Continuous production of minute virus of mice by an untransformed variant of Fisher rat fibroblast (FR3T3)

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Many tumour cells are killed by the lytic replication of the autonomous parvoviruses H-1 and minute virus of mice (MVMp), whereas most untransformed cells (although they take up these viruses efficiently) are resistant, i.e. they do not produce infectious virus and are not lysed. Therefore, cells able to continuously produce large quantities of infectious virus have not yet been described. We have isolated such cells from the resistant cell line FR3T3 (Fisher rat fibroblast). These cells (called FR3T3C) produce infectious MVMp virions without being detectably lysed. Furthermore, a per-

sistently infected population (R100FR3T3C) was generated by repetitive infection of FR3T3C cells with MVMp. Indeed, R100FR3T3C cells were successfully cultivated for two years and continuously produced infectious virus. Seventeen clones of R100FR3T3C cells isolated by limiting dilution produced infectious virions, indicating that in the R100FR3T3C cell population, virus production was not limited to a few cells. These cell lines may be useful for the production of MVMp and for the generation of a cell line for the packaging of recombinant viral genomes.

Introduction

The autonomous parvoviruses H-1 and minute virus of mice (MVMp) are small single-stranded DNA viruses which infect a wide variety of animal species, including humans for H-1 (Siegl, 1984). These viruses are very dependent for their replication on cellular factors which are more specific to transformed cells (Cotmore, 1990; Tattersall & Gardiner, 1990); their replication is lytic and kills the cell (Tattersall & Cotmore, 1990; Rommelaere & Cornelis, 1991). Indeed, the killing by the parvoviruses H-1 or MVMp of a number of human or rodent fibroblasts and epithelial cells increases dramatically when they are transformed *in vitro* by known oncogenes, chemicals or physical agents (Cornelis *et al.*, 1988a; Mousset *et al.*, 1986; Salome *et al.*, 1990; Van Hille *et al.*, 1989). Cell killing has been dissociated from the lytic production of viral particles, suggesting that specific parvoviral products are cytotoxic (Guetta *et al.*, 1990). This cytotoxicity has been shown to be due to the major non-structural protein, NS-1, and is oncogene-

specific (Brandenburger *et al.*, 1990; Caillet-Fauquet *et al.*, 1990; Cornelis *et al.*, 1988b; Salome *et al.*, 1989, 1990; Van Hille *et al.*, 1989). Conversely, most untransformed cells, although they take up virus efficiently, do not produce infectious virus and are not lysed (Cornelis *et al.*, 1988a, 1990; Mousset *et al.*, 1986). Cells able to continuously produce large quantities of infectious virus are therefore difficult to isolate.

Here we report on the characterization of FR3T3C cells, which produce MVMp infectious particles without being lysed. These cells emerged spontaneously from the untransformed FR3T3 (Fisher rat fibroblast) cell line (Salome *et al.*, 1990; Van Hille *et al.*, 1989). When repeatedly infected with MVMp, FR3T3C cells gave rise to a persistently infected cell population (R100FR3T3C) which was successfully cultivated for two years and continuously produced infectious virus.

Methods

Cells. All cells (see below) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal calf serum (FCS; Gibco), 1% glutamine and 12 µg/ml gentamicin. FR3T3 (Fisher rat fibroblast; Salome *et al.*, 1990; Van Hille *et al.*, 1989), FREJ4 cells [FR3T3 cells transformed with the plasmid pSVEJ carrying the c-Ha-ras gene mutated at codon 12 (Van Hille *et al.*, 1989)], FR3T3C cells (variant of FR3T3), NRK cells (Salome *et al.*, 1989), NIH3T3 cells and A9 cells (variant of mouse L cells) were used in the experiments.

