

Age-dependent silencing of globin transgenes in the mouse

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ABSTRACT

Variegation of transgene expression, a heterocellular or mosaic pattern of expression seen in all mice in a given transgenic line, is a frequently observed but unexplained phenomenon. We have encountered variegation with globin transgenes; when *lacZ* expression is driven by globin control elements a proportion of erythrocytes express β -galactosidase (β -gal), while the remaining erythrocytes express none. The percentage of expressing cells is constant within each line (at any particular developmental stage), but varies between lines. Such variation may account for much of the line-to-line variability which has been reported in the expression of a transgene construct. We have now extended these observations by studying expression of several globin/*lacZ* transgenes with increasing age. Expression of β -gal is variegated in all lines in adult mice, including those made with a β -globin promoter and locus control region driving *lacZ*. The extent of variegation differs widely between lines, but in all lines there is a marked decline in the number of erythrocytes expressing β -gal with increasing age. Progression of silencing continues long past the point at which globin switching is complete, suggesting that it is not related to this process. We observe that age-dependent silencing is most severe in high copy number animals. Increasing variegation of transgene expression with ageing of mice is likely to complicate interpretation of the developmental regulation of transgenes. We speculate that it reflects a general mechanism of epigenetic regulation.

INTRODUCTION

Transgenic animals have been widely employed in the analysis of DNA sequences responsible for expression of genes in specific lineages and developmental stages. These mice carry an expression unit as an array containing one to dozens of copies. While

transgenic analysis has proven a powerful method of analysing complex mechanisms of gene regulation, it is clear that transgenes often do not behave as independent units, but rather are significantly and variably influenced by factors such as the site of integration and the number of transgene copies in an array. These influences sometimes lead to marked variations in expression patterns between different transgenic lines carrying the same construct.

Regulation of the globin genes has been intensively and productively studied with transgenic mice, but the problems mentioned above are prominent confounding factors in these studies. Because regulation of the human globin genes in mice follows a developmental pattern similar to that seen in humans (1), transgenic mice have in recent years been used to study the control of this process. The expression of human globin genes follows a coordinated temporal programme in specific erythroid tissues. The embryonic ζ and ϵ genes, from the α and β clusters respectively, are expressed in yolk sac blood islands. Later, when the site of erythropoiesis shifts to the fetal liver, the embryonic globins are replaced by the α - and γ -globins. Finally, in the adult erythropoiesis moves to the bone marrow and spleen, where the α gene continues to be expressed and β gene expression replaces that of γ . Sequences far upstream of the globin structural genes are required for erythroid-specific expression of genes from both the α - and β -globin clusters. The β locus control region (β LCR) spans a region from 6 to 20 kb upstream of the ϵ -globin gene and contains four DNase I hypersensitive sites (HS1–4) (2–4), while the α locus has an analogous element (α HS-40) associated with a hypersensitive site 40 kb upstream of the ζ -globin gene (5,6). Both the β LCR and the α HS-40 confer high level erythroid-specific expression on linked promoters. However, the α HS-40 is apparently not equivalent to the β -globin LCR in its ability to confer position independence or copy number dependence (7–9).

We have previously described variegation in transgenic mouse lines which express β -galactosidase (β -gal) under the control of globin promoters linked to α HS-40 (10). Use of β -gal permitted analysis of transgene expression in individual red blood cells, while nearly all other globin transgenes have been assayed by analysis of RNA from pooled cells. We found that expression of *lacZ* within all mouse lines is heterocellular. Within a given

