JOURNAL OF VIROLOGY, Dec. 1978, p. 992-996
0022-538X/78/0028-0992$02.00/0

Vol. 28, No. 3
Printed in U.S.A.

NOTES

Characterization of the Amino-Terminal Tryptic Peptide of Simian Virus 40 Small-t and Large-T Antigens

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Received for publication 10 July 1978

Simian virus 40 small-t and large-T antigen were synthesized in vitro and labeled with methionine donated by initiator tRNA. Tryptic peptide fingerprinting was used to identify the amino-terminal peptide of the two proteins. Similar fingerprint analysis of small-t and large-T made in vitro in the absence of acetyl coenzyme A showed that the mobility of the amino-terminal peptide was changed under these conditions and suggested that it is acetylated. These data establish that the amino-terminal methionine residue of simian virus 40 small-t and large-T results from an initiation event, not post-translational cleavage, and provides additional evidence that the amino terminus of both proteins is acetylated. The identification of the amino-terminal peptide provides a useful marker for further studies on different forms of T-antigen from cells infected with and transformed by simian virus 40 and related viruses.

At least two forms of tumor antigen (T-Ag) are encoded by the early region of simian virus 40 (SV40). These are called small-t and large-T and have molecular weights in the range 15,000 to 20,000 and 90,000 to 100,000, respectively (3, 9, 12, 14). Peptide fingerprinting data indicate that the two proteins share amino acid sequences (9, 11, 16, 18), and studies with specific deletion mutants of SV40 suggest that the shared region is present at the amino-terminal end of the proteins (3). This has been confirmed by partial sequence analysis of large-T and small-t which shows that they have an identical amino-terminal amino acid sequence (10). The amino acid sequence determined is in agreement with that predicted from the DNA sequence beginning at 0.65 map units (4, 13, 19) and indicates that both forms of T-Ag begin Met-Asp-Lys (10). However, our observation that T-Ag's made in vitro in the presence of acetyl donor are not amenable to sequence analysis suggests that the amino-terminal peptide is acetylated (10). In this report we identify the amino-terminal tryptic peptide of both forms of T-Ag by labeling the proteins made in vitro with methionine from initiator tRNA. We also show that the amino-terminal peptide has an altered mobility when the proteins are synthesized under conditions which prevent acetylation.

SV40 small-t and large-T can be made in response to polyadenylic acid-containing RNA isolated from SV40-infected CV1 cells, using cell-free systems derived from either L-cells (8, 9) or wheat germ (11, 12). [^{35}S]methionine-labeled T-Ag's were synthesized in the L-cell cell-free system, immunoprecipitated with anti-T-serum, and prepared for tryptic peptide analysis as described previously (8, 10, 18). Fingerprints of small-t and large-T using pH 2.1 in the first dimension are shown in Fig. 1a and 2a, respectively. As has been found previously, the small-t fingerprint contains many of the methionine tryptic peptides present in the fingerprint of large-T (9, 18).

Since in our sequence analysis we detected a methionine residue in the amino-terminal position of both small-t and large-T (10), one of the shared methionine-containing tryptic peptides must arise from the amino terminus of the proteins. Assuming that the amino-terminal methionine arises from an initiation event, we attempted to identify this peptide by fingerprinting small-t and large-T synthesized in the wheat germ cell-free system and labeled with methionine donated by wheat germ initiator tRNA (Met-tRNA$_F$) (5, 15, 17). [^{35}S]Met-tRNA$_F$ was prepared by separating tRNA$_F^{Met}$ by chromatography on benzoylated DEAE (BD)-cellulose (17) and charging the purified tRNA with highly purified *Escherichia coli* Met-tRNA synthetase. When [^{35}S]Met-tRNA is prepared using these conditions over 95% of the label is present in

