Differentiation Between SV40 Large-T and U Antigenic Sites

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SUMMARY

Radioimmune precipitation, SDS-polyacrylamide slab gel electrophoresis and fluorography were used to investigate the SV40 large-T and U antigenic sites on species of proteins synthesized during wild type and tsA58 mutant infections in TC7 monkey cells. Wild type infection at 33 and 41.5 °C and the A58 infection at 33 °C produced similar profiles of three species ranging in mol. wt. from 84,000 to 94,000, all of which had both the large-T and U antigenic sites. The A58 infection at 41.5 °C, however, produced an additional four discrete species ranging in mol. wt. from 60,000 to 74,000 that contained the large-T site(s), but not the U site(s). A subpopulation of the 74,000 mol. wt. species contained both sites. Therefore, the region of the A58 mutant 94,000 mol. wt. species containing the U antigenic site(s), the COOH-terminal region, appears to be more sensitive to processing, probably proteolytic cleavage, than does the region containing the large-T antigenic site(s).

Simian virus 40 (SV40) is a small oncogenic papovavirus. The oncogenic potential of this virus is mediated by two proteins, large-T (mol. wt. 94,000) and small-t (mol. wt. 17,000) (Rundell et al. 1977; Brockman, 1978; Prives et al. 1978; Sleigh et al. 1978), which have the same NH2-terminal acid sequence (Crawford et al. 1978; Paucha et al. 1978; Volckaert et al. 1978). The molecular interactions between these two ‘transforming’ proteins and cellular molecules have not been fully defined (Nathans, 1976; Fareed & Davoli, 1977; Weinberg, 1977). Different species of proteins processed from the 94,000 mol. wt. large-T molecule, probably by proteolytic cleavage (Neurath & Walsh, 1976; Tegtmeyer et al. 1977), may reside in different intracellular compartments and have different functions (Carroll et al. 1974; Chou & Martin, 1975; Nakajima & Oda, 1975; Tevethia & Tevethia, 1975; Anderson et al. 1977; Deppert et al. 1977; Jay et al. 1977; Lin et al. 1977; Robb, 1977; Schmidt-Ullrich et al. 1977; Segawa et al. 1977). A species of protein having the U antigenic site(s), but not the large-T antigenic site(s), has been found in the plasma membrane of SV40-transformed hamster and mouse cells, a protein that is not in the plasma membrane of the non-transformed parental cells (Schmidt-Ullrich et al. 1977). I have previously shown that the SV40 U antigenic site(s) is contained in the 94,000 mol. wt. large-T molecule, presumably in the amino acid sequence of the COOH-terminal region that is encoded from o.18 to 0.28 fractional units on the SV40 map. Further definition of the difference between the SV40 large-T and U antigenic sites might help elucidate the formation and/or function of the various early species of SV40-coded protein during productive and transforming infections.

To further define the large-T and U antigenic sites, I have taken advantage of the instability of the mutant 94,000 large-T molecule produced during temperature-sensitive Group A mutant infection in monkey cells at restrictive temperature (Tegtmeyer et al. 1975; Robb, 1977). During these infections and/or extractions the mutant large-T molecule is processed, probably by proteolytic cleavage (Neurath & Walsh, 1976), to at least four discrete species with mol. wt. ranging from 60,000 to 74,000 (Robb, 1977). These four
Fig. 1. Comparison of the molecular species of proteins carrying SV40 large-T and U antigenic sites that are synthesized in wild type (WT) and A58 mutant infected TC7 cells at 41.5 °C. TC7 cells were infected with TC7 cell lysate (mock) or with 100 infectious units/cell of wild type SVS virus or tsA58 mutant virus and incubated at 41.5 °C. At 23 h after infection, the proteins in the cells were radiolabelled with 50 μCi/ml of 35S-methionine in methionine- and serum-free medium for 60 min. The cells were then extracted for protein using NP40 at pH 8.0. The extracted proteins were immune-precipitated using anti-large-T and anti-U IgG. The precipitated radiolabelled proteins were analysed on 10% polyacrylamide slab gels using 20 μl/track. Tracings of the autoradiograms are presented. The wild type and A58 infections at 33 °C gave results similar to the wild type infection at 41.5 °C. The wild type parent of A58, VA 45-54, is not detectably different from the SVS wild type infection at 33 or 41.5 °C. The arrows indicate the four smaller T+U- peaks.