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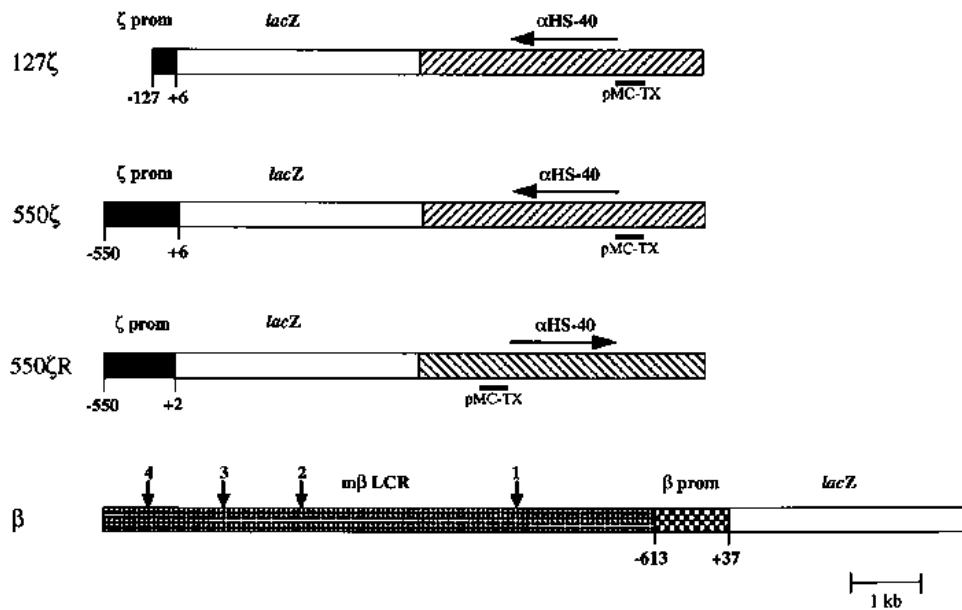


Figure 1. Maps of the ζ promoter/*lacZ*/ α HS-40 and β LCR/ β promoter/*lacZ* constructs used to generate transgenic mice. The fragment containing the α HS-40 element is present in the 550 ζ R construct in the opposite orientation relative to the 550 ζ and 127 ζ constructs and to that found in the α -globin locus on chromosome 16. The position of the core α HS-40 element pMC-TX, as defined by Jarman *et al.* (6), is shown by a black box below the 4.0 kb *Hind*III α HS-40 fragment. The positions of the four DNase I hypersensitive sites within the β -globin miniLCR (2) are shown by arrows.

mouse individual erythroid cells either do not express the transgene at all or express it at a level which is characteristic of each line. The number of β -gal expressing cells varies greatly between different lines of transgenic mice carrying the same construct, but is consistent within a given line, suggesting that the degree of heterocellular expression is determined at least in part by the site of integration. This finding is directly relevant to the interpretation of transgenic studies. The heterocellular expression we observe is similar to position-effect variegation (PEV) seen in *Drosophila*, yeast and in mice with X chromosome translocations (11). In PEV, genes translocated to new chromosomal regions are inactivated in a stochastic manner. This inactivation is associated with proximity to heterochromatic regions, which may spread along a chromosome and exert a repressive effect on the expression of flanking genes, and is also influenced by the *cis*-acting elements flanking the gene (12) and the presence of multiple copies of the transgene (13).

The present study examines the effect of age on transgene expression in mice harbouring *lacZ* transgenes driven by different globin control elements. We find that with all constructs expression is variegated at certain developmental stages, although inclusion of the entire β -globin LCR results in pan-cellular expression at early stages. Most notably there is a progressive decrease in the proportion of expressing erythrocytes in all lines and this decrease continues far past the point at which globin switching is complete, implying that this silencing is not equivalent to normal globin developmental regulation. The degree to which silencing progresses after birth is related to the copy number of the transgene, however, variegation is observed even in low copy number animals. These observations indicate that multiple factors may contribute to variegation of transgene expression, including the site of integration and nature of the transgene array.

MATERIALS AND METHODS

Transgene constructs

All constructs were synthesized by linking portions of the human globin promoters to a construct containing the reporter gene *lacZ*, which produces β -gal (a gift from Dr J. Rossant, Mount Sinai Research Institute, Toronto) (see Fig. 1). The 550 ζ and 127 ζ constructs contain from +6 to -550 and from +6 to -127 respectively of the human ζ -globin promoter. The 4 kb *Hind*III fragment containing the α HS-40 element (6) was inserted into the 550 ζ R construct in the opposite orientation relative to the 550 ζ and 127 ζ constructs. The β construct contained the same *lacZ* reporter gene linked to the β -globin promoter from +37 to -613 and the β -globin miniLCR (2) with HS1-4. Prior to microinjection DNA fragments were excised from vector sequences with *Kpn*I for the 550 ζ R construct, *Not*I/*Kpn*I for the 550 ζ and 127 ζ constructs and *Not*I for the β construct and purified by agarose gel electrophoresis.