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Fig. 1. As shown by RFLP, FR3T3C cells are rat fibroblasts. (a) A mitochondrial DNA fragment (RNA 12S gene) was amplified by PCR from genomic DNA samples isolated from different animal species, using the oligonucleotides located at positions shown by arbitrary numbers and arrows. (b) The PCR-amplified DNA fragments were digested with appropriate restriction enzymes as indicated in (a). The digestion products were separated by electrophoresis in agarose gels and visualized by ethidium bromide staining. Lane p, size marker; lane w, water; lanes m1, NIH3T3 cells; lanes m2, *ras*-transformed NIH3T3 cells; lanes Ra, FR3T3C cells (passage 17); lanes R1, FREJ4 cells; lanes Rb, FR3T3C cells (passage 50); lane R2, FR3T3 cells. Slashes indicate lanes containing non-digested DNA. Restriction enzyme symbols: B, *Bst*XI; H, *Hinf*I; R, *Rsa*I and A, *Alu*I.

Identification of FR3T3C cells

(i) *Karyotypes*. Semi-confluent monolayers of A9, FR3T3C, R100FR3T3C and FR3T3 cells were growth arrested with colcemide (Gibco BRL) at a final concentration of 0.06 µg/ml of medium for 4 h. Cells were treated and processed for R banding (RHG) as described (Dutrillaux & Couturier, 1981). Chromosomal analysis was done on photographed metaphases. Five to ten spreads were analysed per cell line and 25 cells were examined for total chromosome number in each spread.

(ii) *Restriction fragment length polymorphism (RFLP)*. Extraction of total genomic DNA from NIH3T3, FREJ4, FR3T3 and FR3T3C cells was performed by using standard protocols. The PCR reactions were carried out with approximately 1 µg of total genomic DNA in a final

Fig. 2. FR3T3C cells are FR3T3 cell derivatives as shown by RAPD. Genomic DNA isolated from different cell lines was amplified by PCR using the random DNA primer (see Methods). The PCR-amplified DNA fragments were size separated by electrophoresis in agarose gel and visualized by ethidium bromide staining. Lane p, size markers; lane w, water; lane m1, NIH3T3 cells; lane m2, *ras*-transformed NIH3T3 cells; lane Nr, NRK (newborn rat kidney) cells; lane Ra, FR3T3C cells (passage 17); lane R2, FR3T3 cells; lane Rb, FR3T3C cells (passage 50); lane R1, FREJ4 cells.

volume of 50 µl as previously described (Hänni *et al.*, 1995). The primers used to amplify the 12S rRNA mitochondrial genes and the restriction map of the 12S rRNA genes are shown in Fig. 1(a).

The PCR products were digested with the following restriction enzymes: *Bst*XI, *Dde*I, *Hinf*I, *Rsa*I and *Alu*I, the DNA fragments generated were separated on a 2% agarose gel (Fig. 1b).

(iii) *Random amplification polymorphism DNA (RAPD)*. The PCR protocols used in this experiment were adapted from the published RAPD protocols (Hadrys *et al.*, 1992; Welsh *et al.*, 1991). The PCRs were carried out in a total volume of 25 µl containing: 10 mM-Tris-HCl, 0.1% Triton X-100, 1.5 mM-MgCl₂, 0.2 mg/ml BSA, 100 µM-dNTPs, 0.4 µM-primer, 50 ng of cellular DNA and 0.5 U *Taq* DNA polymerase (Applicogene). The primer (5' AACTGACCAACCTGTG 3') was annealed to the genomic DNA at 42 °C for 1 min and then 40 cycles of PCR were performed as follows: elongation for 2 min at 72 °C, denaturation for 1 min at 92 °C. Twenty µl of the PCR products was loaded on a 2% agarose gel (Fig. 2).

