Transformation Phenotype of Polyoma Virus-Transformed Rat Fibroblasts: Plasminogen Activator Production Is Modulated by the Growth State of the Cells and Regulated by the Expression of an Early Viral Gene Function

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The expression of two transformation parameters, namely, ability to grow in agar and plasminogen activator production, was studied in several rat fibroblasts transformed by either wild-type or thermo-sensitive (tsa and ts25) polyoma viruses. The production of plasminogen activator was found to be dependent upon the growth state of the infected cells during a period of several days after infection. The analysis of the transformed phenotype of 25 tsa transformants and of 19 ts25 transformants independently isolated under various growth conditions led to the conclusion that there is no correlation between the regulation processes involved in plasminogen activator production and ability to grow without anchorage. The results obtained also suggested that the production of plasminogen activator is under the control of a functional large T antigen.

Oncogenic transformation of eucaryotic cells is characterized by the expression of specific morphological and biochemical changes which differ from the normal parental cell phenotype. Most of these properties, referred to as transformation parameters, are also common to cells transformed by different oncogenic viruses (see reviews by Tooze [52], Hynes [15], Robbins and Nicolson [43], and Hanafusa [12]). In addition to the study of the possible involvement of these parameters in the establishment of the transformed state, there is a need to better understand the regulatory mechanisms involved in the maintenance of the transformation phenotype. To define the relationships which may preside in the expression of distinct transformation parameters, a comparative study of the ability of polyoma-transformed cells to grow in agar and to produce plasminogen activator (PA) has been undertaken.

After infection of Fisher rat 3T3 fibroblasts with tsa mutant of polyoma virus, at least two types of transformants can be distinguished on the basis of their ability to grow without anchorage (48). In one type, this capacity is expressed in a temperature-dependent way, whereas in the other, it is expressed irrespective of the temperature. Temperature-dependent transformants arise more frequently when the infected cells are kept in an actively growing state for 5 days after infection (36) and when a low multiplicity of infection of 1 to 100 is used (35).

PA production has been reported in various cases to correlate with malignancy (3, 9, 19, 30, 37, 39, 63), as the appearance of PA is closely related to, and an early event in, the transformation process (28, 30, 53, 54). Elevation of PA activity has been reported in several transformation systems from different origins (see reviews by Reich [38], Christman, Silverstein, and Acs [4], and Quigley [34]) and to require the continued expression of a viral function in some transformed cell lines (29, 41, 54, 56, 62).

Both PA production and ability to grow in agar have been found to depend upon the expression of an early viral gene function in some tsa transformants (29). However, it appears that some cell lines or clonal isolates which exhibit several transformation characters, such as altered morphology, increased sugar uptake, and ability to grow in soft agar, do not produce high levels of plasminogen activator (15, 19, 25, 26, 29, 46, 61; B. Perbal, submitted for publication).

Several distinct possibilities have been considered to account for such observations. Besides the existence of cell-secreted inhibitors (21, 44), it has been postulated that transformation of the normal cell might proceed through distinct steps (57). Our recent studies, performed on simian virus 40-transformed cells, revealed the existence of discrete levels of PA production among simian virus 40 transformants (B. Perbal, sub-

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PA production. The qualitative assay for PA production used in this work has been previously described (29). Briefly, colonies of transformed cells were overlayed with a mix containing agarose (0.6%), nonfat dried milk (5%), and purified plasminogen (20 μg). No serum was added (it had been checked that the addition of 10% chicken serum did not change the results obtained). PA activity was estimated by means of scoring plasmin-mediated caseinolysis (clear halos) after 18 to 48 h of incubation at 33 or 41°C, as indicated. All the assays were performed in duplicate.

RESULTS

Proportion of PA producers among polyoma transformants isolated after growth of the infected cells under various conditions. High levels of PA production have been reported in most of the tsa and WT polyoma-transformed rat fibroblasts previously studied (29). Also reported was the importance of the rat cell growth state, early after infection with tsa mutant, in the choice between distinct transformation states (36). We therefore investigated the possibility that the growth state of the infected cells may also be critical for the expression of PA production.

Fisher 3T3 rat cells (48) which do not produce significant amounts of PA (29) were infected with polyoma WT and ts mutants (multiplicity of infection, 100 PFU/cell) under conditions described previously (48). Two distinct mutants, tsa (7) and ts25 (5), which belong to the same complementation group (23) but map in different locations in the early gene region (Fig. 1) were used, in addition to the A2 wild-type strain (Pasadena strain). Infected cells were subjected to different growth conditions before transformants were selected, either on plastic or in agar. Immediately after infection, cells were divided averages from two independent measurements. The mean size of the colonies scored was estimated to range between 0.5 to 1 mm.