Transgenic mouse production

Transgenic mice were generated by microinjection of linear DNA fragments into the pronuclei of fertilized eggs from the outbred P.O. mouse strain by standard procedures (14). Transgenic progeny were identified and copy number was determined by Southern analysis of tail DNA. Hemizygous lines were established by mating transgenic founders to P.O. mice.

β -Galactosidase activity in whole cells

Embryos were obtained 12.5 and 17.5 days post-coitum (d.p.c.) from P.O. females mated to hemizygous transgenic males. After bleeding whole embryos into phosphate-buffered saline (PBS)

erythrocytes were gently spun in a microcentrifuge and then fixed in 0.25% glutaraldehyde, washed and stained with 5-bromo-4-chloro-3-indolyl β -D-galactopyranoside (X-gal) as described (10,15) for at least 24 h at 37°C. We have shown previously that all cells containing β -gal activity can be detected by light microscopy following these staining conditions (10). Adult peripheral blood obtained from hemizygous transgenic mice from 1 week of age onwards was stained with X-gal in a similar way. All the data in this paper has been collected from established transgenic lines.

Table 1. Copy number and site of transgene expression

Construct	Line	Copy number	Erythroid expression	Ectopic expression ^a
127 ζ	127 ζ 1	50–100	–	n.d.
	127 ζ 2	10–15	+	–
	127 ζ 3	3–5	–	n.d.
	127 ζ 4	15–20	+	–
	127 ζ 5	8–10	+	Nose, ventral spinal chord, hindbrain
	127 ζ 6	1	–	–
	127 ζ 7	1	–	–
	127 ζ 8	1	–	–
550 ζ	550 ζ 1	3–5	+	Limb bud (AER,ZPA), notochord, nose
	550 ζ 2	15–20	+	Limb bud (AER)
	550 ζ 3	1–2	+	Midbrain, hindbrain, mouth, limbs, somites
	550 ζ 4	3–5	–	–
	550 ζ R	550 ζ R1	10–15	+
	550 ζ R2	10–15	+	–
	550 ζ R3	10	+	Ear, nose, mouth
	550 ζ R4	10	+	Ear, nose, mouth, mid/forebrain junction
	550 ζ R5	40–50	+	Midbrain
β	β 1	30	+	–
	β 2	3–5	+	–
	β 3	5	+	–
	β 4	15–20	+	–
	β 5	30	+	–

^aEctopic expression was examined by staining 10.5–12.5 d.p.c. embryos with X-gal. AER, apical ectodermal ridge; ZPA, zone of polarizing activity; n.d., not determined.

Flow cytometric analysis of β -gal activity

This was done using a similar method to that reported previously (16,17). Embryos at 12.5 d.p.c. were dissected and bled into PBS to give a cell suspension at $\sim 1\text{--}5 \times 10^7$ cells/ml. Aliquots of 30 μ l of this cell suspension were dispensed into 5 ml polystyrene tubes and incubated for 5 min at 37°C. An aliquot of 50 μ l 4 mM

fluorescein digalactoside (FDG; Molecular Probes, Eugene, OR) in reverse osmosis grade water, pre-warmed to 37°C, was then added to each tube. The tubes were held in a 37°C water bath for 75 s, after which time the tubes were removed from the water bath and quickly filled with ice cold PBS. The cells were pelleted by centrifugation at 4°C for 5 min at 300 g. The cells in each tube were gently resuspended in 0.5 ml ice-cold PBS and held on ice protected from light before analysis in a Becton Dickinson FACsort. Primitive erythrocytes from non-transgenic embryos were also analysed to determine the level of autofluorescence in non-expressing cells. The percentage of β -gal-positive cells in transgenic embryos was determined from the number of cells which showed fluorescence above this background level.

RESULTS

Tissue specificity of transgene expression

Mouse lines (4–8 for each construct) were generated carrying ζ promoter/*lacZ*/ α HS-40 transgenes or β promoter/*lacZ*/ β LCR transgenes (see Fig. 1). When embryos were stained with X-gal to assay for transcription from the *lacZ* reporter transgene β -gal expression was detected in circulating primitive erythrocytes from the majority of lines (Table 1), however, in some lines no expression of the transgene was found, even after careful examination of $\sim 10^6$ erythroid cells. In some lines derived from the ζ promoter constructs β -gal expressing cells were readily detected in a variety of other tissues in a pattern unique to each mouse line (Table 1). Ectopic expression is not unique to mice carrying ζ promoter transgenes; we have found ectopic expression of constructs driven by the α -globin and TK promoters when linked to α HS-40 (H.Sutherland and E.Whitelaw, unpublished data). No lines carrying the β promoter/*lacZ*/ β LCR transgene showed ectopic expression, consistent with the idea that the β LCR, but not the α HS-40, is able to suppress expression in non-erythroid lineages.