MVMp infection and measurement of cell survival after MVMp infection. MVMp (prototype strain) was propagated in A9 cells and purified according to Tattersall *et al.* (1976). MVMp was titrated by plaque assays on A9 indicator cells (Tattersall *et al.*, 1976). Exponentially growing cultures (5×10^5 cells per 60 mm dish) were infected with MVMp at various m.o.i.s (1, 10 and 100 p.f.u. per cell) (Koering *et al.*, 1994). Cells were trypsinized 4 h after mock infection or infection with MVMp and replated at low densities (250 to 500 cells) onto 60 mm dishes. Cell survival was determined by comparing the ability of MVMp-infected and mock-infected cells to form colonies on plastic. Colonies were fixed and stained 5–7 days post-infection (p.i.) as described by Koering *et al.* (1994).

Measurement of MVMp DNA amplification. The amount of total intracellular viral DNA was measured in whole cell lysates by dispersed cell assay (DCA) (Salome *et al.*, 1989). Cultures (5×10^5 cells per 60 mm dish) were infected with MVMp at various m.o.i.s, incubated for 2 h and 30 h, respectively, harvested, and trapped on BA85 nitrocellulose membranes (Schleicher & Schuell). BA85 filters were processed as described by Salome *et al.* (1989). As a probe for MVMp, a 5 kb *Bam*HI fragment excised from pMM984 (Merchinsky *et al.*, 1983) was radiolabelled with [α -³²P]dCTP using the Megaprime DNA labelling system (Amersham). Hybridized radiolabelled DNA was quantified by liquid scintillation spectrometry. MVMp DNA amplification was expressed as a ratio of the amount of hybridized radiolabelled DNA at

Table 1. FR3T3C cells produce infectious MVMp virions

(a) Determination of infectious virus production of infected cultures (m.o.i. = 1) at 30 h p.i. by plaque assay on A9 indicator cells. Viral titre in p.f.u./ml. (average of five independent experiments, SD less than 20%). (b) Comparison of production of infectious virus. MVMp (prototype strain) was propagated in A9 cells and in FR3T3C cells, purified and plaque-titrated by plaque assays on A9 indicator cells (Tattersall *et al.*, 1976). Viral titre in p.f.u./ml. (c) Infectious centres assays. Percentage of infected cells initiating plaque formation (average of four independent experiments in triplicate, SD less than 20%).

Cells	Origin	(a) Production of infectious virus 30 h.p.i.	(b) Production of infectious virus 6 days p.i.		(c) Infectious centres	
			m.o.i. 5×10^{-3}	m.o.i. 10	m.o.i. 1	m.o.i. 10
A9	Derived from mouse L cells	9.5×10^9	1.9×10^9	ND	84 %	68 %
FR3T3C	FR3T3 cell variant	6.9×10^6	10^4	2.5×10^9	2 %	67 %
R100FR3T3C	FR3T3C persistently infected cell	0.6×10^6 – 1.6×10^8 *	ND	ND	21 %–64 %	21 %–64 %

* Persistently infected cells. ND, not determined.

30 h p.i. compared to that at 2 h (input) p.i. for the different m.o.i.s (0.1, 1 and 10).

Production of infectious MVMp virions. A9 cells infected at an m.o.i. of 5×10^{-3} and FR3T3C cells infected at an m.o.i. of 5×10^{-3} or 10, were compared quantitatively for their production of infectious virus by the standard method of propagation and purification (Tattersall *et al.*, 1976). Viral particles produced were titrated by plaque assay on A9 cells (See Table 1b).

For determination of infectious virus production, infected cultures (m.o.i. = 1) were frozen at 30 h p.i. and subjected to three freeze-thaw cycles. The suspensions were diluted in culture medium without serum and the released particles were titrated by plaque assay on A9 indicator cells (See Table 1a).

Production of virions was also evaluated by the infectious centre assay. Appropriate numbers of persistently infected R100FR3T3C and virus-infected (m.o.i. = 1 or 10) A9 and FR3T3C cells were treated as described by Salome *et al.* (1989) and the proportion of productively infected cells was determined 6 days later, as the fraction of seeded cells producing plaques (See Table 1c).