MATERIALS AND METHODS

Cells and virus strains. Cells were grown in Dulbecco-modified Eagle medium (GIBCO laboratories, Grand Island, N.Y.) as previously described (29). The growth properties of normal Fisher rat 3T3 cells have already been reported (48). Wild-type (WT) polyoma virus (A2 strain), the tsa mutant (7), and the ts25 mutant (5) were plaque purified and grown in secondary mouse embryo cells infected at a multiplicity of 0.01 to 0.05 PFU/cell.

Transformation. Purified agarose (Agarose A 37 Indubiose, Industrie Biologique Française) was used instead of bacteriological agar for selection of transformants (24) from infected cells grown as described above. The multiplicities of infection routinely used were 100 PFU/cell for both WT, tsa, and ts25 viruses.

Ability to grow in agar. Ability to grow without anchorage was measured at both high and low temperature by seeding 10⁴ cells in agarose plates. The cloning efficiency is expressed as the number of colonies which arose per plate. The values given represent

![Fig. 1. Location of tsa and ts25 mutants on the polyoma genome. The linear representation of the polyoma DNA genome shows the location of tsa and ts25 mutants as determined by marker rescue using WT restriction endonuclease fragments (23). The early and late mRNA transcripts are demarcated under their respective HpaII restriction nuclease fragments. OR designates the origin for DNA replication.](http://jvi.asm.org/article-pdf/42/4/421/2372588/pjvi-42-4-421.pdf)
in five subsets which were grown as follows. (i) Set a, infected cells were seeded directly in agar (2 × 10^4 cells per 5-cm plate) and incubated for 22 days at 33°C. Under these conditions, only transformed cells are able to grow. (ii) Set b, plastic dishes (5-cm diameter) were seeded with a mixture of infected cells (2 × 10^6 cells) and uninfected cells (10^6 cells) before incubation at 33°C for 9 days, with regular medium changes. (iii) Sets c, d, e, the petri dishes (5-cm diameter) were seeded with 2 × 10^4 infected cells, which were subsequently kept in an actively growing state for increasing periods of time. In set c, the cells were trypsinized once (at day 3 postinfection), replated at a density of 2 × 10^6 cells per dish, and left to reach confluency at 33°C. These cells were kept at confluency until day 9 postinfection. Cells from set d were treated in the same way, except that they were trypsinized twice (at day 3 and day 6 postinfection). In set e, cells were always kept actively growing and were therefore trypsinized at days 3, 6, and 9 postinfection, to avoid them reaching confluency at any time of the experiment.

At day 9 postinfection, cells from sets b, c, d, and e were seeded in agar (2 × 10^6 cells per plate) for transformant selection. Transformation frequencies were found to be the same for ts25 (0.12%), ts25 (0.15%), and WT (0.15%) polyoma viruses and similar to those already reported (17, 31, 48).

The proportion of PA producers among transformants isolated under the conditions described above was determined by the mean of the qualitative assay used before (29). The proportion of PA producers found in transformants from set a (Table 1) is very close to values obtained before under similar conditions for ts25 and WT transformants (29), suggesting that transforming abilities of ts25 and ts25 polyoma mutants are not very different.

The effect of the infected cells growth state in the expression of the final transformed phenotype is exemplified by the various proportions of PA producers found in the other sets. When infected cells are kept in a confluent state before the selection of transformants, the proportion of PA producers obtained with the three viruses used dropped to values (10 to 20%) considerably less than those obtained in set a (85 to 90%) where growth was arrested by seeding in agar. Furthermore, it appeared that active growth of the infected cells might counterselect the expression of PA. For example, the proportion of PA producers in ts25 transformants dropped from 58% in set c (active growth for 3 days postinfection) to 3.5% in set e (actively growing cells for 9 days postinfection). It should be emphasized that the overall behavior of ts25, ts25, and WT polyoma viruses was not significantly different with respect to PA production under the conditions used.