Expression of globin/*lacZ* transgenes in erythrocytes is heterocellular

Traditionally analysis of globin transgene expression has involved the measurement of mRNA levels in cell lysates, which gives an average of expression in all cells. We developed a technique for staining circulating erythrocytes obtained from dissected embryos and adults permitting the detection of β -gal in individual erythrocytes and found that mice carrying a hybrid γ/ζ -globin promoter/*lacZ* construct expressed the transgene in only a portion of erythrocytes. Moreover, the percentage of positive cells varied markedly between transgenic lines (10). We have expanded this analysis with the constructs shown in Figure 1. These contain the ζ -globin promoter truncated to –127 or –550 or the β -globin promoter to truncated to –600. The ζ -globin promoter constructs also contain the α HS-40 element and the β -globin promoter construct contains the miniLCR cassette, which consists of the four upstream DNase I hypersensitive sites of the human β -globin locus. When peripheral blood from 12.5 d.p.c. embryos carrying the 127 ζ promoter transgene was stained with X-gal it was found that not all cells expressed β -gal. Moreover, the percentage of positive cells varied markedly between transgenic lines (carrying the same construct) (Table 2). Within a given transgenic line, however, the percentage of positive cells did not vary significantly between individual mice (Table 2). This can be seen by the relatively small standard errors

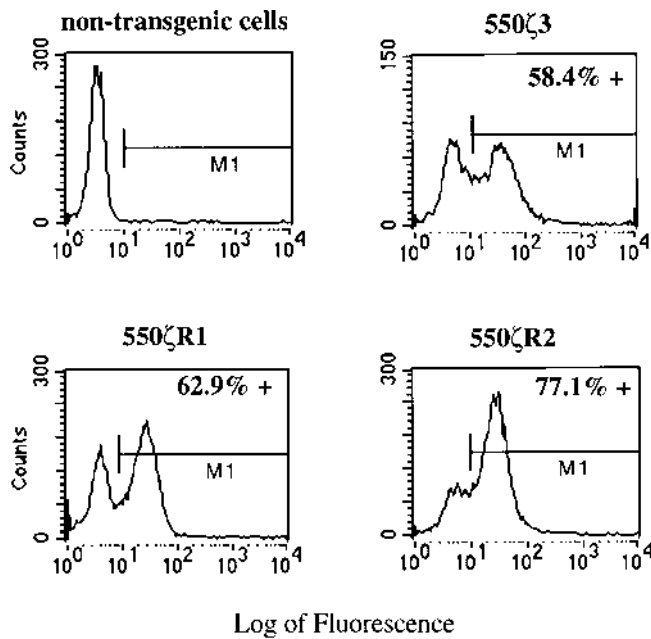


Figure 2. Flow cytometric analysis of β -gal expression in 12.5 d.p.c. erythrocytes stained with FDG. Representative analyses of lines 550 ζ R1 and 550 ζ R2 with the 550 ζ R construct and line 550 ζ 3 with the 550 ζ construct. Erythrocytes from a non-transgenic embryo were also analysed as a negative control. The x-axis depicts the fluorescence produced by hydrolysis of FDG by β -gal and thus represents β -gal activity in individual cells. Two separate populations of cells are evident, one is negative for β -gal and the other has a high level produced by the *lacZ* transgene. The percentage of cells in the latter population is given for each line.

for each data point. These findings are consistent with our previous observations with a different transgene construct (10). Since the variation from line to line does not correlate with copy number (compare Tables 2 and 1), we conclude that these transgenes are being influenced by the site of integration. Thus the short ζ promoter (127 ζ) construct directs expression in erythroid cells, but expression is heterocellular and clearly influenced by position effects. In an attempt to overcome these effects we went on to produce mice with a larger promoter (550 ζ) and with the α HS-40 in the opposite orientation (550 ζ R). However, mice expressing these transgenes also did so in a heterocellular manner (Table 2). Finally, we produced mice with a β promoter/ β LCR construct and these did show pancellular expression at this early stage of development. Pancellular expression is consistent with previous reports that the β LCR confers integration site independence (3). In one of the lines carrying the β construct, β 1, 56% of erythroid cells are expressing, but the transgene in this line has integrated into the X chromosome and so the mosaic expression is presumably a result of random X inactivation. All positive embryos analysed here were female, since they resulted from the mating of a transgenic male with a wild-type female.

