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LacZ transgene expression as a cell marker to analyse rescue from the 2-cell block in mouse aggregation chimeras

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Summary

Embryos from certain mouse strains are arrested at the 2-cell stage in cell culture ('2-cell block'), whereas those from other strains develop to the blastocyst stage under the same conditions. It was previously shown that blocking embryos can be rescued in culture by aggregation with an excess of 2-cell stages of a non-blocking strain such as CBA × C57BL/6 F_2. Here we have employed a *LacZ* transgene in a blocking strain (NMRI) to follow the fate of rescued blastomeres up to the blastocyst stage. We found that rescued blastomeres can participate in both inner cell mass and trophoblast formation, thus completely overcoming the 2-cell block.

Keywords: Aggregation chimeras, 2-cell block, Cell labelling, *LacZ* transgene expression, Preimplantation development

Introduction

Under culture conditions, preimplantation embryos originating from several mouse inbred and outbred strains show a characteristic arrest of development, which was first described by Biggers (1971) and subsequently termed the '2-cell block' (Goddard & Pratt, 1983). The time of arrest corresponds to the switching from maternal to embryonic gene expression, i.e. late G_2/M phase of the second cell cycle. A block during preimplantation development occurs not only at the 2-cell stage in mouse, but also in hamster 2-cell (Carney & Bavister, 1987), human 4- to 8-cell (Braude *et al.*, 1988), sheep and goat 8- to 16-cell (Sakkas *et al.*, 1989), cattle 4- to 8-cell (Camous *et al.*, 1984) and pig 4-cell stage embryos (Davis, 1985). In the mouse, the blocked embryos remain macroscopically and ultrastructurally intact for more than 48 h (Biggers, 1971; Goddard & Pratt, 1983).

In previous investigations we found that under our culture conditions BALB/c embryos display the blocking phenotype when explanted prior to the late 2-cell stage, i.e. prior to the phase of major genome activation. These blocking embryos were rescued by aggregation with embryos competent to develop in culture into blastocysts (Sekirina & Neganova, 1995).

In the present study we combined non-blocking embryos of the 2-cell stage with blocking embryos expressing a β-galactosidase transgene to trace descendants of transgenic blastomeres in aggregation chimeras during preimplantation development. We made use of this cell-labelling technique to study the fate up to the blastocyst stage of blocking blastomeres after rescue by aggregation with blastomeres from a non-blocking strain. A preliminary report of this work has been given (Neganova *et al.*, 1996).

Materials and methods

Mice and embryos

NMRI, MF1 and CBA × C57BL/6 F_1 mice were purchased from Harlan Winkelmann, Borchen, Germany; TgR[ROSA26]26Sor (*LacZ*/*LacZ*; Friedrich & Soriano, 1991) mice were obtained from Dr E.-M. Füchtbauer,

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Max Planck Institute for Immunology, Freiburg, Germany. NMRI (wild-type '0/0') females were mated to transgenic TgR[ROSA26]26Sor (*LacZ*/*LacZ*) males. The resulting F_1 individuals (*LacZ*/0) were backcrossed to NMRI to generate the blocking embryos (genotype *LacZ*/0 or 0/0) used for aggregation. Embryos of this type were designated NI-ROSA26. Fifty per cent of the NI-ROSA26 backcross mice carry a transgenic copy of the *E. coli* β-galactosidase (*LacZ*) gene, the product of which can be visualised by X-gal staining. Some backcrosses were done with MF1 instead of NMRI. In this case *LacZ* expression started later in the embryos (late 2-cell stage onwards). However, in embryo-aggregates *LacZ* expression was as in NMRI backcrosses.

Female mice were maintained on a constant light–dark cycle (light 0600–1800 hours). Superovulation of 4- to 8-week-old-females was done by intraperitoneal injections of 7.5 IU PMS (Interogonan, Vemie Chemie, Tönisvorst, Germany) between 1400 and 1500 hours, and 7.5 IU hCG (Predalon, Organon, Oberschleissheim, Germany) 46 h later, between 1200 and 1300 hours. Blocking NI-ROSA26 embryos were recovered 32–34 h post-hCG (early 2-cell stage), non-blocking CBA × C57BL/6 F_2 embryos at 40–42 h post-hCG.