It has already been suggested that proteases may induce modifications of the cellular membrane (2, 13, 14). We therefore examined whether the decrease in the proportion of PA producers observed after active growth (set e) was due to the successive trypsinizations. For this purpose, 2 × 10^6 cells from set c (ts25, ts25, and WT) were incubated in the presence of 10^6 uninfected cells and trypsinized three times, in the same way as cells from set e. The frequency of PA producers was not found to be sensitive to trypsin per se since the values obtained were the same as those in set c (data not shown).

Regulation of ability to grow in agar and PA production by distinct control mechanisms in both ts25 and ts25 polyoma transformants. Previous studies have shown that when ts25 polyoma-infected cells are maintained under growth-inhibiting conditions, such as confluency or absence of anchorage in agar, during

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Frequency (%) among transformants obtained with following virus strain:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ts25</td>
</tr>
<tr>
<td>Growth arrested by seeding in agar (set a)</td>
<td>87.5 (70/80)</td>
</tr>
<tr>
<td>Growth arrested at confluency (set b)</td>
<td>20 (23/114)</td>
</tr>
<tr>
<td>Continuous growth at low cell density</td>
<td></td>
</tr>
<tr>
<td>for 3 days (set c)</td>
<td>58 (36/62)</td>
</tr>
<tr>
<td>for 6 days (set d)</td>
<td>41 (37/90)</td>
</tr>
<tr>
<td>for 9 days (set e)</td>
<td>3.5 (5/135)</td>
</tr>
</tbody>
</table>

*The numbers in parentheses refer to the absolute number of positive halos scored among the total number of isolated clones tested.*
the first days after infection, a greater number of
temperature-independent clones (up to 90%)
is found among the isolated transformants (36).

To determine whether PA production and
ability to grow in agar are regulated by unique
control processes, several cell lines were estab-
lished from different clones isolated under the
conditions described in the preceding section.
Their ability to grow without anchorage was
measured under conditions previously described
(36) at both restrictive and permissive tempera-
tures for tsa and ts25 mutations (41 and 33°C)
by seeding plates in triplicate with 10⁴ cells each.
One set of plates was immediately incubated at
41°C, and the other two were incubated at 33°C.
After 3 weeks of incubation, the number of trans-
formed clones which had appeared on all plates
was scored, and one of the two sets which were
incubated at 33°C was shifted to 41°C for 48 h.
At this time, PA production was measured, as
described above. An overlay containing the ca-
sein-plasminogen mix was also poured on plates
left at 33°C in order to measure PA production
at the low temperature. Scoring of caseinolyis
halos was performed after 18 and 48 h of incu-
bation at either 33 or 41°C. In no case was the
number of halos found to change with time of
incubation.

(i) tsa-transformed cell lines. Table 2 shows
the results obtained both for the ability
to grow in agar and PA production of tsa-trans-
formed cell lines.

When infected cells were kept actively grow-
ing for at least 3 days after infection (sets c, d,
e), most of the resulting transformants appeared
to be temperature sensitive for their ability to
grow in agar (10 lines out of 11). Among them,
six clones were also temperature sensitive for
PA production, whereas two lines did not pro-
duce detectable amounts of PA at either 33 or
41°C, and three lines were temperature inde-
pendent for PA production.

The relative proportion of temperature-sensi-
tive clones for growth in agar (10 out of 11) was
in agreement with the values previously re-
ported (36).

The only clone which grew with the same
efficiency at both 33 and 41°C (py tsa 13-2) was
also found to produce PA irrespective of the
temperature.

Three transformed cell lines established from
cells kept under growth-inhibiting conditions
(confuency on solid substrate, set b) after tsa
polyoma infection were found to express in a
temperature-independent way both the ability
to grow in agar and PA production. These ob-
servations were in agreement with those previ-
ously reported (36).