Flow cytometric analysis of β -gal expression

In earlier work we used an indirect method to establish that non-staining cells are truly negative, rather than simply falling below some threshold of visibility with X-gal staining (10). As a direct confirmation of this result we have used a fluorescent substrate for β -gal (FDG) in conjunction with flow cytometric

analysis. This assay (FACS-Gal) is far more sensitive than X-gal detection by light microscopy and can detect levels of β -gal expression as low as 5 molecules/cell (16,17). Erythroid cells from 12.5 d.p.c. transgenic embryos assayed with FACS-Gal fall into two distinct populations, one of which has the same level of fluorescence as cells from a non-transgenic embryo (Fig. 2). This confirms the notion that cells are either expressing the transgene or are completely silent. The numbers of cells containing levels of fluorescence above background at 12.5 d.p.c. for lines 550 ζ R1, 550 ζ R2 and 550 ζ 3 were 63, 77 and 58% respectively (Fig. 2) and these numbers are similar to those obtained with X-gal (Table 2). Together with our observation that a plateau in the number of stained cells is reached under non-limiting conditions (10), this result confirms that for most lines there is a characteristic number of cells that do not contain β -gal activity.

Table 2. Percentage of expressing cells during development and after birth

Construct	line	12.5 d.p.c.	17.5 d.p.c.	1 W	8 W	
127 ζ	127 ζ 2	100	72	56 \pm 0.6	6.4 (5.5-7.7)	
	127 ζ 4	59 \pm 13	4 \pm 1.4	4 \pm 2.4	0.1	
	127 ζ 5	27 \pm 9	3	9.4 \pm 2.8	5.9 \pm 1.7	
	550 ζ	550 ζ 1	100	29 \pm 1.7	14.6 \pm 1.1	2.5 \pm .67
	550 ζ 2	74 \pm 1.2	10	10 \pm .73	0.22 \pm .11	
550 ζ R	550 ζ 3	77 \pm 0	14.5 (14-15)	3.7 \pm 0.36	7.3 \pm 2.2	
	550 ζ R1	59 \pm 10	1		\approx 0.001	
	550 ζ R2	65 \pm 13.5	1 (0.9-1.2)	0.2 \pm .09	\approx 0.01	
	550 ζ R3	36 \pm 4.5		0.2	0.1	
	550 ζ R4	50 \pm 2.3	0.2 \pm .07		0.02	
β	550 ζ R5	90 \pm 0.6	2.0	0.15 \pm .04	\approx 0.01	
	β 1	56 \pm 8.2	5.4 \pm 1.6	5.4 \pm 0.3	5 \pm 0.9	
	β 2	100	100	93 \pm 3.4	42 \pm 3.8	
	β 3	100	94 \pm 2.5	82 \pm 2.2	21 \pm 5.2	
	β 4	100	81	86	9.7 \pm 1.2	
β 5	95 \pm 1.5	5.5 \pm 0.4	3.8 (3.7-4.0)	\approx 0.1		

The percentage of expressing cells was determined by staining blood from transgenic embryos at 12.5 and 17.5 d.p.c. or adult mice at 8 weeks with X-gal and is given as the mean \pm SD, except where only two animals were used and the data is presented as the mean with range. Where the values are very low an approximate value is given.