Aggregation of embryos

The zona pellucida was lysed with pronase (D-5147, Sigma, Deisenhofen, Germany) and embryos subjected to assisted aggregation with 400 µg/ml phytohaemagglutinin (PHA, Sigma L-9132) as described (Mintz, 1971; Mintz *et al.*, 1973). Special care was taken to arrange embryos so that one NI-ROSA26 embryo was enclosed by four 'carrier' embryos (Fig. 1). Aggregates were cultured under standard conditions (Hogan *et al.*, 1994) in M16 medium (Whittingham, 1971) with 4 mg/ml of bovine serum albumin (BSA) under mineral oil (Sigma M 8410).

Histology and histochemistry

The embryos were fixed for 5 min in freshly prepared 0.25% glutaraldehyde in phosphate-buffered saline (PBS). Fixed embryos were rinsed three times in PBS with 1% (v/v) BSA. Staining was performed overnight in a solution of 1 mg/ml 5-bromo-chloro-indolyl-β-d-galactopyranoside (X-gal, B 4252, Sigma), 1 mM $MgCl_2$, 10 mM potassium ferrocyanide, 10 mM ferricyanide in PBS at 37 °C. After staining the embryos were rinsed in PBS with 1% BSA and postfixed in 4% paraformaldehyde in PBS at 4 °C for 10 min. Embryos used for sectioning were embedded in Paraplast.

Results and conclusions

Pattern of ROSA *LacZ* expression in preimplantation embryos

In adult NI-ROSA26 mice *LacZ* transgene expression is limited to some tissues, such as a proportion of follicle