Direct selection of transformants in agar (set

<table>
<thead>
<tr>
<th>Cell line</th>
<th>PA production(^\text{a}) at:</th>
<th>Cloning efficiency(^\text{b}) in agar at:</th>
<th>Growth conditions(^\text{c}) after infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>33°C  41°C</td>
<td>33°C  41°C</td>
<td></td>
</tr>
<tr>
<td>py tsa 1-2</td>
<td>+    -</td>
<td>10   4</td>
<td>Set d</td>
</tr>
<tr>
<td>py tsa 6-2</td>
<td>+    -</td>
<td>60   30</td>
<td>Set d</td>
</tr>
<tr>
<td>py tsa 8-2</td>
<td>+    -</td>
<td>20   0</td>
<td>Set c</td>
</tr>
<tr>
<td>py tsa 9-2</td>
<td>+    -</td>
<td>30   0</td>
<td>Set c</td>
</tr>
<tr>
<td>py tsa 12-2</td>
<td>+    -</td>
<td>10   0</td>
<td>Set c</td>
</tr>
<tr>
<td>py tsa 18-2</td>
<td>+    -</td>
<td>55   0</td>
<td>Set e</td>
</tr>
<tr>
<td>py tsa 4-2</td>
<td>+    +</td>
<td>15   1</td>
<td>Set d</td>
</tr>
<tr>
<td>py tsa 7-2</td>
<td>+    +</td>
<td>10   0</td>
<td>Set c</td>
</tr>
<tr>
<td>py tsa 13-2</td>
<td>+    +</td>
<td>50   50</td>
<td>Set e</td>
</tr>
<tr>
<td>py tsa 14-2</td>
<td>+    +</td>
<td>90   90</td>
<td>Set b</td>
</tr>
<tr>
<td>py tsa 15-2</td>
<td>+    +</td>
<td>40   40</td>
<td>Set b</td>
</tr>
<tr>
<td>py tsa 16-2</td>
<td>+    +</td>
<td>60   60</td>
<td>Set b</td>
</tr>
<tr>
<td>py tsa 11-2</td>
<td>-    -</td>
<td>18   0</td>
<td>Set c</td>
</tr>
<tr>
<td>py tsa 17-2</td>
<td>-    -</td>
<td>40   0</td>
<td>Set e</td>
</tr>
<tr>
<td>py tsa A61/2</td>
<td>+    +</td>
<td>13   2.4</td>
<td>Set a</td>
</tr>
<tr>
<td>py tsa A81/2</td>
<td>+    +</td>
<td>32   40</td>
<td>Set a</td>
</tr>
<tr>
<td>py tsa A91/2</td>
<td>-    -</td>
<td>5    2</td>
<td>Set a</td>
</tr>
<tr>
<td>py tsa A91/1</td>
<td>+    +</td>
<td>95   12</td>
<td>Set a</td>
</tr>
<tr>
<td>py tsa A131/2</td>
<td>+    +</td>
<td>95   95</td>
<td>Set a</td>
</tr>
<tr>
<td>py tsa A11/2</td>
<td>+    +</td>
<td>60   95</td>
<td>Set a</td>
</tr>
<tr>
<td>py tsa A221/2</td>
<td>+    +</td>
<td>16   14</td>
<td>Set a</td>
</tr>
<tr>
<td>py tsa A201/2</td>
<td>+    +</td>
<td>65   95</td>
<td>Set a</td>
</tr>
<tr>
<td>py tsa A121/1</td>
<td>-    -</td>
<td>24   24</td>
<td>Set a</td>
</tr>
<tr>
<td>py tsa A241/1</td>
<td>+    +</td>
<td>60   95</td>
<td>Set a</td>
</tr>
<tr>
<td>py tsa A241/1</td>
<td>+    +</td>
<td>95   0</td>
<td>Set a</td>
</tr>
</tbody>
</table>

\(^\text{a}\) PA production was measured by the qualitative
assay described in the text. The production of PA has
been scored (+) when a clear halo was observed, on
plastic as well as in agar, within 18 to 48 h of incuba-
tion, whereas it has been scored (−) when no halo was
observed after 4 days of incubation under the condi-
tions used.

\(^\text{b}\) A total of 10⁴ cells per plate were seeded. The
average number of colonies per 5-cm petri dish, ob-
tained in two independent determinations, is given.

\(^\text{c}\) Growth was arrested by seeding in agar (set a) or
at confluence (set b). Other cultures were subjected to
continuous growth for 3 days (set c), 6 days (set d), or
9 days (set e). In each of the last three sets, the number
of cells was found to increase and reach 10⁶ cells per
plate at confluence.
dent way although the ability to grow in agar did not seem to be affected by the temperature. 

(ii) ts25-transformed cell lines. Reported in Table 3 are the results obtained with ts25-transformed cell lines.

Seven out of eight lines established from cells kept in a growing state after infection (sets c, d, e) were temperature sensitive with respect to their ability to grow in agar. Four of them also expressed PA production in a temperature-dependent way, whereas two others produced PA at both high and low temperatures. The two remaining lines did not produce any detectable PA. It therefore appeared that the ts25 polyoma virus mutant might be responsible for a temperature-dependent transformation phenotype in rat fibroblasts similar to that reported for tsa mutant (48).