The proportion of expressing cells decreases with age

When we determined the percentage of expressing cells at later stages of development we found that the proportion of expressing cells decreased in mice carrying both ζ and β transgenes (Table 2). This decrease in the ζ promoter transgenic mice could be interpreted as being consistent with correct developmental stage-specific regulation at the promoter. However, in this case we would expect expression to be off in all lines after birth and this is not the case: 550 ζ 1, 550 ζ 3, 127 ζ 2 and 127 ζ 5 still have significant numbers of expressing erythroid cells in the adult. Furthermore, the erythroid cells from mice carrying the β promoter also show a significant drop in the percentage of 'on' cells as the animals age and this is clearly inconsistent with the behaviour of the

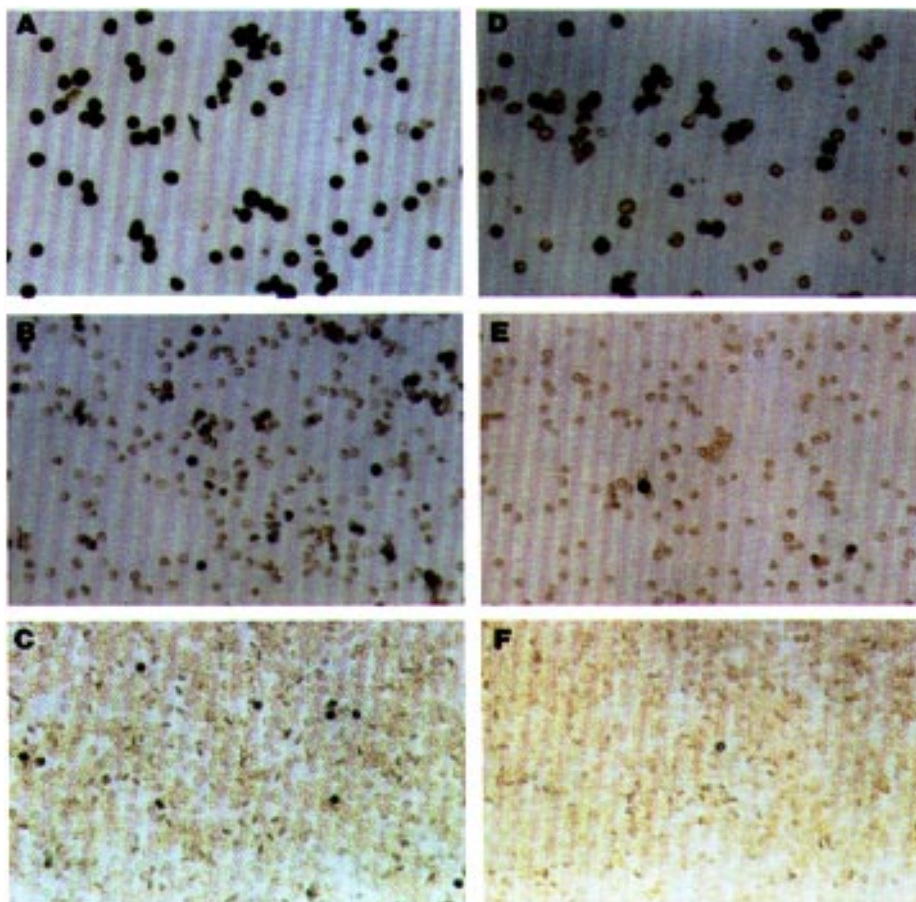


Figure 3. Heterocellular expression of the *lacZ* transgene in erythrocytes. X-gal staining of peripheral blood from embryos and adults from lines 550 ζ 1 (A–C) and 550 ζ R4 (D–F) showed variability in the percentage of expressing cells at 12.5 (A and D) and 17.5 d.p.c. (B and E) and 8 weeks (C and F). At 12.5 d.p.c. all large nucleated primitive erythrocytes stain blue with X-gal for line 550 ζ 1 (A), whereas there are clearly some non-staining cells from line 550 ζ R4 (D). At 17.5 d.p.c., when the majority of cells are smaller non-nucleated definitive erythrocytes derived from the fetal liver, there are fewer blue cells from both lines. This is particularly evident in 550 ζ R4 (E), where a major proportion of positive cells are larger primitive erythrocytes which have remained in circulation, with very few definitive cells staining blue. In adult mice from both lines (C and F) even less cells are blue and a greater difference between lines is apparent, with many similar fields examined to find these rare blue cells in line 550 ζ R4 (F).

endogenous adult β -globin gene. Most previous studies of the behaviour of β -globin transgenes in mice have not investigated transgene activity beyond the late fetal stage, because high levels of transgene expression lead to globin chain imbalance, thalassaemia and fetal death. By using the *lacZ* reporter gene we can follow expression throughout the life of the mouse.