R100FR3T3C cells and clonal populations of R100FR3T3C cells selected by limiting dilution, were analysed for their capacity to produce infectious virus by the modified plaque assay (Faiss *et al.*, 1989). The last wash of R100FR3T3C cells before plating for limiting dilution was added to A9 cells and did not detectably induce cell mortality after 1 week in culture. A9 cultures (3×10^5 cells) were infected with MVMp virus produced by FR3T3C cells or by A9 cells (m.o.i. = 10^{-4} or 10^{-2}) with 0.5 ml of filtered (Minisart NML 0.20 μ m pore size; Sartorius) lysates of 10^6 cells of R100FR3T3C cells and clones. The production of infectious viruses in A9 cells was evaluated 30 h p.i. by the modified plaque assay on BA85 filters as described by Faiss *et al.* (1989). As a probe for MVMp, the 5 kb *Bam*HI fragment excised from pMM984 was radiolabelled with [α - 32 P]dCTP. Hybridized radiolabelled DNA was quantified by liquid scintillation spectrometry.

Results and Discussion

FR3T3C cells emerged spontaneously from a culture of FR3T3 cells (Fisher rat fibroblast) and were phenotypically indistinguishable from FR3T3 cells, i.e. they had a similar morphology, the same doubling time and did not grow in semi-solid medium or as tumours in nude

mice (not shown). However, FR3T3C cells differed from FR3T3 cells by their karyotype and their ability to amplify the MVMp genome and to produce infectious virions.

FR3T3C cells have a different karyotype than FR3T3 cells but are Fisher rat fibroblasts

In order to characterize FR3T3C cells, karyotypes were made. As compared to FR3T3 cells which have 42 chromosomes, FR3T3C cells had an unusual number of chromosomes, which varied from 66 to 74, the average number being 72 for FR3T3C cells. However, we did not find a single FR3T3C cell with 42 chromosomes (not shown).

In order to ascertain the origin of FR3T3C cells [and to rule out any possibility of cell contamination (Nelson-Rees & Flandmeyer, 1977)] we first verified that they were rat cells by RFLP (Hänni *et al.*, 1995) on mitochondrial DNA (12S rRNA). A mitochondrial DNA fragment (12S RNA gene; Fig. 1a) was amplified by PCR from total DNA isolated from FR3T3C, NIH3T3 (mouse), FR3T3 (rat) and FREJ4 (rat) cells. The PCR-amplified DNAs were digested with the enzymes *Bst*XI, *Hinf*I, *Rsa*I or *Dde*I which allow discrimination between human, mouse, rat and hamster species. As a control we used the *Alu*I enzyme cutting sites. The comparison of the digestion patterns of the PCR-amplified DNA fragments demonstrated that FR3T3C were rat cells [Fig. 1b; the *Dde*I digestion clearly showed that FR3T3C cells are not hamster cells (not shown)].

We next ascertained by RAPD (Hadrys *et al.*, 1992; Welsh *et al.*, 1991) that FR3T3C cells were indeed FR3T3 cell derivatives. We therefore compared the

Fig. 3. FR3T3C cells amplify MVMp DNA. FR3T3C cells (●), A9 cells (▲) or FR3T3 cells (□) were infected with MVMp virions at an m.o.i. of 0.0, 0.1, 1.0 or 10 p.f.u./cell. The MVMp DNA amplification factor was determined as described in Methods.

RAPD patterns obtained with the DNA isolated from FR3T3C cells to the RAPD patterns obtained with the DNA isolated from NIH3T3 mouse cells, NRK, FREJ4 and FR3T3 rat cells used as controls. The RAPD patterns obtained with the DNA of FR3T3C, FR3T3 and FREJ4 cells were similar but different from those obtained with the DNA of NIH3T3 and NRK cells (Fig. 2). These results strongly suggest that FR3T3C cells are FR3T3 cell derivatives.