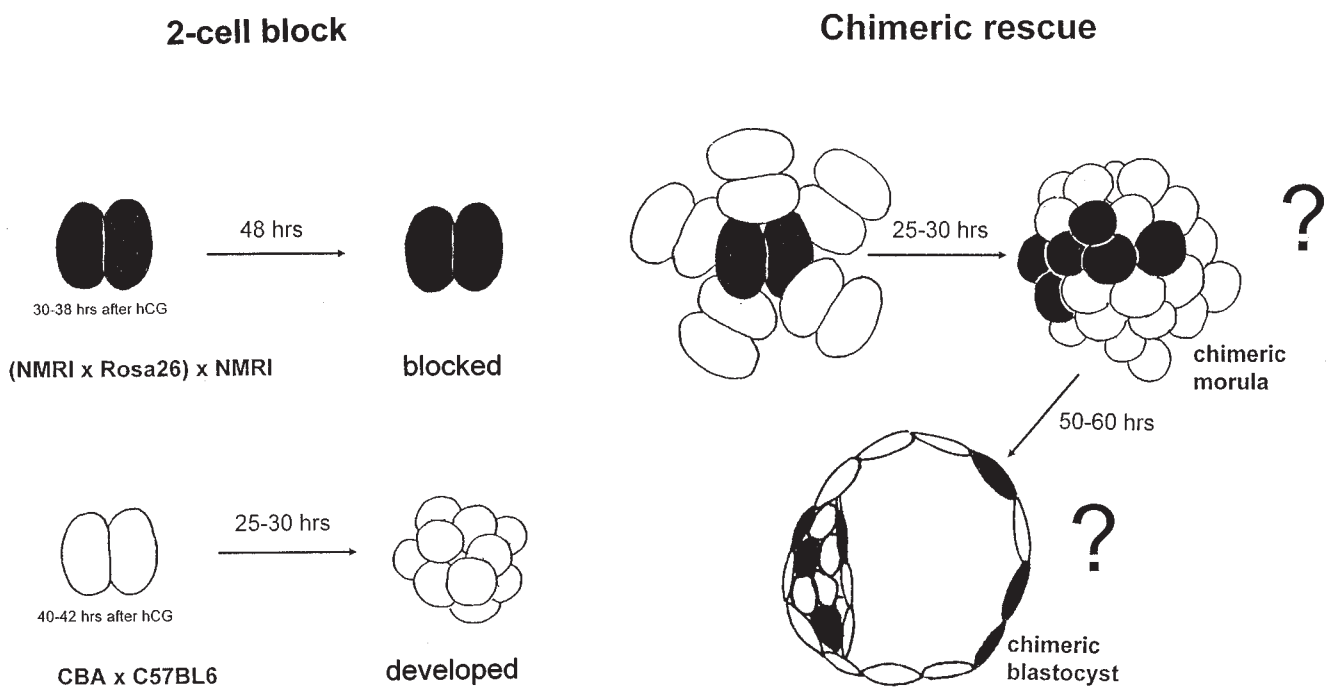

Figure 1 Design of embryo aggregation experiments to rescue blocking embryos. Blastomeres marked in black are β-galactosidase positive. Fifty per cent of the blocking (NMRI x Rosa26) x NMRI embryos carry the *LacZ* transgene and express β-galactosidase. Only the *LacZ*-positive embryos are shown, because only these yield informative developmental stages.

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cells (Augustin *et al.*, unpublished observation). This lack of expression in most adult tissues indicates developmental regulation of the yet unknown promoter downstream to which the transgene had been integrated. The original observation of ubiquitous expression of the *LacZ* in dE 7, dE 9 and dE 12 embryos had suggested that it might be a reporter for a dispensable house-keeping gene, as homozygous transgenic individuals displayed no pathological phenotype (Friedrich & Soriano, 1991).

During preimplantation development of NI-ROSA26 *LacZ*/0 embryos, the transgene is expressed in all blastomeres and later in the inner cell mass and trophoblast. When transgenic insertion was maternally derived, *LacZ* expression was recognised as a dot from the 1-cell stage onward, i.e. earlier than with paternal transmission (not shown; Neganova *et al.*, in preparation). Independently of the parental origin of the *LacZ* transgene, blastomeres of both late 2-cell embryos and early 2-cell embryos that had overcome the block showed overall X-gal staining (Plate 1*A*, facing p. 224). Therefore this labelling technique can be used for cell fate analysis in aggregation chimeras.

Rescue of blocking embryos by co-aggregation with non-blocking embryos

The principal design of this experiment was as described (Sekirina & Neganova, 1995), but the *LacZ* labelling of blastocysts introduced here allowed for an extended observation period (Fig. 1). Ninety-six embryo co-aggregations with blocking NI-ROSA26 and non-blocking CBA × C57BL/6 F_2 embryos were performed. After 25–30 h 82 aggregates developed to morula, and after a further 40 h, 74 aggregates developed to blastocyst stages. As expected for the two possible allelic situations with respect to the transgene in NI-ROSA26 backcross embryos (*LacZ*/0 and 0/0), about 50% of these blastocysts (35/74) showed the positive X-gal staining required for evaluation.

Participation of rescued blastomeres in inner cell mass and trophoblast formation

In aggregation chimeras with blocking NI-ROSA26 and non-blocking CBA × C57BL/6 F_2 embryos *LacZ*-positive cells were recognised in morula stages (Plate 1*B*, arrowheads) and in blastocysts. Label was found in both the inner cell mass and the trophoblast (Plate 1*C* and *D*, arrowheads).

There are two major problems that interfere with the investigation of participation of rescued NI-ROSA26 cell descendants during later embryogenesis: the preponderance of CBA × C56BL/6 cells resulting from numerical matching of embryos (4 non-blocking to 1 blocking) in the aggregation set-up and the prolonged culture time required to obtain morulas or blastocysts (5.5 days *p.c. in vitro* vs 4.5 days *p.c. in utero*). After reimplantation, development of blastocysts *in utero* is adversely influenced by the prolonged time in culture (Bowman & McLaren, 1970).

Traditionally, rescue by aggregation due to the allophenic situation in chimeras had been applied to parthenogenetic embryos (Kaufman, 1983; Stevens, 1978; Stevens *et al.*, 1977) or to those that carry mutations which normally cause lethality in the homozygous state, such as *Jimpy* (Sidman *et al.*, 1964) or T^{hp} (Bennett *et al.*, 1975). These embryos did not require support by other embryos at the time of aggregation, but blocking 2-cell embryos do. The latter type of aggregation rescue is more comparable to the situation of generating chimeras with teratocarcinoma and embryonic stem cells that are dependent on the microenvironment provided by normal embryos to contribute to embryo- and organogenesis.