Photographs of the erythroid cells purified at different stages of development from two lines are shown in Figure 3. At 12.5 d.p.c. in line 550 ζ 1 (Fig. 3A) all cells were stained blue, while in line 550 ζ R4 (Fig. 3D) there were clearly unstained primitive erythrocytes. At later stages of development and after birth mice from both lines showed a lower percentage of erythroid cells which stained blue. In fact, at 8 weeks in line 550 ζ R4 there were very few expressing cells (Fig. 3F), with examination of many similar fields of view needed to find a positive cell. Thus in both lines the number of expressing cells declines with age.

Progressive silencing in adult stage erythrocytes is linked to high transgene copy number

The rate at which the transgene is switched off during adult life varied from line to line; 127 ζ 5 dropped only 2-fold from 1 to 8

weeks of age, while another line with the same construct, 127 ζ 4, dropped 40-fold over the same period. In the β promoter/*lacZ*/ β LCR lines there was also a large but highly variable decline in the adult stage; line β 5 dropped 40-fold between 1 and 8 weeks, while line β 3 dropped only 4-fold. In Figure 4 the decline in the percentage of β -gal expressing cells between 1 and 8 weeks for the 550 ζ , 127 ζ and β lines is compared with their copy number. The decline was greatest for lines which have the highest copy number. All the lines with the 550 ζ R construct had already declined to a low percentage by birth and so were not included in this comparison.

DISCUSSION

We have extended our studies of variegated transgene expression in mice and confirmed that this phenomenon is prevalent in globin transgenes. All lines in which expression of the *lacZ* transgene occurred, including those in which expression was driven by the β -globin LCR, exhibited some degree of variegation (heterocellular or mosaic expression) at some developmental stage. Moreover, we observed a general tendency for expression of the transgene to decline with age, well past the point at which

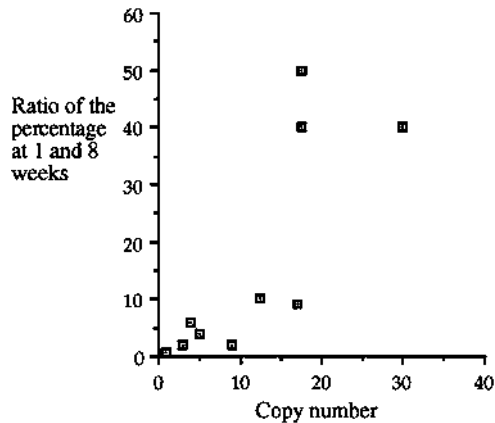


Figure 4. Relationship between the rate of decline of transgene expression after birth and copy number. The ratio of the percentage of expressing cells at 1 and 8 weeks after birth (from Table 2) is plotted against copy number (from Table 1) of each line carrying the 127 ζ , 550 ζ and β constructs. The lines with the highest copy number show the greatest rate of decline between 1 and 8 weeks.

definitive erythrocytes have replaced those of earlier stages and globin switching is complete. The extent of the decline was greatest in lines with large numbers of copies of the transgene. These findings of variegation and a correlation with copy number are similar to a large number of observations made in other systems (13) and suggest that the interpretation of transgene activity requires consideration of these factors.

Variegation of transgene expression has been widely observed in mice, although it has been commented on very little (10). Classical PEV was described in chromosomal translocations that place a marker gene near constitutive heterochromatin, resulting in stochastic and clonally heritable inactivation of the marker (11). Related phenomena have been described when genes are placed in or near inactive chromatin at telomeres, the X chromosome and the mating type loci in yeast and when plasmids are randomly integrated in mammalian cell lines, and have also been described in plants (18–23). When this extensive evidence for stochastic silencing of transgene expression is considered, variegation of globin transgenes is not surprising. Other work suggests that this phenomenon is not confined to *lacZ* constructs. Heterocellular expression has been observed in mice carrying human and murine β LCR elements linked to human globin genes (24,25). In studies of inbred albino and black mice carrying transgenes with a tyrosinase cDNA a genetically stable pattern of coat colour variegation was observed (26,27). Other examples of transgenes that exhibit variegated expression include myelin basic protein antisense cDNA (28) and a hypoxanthine phosphoribosyltransferase transgene on the Y chromosome (29). The globin/*lacZ* transgene permits convenient observation and quantification of expression in individual erythrocytes, as does another recently described system that uses expression of human CD2 in T cells (30). These transgenes may be useful in analysing the factors responsible for variegation. Transgene silencing may be associated with methylation of the DNA and we are currently investigating this possibility. The factors responsible for variegated transgene silencing are likely to be considerably more complex than the effect of flanking heterochromatin and include the *cis*-acting control elements flanking the transgene (which suppress silencing) (12), the lineage in which the gene is