FR3T3C cells amplify MVMp DNA

A9 cells, FR3T3 cells and FR3T3C cells were infected at m.o.i.s of 0.1, 1 and 10 p.f.u./cell with MVMp virions produced by A9 cells (see Methods). When the amount of intracellular viral DNA was measured at 2 h p.i. and 30 h p.i. in whole cell lysates by DCA, we found that the viral DNA was amplified similarly in FR3T3C cells and in the permissive A9 cells, but was not amplified in FR3T3 cells (Fig. 3). These results indicated that the viral DNA was replicated in FR3T3C cells.

FR3T3C cells produce infectious MVMp virions

We next tested the capacity of FR3T3C cells to produce infectious virions 6 days p.i. by plaque assay on A9 cells indicator cultures. The results presented in Table 1(a) demonstrate that FR3T3C were able to produce infectious virus. However, FR3T3C cells had to be infected at an m.o.i. of 10 (Table 1b) as compared to an m.o.i. of 5×10^{-3} for A9 cells, to produce at 6 days p.i. an infectious virus titre of 10^8 – 10^9 p.f.u./ml. This observation was confirmed by infectious centres assays (Table 1c).

The results described above strongly suggest that the

Fig. 4. Transformed cells are infected and killed by MVMp virions produced by FR3T3C cells. A9 mouse cells (a) or FREJ4 rat cells (b) were infected at m.o.i. of 0.0, 1.0 or 10 with MVMp virions produced either by A9 cells (black bars) or FR3T3C cells (open bars). The percentage of cells surviving infection was determined as described in Methods. (a and b represent the average of three independent experiments in triplicate, SD less than 15%).

rat cells FR3T3C produce MVMp virions able to infect and kill A9 mouse cells. However, it remained to be established whether MVMp virions produced by FR3T3C cells could infect and kill permissive rat cells. To do so, we compared the survival of A9 mouse cells and FREJ4 rat cells [*ras*-transformed FR3T3 cells highly sensitive to infection by MVMp (Salome *et al.*, 1990; Van Hille *et al.*, 1989)] infected by MVMp virions produced either by A9 cells or FR3T3C cells. The percentage of A9 cells and FREJ4 cells killed by MVMp infection was similar whether the MVMp virions used were produced by FR3T3C cells or by A9 cells (Fig. 4a, b).

R100FR3T3C cells produce MVMp virions continuously

Since most of the cells producing infectious MVMp virions are killed and cannot be kept in long-lasting cultures, we next evaluated whether FR3T3C cells survived MVMp production and whether MVMp-producing FR3T3C cell lines could be established.

When compared to FR3T3 cells which are resistant to MVMp killing (65.4% of cells survive at day 6 p.i. at an m.o.i. of 10; Fig. 5) and to A9 cells which are highly sensitive to MVMp killing (0% cells survive at day 6 p.i. at an m.o.i. of 10; Fig. 5) FR3T3C cells were highly resistant to MVMp killing [95.8% cells survive at day 6 following an infection at an m.o.i. of 10 (Fig. 5) and more than 87% of the cells survived an infection at an m.o.i. of 100 (data not shown)]. Accordingly, it was possible to establish a cell line of FR3T3C cells (R100FR3T3C) following two subsequent rounds of infection (m.o.i. = 10 then m.o.i. = 100). This cell line has been cultivated for about two years and still produces infectious MVMp virions (Table 1a*). Indeed, infection of A9 indicator cells with the filtered supernatants of R100FR3T3C cultures (modified plaque assay) showed

Fig. 5. The production of infectious MVMp virions by FR3T3C cells is not lytic. FR3T3C cells (black bars), FR3T3 cells (open bars), A9 cells (grey bars) were infected with MVMp virions at m.o.i. 0.0, 1.0 or 10. The percentage of surviving cells was determined as described in Methods (average of 6 independent experiments in triplicates, sd less than 15%).

that, independent of the number of passages, infectious MVMp virus was produced by R100FR3T3C cells (not shown). It must be noticed that R100FR3T3C cells did not withstand transformation by the oncogene *ras*. Indeed, it was not possible to obtain persistently infected clones expressing the p21^{ras} protein from R100FR3T3C cells transfected with pSVEJ (Van Hille *et al.*, 1989) and selected by G418 (data not shown). These last results, in accordance with all previous reports, strongly suggest that R100FR3T3C cells are sensitized to MVMp lytic effect by transformation.

