Reversion of Temperature Sensitive Transformation Mutants of Rous Sarcoma Virus and its Effect on the Expression of Tumour Specific Surface Antigen

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SUMMARY

Rous sarcoma virus (RSV) mutants, which bear temperature sensitive (ts) defects in both the maintenance of cell transformation and the expression of tumour specific cell surface antigen(s) (TSSA), have yielded a number of revertants. In seven revertants studied, the acquisition of wild type transforming capacities is always accompanied by a wild type TSSA expression. This strongly indicates that transformation and TSSA expression in RSV are affected by the same mutation.

Transformation of both avian and mammalian cells by avian sarcoma viruses (ASV) is mediated by the virus src gene(s) and is invariably accompanied by the appearance of a tumour specific cell surface antigen or antigens (TSSA; reviewed by Kurth & Bauer, 1975; Kurth, 1976; Wainberg & Phillips, 1976). Since this antigen is not detected in fibroblasts infected with either avian leukemia viruses or transformation defective derivatives of ASV (Gelderblom et al. 1972; Kurth & Bauer, 1972) it has been proposed that TSSA expression is a necessary concomitant of virus transformation. To test more rigorously the possible causal relationship between the expression of TSSA and the transformed phenotype of the cell, the antigen was studied in both avian and mammalian cells infected with ASV mutants which have a temperature sensitive (ts) defect in the maintenance of cell transformation (Toyoshima & Vogt, 1969; Graf & Friis, 1973; Wyke, 1973). It was found that all these mutants induced TSSA, detectable by labelling with iodinated anti-TSSA antibodies, in transformed cells at permissive temperature (35 °C; Kurth, 1975; Kurth et al. 1975, 1976). At restrictive temperature (40 to 41 °C) the cells were phenotypically untransformed and six mutants induced an undetectable or much reduced level of TSSA. However, in cells infected with three other ts transformation mutants the TSSA levels at restrictive temperature were as high as those at permissive temperature.

These findings questioned the relationship between transformation and TSSA expression, for they allow three possible interpretations.

(1) TSSA expression and cell transformation are not causally related and their frequently demonstrated association is thus fortuitous. Many mutants which cannot transform cells (either through loss of, or mutation in, the src region of the genome) happen to have lost the ability to induce TSSA. However, TSSA induction has been maintained by three ts src mutants as described above.

(2) TSSA expression is concomitant with other parameters of transformation, to which it is related as either a cause or a consequence. However, its presence is not sufficient to account for the appearance of all these parameters. In phenotypically untransformed cells infected by some ts mutants TSSA expression may indicate residual transforming capabilities of these mutants, either because more than one virus protein is encoded in the src region or because a single src-coded protein possesses more than one function.
(3) TSSA expression is both necessary and sufficient for transformation. If this is so, the TSSA detected in phenotypically untransformed cells, though still antigenic, must be non-functional. This could be because it is incorrectly inserted in the cell membrane or because it is itself, in whole or in part, the src gene product.

To attempt to distinguish these possibilities we have isolated wild type revertants from mutants whose transformation and TSSA expression are both ts. The revertants were selected for their ability to induce transformation at restrictive temperature. If transformation and TSSA are unrelated it is unlikely that such viruses would show an accompanying reversion for TSSA expression. However, if transformation and TSSA are causally associated, the revertants should always show a co-ordinate reversion of both parameters.

The Rous sarcoma virus (RSV) mutants ts LA29 and ts LA33 (Wyke, 1973) show a temperature sensitive expression of TSSA (Kurth et al., 1975) and were used as a source of revertant viruses. To avoid repeated isolation of the same revertant, the mutants were first cloned and the clones were shown to be temperature sensitive for transformation, focus formation at 35 °C being 10^8 to 10^4 times the level at 41 °C. Several potential revertants were then isolated from each of a number of ts clones by isolating foci produced at restrictive temperature. Because focus production at 41 °C by the ts clones was very low it was likely that foci isolated at this temperature would be contaminated by parental ts virus. For this reason all potential revertants were recloned before testing their temperature sensitivity. All confirmed revertant viruses produced more foci at 41 °C than at 35 °C, in some cases the titre at 41 °C being severalfold that at permissive temperature. Out of a total of 89 foci isolated at 41 °C, 8 contained ts virus only and were possibly produced by leakiness rather than reversion of the mutant function.