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References

Bennett, D., Dunn, L.C., Spiegelman, M., Artzt, K., Cookingham, J. & Schermerhorn, E. (1975). Observations on a set of radiation-induced dominant T-like mutations in the mouse. *Genet. Res.* **26**, 95–108.

Biggers, J.D. (1971). Metabolism of mouse embryos. *J. Reprod. Fertil. Suppl.* **14**, 41–54.

Bowman, P. & McLaren, A. (1970). Viability and growth of mouse embryos after *in vitro* culture and fusion. *J. Embryol. Exp. Morphol.* **23**, 693–704.

Braude, P., Bolton, V. & Moore, S. (1988). Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. *Nature* **332**, 459–61.

Camous, S., Heyman, Y., Meziou, W. & Menezo, Y. (1984). Cleavage beyond the block stage and survival after transfer of early bovine embryos cultured with trophoblastic vesicles. *J. Reprod. Fertil.* **72**, 479–85.

Carney, E.W. & Bavister, B.D. (1987). Stimulatory and inhibitory effects of amino acids on the development of hamster eight-cell embryos in vitro. *J. In Vitro Fertil. Embryo Transfer* **4**, 162–7.

Davis, D.L. (1985). Culture and storage of pig embryos. *J. Reprod. Fertil. Supp.* **33**, 115–24.

Friedrich, G. & Soriano, P. (1991). Promoter traps in embryonic stem cells: a genetic screen to identify and mutate developmental genes in mice. *Genes Dev.* **5**, 1513–23.

Goddard, M.J. & Pratt, H.P.(1983). Control of events during early cleavage of the mouse embryo: an analysis of the '2-cell block'. *J. Embryo. Exp. Morphol.* **73**, 111–33.

Hogan, B., Beddington, R., Constantini, F. & Lacy, E. (1994). *Manipulating the Mouse Embryo*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Kaufman, M.H. (1983). *Early Mammalian Development*. Cambridge: Cambridge University Press.

Mintz, B. (1971). Allophenic mice of multi-embryo origin. In *Methods in Mammalian Embryology*, ed. Joseph C. Daniel Jr, San Francisco: W.H. Freeman. pp. 186–214.

Mintz, B., Gearhart, J.D. & Guymont, A.O. (1973). Phytohaemagglutinin-mediated blastomere aggregation and development of allophenic mice. *Dev. Biol.* **31**, 195–9.

Neganova, I.E. & Sekirina, G.G. (1995). Block and viability of BALB/c mouse embryos after explantation during the second cell cycle of cleavage division. *Ontogenez (Russian Journal of Developmental Biology)* **27**, 1–12.

Neganova, I., Sekirina, G., Augustin, M. & Jockusch, H. (1996). Early development marker to analyze rescue from the 2-cell block in aggregation chimeras. *Eur. J. Cell Biol.* (Suppl. 42) **69**, 102.

Sakkas, D., Batt, P.A. & Cameron, A.W. (1989). Development of preimplantation goat (*Capra hircus*) embryos *in vivo* and *in vitro*. *J. Reprod. Fertil.* **87**, 359–65.

Sekirina, G.G. & Neganova, I.E. (1995). The microenvironment created by non-blocking embryos in aggregates may rescue blocking embryos via cell–embryo adherent contacts. *Zygote* **3**, 313–24.

Sidman, R.L., Dickie, M.M. & Appel, S.A. (1964). Mutant mice (quaking and jimpy) with deficient myelination in the central nervous system. *Science* **144**, 309–11.

Stevens, L.C. (1978). Totipotent cells of parthogenetic origin in a chimaeric mouse. *Nature* **276**, 266–7.

Stevens, L.C., Varnum, D.S. & Eicher, E.M. (1977). Viable chimaeras produced from normal and parthenogenetic mouse embryos. *Nature* **269**, 515–17.

Whittingham, D.G. (1971). Culture of mouse ova. *J. Reprod. Fertil. Suppl.* **14**, 7–21.