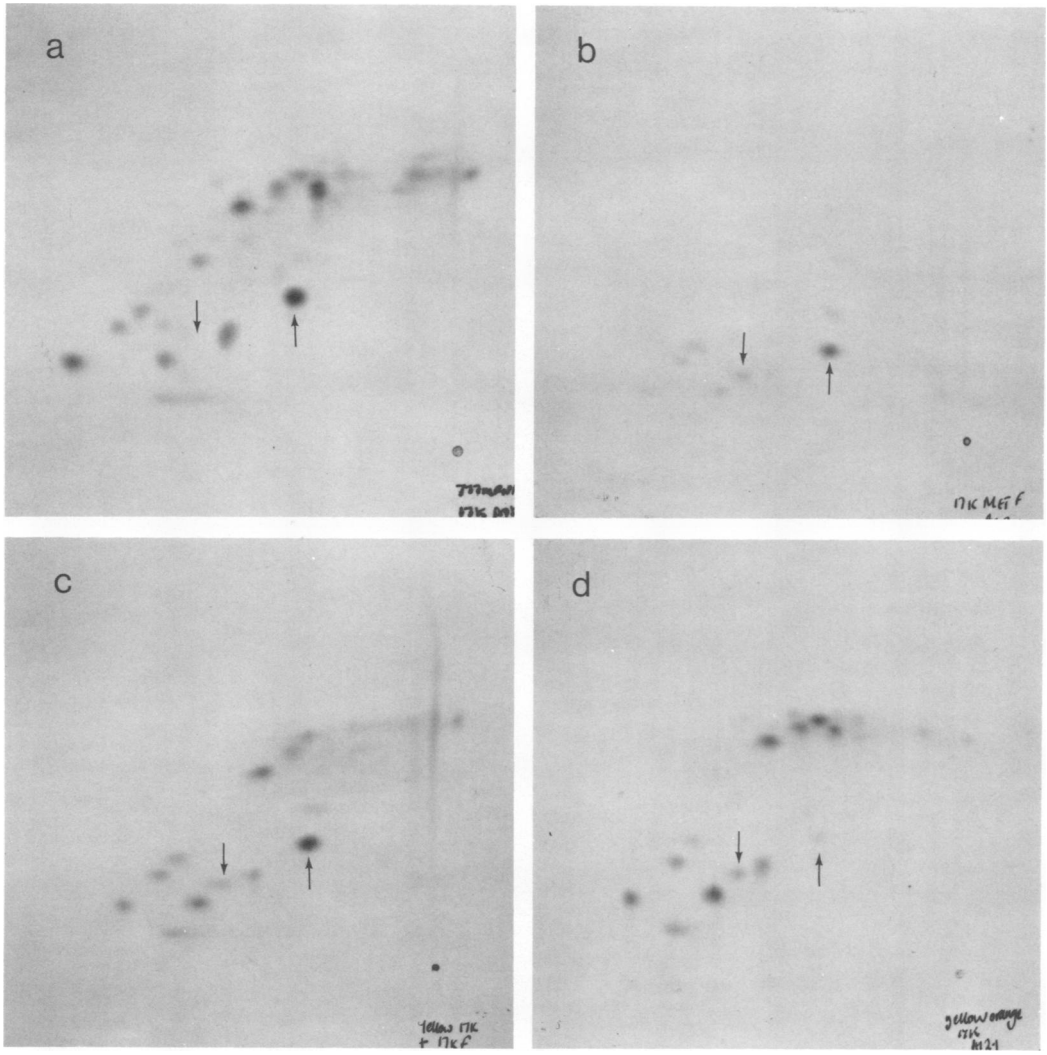

FIG. 1. *Tryptic peptide fingerprints of [^{35}S]methionine-labeled SV40 small-t synthesized in vitro. Conditions for cell-free synthesis (8), purification of initiator tRNA (15, 17), pretreatment to remove acetyl coenzyme A (6, 10), immunoprecipitation (8), and fingerprinting (18) have all been described in detail. Electrophoresis in the first dimension was at pH 2.1. (a) Small-t (20,000 cpm) synthesized in the L-cell cell-free system, using [^{35}S]methionine as label and standard cell-free conditions (8). (b) Small-t (5,000 cpm) synthesized in 500 μl of wheat germ extract containing 25 × 10^6 cpm of wheat germ [^{35}S]Met-tRNA$_F$ (15). (c) Mixture of 15,000 cpm of sample a with 4,000 cpm of sample b. (d) Small-t (25,000 cpm) synthesized in an L-cell cell-free system, previously treated with citrate synthase and oxaloacetate to deplete the endogenous pool of acetyl coenzyme A (6, 10). Autoradiography was for 7 to 14 days. The upward pointing arrow indicates the position of the peptide identified here as the amino-terminal peptide of small-t (N-acetyl-Met-Asp-Lys); the downward pointing arrow indicates the position of the peptide which is probably the non-acetylated form of the amino-terminal peptide.*

Met-tRNA$_F$ (15). Incubations using [^{35}S]Met-tRNA$_F$ also contained unlabeled methionine as a source of internal methionine residues.

