Use of puromycin N-acetyltransferase (PAC) as a new reporter gene in transgenic animals

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Puromycin N-acetyltransferase (PAC) is a bacterial enzyme produced by Streptomyces alboniger, which has been purified and well characterized (1). PAC inactivates puromycin by acetylating the amino position of its tyrosinol moiety. Its gene has been cloned (2), and its use as a reporter gene in transient expression of transfected cells has been described (3, 4).

We have made different lines of transgenic mice with pac as a reporter gene. These lines have been made with different parts of the uteroglobin 5'-flanking region (5), the pac coding sequence and the adjacent regions as controls were cut and counted. After a microfuge centrifugation (3', 4°C) supernatants were employed for PAC assay.

Two kind of controls were applied to avoid variations among different experiments on different days: Non transgenic tissues were employed in all PAC assays and two samples of each homogenate, with and without the substrate puromycin, were always used. The reaction mixture for PAC assays contains: 10 ^tl of an acetyl CoA mix (for 100, 50; 1 ml Tris-HCl pH 8.0, 35 µl 2 mM acetyl CoA and 15 µl [3H]acetyl CoA); 10 µl 10 mM ATP; 2.5 µl 100 mM MgCl2; 0.3 units of acetyl CoA synthetase; 10 µl tissue homogenate; +/− 5 µl 2 mM puromycin and H2O to get a final volume of 50 µl. The reaction was performed at 33°C for 1 hour.

Two main methods have been used for CAT assay and both of them can be used with PAC as well: A classic chromatographic method (6) and a recently called two phase partition assay (7).

Transgenic mice were detected by slot-blot of genomic DNA and their tissues were extracted, minced and frozen at −80°C until they were used. Tissue homogenization was made in 200 µl of TGE buffer. After a microfuge centrifugation (3', 4°C) supernatants were employed for CAT assay.

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For the chromatographic assay, the N-acetylpuromycin was extracted twice with 500 µl ethyl acetate. After partial evaporation, volumes of 20 /tl were applied to a TLC aluminum sheet of silica gel 60F 254 which were developed with 254 nm UV light.

For non chromatographic assay, 200 µl of 5 M NaCl-0.1 M Na2B4O7 and 1 ml of scintillation liquid were added and counted for 5 min.

Both assays work equally well in our hands, although the former allows more confidence when measuring low PAC activity.

The system described here functions in a reproducible fashion and is quite sensitive, even working with the not very strong uteroglobin promoter (see table below).

The use of pac as a reporter gene for transgenic animals, reported here for the first time, could be based on different aspects: (i) PAC exhibits an enzymatic activity not present in eukaryotic systems, at least in the different tissues analyzed in control animals (brain, lung, heart, liver, intestine, kidney, spleen, skeletal muscle, oviduct, uterus, testis and male tract). (ii) The product of the reaction, N-acetylpuromycin, shows a chromatographic behavior different from the non acetylated form. This characteristic allows the use of similar techniques than those applied to detect chloramphenicol acetyltransferase (CAT) activity in cultured cells and in tissues of transgenic animals. (iii) As puromycin is toxic for animal cells, its inactivation by PAC could be used to select cells expressing PAC, i.e. embryonic stem cells transfected with PAC constructs, and use them to create transgenic animals by microinjection to the blastocyst, as it has been described before for gene constructs bearing the neomycin resistance gene (8). The use of PAC in this task is simpler compared to neo constructs as it allows to use just one gene both as a reporter and to confer antibiotic resistance. (iv) The use of PAC might bypass some new problems found with the use of CAT since a CAT-like activity has been recently described in eukaryotic cells (7, 9).

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REFERENCES


Table 1. Tissue-specific expression of PAC in different animals bearing CHI construct.

<table>
<thead>
<tr>
<th>Animal Line</th>
<th>Tissue</th>
<th>PAC Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Kidney</td>
<td>N.D.²</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>Uterus</td>
<td>N.D.</td>
</tr>
<tr>
<td>EGCIII.11</td>
<td>Kidney</td>
<td>N.D.</td>
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<tr>
<td></td>
<td>Liver</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>Uterus</td>
<td>20</td>
</tr>
</tbody>
</table>

¹pmol of acetyl puromycin/mg of tissue protein.
²Not detectable.