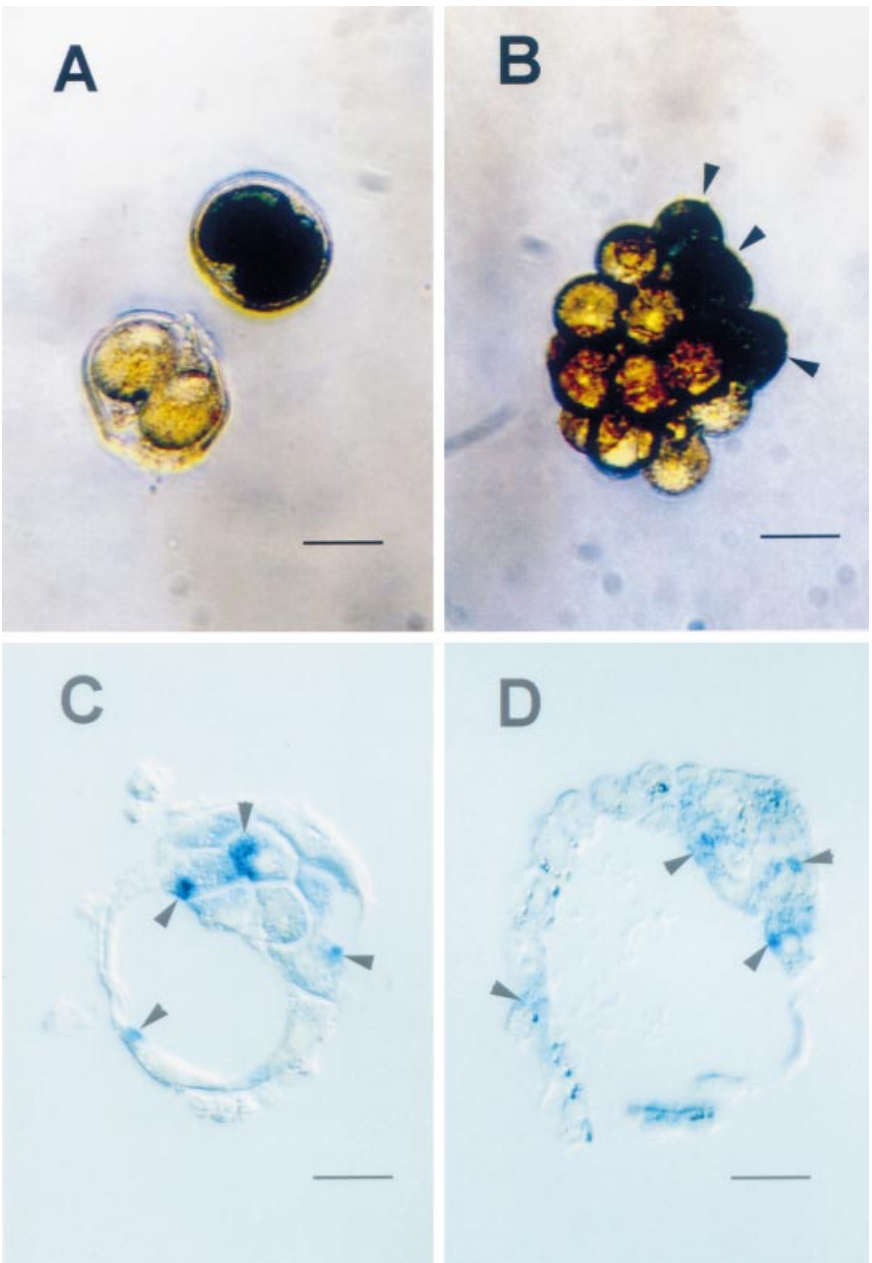

Plate 1 *LacZ* labelling to demonstrate rescue of blocking embryos. (*A*) Two 2-cell stages of (NMRI × Rosa26) × NMRI embryos, one of which is *LacZ*-negative, one *LacZ*-positive. (*B*) Morula derived from an aggregate of a blocking *LacZ*-positive 2-cell stage with *LacZ*-negative non-blocking embryos. *LacZ*-positive, 'rescued' blastomeres are marked by arrowheads. (*C*), (*D*) Sections through chimeric blastocysts. Examples of *LacZ*-positive inner cell mass and trophoblast cells are indicated by arrowheads. Scale bars: A, B, 40 µm; C, 25 µm; D, 20 µm.

(Facing p. 224)

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