The isolation of two PA nonproducers among the six established lines obtained after ts25 infection would suggest that the proportion of PA producers found among ts25 transformants might be lower than in the case of tsa infections where 90 to 100% of the clones were found to produce PA under the same conditions (see above). Two of the three lines isolated from cells which have been kept at confluency after ts25 infection (set b) were found to grow as well at 41 or 33°C. Again, no correlation was observed between ability to grow in agar at the high temperature and PA production, since one line did not produce PA at 41°C. The third line which was isolated under these conditions expressed both ability to grow in agar and PA production irrespective of the temperature.

The behavior of ts25 transformants grown directly in agar after infection (set a) was totally unexpected. All the PA producer lines expressed this character in a temperature-dependent way, regardless of their ability to grow in agar. Interestingly, the proportion of cell lines whose ability to grow in agar was temperature dependent was found to be much higher among ts25 transformants (50%) than among tsa transformants (10%). The two PA nonproducer lines were found to grow equally well at high or low temperature.

**DISCUSSION**

PA production has been shown to be associated with polyoma transformation of rat fibroblasts and was therefore considered as a parameter of the transformed phenotype expressed by such transformed cells (29). The experiments described in this paper show that PA production by polyoma-transformed cells is modulated by the growth state of the infected cells and that ability to grow without anchorage and PA production are regulated by distinct control processes.

Two distinct types of transformants have been described after infection of Fisher 3T3 rat cells with tsa mutant of polyoma (48). In one type (called N), ability to grow without anchorage is expressed in a temperature-dependent way, whereas in the other (called A), this capacity is expressed irrespective of the temperature. Already reported was the critical effect of infected cell growth state in the choice between these two transformation states (36). The study of PA production by polyoma-transformed cells isolated under selected growth conditions has shown that the expression of this transformation parameter is also dependent upon the growth state of the infected cells (either with WT or ts mutants) during a period of several days after infection. It therefore appears that conditions which lead preferentially to the isolation of so-called N-type transformants, i.e., active growth after infection (36), may in fact counterselect the PA-producing transformants (Table 1).

Several hypotheses can be put forward to account for these observations. First, although it has been shown that PA is not weakly bound to the cell membrane but is closely associated with plasma membrane-like or Golgi membrane-

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**TABLE 3. Ability to grow in agar and PA production in ts25-transformed cell lines isolated under various growth conditions**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>PA production* at:</th>
<th>Cloning efficiency* in agar at:</th>
<th>Growth conditions after infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>33°C</td>
<td>41°C</td>
<td>33°C</td>
</tr>
<tr>
<td>py ts 25-1</td>
<td>+ -</td>
<td>10 1</td>
<td></td>
</tr>
<tr>
<td>py ts 25-2</td>
<td>+ -</td>
<td>20 6</td>
<td></td>
</tr>
<tr>
<td>py ts 25-3</td>
<td>+ -</td>
<td>32 3</td>
<td></td>
</tr>
<tr>
<td>py ts 25-5</td>
<td>+ -</td>
<td>46 11</td>
<td></td>
</tr>
<tr>
<td>py ts 25-4</td>
<td>+ +</td>
<td>20 18</td>
<td></td>
</tr>
<tr>
<td>py ts 25-6</td>
<td>+ +</td>
<td>10 0</td>
<td></td>
</tr>
<tr>
<td>py ts 25-7</td>
<td>- -</td>
<td>95 50</td>
<td></td>
</tr>
<tr>
<td>py ts 25-8</td>
<td>- -</td>
<td>80 30</td>
<td></td>
</tr>
<tr>
<td>py ts 25-13</td>
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<td>30 30</td>
<td></td>
</tr>
<tr>
<td>py ts 25-15</td>
<td>+ -</td>
<td>12 2</td>
<td></td>
</tr>
<tr>
<td>py ts 25-16</td>
<td>+ +</td>
<td>16 13</td>
<td></td>
</tr>
<tr>
<td>py ts 25-A9</td>
<td>+ -</td>
<td>9 7</td>
<td></td>
</tr>
<tr>
<td>py ts 25-A12</td>
<td>+ -</td>
<td>15 0</td>
<td></td>
</tr>
<tr>
<td>py ts 25-A5</td>
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<td>6 5</td>
<td></td>
</tr>
<tr>
<td>py ts 25-A1</td>
<td>+ -</td>
<td>4 4</td>
<td></td>
</tr>
<tr>
<td>py ts 25-A13</td>
<td>+ -</td>
<td>25 42</td>
<td></td>
</tr>
<tr>
<td>py ts 25-A6</td>
<td>- -</td>
<td>48 0</td>
<td></td>
</tr>
<tr>
<td>py ts 25-A11</td>
<td>- -</td>
<td>84 7</td>
<td></td>
</tr>
</tbody>
</table>

