Zygote **9** (August), pp 219–228. © 2001 Cambridge University Press Printed in the United Kingdom

Maternal versus paternal expression of a *LacZ* transgene in preimplantation mouse embryos: effects of genetic background and 2-cell block

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Date submitted: 11.1.01. Date accepted: 23.3.01

Summary

The expression of a transgene NI-ROSA *LacZ* (*LacZtg*) trapped into the genes for two presumably untranslated, ubiquitously expressed RNAs, was studied in preimplantation mouse embryos with respect to penetrance (fraction of expressing embryos) and to localisation of β-galactosidase activity. With maternal origin in NMRI mice β-galactosidase was first detected within one dot in the cytoplasm of zygotes at 30 h post-hCG. The staining pattern progressed to small clusters and to dense, homogeneous staining of the entire cytoplasm during further development. Within the NMRI background, penetrance *in utero* was delayed by at least 6 h when the transgene was of paternal as compared with maternal origin. Paternal transgene expression increased marginally during culture to 50 h after explantation of embryos at 30–48 h post-hCG and remained low or decreased in the '2-cell block'. Expression of a paternal transgene in preimplantation embryos developing *in utero* was further delayed in the maternal MF1 as compared with the NMRI background. In contrast to NMRI × NMRI embryos with paternally derived transgene, expression increased with time during the 2-cell block in MF1 × NMRI embryos. Thus, in the earliest phase of mammalian development expression of this *LacZtg* is influenced by parental origin, maternal genetic background and environment. The spatial distribution of the gene product is developmentally controlled.

Keywords: 2-cell block, Expression, Imprinting, *LacZ* transgene, Spatial distribution

Introduction

Transgenes fused to defined or unknown regulatory sequences have been used frequently as 'reporters' for temporal and spatial patterns of gene expression and function (e.g. Kothary *et al*., 1989; Fleischmann *et al*., 1998; Zambrowicz & Friedrich, 1998; Skynner *et al*., 1999; Acampora *et al*., 1999), or to study parental effects on penetrance of the phenotype mediated by methylation of the DNA, modifiers and chromatin structure (Sapienza *et al*., 1989; Allen *et al*., 1990; Howell *et al*., 1998; Szabom *et al*., 1998; Hershko *et al*., 1999; Brenton *et al*., 1999). For developmental studies in mammals, the preimplantation embryo is of special interest since little is known about the spatial organisation of gene expression. A commonly used transgene is the bacterial Z-gene of the Lac operon, the product of which, β-galactosidase, can be demonstrated by histochemical reactions using the synthetic substrate X-gal. In cells with an integrated *LacZ* gene, temporal (e.g. stage of expression) and spatial expression pattern (e.g. tissue distribution) directed by a specific promoter and/or other regulatory elements can be easily monitored during embryogenesis. In addition, information on the distribution of gene product within a cell (e.g. localised versus evenly distributed) may be obtained.

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https://doi.org/10.1017/S096719940100123X Published online by Cambridge University Press

In the present work we have used a *LacZ* transgene, NI-ROSA26LacZ, of the mouse (Zambrowicz & Friedrich, 1998) which has been inserted into a region of DNA on mouse chromosome 6 that produces three transcripts (Zambrowicz *et al*., 1997) from a common promoter. One gene, untrapped by the insertion (since it is in opposite orientation) codes for a protein of unknown function. The ROSAβgeo provirus vector integrated into the first exon of the two other transcripts, and their message could not be detected by Northern blot in tissues of homozygous transgenic animals yet there was no phenotypic difference compared with wild-type mice (Zambrowicz *et al*., 1997). This may be due to the fact that neither of the two disrupted transcripts contains a significant open reading frame. However, the reporter gene displayed expression in many organs in embryonic and adult, transgenic animals, e.g. in the haematopoietic and haematolymphoid compartment, in head, body, visceral yolk sac and feet (West, 1999) as well as in lung, kidney and testis (Zambrowics *et al*., 1997). In fact, *LacZ*-expressing cells from ROSA26 mice have been used in chimera experiments to follow cell fate (Freidrich & Soriano, 1991; Neganova *et al*., 1998; West, 1999). In this work we studied expression of this transgene in preimplantation embryos with regard to the distribution of gene production in early 2-cell embryos up to the blastocyst stage, and penetrance relative to parental origin, genetic background and development of embryos in culture versus *in utero*, especially in relation to their arrest in the '2-cell block' (Goddard & Pratt, 1983; Biggers, 1998; Neganova *et al*., 1998) where full zygotic gene activation may be affected (Schultz *et al*., 1999).

Materials and methods

NMRI and MF1 mice were purchased from Harlan Winkelmann, Borchen, Germany and bred in our animal facilities under 12 h/12 h light/dark cycles. NI-ROSA26 (*LacZ*/0) mice were derived by mating NMRI females to transgenic TgR[ROSA26]26Sor (*LacZ*/*LacZ*) males (kindly provided by Dr H.-M. Füchtbauer, now University of Aarhus, Denmark) as described previously (Neganova *et al*., 1998). Fifty per cent of NI-ROSA26 backcross embryos are expected to contain the *E. coli* β-galactosidase (*LacZ*) gene.

Superovulation was achieved by intraperitoneal injections of 7.5 IU PMSG (Interogan, Vemie Chemie, Tönisvoerst, Germany) and 7.5 IU hCG (Predalon, Organon, Oberschleissheim, Germany) 46 h later.

Embryos ('*in utero*' group) were flushed from the ampullae at 2 h intervals between 30 h and 48 h post-hCG (corresponding to late 1-cell embryos/zygotes to late 2-cell stages).