Finally, we evaluated whether individual R100FR3T3C cells isolated by limiting dilution would give rise to clonal populations producing infectious viruses. Seventeen clonal populations were analysed for MVMp production by modified plaque assay on A9 cells. All clones produced infectious virus (not shown). This suggests that in the R100FR3T3C cell line, the production of MVMp virions is not limited to a few cells.

In conclusion, this is the first report on untransformed cells continuously producing infectious parvoviruses. We must stress the fact that FR3T3C could also amplify H-1 viral DNA and produce infectious H-1 viruses non-lytically (not shown). At present we do not know why FR3T3C cells produce MVMp virions while the parental untransformed FR3T3 cells do not. Nevertheless, the FR3T3C cells are of high potential interest for the production of large quantities of infectious MVMp and H-1 virions, or to be used as packaging cells lines to generate recombinant parvoviruses.

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References

- BRANDENBURGER, A., LEGENDRE, D., AVALOSSE, B. & ROMMELAERE, J. (1990). NS-1 and NS-2 proteins may act synergistically in the cytopathogenicity of parvovirus MVMp. *Virology* **174**, 576–584.
- CAILLET-FAUQUET, P., PERROS, M., BRANDENBURGER, A., SPEGELAERE, P. & ROMMELAERE, J. (1990). Programmed killing of human cells by means of an inducible clone of parvoviral genes encoding non-structural proteins. *EMBO Journal* **9**, 2989–2995.
- CORNELIS, J. J., BECQUART, P., DUPONCHEL, N., SALOME, N., AVALOSSE, B. L., NAMBA, M. & ROMMELAERE, J. (1988a). Transformation of human fibroblasts by ionizing radiation, a chemical carcinogen, or simian virus 40 correlates with an increase in susceptibility to the autonomous parvoviruses H-1 virus and minute virus of mice. *Journal of Virology* **62**, 1679–1686.
- CORNELIS, J. J., SPRUYT, N., SPEGELAERE, P., GUETTA, E., DARAWSHI, T., COTMORE, S. F., TAL, J. & ROMMELAERE, J. (1988b). Sensitization of transformed rat fibroblasts to killing by parvovirus MVM correlates with an increase in viral gene expression. *Journal of Virology* **62**, 3438–3444.
- CORNELIS, J. J., CHEN, Y. Q., SPRUYT, N., DUPONCHEL, N., COTMORE, S. F., TATTERSALL, P. & ROMMELAERE, J. (1990). Susceptibility of human cells to killing by the parvoviruses H-1 and minute virus of mice correlates with viral transcription. *Journal of Virology* **64**, 2537–2544.
- COTMORE, S. F. (1990). Gene expression in autonomous Parvoviruses. In *Handbook of Parvoviruses*, vol. I, pp. 141–154. Edited by P. Tijssen. CRC Press: Boca Raton, USA.
- DUTRILLAUX, B. & COUTURIER, J. (1981). *Technique de laboratoire, pratique de l'analyse chromosomique*, pp. 40–45. Paris: Masson.
- FAISST, S., SCHLEHOFER, J. R. & ZUR HAUSEN, H. (1989). Transformation of human cells by oncogenic viruses supports permissiveness for parvovirus H-1. *Journal of Virology* **63**, 2152–2158.
- GUETTA, E., MINCBERG, M., MOUSSET, S., BERTINCHAMPS, C., ROMMELAERE, J. & TAL, J. (1990). The selective killing of transformed rat cells by minute virus of mice does not require infectious virus production. *Journal of Virology* **64**, 458–462.
- HADRY, H., BALICK, M. & SCHIERWATER, B. (1992). Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Molecular Ecology* **1**, 55–63.
- HÄNNI, C., LAUDET, V., BARRIEL, V. & CATZEFLIS, F. M. (1995). Evolutionary relationships of *Acomys* and other murids (Rodentia, Mammalia) based on complete 12S rRNA mitochondrial gene sequences. *Israel Journal of Zoology* **41**, 131–146.
- KOERING, C. E., DUPRESSOIR, T., PLAZA, S., STEHELIN, D. & ROMMELAERE, J. (1994). Induced expression of the conditionally cytotoxic herpes simplex virus thymidine kinase by means of a parvoviral regulatory circuit. *Human Gene Therapy* **5**, 457–463.
- MERCHLINSKY, M. J., TATTERSALL, P., LEARY, J. J., COTMORE, S. F., GARDINER, E. M. & WARD, D. C. (1983). Construction of an infectious molecular clone of the autonomous parvovirus minute virus of mice. *Journal of Virology* **47**, 227–232.
- MOUSSET, S., CORNELIS, J. J., SPRUYT, N. & ROMMELAERE, J. (1986). Transformation of established murine fibroblasts with an activated cellular Harvey *ras* oncogene or the polyoma virus middle T gene increases cell permissiveness to parvovirus minute virus of mice. *Biochimie* **68**, 951–956.
- NELSON-REES, W. A. & FLANDERMAYER, R. R. (1977). Inter- and intra-species contamination of human breast tumor cell lines HBC and BrCa5 and other cell cultures. *Science* **185**, 1343–1344.
- ROMMELAERE, J. & CORNELIS, J. J. (1991). Antineoplastic activity of parvoviruses. *Journal of Virological Methods* **33**, 233–251.