species contain the large-T antigenic site(s), but not the U antigenic site(s) which was either removed, or modified in its conformation so that it was no longer recognized by anti-U IgG.

The TC7 monkey cells, small plaque (SVS) wild type SV40, tsA58 SV40 mutant, conditions of infection, antisera and the radioimmune precipitation, SDS-polyacrylamide slab gel electrophoresis and fluorography techniques are the same as previously described (Robb, 1977) with the exception that equal volumes (20 μl) of radioimmune precipitate were loaded on to each track for slab gel electrophoresis rather than equal radioactivity. The anti-large-T IgG used in these experiments does not precipitate detectable quantities of small-t antigen because the anti-small-t titre is too low (K. Rundell, personal communication).

The data in Fig. 1 demonstrate five features. First, the major capsid protein of mol. wt. 45000, VP1, was not detected in the A58 infection. The VP1 precipitated during the wild
type infection by the anti-T and anti-U sera was due to autoprecipitation rather than specific recognition of VP1 sequences because the anti-T IgG does not recognize purified VP1 (H. Kasamatsu, personal communication). Second, only three species of large-T antigen were observed in the wild type infection. The 94000 mol. wt. species (94K) was degraded into the 89000 and 84000 species during extraction (Tegtmeyer et al. 1977), but all three still retained the large-T antigenic site(s). Third, in addition to the three large-T species produced during wild type infection, at least four discrete species from mol. wt. 74000 to 60000 were formed during the A58 infection at 41.5 °C. Only the three 94 to 84K wild type species are formed during the A58 infection at 33 °C. The largest of the A58 peaks, mol. wt. about 74000, is probably heterogeneous due to its width and sub-population of U-positive molecules. The formation of at least one of these four species, the 60K species, is dependent upon the Group A mutant used for the infection (Robb, 1977). Fourth, the majority of the 74K species and essentially all of the other three A58 specific species containing the large-T site(s) did not contain the U site(s). There is about threefold more radioactivity precipitated by the anti-T IgG than by the anti-U IgG in the 74 to 60K region when the areas under the peaks are corrected for the mock infection and normalized so that the 94 to 84K peaks are equivalent for the T and U antigen curves. (This normalization corrects for the titre differences). Fifth, the amount of 94 to 84K and 74 to 60K large-T species produced during the A58 infection was about sixfold greater than the large-T species produced during the wild type infection as observed previously (Tegtmeyer et al. 1975). Two independent experiments gave similar results.

The 89K and 84K species of large-T antigen that are 'processed' degradation products of the 94K molecule contain both the large-T and U antigenic sites. Further processing of any or all of these three species, however, appears to remove or modify the U antigenic site(s) in species that retain the large-T antigenic site(s). At least three groups of processed molecules could not have been detected in these experiments if they had been formed. First, species whose large-T and U sites were both lost or modified. Second, species that contained large-T and/or U antigenic sites, but did not contain methionine. Third, species that had a conformational change in large-T and/or U sites, but did not have a sufficient change in their mol. wt. to separate them as discrete species.

Further analysis of the various discrete species of molecules containing large-T and/or U antigen sites formed during wild type and mutant SV40 productive and transforming infections should be helpful in explaining the formation and function of the SV40-coded proteins that are present in different intracellular compartments. Such studies might also help explain the temperature-dependent regulation of a least some parameters of the SV40 transformed phenotype at restrictive temperature in cells transformed by Group A mutants (Tegtmeyer et al. 1977; Butel & Soule, 1978).

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