed to T Ag at the nonpermissive temperature (Fig. 4*D*) than at the permissive temperature (Fig. 4*C*). It is striking that although the total [^{3}H]methionine in T Ag at the nonpermissive temperature is 93% that of T Ag at the permissive temperature, the total [^{3}H]methionine in the 48,000 HTA at the nonpermissive temperature is only 38% of that of 48,000 HTA at the permissive temperature. The overall ^{32}P/^{3}H ratio of the 48,000 HTA is the same at both the permissive and nonpermissive temperatures, in contrast to the effect on T Ag.

Correlation of the ^{32}P/^{3}H Ratio of T Ag with its Affinity for Host Chromatin. In order to determine if T Ag subfractions differing in their affinities for host chromatin differed in their ^{32}P/^{3}H ratios, SVA31E7 cells were again double labeled with [^{3}H]methionine and [^{32}P]orthophosphate. The nuclei of these cells were then isolated and serially extracted with buffers of increasing pH. The various extraction supernatants were then immunoprecipitated with hamster anti-tumor serum and the immunoprecipitates were separated by NaDodSO$_4$/polyacrylamide gel electrophoresis (Fig. 5*A*). It can be seen that both T Ag and the 48,000 HTA eluted from host chromatin at pH 7.0, 8.0, and 9.0 but not at pH 6.0. Small amounts of both proteins were found in the cytoplasmic fraction and may have eluted from nuclei under the conditions employed for nuclear isolation. Determination of ^{32}P and ^{3}H contents in the various fractions of T Ag revealed that although the major fraction of T Ag eluted at pH 8.0, T Ag eluting at pH 9.0 contained the most phosphate. The ^{32}P/^{3}H ratio of T Ag increased with its affinity for host chromatin (Fig. 5*B*).

DISCUSSION

This work indicates that a sequence of reactions takes place between T Ag and the 48,000 HTA, involving phosphorylation of T Ag and leading to subsequent multimeric association and

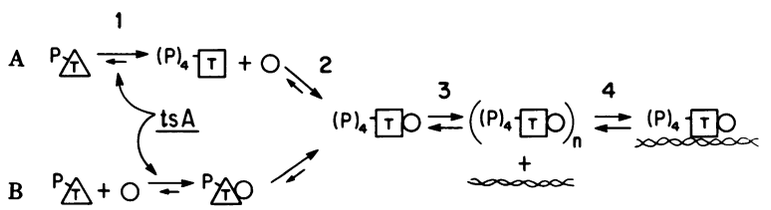

FIG. 6. Proposed reaction sequence for T Ag–HTA–DNA interactions affected by the phosphorylation of T Ag. Triangles represent minimally phosphorylated molecules of T Ag. Squares represent maximally phosphorylated molecules of T Ag. Circles represent the 48,000-dalton HTA. Double-stranded DNA is represented by a double helix. See text for discussion.

chromatin binding by the T Ag·48,000 HTA complexes. This reaction sequence is schematized in Fig. 6.

Reactions 1 and 2: Phosphorylation and 48,000 HTA Binding. The close, perhaps causal, relationship between the level of phosphorylation of T Ag and its ability to complex 48,000 HTA suggests two possibilities: (pathway A) progressive phosphorylation of T Ag enhances its binding affinity for 48,000 HTA or (pathway B) T Ag must first be complexed to 48,000 HTA for maximal phosphorylation to occur. The minimal phosphorylation of monomeric T Ag is rapid, whereas the progressive phosphorylation accompanying complex formation with the 48,000 protein is slow. This might suggest that two different functional types of phosphate exist on the T Ag molecule. However, our results indicate that the ratio of phosphoserine to phosphothreonine is uniform throughout the gradient but that an increase in total phosphorylation correlates with aggregation.

The ability of ts*A* locus mutations to inhibit both the phosphorylation of T Ag and its ability to complex the 48,000 HTA indicates that these activities may be important in affecting one or more functions of the *A* locus.

Reaction 3: Association to Multimers. The smallest T Ag·48,000 HTA complexes sediment at 15–16 S; T Ag in these complexes is half as phosphorylated as the maximally phosphorylated T Ag of larger complexes. The smallest complexes containing maximally phosphorylated T Ag sediment at 24 S. In addition to containing T Ag twice as phosphorylated as that in the 15–16S complex, the 24S complex has twice the ratio of 48,000 HTA to T Ag as the 15–16S form. Absolute stoichiometries cannot be derived at this time, however, because the specific activities of ^{3}H in the two proteins is not known. Multimeric complexes sedimenting faster than 24S have the same ratio of 48,000 HTA to T Ag and contain T Ag phosphorylated to the same extent as in the 24S form. Ratios of 48.000 HTA to T Ag are the same whether immunoprecipitations are performed with antitumor or monospecific anti-94,000 T Ag serum. This indicates that the ratios are those of true complexes and are not derived from cosedimentation of homopolymers of T Ag and 48,000 HTA.

In time course studies, monomeric T Ag appears as a significant fraction of T Ag in cells transformed by wild-type SV40 only upon labeling for short periods of time (Fig. 2). The conversion of minimally phosphorylated, monomeric T Ag to more highly phosphorylated, complexed forms, therefore, either is unidirectional or involves equilibrium strongly favoring formation of highly phosphorylated complexed T Ag. We have found that the maximally phosphorylated species of T Ag in rapidly sedimenting complexes (≥24S) contains about 4 times the phosphate of monomeric T Ag. Walter and Flory (30) have determined that T Ag of SV40-infected cells contains an average of 4 phosphates per molecule. Our results are, therefore, consistent with the existence of highly phosphorylated T Ag in high molecular weight complexes, which represent the predominant form of T Ag in SV40-transformed cells and have 3–4 phosphates per molecule.

Reaction 4: Chromatin Binding. T Ag binds DNA in the absence of other proteins (9, 11). It has yet to be determined if the 48,000 HTA can bind DNA in the absence of T Ag, although it is found in the extranucleolar nucleoplasm of methylcholanthrene-transformed cells in the absence of SV40 T Ag (31). However, the finding that the most phosphorylated form of T Ag both complexes the 48,000 HTA and binds with greatest affinity to host chromatin suggests that high molecular weight T Ag·48,000 HTA complexes represent the major form of T Ag bound to host chromatin *in vivo*. This hypothesis is supported by immunofluorescence studies in which T Ag and 48,000 HTA were found to have the same distribution in the nuclei of SV40-transformed cells (19). Moreover, T Ag and 48,000 HTA have been shown to cochromatograph, as complexes, on DNA-cellulose columns (19). Other groups have reported that ts*A* lesions impair the ability of T Ag to bind host chromatin (13) and SV40 DNA (14). We propose this may result from a prior effect of the ts*A* lesion on the phosphorylation of T Ag and its ability to complex host tumor antigens (reactions 1 and 2, Fig. 6). Thus, ts*A* inhibition of the phosphorylation of T Ag and its ability to complex HTAs would interfere with *A* locus functions requiring T Ag–HTA interactions with host chromatin, including (*i*) initiation of replication, (*ii*) control of transcription, and (*iii*) establishment and maintenance of viral transformation.

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