Influence of culture on gene expression ('*in vitro*' group) was studied in embryos recovered between 30 and 48 h post-hCG and cultured in 20 µl drops of M16 medium (Whittingham, 1971) with 4 mg/ml bovine serum albumin (BSA; Sigma) under mineral oil (Sigma) at 37 °C with 5% CO_2 in air until 50 h, or when they were in a 2-cell block, after isolation at 32 h, 38 h or 42 h and culture up to 68 h or 92 h post-hCG, designated as '1 day of block' or '2 days of block', respectively. Expression was also studied in embryos that escaped the 2-cell block. For this, late 2-cell stages (at 48 h post-hCG) were explanted and cultured for 52–54 h, when they had reached the 4-cell stage, or 72 h or 110 h post-hCG, when they were in the 8-cell or blastocyst stage of development.

Histological and histochemical investigation of embryos was done as previously described (Neganova *et al*., 1998). Images observed with a Zeiss Axiophot microscope were recorded on Alfo-slide film (64 ASA), scanned and electronically processed.

Results

Our observations on β-galactosidase activity, i.e. the expression of the NI-ROSA26 *LacZ* transgene (subsequently abbreviated as *LacZtg*), is based on the standard pattern observed *in utero* when the *LacZtg* is maternally derived and both parents are of the NMRI strain, i.e. the spatial distribution in *LacZ* embryos derived from the cross NI-ROSA26 × NMRI. Transgene expression was studied with respect to 'penetrance', which refers to the fraction of embryos showing any pattern of β-galactosidase staining and to staining of somatic (cumulus) cells. Secondly, the spatial pattern of β-galactosidase activity was analysed, and lastly the frequencies of the different patterns observed at subsequent stages of development and in embryos arrested in the 2-cell block were recorded.

None of the embryos with a maternally derived *LacZtg* stained positive for β-galactosidase at 26 h post-hCG. The principal staining patterns in more advanced embryos are shown schematically and in selected micrographs in Fig. 1. Some of the 1-cell embryos of NI-ROSA26 × NMRI contained only one intensely stained dot of reaction product at 30 h of *in utero* development (Fig. 1*C, D*), a pattern never observed past the 2-cell stage. However, a single dot was found in an otherwise homogeneously stained embryo arrested at the 2-cell stage in a 'deep block' (92 h post-hCG; Fig. 1*K*) after explanation at 32–34 h, before major zygotic gene activation is usually completed, and culture to 92 h. Two-cell blocked embryos explanted later, at 42 h post-hCG, when the major phase of zygotic gene activation presumably has been completed, never exhibited pattern 1 of staining, even when cultured up to 92 h.

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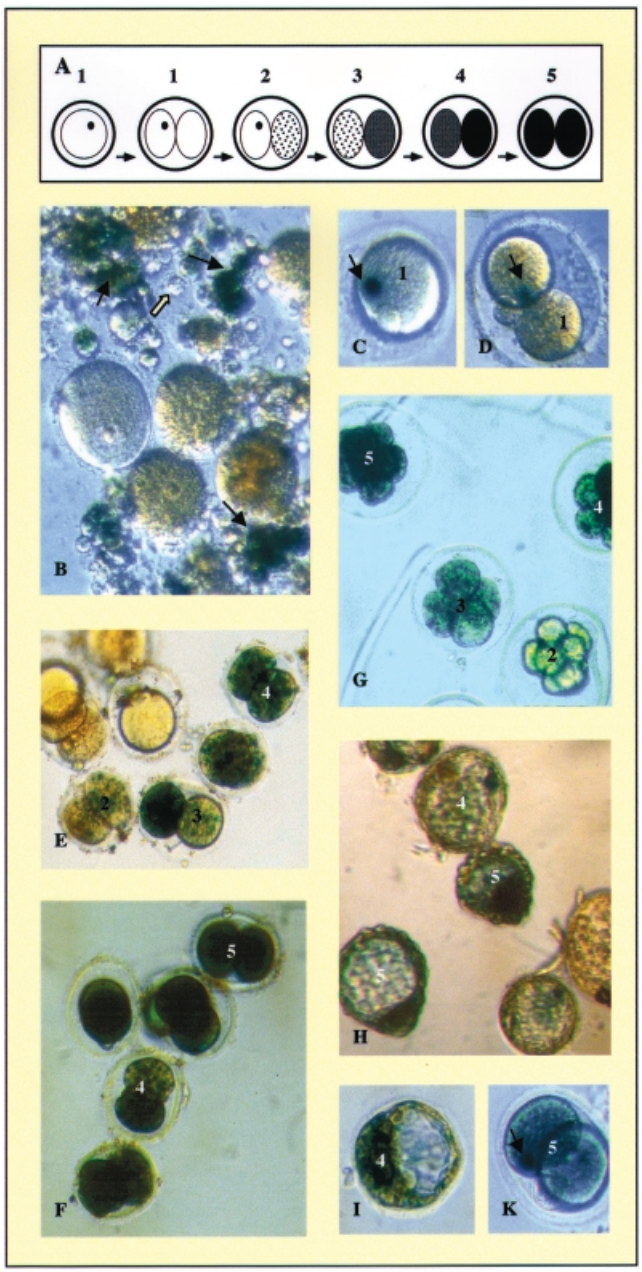