Figures 1b and 2b show the fingerprints of small-t and large-T, respectively, labeled with methionine from initiator tRNA. One predominant labeled peptide is present in both fingerprints, and this migrates in the position of one of the methionine peptides already identified as common to both small-t and large-T (marked with an upward pointing arrow in Fig. 1a and 2a). This was confirmed by mixing samples of the digests shown in Fig. 1a and 1b before fractionation. Figure 1c shows that the intensity of

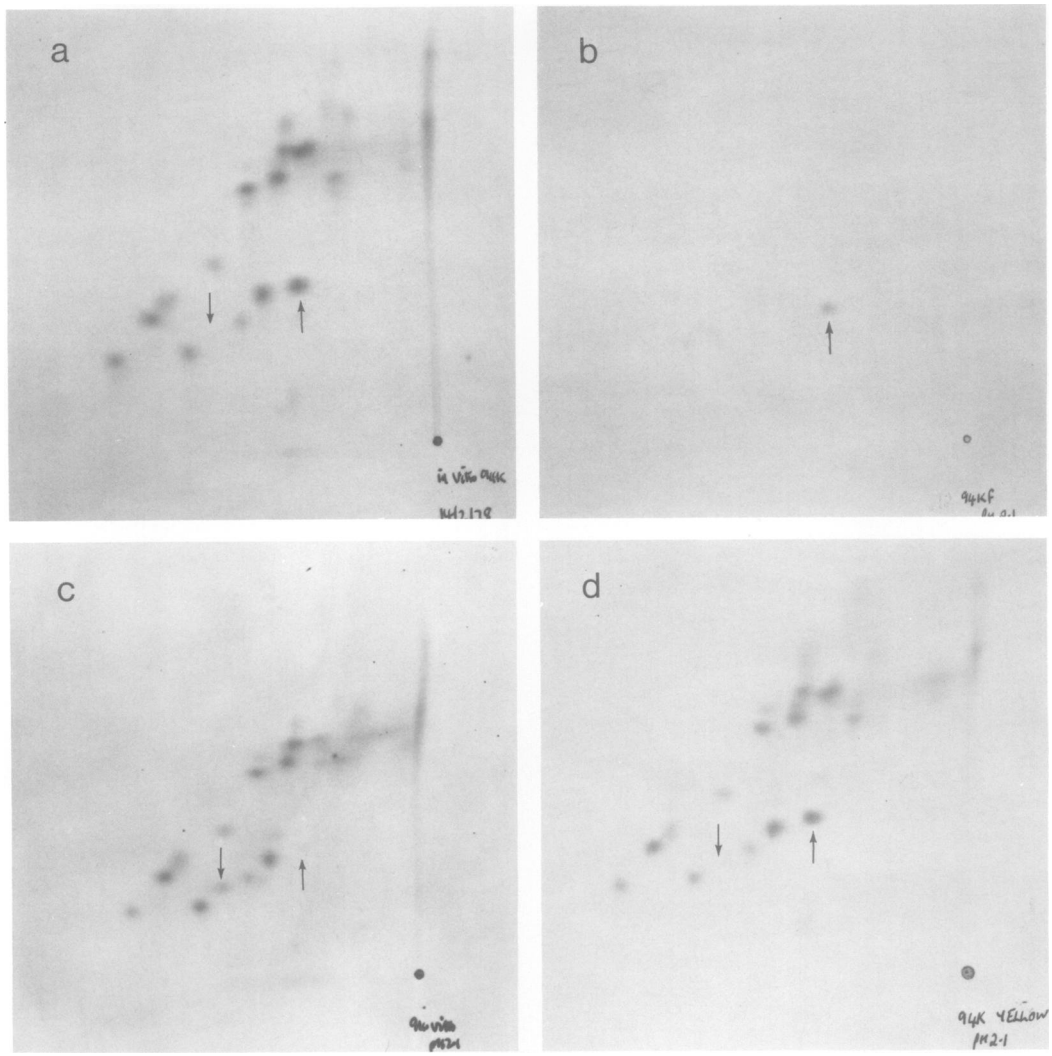

FIG. 2. *Tryptic peptide fingerprints of [^{35}S]methionine-labeled SV40 large-T. The procedures used were exactly similar to those described in the legend to Fig. 1. (a) Large-T (20,000 cpm) synthesized under standard conditions in the L-cell system. (b) Large-T (2,500 cpm) synthesized in a wheat germ cell-free system containing [^{35}S]Met-tRNA$_F$. (c) Large-T (15,000 cpm) synthesized in an L-cell system, previously treated to remove endogenous acetyl coenzyme A. (d) Large-T (20,000 cpm) isolated from SV40-infected CV1 cells. Autoradiography was for 7 to 21 days. The arrows indicate the position of the amino-terminal peptide in its acetylated (upward pointing) and possible non-acetylated (downward pointing) forms.*