* See Table 2, footnotes a, b, and c.
like elements of the cell (32), we considered the possibility that the repeated trypsinizations applied to the cells to keep them in an actively growing state might remove some of the cell-associated PA. Two lines of evidence argue against this hypothesis: (i) the assay used detects activator(s) excreted in the medium as well as with the external cell-associated PA (29); (ii) infected cells whose growth was rapidly arrested by confluency (as in set c) were trypsinized in the same way as actively growing cells (set e), except that they were replated each time at confluency, to avoid any more growth. No decrease in the proportion of PA producers among the resulting transformed clones was observed (data not shown). It therefore appears that the lack of PA production observed in cells kept in an active growing state after infection is probably not due to a trivial effect of trypsin.

On the other hand, a growing body of evidence indicates that the level of PA produced by transformed cells is predominantly determined by the cellular genome (62) and may be influenced by expression of viral genes (29, 41, 54). The levels of PA production by some normal cells were found to be subject to the physiological and developmental state of the cells (1, 40, 51, 53) and modulated by several different compounds, such as steroid and polypeptide hormones, lectins, cyclic nucleotides, glucocorticoids, retinooids, lymphoid products, and growth factors (1, 11, 20, 47, 49, 50, 55, 58–60). This may explain why some mammalian cells do not always produce increased levels of PA after transformation or differ in their response to different viruses (10, 16, 26, 39). It may also clarify the differential pattern of PA production by polyoma-transformed cells which we report in this work to be associated with different cellular growth states of the infected cells.

A similar decline of both cell-associated PA and excreted PA has been shown to occur with increased passaging of normal cells (45) and of some polyoma transformants (B. Perbal, unpublished data).

Another hypothesis is that the PA nonproducer transformants are genetically defective. The presence of chromosome 6 has been reported to be necessary for the production of human PA since this activity was not detected in the absence of this chromosome (18). It would be interesting to compare the karyotype of several polyoma transformants isolated under the different conditions used in our study to determine whether a similar conclusion can be obtained with rat cells.

It would appear that the transformed cells which are selected from infected cells kept in an actively growing state after infection may reflect a particular transformation state which may not be related to the state of the transformants isolated directly in agar. A preliminary study of simian virus 40-transformed rat cells isolated under similar conditions has led to similar conclusions (B. Perbal, unpublished data).

The use of different thermo-sensitive mutants of polyoma virus which belong to the same complementation group (23) allowed us to undertake a comparative study of the regulation process involved in PA production and ability to grow in agar. It has been previously reported that PA production and ability to grow in agar were under a unique viral control process in two ts a N polyoma transformants (29).

The results obtained in the present study confirm that it is possible to obtain thermo-sensitive transformants, for growth in agar, with either tsa or ts25 mutants of polyoma virus. However, the analysis of the transformed phenotype of 25 tsa transformants and 19 ts25 transformants isolated under various conditions led to the conclusion that there is little or no correlation between PA production and ability to grow without anchorage. Furthermore, it has been possible to isolate many transformants which expressed a temperature-dependent PA production, whereas their ability to grow in agar was not affected by incubation at the restrictive temperature (41°C).

These results suggested that the distinction between the N and A transformation states (48) might not apply to transformation parameters other than growth properties and led us to consider that PA production is regulated by a viral gene function, independent of the cell growth. A similar conclusion was obtained from a recent study of simian virus 40 tsA30-transformed rat cells (B. Perbal, submitted for publication).

The proportion of temperature-dependent PA producer among ts25-transformed cell lines (12 out of 15) would strongly suggest that the expression of PA production is under the direct control of large T antigen. The lower proportion of temperature-dependent PA producer lines obtained after tsa infection (10 out of 21) could be due either to the known leakiness of the tsa mutation (8) or to the different location of tsa and ts25 mutations on the polyoma genome (see Fig. 1), which could result in different heat sensitivity of the corresponding polypeptides.

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LITERATURE CITED