Figure 1 Patterns of ROSA26 (*LacZ*) transgene (*LacZtg*) expression in preimplantation embryos. (*A*) Schematic drawing of progressive patterns of X-gal staining, numbered 1 to 5: 1, one intensely stained spot; 2, several spots in one or a few blastomeres; 3, numerous dots in blastomeres; 4, only one or some of the blastomeres are stained homogeneously and intensely; 5, all blastomeres in the embryo are stained homogeneously and intensely. (*B*)–(*I*) Examples of *LacZ* staining patterns observed from zygote to blastocyst stages. Numbers refer to staining patterns as defined in (*A*). (*B*) β-gal negative zygotes and β-gal positive cumulus cell masses (black arrows) and the same without reaction product (white arrow) from NI-ROSA26 × NMRI embryos explanted at 27 h post-hCG. (*C*) Late zygote (30 h post-hCG) with pattern 1 of *LacZ* expression and reaction product (arrow) close to the polar body. (*D*) *LacZ* transgene expression according to pattern 1 in blocked (2 days in block) 2-cell stage embryo from NRMI × NI-ROSA26 embryos with reaction product close to second polar body (arrow). (*E*) Patterns 2, 3 and 4 of *LacZ* transgene expression in a NMRI × NI-ROSA26 2-cell embryo after explantation at 38 h post-hCG and culture until the end of the 2-cell stage (48 h). (*F*) Two-cell stage embryos from NI-ROSA26 × NMRI explanted at 38 h post-hCG and cultured to 48 h with patterns 4 and 5, according to a high level of transgene expression. (*G*) Patterns from 2 to 5 in β-gal positive embryos from an NMRI × NI-ROSA26 cross developed in culture from 48 h to 62 h post-hCG. (*H*) Blastocysts (110 h post-hCG) stained with patterns 4 and 5 from NI-ROSA26 × NMRI crosses developed in culture from 48 h. (*I*) Blastocyst with pattern 4 of *LacZ* transgene activity from an NI-ROSA26 × NMRI just after explantation at 110 h post-hCG. (*K*) Two-cell stage MF1 × NI-ROSA 26 embryo stained according to pattern 5 after 2 days in block. Scale: Long axis of 2-cell stages corresponds to 80 μm and diameter of blastocysts to 100–120 μm.

With an overall increased staining intensity in advanced stages of embryogenesis, the distribution of reaction product changed from a single, larger dot to a distinct granular staining of relatively smaller spheres and finally to a homogeneous dark blue appearance of the entire cytoplasm. The staining pattern was often mosaic with respect to the distribution and density of reaction product. Thus blastomeres of *in utero* or in culture developed embryos often exhibited heterogeneous staining patterns: e.g. one blastomere appeared only light blue while the other was homogeneously stained darkly (Fig. 1*E*, *F*). Occasionally 6- to 8-cell embryos were seen with three or four blastomeres exhibiting dark staining, others only intermediate types of staining (consistent with pattern 4: see Fig. 1*G*, right-hand side). Heterogeneity existed also for expression of the transgene in specific somatic cells of the ovary since some corona cells surrounding ovulated oocytes and early preimplantation embryos were stained (black arrow in Fig. 1*B*) while others did not contain reaction product (white arrow in Fig. 1*B*). Thus, penetrance may not be 100% in all somatic cells of the heterozygous mother (*LacZ*/0). However, cells of the trophectoderm as well as the inner cell mass expressed the transgene in developing embryos (Fig. 1*H*), as previously described for aggregation chimeras (Neganova *et al*., 1998).

https://doi.org/10.1017/S096719940100123X Published online by Cambridge University Press

Expression of maternally derived *LacZtg* in NMRI mice

With a maternally derived transgene (NI-ROSA26 × NMRI) and development *in utero* there was no staining in any embryo at 26 h post-hCG. Unstained embryos from heterozygous females (Fig. 1*B*) may lack transgene or pools of maternal protein are depleted. The onset of zygotic *LacZtg* expression was recognised in 46% of the embryos (Fig. 2) at the late zygote stage (30 h post-hCG; Fig. 1*B*) with only a maternally derived transgene (NI-ROSA26 × NMRI) – close to the 50% expected when the single maternal *LacZtg* segregates randomly during oogenesis and penetrance is 100%. At this stage all embryos expressed the *LacZtg* according to pattern 1, with a single darkly stained dot in the cytoplasm (Fig. 3*A*, upper panel). From the early 2-cell stage (32 h post-hCG) up to 48 h of *in utero* development, the number of β-gal positive embryos stayed at around 50% with a maternal transgene (Fig. 2). Slightly higher frequencies were seen at the end of the second cell cycle (42 h and 48 h).

Embryos which displayed a few small spots within one or two blastomeres, corresponding to pattern 2, were first observed at 32 h post-hCG with only one maternal copy of the transgene (Fig. 3*A*). By 34 h already 13% of the embryos were stained according to pattern 4, corresponding to a much higher level of enzyme activity. In the course of the second cell cycle, the fraction of embryos with an intermediate type of transgene expression (pattern 3 or pattern 4) increased steadily, and most of the embryos had pattern 5 of *LacZtg* expression at 42 h post-hCG and at the late 2-cell stage, shortly before the next embryonal division (48–50 h) (Fig. 3*A*).

In embryos developed *in vivo* between 30 and 48 h (Fig. 3*A*, left-hand panel, '*in utero*'), and those explanted at various times, from 30 h to 48 h, and cultured to 50 h post-hCG (Fig. 3*A*, right-hand panel, '*in vitro*'), there was a general tendency for *LacZtg* expression to shift to more advanced patterns and higher expressions levels. However, the expression patterns reached in embryos explanted at 30–38 h and subsequently cultured to 50 h differed substantially from embryos developed *in utero* to 48/50 h post-hCG (Fig. 3*A*). The developmentally regulated increase in expression of transgene was therefore significantly retarded when embryos were cultured. Only when explantation was performed at 38 h or later, a time corresponding to the middle of S-phase of the 2-cell stage, where initiation of major zygotic gene activation has occurred (e.g. Schultz *et al.*, 1999), and the embryos then cultured to 50 h, were expression patterns similar between the *in utero* group and the *in vitro* developed embryos (Fig. 3*A*).