label in the peptide arrowed in Fig. 1 is specifically enhanced in the fingerprint of the mixture. Since initiator tRNA only donates methionine into amino-terminal positions (17), this result strongly suggests that the labeled peptide is the amino-terminal tryptic peptide of both small-t and large-T. This conclusion is also supported by fingerprint analysis of small-t labeled with methionine from purified Met-tRNA$_M$ (data not shown). This tRNA donates methionine exclusively into internal positions of proteins, and the fingerprint of small-t labeled with methionine from Met-tRNA$_M$ specifically lacks the peptide labeled with methionine from initiator tRNA. All of the fingerprints described have also been performed using electrophoresis at pH 6.5 in the first dimension, and the results obtained confirmed those shown here.

If the peptide we have identified as the amino-terminal peptide of both small-t and large-T is acetylated, then the mobility of this peptide should be affected in fingerprints of the proteins

synthesized under conditions which prevent acetylation. To test this prediction, we fingerprinted small-t and large-T made in a cell-free system from L-cells which had been pretreated with citrate synthase and oxaloacetate to deplete the endogenous pool of acetyl coenzyme A (6, 10). The fingerprints of the small-t and large-T synthesized under these conditions are shown in Fig. 1d and 2c, respectively. One peptide is absent from the fingerprints of the proteins made in the absence of acetyl coenzyme A, and this peptide corresponds to the peptide labeled with methionine from initiator tRNA (marked with an upward pointing arrow in Fig. 1 and 2). From this result we conclude that the amino-terminal peptides of small-t and large-T are altered when they are synthesized in the presence of acetyl donor. Our sequencing data suggest that the alteration blocks the amino terminal of the proteins, and taken together these results strongly suggest that the amino-terminal tryptic peptide is *N*-acetyl-Met-Asp-Lys. Since a peptide of identical mobility is present in fingerprints of large-T (Fig. 2d) and small-t (see Fig. 5 of reference 18) isolated from cells, it is very likely that the cellular forms of these proteins have the sequence *N*-acetyl-Met-Asp-Lys at their amino terminus.

The fingerprints of small-t and large-T which were synthesized under conditions preventing acetylation contain a new labeled peptide (marked with a downward pointing arrow). This peptide is more positively charged at pH 2.1 than *N*-acetyl-Met-Asp-Lys, and this is consistent with it being the non-acetylated form of the amino-terminal peptide. This peptide is not present in the fingerprints of small-t (Fig. 1a) or large-T (Fig. 2a) made under normal conditions in the L-cell system or of small-t (data not shown) or large-T (Fig. 2d) isolated from cells, but it is present in the fingerprint of small-t synthesized in the wheat germ cell-free system and labeled with methionine from initiator tRNA (Fig. 1b). This possibly indicates that acetylation is incomplete in the wheat germ cell-free system.

Acetylation of the amino terminal of proteins appears to be fairly widespread in nature, and many examples of its occurrence have been compiled (1). Relatively few amino acids are commonly found in the amino-terminal position of such blocked proteins, and these include methionine, alanine, and serine. The functional significance of acetylation is unclear. The retention of the amino-terminal methionine residue donated by initiator tRNA, even in the absence of acetylation, was at first unexpected because, although methionine initiates the synthesis of all proteins in eucaryotes, this residue is commonly removed from the nascent polypeptide chain (5, 15, 17). However, recent sequencing studies of many different proteins have shown that in several cases the amino-terminal methionine is retained, at least in vitro (1, 2, 7). This is particularly common in cases where the second amino acid carries a large or polar side chain, for example, aspartic acid, lysine, and arginine.

The data presented here show that the amino-terminal residue of SV40 small-t and large-T is a methionine donated by initiator tRNA. This formally establishes that the site mapped on SV40 DNA as coding for the amino terminal of these proteins (10) corresponds to the site at which their synthesis is initiated and excludes the possibility that T-Ag's are synthesized via a precursor molecule which is subsequently subject to proteolytic cleavage at the amino-terminal end. The identification of the amino-terminal tryptic peptide of large-T and small-t provides a useful marker for further studies on these proteins. The presence or absence of this peptide from different forms of T-Ag's, particularly those from mutant-infected and different transformed cells, will be of interest.

We thank R. Smith for help with the fingerprints, Eva Paucha for SV40 T-antigens synthesized in vitro, and C. Bruton for purified Met-tRNA synthetase. We are also grateful to P. Bourgaux and H. H. M. Dahl for help with the manuscript and to Maria Brennan for its preparation.

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