expressed and the presence of genetic modifiers (11). Variegation has also been found to be associated with tandem arrays of an integrated construct, which may form foci of heterochromatinization (13,31,32), and this is consistent with our observation that age-related transgene silencing is greatest in lines with higher transgene copy number.

This general decrease in transgene expression during adult life is the most dramatic observation made in this study. Only a few studies (7,8,33) have looked in detail at globin transgene expression in older adults and in all of these a decline in expression was observed with increasing age. The percentage of erythroid cells expressing a γ -globin transgene was found to decrease by 50% between 2 and 6 months after birth (33). Since these studies used globin coding sequences and not *lacZ*, this implies the phenomenon is not an artifact of the *lacZ* gene. One trivial explanation for the decrease which we see in the percentage of expressing cells with age would be that the transgene array is unstable in somatic cells. However, detailed Southern transfer analysis and sequencing of PCR-amplified transgene DNA using tail DNA from mice at different ages suggests that this is not the case (34; data not shown). The fact that the rate of silencing during adult life varies from line to line suggests to us that it may depend upon the site of integration and/or the nature of the transgene array, however, we see no correlation between the rate of decline and the initial level of heterocellular expression (percentage of 'on' cells at 12.5 d.p.c.), suggesting that age-dependent silencing is independent of site of integration. We do see a correlation between the transgene copy number and the rate of silencing; the higher the copy number the faster the silencing. Lines with very low copy number show little decline in numbers of expressing cells after birth, but expression is variegated. This suggests that the integration site may exert a constant influence on the probability of expression, while large arrays are progressively silenced. It is worth noting here that we see no correlation between copy number and level of expression in each 'on' cell (10), excluding the possibility that silencing is greater in high copy number mice simply because higher levels of β -gal place the 'on' cells at a selective disadvantage.

Silencing of transgene expression as a result of multicopy arrays is a widespread phenomenon and has recently been reviewed at length by Dorer (13). Observations similar to ours have been made in both *Drosophila* (31) and plants (35). Expression of most mouse transgenes is analysed with methods that produce an average of expression in a whole tissue or lineage. Silencing associated with high copy number would in these systems appear as a lower expression per copy with increasing copy number, and this correlation has been observed (7,8,9,36). We suggest that the late silencing of *lacZ* expression in this study is due to progressive heterochromatinization of larger transgene arrays. In a previous study (33) in which γ -globin transgene expression was found to be heterocellular the rate of transgene silencing was found to be relatively low (2-fold over a 4 month period). Interestingly, these mice contained only three copies of the transgene.

In summary, we have shown that erythroid cells from mice carrying the human ζ - or β -globin promoter attached to the *lacZ* gene display variegated silencing and that the number of expressing cells declines with age. The extent of silencing varies from line to line, even between lines with the same transgene copy number, suggesting that it is at least in part dependent on the site of integration of the transgene. The fact that variegation occurs in

all of the lines we have made suggests that the *cis*-acting control elements in our constructs (even the β -globin miniLCR) are sufficiently weak that they are unable to overcome repressive chromatin effects in all cells. Presumably, the endogenous globin genes, like most other genes, lie within a chromosomal region in which sufficient regulatory elements ensure that transcription will occur. The expression of globin/*lacZ* transgenes in erythroid cells permits the convenient quantification of variegation in a mammalian tissue during development. Coupled with the ability to separate expressing from non-expressing cells by FACS-Gal, this system should enable study of the factors involved in transgene silencing. It also provides an assay to test the ability of elements such as 'insulators' and 'boundary elements' that lie within or flank a transgene to protect against general transgene silencing in mammals. Variegated silencing and the influence of multicopy arrays should be taken into account when analysing transgene expression, even with transgenes that are not amenable to single cell analysis. An understanding of this phenomenon has far reaching implications for the stability of novel phenotypes created by genetic engineering.

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