Comparison of patterns of transgene expression between embryos explanted at 32–48 h and cultured to 50 h (Fig. 3*A*, *in vitro* group) and those explanted at

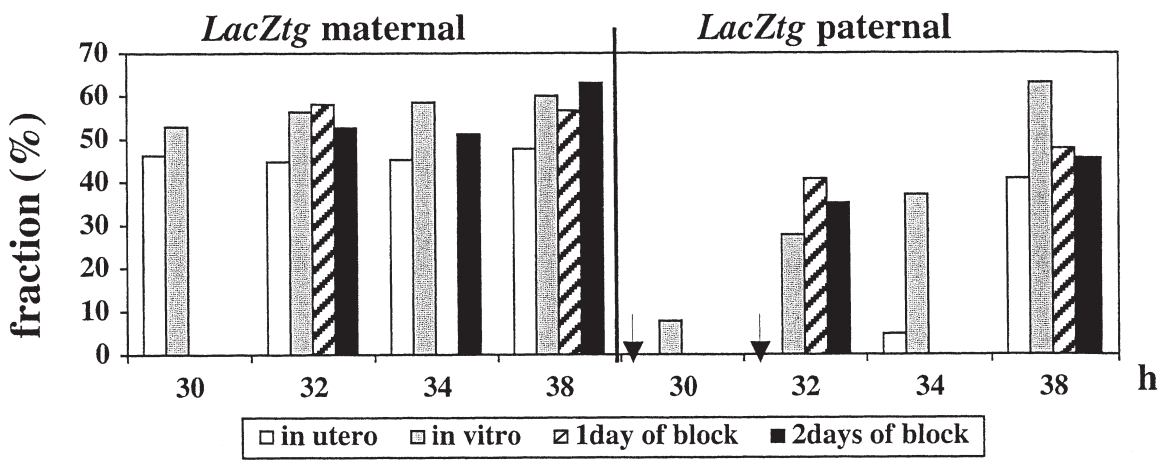

Figure 2 Frequency ('penetrance') of *LacZ* transgene expressing embryos according to parental origin. The percentage of embryos expressing any pattern of the transgene after development *in utero*, until 30–38 h post-hCG, or after explantation at 30–38 h post-hCG and culture until 50 h ('*in vitro*'), until 68 h ('1 day of block') or 92 h post-hCG ('2 days of block'). Left-hand panel: NI-ROSA × NMRI cross, *LacZ* transgene of maternal origin. Number of embryos isolated from the ampullae/explanted at 30 h, 32 h, 34 h and 38 h, and viewed directly (*in utero* group) or after culture to 50 h post-hCG (*in vitro* group), was 63/61, 96/61, 51/65 and 80/60, respectively. The numbers of embryos 1 or 2 days in block from this cross was 69/50, 0/51 and 53/27 for explantation at 32 h, 34 h and 38 h, respectively. Right-hand panel: NMRI x NI-ROSA cross, *LacZ* transgene of paternal origin. The number of embryos isolated from the ampullae/explanted at 30 h, 32 h, 34 h and 38 h, and viewed directly (*in utero* group) or after culture to 50 h post-hCG (*in vitro*), was 55/67, 60/58, 63/32 and 44/27, respectively. The number of embryos 1 or 2 days in block from this cross was 42/17 and 23/11 for explantation at 32 h and 38 h, respectively.