- SALOME, N., VAN HILLE, B., DUPONCHEL, N., MENEGUZZI, G., CUZIN, F., ROMMELAERE, J. & CORNELIS, J. J. (1990). Sensitization of transformed rat cells to parvovirus MVMp is restricted to specific oncogenes. *Oncogene* **5**, 123–130.
- SALOME, N., VAN HILLE, B., GEUSKENS, M. & ROMMELAERE, J. (1989). Partial reversion of conditional transformation correlates with a decrease in the sensitivity of rat cells to killing by the parvovirus minute virus of mice but not in their capacity for virus production: effect of a temperature-sensitive *v-src* oncogene. *Journal of Virology* **63**, 4797–4807.
- SIEGL, G. (1984). Biology and pathogenicity of autonomous parvoviruses. In *The Parvoviruses*, pp. 297–362. Edited by K. I. Berns. New York: Plenum Press.
- TATTERSALL, P. & COTMORE, S. F. (1990). Reproduction of autonomous parvoviruses. In *Handbook of Parvoviruses*, vol. I, pp. 123–140. Edited by P. Tijssen. CRC Press: Boca Raton, USA.
- TATTERSALL, P. & GARDINER, E. M. (1990). Autonomous parvovirus-host-cell-interactions. In *Handbook of Parvoviruses*, vol. I, pp. 111–121. Edited by P. Tijssen. CRC Press: Boca Raton, USA.
- TATTERSALL, P., CAWTE, P. J., SHATKIN, A. J. & WARD, D. C. (1976). Three structural polypeptides coded for by minute virus of mice, a parvovirus. *Journal of Virology* **20**, 273–289.
- VAN HILLE, B., DUPONCHEL, N., SALOME, N., SPRUYT, N., COTMORE, S. F., TATTERSALL, P., CORNELIS, J. J. & ROMMELAERE, J. (1989). Limitations to the expression of parvoviral nonstructural proteins may determine the extent of sensitization of EJ-*ras*-transformed rat cells to minute virus of mice. *Virology* **171**, 89–97.
- WELSH, J., PETERSEN, C. & MCCLELLAND, M. (1991). Polymorphisms generated by arbitrarily primed PCR in the mouse: application to strain identification and genetics. *Nucleic Acids Research* **19**, 303–306.

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