As a separate assessment of the wild phenotype of revertant viruses, the ability of infected chick embryo fibroblast (CEF) cells to transport labelled 2-deoxyglucose was determined as described previously (Hynes & Wyke, 1975). TSSA expression in infected cultures was assessed by the indirect radioiodine-labelled antibody technique exactly as described by Kurth (1975) and is presented as the number of specifically absorbed immunoglobulins per cell. The specificity of the serum used to demonstrate TSSA is of the utmost importance and for these studies serum No64 was used (characterized by Kurth, 1975). This serum was prepared by immunization of chickens with subtumorigenic doses of the Schmidt–Ruppin strain H (subgroup D) of RSV. In addition to TSSA, the serum was shown to recognize embryonic antigens and group- and subgroup-specific determinants on virus envelope glycoproteins (Kurth, 1975; Rohrschneider et al., 1975; Hayami et al., 1977). To remove antibodies with the latter specificities, the antiserum was routinely absorbed with chicken embryo fibroblasts infected with Rous associated virus-1 (subgroup A). These cells are known to express embryonic antigens (Kurth & Bauer, 1973) as well as group- and subgroup-specific virus envelope antigens (Kurth, 1976; A. Huesgen and R. Kurth, unpublished data), which they share with cells infected with LA29 and LA33 (also in subgroup A).

The results of the indirect antibody technique applied to normal CEF and CEF infected by ts and revertant viruses at permissive and restrictive temperature are shown in Fig. 1. Whereas the ts virus-infected cells show low TSSA expression at 41 °C, the cells infected by two revertants show a TSSA level at 41 °C comparable to that at 35 °C. Table 1 shows the TSSA expression at permissive and restrictive temperature of LA29, LA33 and seven separate revertants isolated from these mutants. The revertants are all wild type for transformation as judged by focus formation (see above) and sugar transport (Table 1), and they all show a wild type expression of TSSA. These results make it extremely unlikely that TSSA expression and transformation are unrelated. We must thus explain the differential expression
Fig. 1. Indirect antibody technique: detection of tumour specific cell surface antigen(s) (TSSA). Absorption of antibodies from normal and anti-TSSA chicken immunoglobulin preparations to parallel cultures of CEF infected with ts transformation mutants of RSV and revertants derived from them. Target cells were grown at either permissive (-----) or restrictive (------) temperature. Absorption was measured by staining the chicken immunoglobulins with 125I-labelled rabbit anti-chicken IgG immunoglobulin. Specific absorption = binding of antibodies from anti-TSSA serum minus binding of antibodies from normal chicken serum. For further details see Kurth (1975). 
(a) △, LA29, clone 8, ts; ▲, LA29, clone 8, revertant 1. (b) Data from same experiment as (a): ○, LA33, clone 1, ts; ●, LA33, clone 1, revertant 5; ■, uninfected CEF.

Table 1. Reversion of RSV ts mutants LA29 and LA33: TSSA induction and sugar uptake

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Induction of TSSA*</th>
<th>Uptake of 2-deoxyglucose: ratio of cts/min incorporated 35°C/41°C†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected CEF</td>
<td>—</td>
<td>1.6</td>
</tr>
<tr>
<td>CEF infected by:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA29, clone 8, ts</td>
<td>++</td>
<td>—</td>
</tr>
<tr>
<td>LA29, clone 6, ts</td>
<td>NT‡</td>
<td>NT</td>
</tr>
<tr>
<td>LA29, clone 4, revertant 2</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>LA29, clone 5, revertant 2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LA29, clone 6, revertant 4</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>LA29, clone 8, revertant 1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LA33, clone 1, ts</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>LA33, clone 1, revertant 5</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>LA33, clone 4, revertant 1</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>LA33, clone 5, revertant 5</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Specific absorption of anti-TSSA antibodies: —, 0 to 10⁴ antibody molecules per cell; ++, 10⁴ to 5 × 10⁴ antibody molecules per cell; +++, 5 × 10⁴ to 8 × 10⁴ antibody molecules per cell.
† Cell cultures whose transformation is ts show a high ratio of 35°C/41°C incorporation (> 5). Non-ts cultures, whether uninfected or infected by wild type virus, show a low ratio of 2-deoxyglucose incorporation (< 3).
‡ NT, not tested.
of TSSA in \textit{ts} mutant-infected cells at restrictive temperature in terms of possibilities (2) and (3) outlined above. Distinction between these two possibilities will require knowledge of the function of TSSA and the small quantities of antigen found in the cells makes such a distinction difficult at present.

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\textbf{REFERENCES}


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