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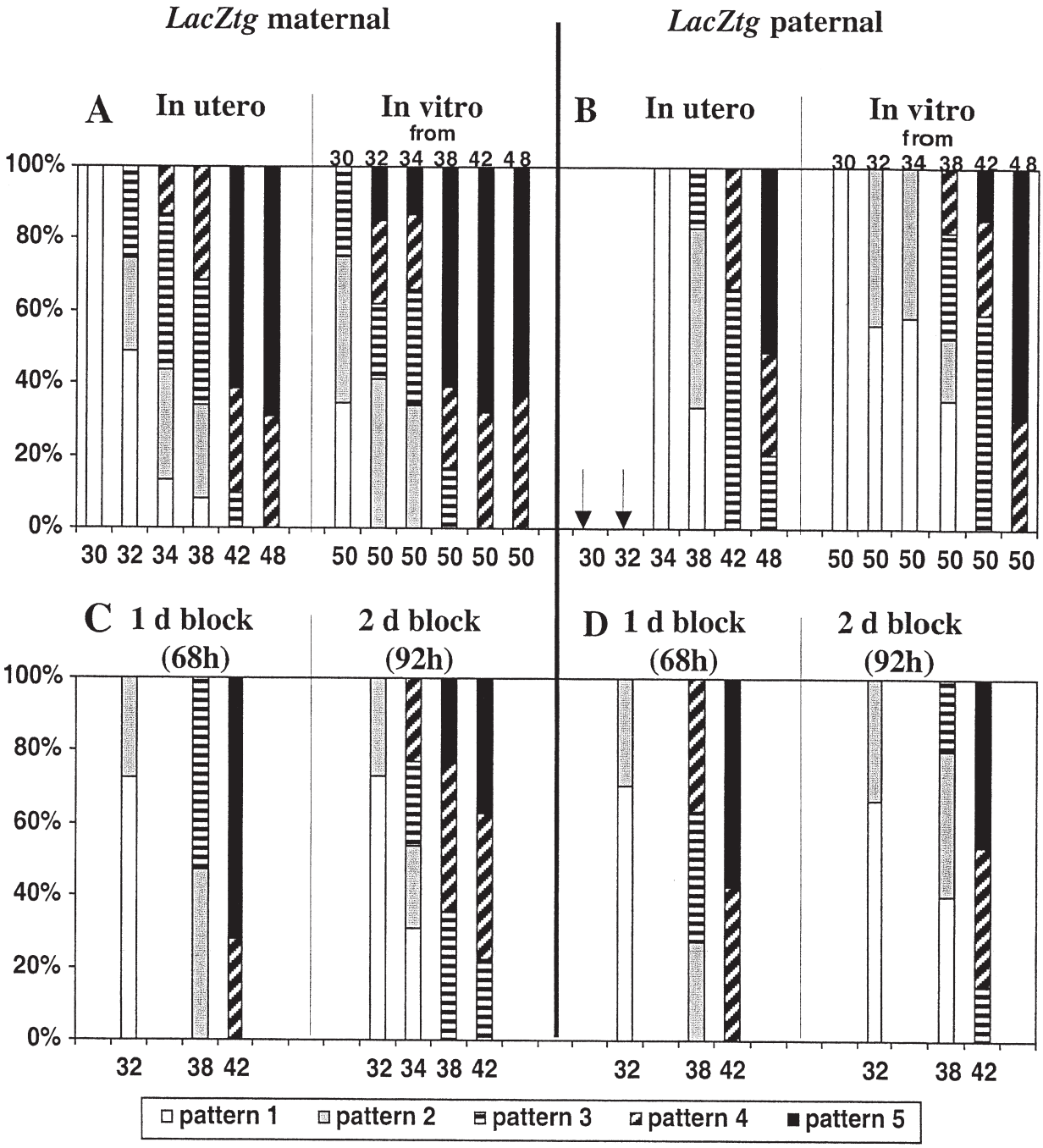

Figure 3 Dependence of *LacZ* transgene expression patterns on parental origin and development *in utero* versus in culture until 50 h post-hCG ('*in vitro*'), or longer, during 2-cell block. For the key for patterns 1 to 5, see Fig. 1*A*. Patterns are shown of *LacZ* transgene expression in embryos from NI-ROSA × NMRI (*LacZtg* maternal, left) and NMRI × NI-ROSA (*LacZtg* paternal, right) after development *in utero*, or after explantation at 30–48 h and culture to 50 h (*in vitro* group in *A* and *B*), or after explantation at indicated times and culture to 68 h ('1 d block') or 92 h ('2 d block'). (*C*, *D*) Arrows indicate that no embryo expressing transgene was observed at the time of isolation (penetrance zero).

32–42 h post-hCG and arrested in the 2-cell block after culture until 66 h or 92 h (Fig. 3*C*, 1 d/2 d block) revealed that transgene expression has a tendency to shift from more advanced to earlier patterns and, accordingly, to reduced levels of expression upon prolonged culture during a developmental block. For instance, about 70% of *in utero* developed embryos expressed pattern 5 at 48 h of development (Fig. 3*A*, *in*

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utero). Explantation between 32 to 38 h and culture to 66 h or 92 h post-hCG (Fig. 3*C*, 1d /2 block) resulted in a maximum of 23.5% of embryos expressing *LacZtg* according to pattern 5 (explantation at 38 h). Patterns of expression similar to those found in embryos developed *in utero* for 48 h were only observed in blocked embryos explanted as late as 42 h post-hCG and cultured for 24 h or 58–50 h, respectively. Developmental arrest in deep block (culture to 92 h post-hCG or longer; 2 d block in Fig. 3*C*) resulted in a substantial reduction in *LacZtg* expression within the NMRI background and a maternally derived transgene in relation to arrest for 1 day (1 d block). From this it appears that penetrance of the transgene is 100% with respect to embryos expressing the *LacZtg in utero* and *in vitro* (Fig. 2, left-hand panel), while explantation in early to middle S-phase of the 2-cell stage and embryo culture *in vitro* results generally in lower expression rates due to delayed increases in expression levels, especially when embryos become developmentally arrested in the 2-cell block. Near-normal levels of expression in block correspond to explantation at middle to late S-phase of the 2-cell stage when the major phase of zygotic gene activation has already been initiated.

As expected from *in utero* observations, around 50% of all embryos escaping the block after explantation at 48 h and culture to the 2- to 8-cell or the blastocyst stage (up to 3 days of culture) expressed the transgene, when it was maternally derived (Fig. 4), suggesting 100% penetrance. In all advanced embryonic stages (4-cell to blastocyst) patterns 3 to 5 were observed but there was a uniform tendency of a lower expression later since pattern 5 decreased while pattern 3 was predominant with development to the blastocyst stage.

When both parents were heterozygous with respect to the *LacZtg* only 31% of early 2-cell embryos were stained at 30 h and 45% were expressing the transgene in embryos of NI-ROSA26 × NI-ROSA26 crosses at 30 h post-hCG (data not shown). This may parallel the situation in adult mice where, for unknown reasons, significantly fewer than expected homozygotes were discovered in crosses between two heterozygote parents (Zambrowicz *et al*., 1997). At 32 h 36% of all embryos had pattern 1 (data not shown), similar to what can be seen with only a maternally derived transgene (Fig. 3*A*).

