Kinetics of Synthesis and Rate of Degradation of T Antigen Induced by Polyoma Virus

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Polyoma virus can follow two distinct paths according to the nature of the cells supporting its development: a lytic or productive cycle in permissive cells and a transforming cycle either abortive or complete. Virus infection always induces the production of the same new antigen which, according to Huebner's terminology (1967), is the T antigen in the lytic cycle and the tumour antigen in the transforming cycles. In the lytic cycle, with a high enough infection multiplicity, the T antigen appears early and disappears when the structural antigens of the virus are synthesized (Takemoto, Malmgren & Habel, 1966). We studied the kinetics of synthesis and the renewal rate of T antigen in mouse embryo fibroblasts infected with polyoma virus (lytic cycle) after labelling the T antigen with $[^3H]$-leucine or with a mixture of $[^14C]$-amino acids. After fractionation of the nuclei, the T antigen in the nuclear proteins was titrated by a radio-immunological method.

The small-plaque TORONTO strain of the virus (concentration: $10^8$ p.f.u./ml.) was allowed to adsorb for 1 hr at 37° to a monolayer of mouse embryo fibroblasts at an m.o.i. of 100. The cells were then overlayed with Eagle's minimal essential medium containing one-tenth the usual concentration of amino acids and $[^3H]$-leucine (specific activity 20 c/m-mole, CEA) at a concentration of 1 mc/ml. Identical results were obtained with a medium containing a hydrolysate of proteins labelled with $[^14C]$ (specific activity 0·5 mc/mg., CEA) at a concentration of 0·1 mc/ml. After 2 hr the cells were harvested, washed and the nuclei were separated by Chauveau's method (Wang, 1967) in $4·5$ ml. of $2 M$-sucrose + $3·3 mM$-CaCl$_2$, after bursting by 25 strokes in a Dounce homogenizer and centrifugation in 3 × 5 ml. swinging buckets at 4000g for 1 hr.

The nuclear pellet was resuspended in 0·5 ml. of hypotonic buffer (0·01 m-NaCl + 0·015 m-MgCl$_2$ + 0·01 m-tris, pH 7·4). The nuclear proteins were solubilized after freezing and thawing (−70° to 37°) by the method of Summers, Maizel & Darnell (1965) and the T antigen was identified and titrated by the radio-immunological method.

Antiserum to T antigen was obtained from hamsters carrying tumours induced by polyoma virus and was kept as −70° until used. The hamster antoglobulin sera were commercial sera prepared in rabbits (Pasteur Institute). The anti-T sera were titrated by complement fixation. The quantity of anti-T serum introduced into the reaction was determined by titration conditions described below. It was usually between 30 and 50 μl. The test itself was made in polypropylene tubes as follows. A previously determined quantity of anti-T serum was added to increasing doses of radioactive proteins varying from 10 to 50 μl. and the volume was adjusted to 90 μl. with 0·15 m-NaCl. After 2½ hr incubation at 37°, 50 μl. of antoglobulin hamster serum were added to the tubes and incubation continued. The precipitates were collected by filtration on fibre glass filters (GF/C, Whatman), washed with 0·15 m-NaCl (10 ml.) and then with ethanol at 70° (2 ml.). Each filtrate was placed in 10 ml. of a scintillation mixture (toluene + PPO + POPP, 1·000 ml.: 5 g.: 0·1 g.) and was counted in a liquid scintillation counter (LS 150 Beckman) at 20°. Two controls were used to ensure the specificity of the antigen: either extracts of mock-infected cells or extracts of mouse embryo cells infected with SV 40. Three control sera were used in place of anti-T serum, hamster anti-
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Fig. 1 Radio-immunological titration of T antigen. - , Nuclear proteins; O -- O, cytoplasmic proteins.

Fig. 2. Kinetics of synthesis of T antigen. Cells infected at zero time. Pulse of [3H]-leucine 2 hr before harvesting the cells. Results (mean of two experiments) are expressed as % of the maximum rate of synthesis.
globulin serum or the two antisera. The total specific activity of the sample was measured after precipitation with 10% trichloroacetic acid (Fig. 1). A weak activity was noted in the cytoplasm, which was unrelated to the quantity of antigen used and was 12% of the immunoprecipitable nuclear specific activity; 65% of the nuclear activity was found in the trichloroacetic acid precipitate.

Synthesis of T antigen began before the 10th hr, reached its maximum at the 18th hr, and decreased rapidly after the 20th hr (Fig. 2). This confirmed the classical data obtained by immunofluorescence (Takemoto et al. 1966).

Table 1. Rates of degradation of T antigen

<table>
<thead>
<tr>
<th>Time of harvest (hr)</th>
<th>Specific activity* (hr after labelling)</th>
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<tbody>
<tr>
<td></td>
<td>16</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>44</td>
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<tr>
<td>Half-life (hr)</td>
<td>3½</td>
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* Residual specific activity as a % of initial specific activity measured at the 16th, 18th and 21st hr after infection (mean of 3 experiments).

The degradation rate was calculated by following the disappearance of the specific activity after a 2 hr labelling period with [3H]-leucine followed by a ‘chase’ of ‘cold’ leucine, at a concentration 10 times greater than that in Eagle’s medium. Equal quantities of cells were harvested at the end of the pulse, zero time, and 2 and 4 hr after the chase: these measurements were made at the 16th, 18th and 21st hr of the cycle, measured at the end of the pulse (Table 1). The mean half-life was between 3 and 3½ hr. There was little variation between the 16th and 21st hr.

The synthesis of T antigen seems to be linked to the translation of early messenger RNA corresponding to 37% of the virus genome (Benjamin, 1966). However, despite the increase of normal cell syntheses which is characteristic of oncogenic viruses, T antigen represents the main fraction of the newly synthesized nuclear proteins. The function of this T antigen is still unknown. It has not been identified with any early enzyme (Kit et al. 1967), but the fact that it is a protein with a high turnover rate seems to imply that it plays an important role in the infectious cycle. The hypothesis that it may be a precursor of an early functional protein is now being investigated in our laboratory.

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