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Transient transfection of ecotropic retrovirus receptor permits stable gene transfer into non-rodent cells with murine retroviral vectors

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Retroviral vectors are useful for efficient, stable and single copy transfer of foreign genes into animal cells. However, the low risk murine ecotropic vectors can only be used with rodent cells due to their restricted host range. When using cells from other species, in particular human, amphotropic retroviral vectors and the corresponding packaging cell lines are required. Given the wide host range of these vectors, they are classified as higher biological risk and high biological containment (L2 or even L3) is mandatory in many countries. To reduce the risk to the experimenter while working with non-rodent cells, we considered a strategy based on transient expression of the recently cloned murine ecotropic retrovirus receptor (1) to allow the use of safer murine retroviral vectors.

Murine retroviruses enter target cells by interaction of the viral envelope glycoprotein with a specific cell-surface receptor, a basic amino acid transporter (2,3). The retroviral host range is determined by the species- and/or tissue-specific expression of the appropriate receptor. To extend the host range of the rodent-specific Moloney murine leukemia virus we transiently transfected the expression vector for the virus receptor, pJET (1), into endometrial epithelial cells from rabbit, RBE7 (4), or human, Ishikawa (5), via the calcium phosphate precipitate technique. Transfection efficiencies of 5–10% were reached as determined by co-transfection of a RSV-LacZ vector followed by cytochemical staining for β-galactosidase activity. Following removal of the precipitate, 16 h after transfection, the cells were co-cultivated with the packaging cell line psi2 expressing v-Ha-*ras* and the neo gene as described (6). After 3–5 days of exposure to high virus titres (10^6 c.f.u./ml), the cells were selected for 4–6 weeks on G418 and the number of transformed clones was counted. Whereas in mock transfected cells no foci were detected, a large number of foci was generated in cells transfected with the murine ecotropic vector prior to infection. The number of foci obtained with 1×10^6 cells was in the range of 400 for RBE7 cells and 50 for Ishikawa cells. These transformation efficiencies are within the range obtained with a rat cell line of endometrial origin, RENT4 (6), infected with the same protocol. Southern blot analysis of five independent RBE7 foci demonstrated single copy retroviral integration (Fig. 1B). Homogeneous expression of the v-Ha-*ras* oncogene was demonstrated by immunofluorescence with anti Ha-*ras* antibodies, which decorated the cytoplasm only in transformed cells but not in control cells (Fig. 2).

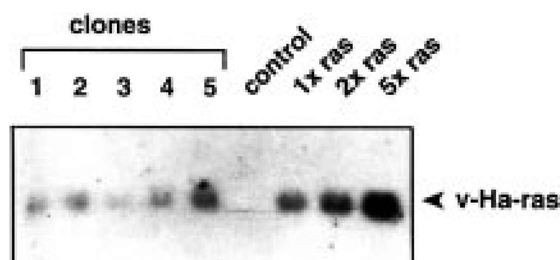

Figure 1. Southern blot analysis of integrated recombinant retrovirus sequences in isolated transformants. Genomic DNA (15 µg) was digested with *Bam*HI, fractionated on a 1.2% agarose gel, transferred to nitrocellulose and hybridized with a ^{32}P-labelled v-Ha-*ras* probe (6). Lanes 1–5: DNA from independent G418 resistant transformants. Control and lanes 1× ras, 2× ras and 5× ras contain RBE7 DNA supplemented with 0, 35, 70 and 175 pg of Zip-ras-6 plasmid (7) respectively, corresponding to 0, 1, 2 and 5 copies per haploid genome.

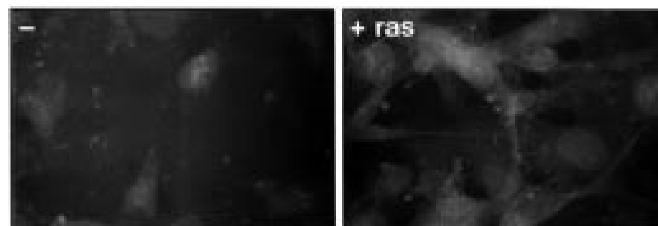

Figure 2. Immunofluorescence staining of a v-Ha-*ras* transformant clone (+ ras) compared with the uninfected RBE7 cell line (–). A combination of anti-v-Ha-*ras* monoclonal antibody (Dianova) and anti-rat-IgG FITC conjugate (Sigma) was used. V-Ha-*ras* expressing cells are uniformly stained, whereas RBE7 cells show only unspecific nuclear staining.

These results show that rabbit and human cells can be stably transformed with murine retrovirus as efficiently as rodent cells, simply by transient transfection of the murine ecotropic retrovirus receptor. This relatively simple and safe procedure could replace the hazardous use of amphotropic retroviral vectors and thus increase the safety of gene transfer procedures. This aspect could be

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particularly relevant in the context of human gene therapy as it would reduce the biological containment measurements required in hospital laboratories. Moreover, this technique could be used for the establishment of stable cell lines from primary cells of non-rodent origin. The basic principle could be extended to the use of other viral vectors which surface receptors have been cloned.

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