Expression of paternally derived *LacZtg* in NMRI mice

Unlike the situation with a maternally derived transgene, β-gal positive embryos from NMRI × NI-ROSA26 crosses with a paternally inherited transgene were first observed at 34 h post-hCG when development occurred *in utero* (Fig. 2, right-hand panel; Fig. 3*B*). In accordance with delayed expression of a paternally derived transgene, the fraction of embryos expressing the transgene reached 50% only at 38 h post-hCG, as expected in the case of 100% penetrance of the transgene. From 38 h onwards about 50% of all embryos of the *in utero* group were β-gal positive. The fraction of stained embryos in block, 66 h or 92 h post-hCG, was around 50% as expected (Fig. 2).

As in the case of a maternally derived transgene, patterns of staining progressed from pattern 1 towards pattern 5 in embryos expressing a paternally derived *LacZtg* upon prolonged *in utero* development of these embryos (Fig. 4*B*). Unlike with the maternally derived transgene, the first β-gal positive embryos were found only at 34 h. In this group 100% of all positive embryos with a paternal transgene exhibited pattern 1 staining. Pattern 2 was first found at 38 h of development post-hCG, in contrast to the maternally derived transgene expressed as pattern 2 already at 32 h post-hCG (compare Fig. 3*A* and *B*, *in utero* groups). This suggests that transgene expression is delayed by at least 6 h in case of a paternal relative to a maternal transgene.

As with the maternally derived transgene, explantation at 30–48 h followed by culture to 50 h post-hCG (Fig. 3*B*, *in vitro* group) resulted in a relatively minor increase in the percentage of embryos with more advanced staining patterns, suggesting only moderate increases in expression during embryo culture. For

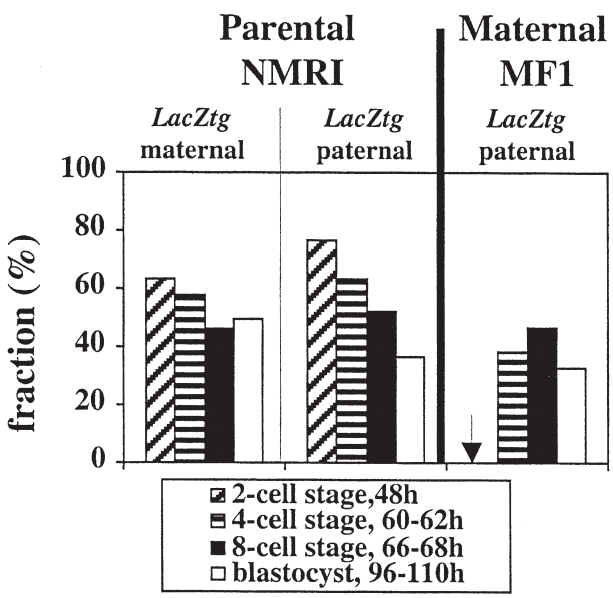

Figure 4 Penetrance of *LacZ* transgene expression at later stages of development: dependence on parental origin and paternal background. Crosses between NI-ROSA26 × NMRI (*LacZtg* maternal, left) and NMRI × NI-ROSA26 (*LacZtg* paternal, centre) and MFI × NI-ROSA (*LacZtg* paternal, right) were analysed. Numbers of embryos included in the analysis were 26/34/38, 59/67/61 and 38/42/38 in 4-cell stage/8-cell stage/blastocysts in (*A*), (*B*) and (*C*), respectively. Arrow indicates penetrance zero.

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instance, embryos explanted at 30 h post-hCG and cultured to 50 h stained according to pattern 1, unlike the absence of staining in the control embryos isolated at 30 h and developed *in utero*. However, earlier explantation times plus culture to 50 h never allowed embryos to express transgene pattern 5, which is predominantly found in the *in utero* group at 48 h post-hCG (Fig. 3*B*). Prolonged culture in 2-cell block to 68 h (Fig. 3*D*) leads to further advanced patterns/higher expression levels (e.g. compare 42 h, *in utero* in Fig. 3*B* with 42 h, 1 d block in Fig. 3*D*), unlike the situation with the maternally derived transgene. Later (2 d block), a slight decrease in the percentage of embryos with the most intense staining pattern was observed (Fig. 3*D*).

The fraction of *LacZtg*-expressing embryos with a paternally derived transgene was about 50% and more in the 2- to 8-cell embryos (Fig. 4, middle panel), and about 40% in blastocysts. Therefore on average 50% of all embryos appear to be *LacZtg* positive for expression, as was observed with the maternally derived transgene. More than 50% of embryos of a NI-ROSA26 × NI-ROSA26 cross were β-gal positive at the 4- to 8-cell stage (data not shown), consistent with expression from the paternal as well as the maternal transgene at these stages of development. Later, at the blastocyst stage, only about 50% of embryos from crosses between heterozygotes were stained – less than the expected three-quarters. Possibly, homozygous embryos may cease to develop or expression of the transgene may decrease towards compaction for unknown reasons (Zambrowicz *et al.*, 1997).

Consistent with this last hypothesis, in the cross between a NMRI female and a heterozygous male (Fig. 5, left-hand panel) the overall percentage of embryos expressing the transgene according to pattern 4 also became lower while that with pattern 2 increased from the 4-cell stage to the blastocyst under conditions with only one paternally derived *LacZtg*. This corresponds to reduced expression levels in the maternally derived *LacZtg* situation. Pattern 5, the highest expression pattern, where all cells of the embryo stain darkly and uniformly for β-gal, was never observed with paternal inheritance, unlike with the maternal transgene (Fig. 5). This supports the notion that penetrance of the transgene and strength of expression is epigenetically modulated and is lower in preimplantation embryos with a paternally inherited as compared with a maternally inherited transgene.

Expression of paternally derived *LacZtg* in a maternal MF1 genetic background

There was no expression of a paternally derived *LacZtg* in *in utero* developed embryos from fertilised MF1 oocytes during the early 2-cell stage up to 50 h post-hCG. The first β-gal positive embryos were identified only at the 4-cell stage, after 60–62 h of development with MIF1-derived ooplasm (Figs 4 and 5, right-hand side). Penetrance of the transgene appears to be much lower compared with the NMRI strain within the maternal MF1 genetic background, since consistently less than 50% of the 4- to 8-cell embryos and blastocysts with a paternally derived transgene stained for β-gal (Fig. 4).

Similar to the situation with the paternal *LacZtg* in the NMRI background, staining was exclusively according to pattern 2 to 4 and not pattern 5 (Fig. 5, right-hand panel). However, the advanced patterns increased somewhat and did not decrease as in the NMRI × NI-ROSA26 matings during development from the 4-cell embryo to the blastocyst stage.

In contrast to observations in crosses with NMRI background, explantation at 32 h or 38 h post-hCG and culture of 2-cell blocked embryos until 68 h or 92 h post-hCG (1 d/2 d block) released embryos derived from MF1 oocytes from suppression of paternal transgene expression and induced a shift from lower expression patterns to transgene expression pattern 5 (Fig. 5, right-hand panel). Therefore exposure of embryos to an altered environment upon explantation may inhibit full zygotic expression of a paternally

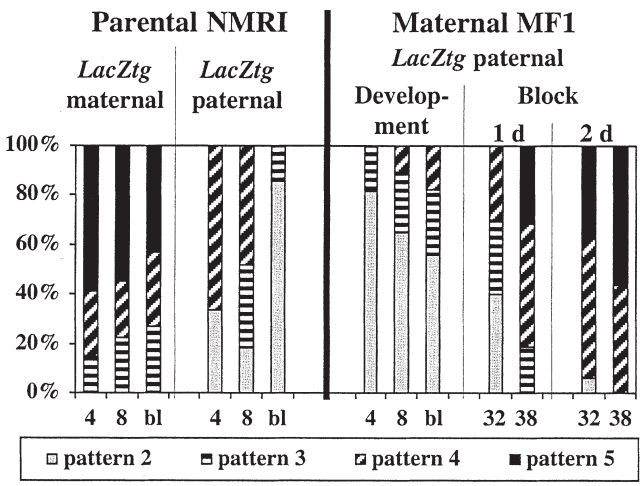

Figure 5 Patterns of *LacZ* transgene expression at later stages of development: dependence on parental origin and maternal background. Patterns are shown of transgene expression in transgene positive 4-cell embryos 60–62 h post-hCG ('4'), 8-cell embryos 66–68 h post-hCG ('8') and blastocysts 96–100 h post-hCG ('bl'). Culture was of 2-cell arrested embryos ('Block') to 68 h (1 day of block: '1 d') or to 92 h (2 days of block: '2 d'), or development of embryos ('Development') to 4-cell, 8-cell or blastocyst stage ('4', '8', 'bl', respectively). The number of embryos with parental NMRI background is given in Figure 4. The number of embryos with maternal MF1 background was 55/79/103 for 4-cell, 8-cell and blastocyst embryos, respectively. The number of embryos in block was 48/35 and 46/38 for 32 h/38 h of explantation and 1 or 2 days in block, respectively.

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derived gene as implicated by staining of NMRI × NI-ROSA26 embryos, but may also overcome a suppression of expression by epigenetic factors intrinsic to the maternal genetic background of a paternally inherited gene in the early preimplantation embryo as observed in the fertilised MF1 embryos.

Discussion

Our data imply that the maternal but not the paternal allele of the gene with the NI-ROSA *LacZ* transgene insertion is expressed during the early cleavage stages. Paternal NI-ROSA26 *LacZ* expression appears initially repressed by maternal and by strain-specific factors. Expression of the paternal allele of the inserted gene is delayed but still reaches about 100% penetrance at the 2- to 4-cell stage, irrespective of maternal genotype. Differences in transgene expression depending on the parental origin may reflect different methylation patterns transmitted by the respective germ lines (e.g. Hershko *et al*., 1999), the effect of silencer elements (e.g. Brenton *et al*., 1999) or alterations in chromatin structure (e.g. Szabo *et al*., 1998), but may also involve epigenetic cytoplasmic factors in oocytes (Sapienza *et al*., 1989; Latham, 1994). We observed heterogeneity and asynchrony of expression of the transgene within populations of genotypically identical heterozygous embryos. Heterogeneity of expression was also implicated by the variability in pattern, ranging from pattern 1 to 4 or from pattern 2 to 5 in individual embryos within one experimental group. Physiological heterogeneity in transgene expression in 2-cell stage mouse embryos does not necessarily depend on heritable modifications (e.g. methylation) of the transgene locus (Kothary *et al*., 1992); it may simply reflect a high inter-embryo variability of the time and strength of zygotic gene activation. Accordingly, we found that the *LacZ* transgene became fully expressed with maternal as well as paternal inheritance once embryos had reached the 4- to 8-cell stage of development when the major phase of zygotic gene activation is completed. Penetrance of *LacZtg* expression was also close to 80% (about 40% of embryos β-gal positive) in the MF1 × NI-ROSA26 embryos at this time.

From the differential pattern of expression of a paternally derived versus a maternally derived transgene and of a paternal transgene in the cytoplasm of embryos with different genetic background (NMRI/MF1), it can be deduced that there is imprinting of the locus into which the transgene was integrated such that penetrance is modulated according to the parental origin as well as the oocyte's genotype. It is of interest that other genes on mouse chromosome 6, such as the recently discovered *Mit1/Lb9* gene (Lee *et al*., 2000), are also imprinted with partial imprinting in embryos and differences in imprinting strength in embryonic and somatic tissues according to the strain-specific genetic background. It was suggested that expression patterns for *Mit1/Lb9* are epigenetically modulated (Lee *et al*., 2000), comparable to expression patterns found for the presently studied *LacZtg*. The genes which were trapped by viral integration of *LacZtg* neither possess an open reading frame nor probably function at the RNA level, similar to other imprinted genes such as *Mit1/Lb9* (Lee *et al*., 2000), *H19* (Leighton *et al*., 1995) and *Igf2rAS* (Wutz *et al*., 1997). Thus, imprinting of regulatory RNAs and, in particular, anti-sense RNAs with currently unknown functions (Altuvia & Wagner, 2000), may be a common feature in early mammalian development.

One aim of the study was to assess the influence of environment and the 2-cell block on expression of the transgene. An important switch in expression patterns is found after cytokinesis, when the 'major' phase of zygotic gene activation occurs at the early to late S-phase of the 2-cell stage in the mouse (e.g. Aoki *et al*., 1997). Earlier work suggested that zygotic gene expression cannot be fully induced in the 2-cell block (Kothary *et al*., 1992). In accordance with this, explantation at 30–38 h resulted in reduction of expression of the maternally derived *LacZtg* upon 2-cell block. Oxidative stress and other parameters affected by culture may cause the developmental arrest after explantation (Nasr-Esfahani *et al*., 1990; Biggers, 1998). From studies of preimplantation embryo development *in vitro* in several species it is known that culture conditions may influence gene expression subtly and in a long-lasting fashion in the embryo without causing developmental arrest (e.g. Niemann & Wrenzycki, 2000).

The expression of the paternally derived transgene *in utero* appeared delayed and may thus be temporally repressed by maternal factors. Repression of genes at the 2-cell stage may be linked to chromatin structure (Thompson *et al*., 1995), culture conditions (Christians *et al*., 1995) and cytoplasmic factors appearing during formation of a 2-cell embryo that can repress promoter activities or activate enhancer activity (Henery *et al*., 1995; Nothias *et al*., 1995). Generally, patterns of transgene expression found in blocked embryos resembled those found at the day of explantation in the NMRI strain, irrespective of maternal or paternal origin of the transgene. There was enhanced, rather than reduced expression of the paternal transgene upon 2-cell block in a maternal MF1 background. This may be due to the continued and strain-specific release of repression by maternal modifiers during the developmental block (Allen *et al*., 1990; Surani, 1991). Expression levels according to pattern 5 were higher in such blocked MF1 × NI-ROSA26 embryos than those found in *in utero* developed blastocysts at 110 h of development.

https://doi.org/10.1017/S096719940100123X Published online by Cambridge University Press

Expression of the same gene can therefore become either increased or decreased with respect to equivalent developmental stages or times of development by identical environmental factors, when the epigenetic regulation transmitted by imprinting from maternal cytoplasm is modulated differently accordingly to the maternal genotype.

Bacterial β-galactosidase appears homogeneously distributed in transgenic cells when expressed from other integration sites (e.g. Kothary *et al*., 1992). The basis of the distinct patterns of β-galactosidase staining in the present study, i.e. dot-like versus homogeneous localisation, is unknown. It may be related to polarisation of the oocyte and embryo (Gardner, 1997; Antczak & van Blerkom, 1997; recently reviewed by Gardner, 1999) and/or to cytoplasmic regions such as 'nuage' or 'sponge-like structures' (Wilsch-Brauninger *et al*., 1997). We observed the 'one dot' staining pattern exclusively in embryos up to the 2-cell stage. In the case of a paternally derived transgene in the maternal MF1 background and delayed expression, there was no such staining pattern. This implies that protein localisation is developmentally regulated and restricted to early embryos. Asymmetry in β-gal staining has also been reported in primordial germ cells of embryos derived from mothers carrying the same ROSA β-geo26 insertional mutation as used in this study (Narasimha *et al*., 1998). This suggests that the locus involved in the polarised expression of the transgene may influence the intracellular distribution of proteins in the germ line and contribute to determination processes (Narasimha *et al*., 1998). Directional transport and localisation in one spot or in distinct granules in oocytes and embryos cannot be based on transport of a fusion protein consisting of the LacZ and another truncated protein since the *LacZtg* is integrated into DNA which is transcribed in the undisrupted gene into two presumably untranslated mRNAs. However, spatio-temporal RNA localisation can involve sequences in the 5′ and 3′ untranslated sequence of developmentally expressed RNAs (e.g. Thio *et al*., 2000). Generally there is evidence from many diverse species that localisation of RNAs and proteins in oocytes and embryos is important or spatio-temporal regulation of expression (e.g. Schnorrer *et al*., 2000), for determination processes (e.g. Micklem *et al*., 2000) and for the developmental potential of an embryo (Antczak & van Blerkom, 1997).

Preliminary electron microscopy studies did not reveal any distinct cytoplasmic organelle in association with transgene product. Future studies have to show whether the 'dot' reflects primarily the localisation of the *LacZ* message, or rather an accumulation of protein unrelated to vectorial RNA transport.

Acknowledgements

This work was supported by the DFG (SFB 223 and 436 RUS 113/228) and the EU (ENV4-CT97-0471). The expert technical assistance of Ilse Betzendahl is gratefully acknowledged.

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