Sequence and Chromosomal Context Effects on Variegated Expression of Keratin 5/lacZ Constructs in Stratified Epithelia of Transgenic Mice

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Manuscript received June 1, 2000 Accepted for publication January 30, 2001

ABSTRACT

The expression of transgene loci in mammals often occurs in a heterocellular fashion resulting in variegated patterns of expression. We have examined the effect of chromosomal integration site, copy number, and transcriptionally activating sequences on the variegation of a keratin 5-lacZ (K5Z) construct in the stratified epithelia of transgenic mice. lacZ expression in these mice is always mosaic, and the β-gal activity per cell is usually higher in the lines with a higher proportion of expressing cells. Similar constructs, in which cDNAs were exchanged by lacZ sequences, showed no variegation. Also, when a strongly active, nonvariegating construct was co-injected with K5Z, most transgenic lines showed an almost homogeneous lacZ expression. The comparison of transgene arrays of different copies inserted at the same locus (obtained by using a lox/Cre system) showed that the reduction of copy number does not lead to an increase in the proportion of cells that express the transgene. Finally, in most of the variegating or nonexpressing lines the transgenes were located both at intermediate positions and at peritelomeric regions in the long chromosome arms. These findings suggest that the probability and efficiency of expression of K5Z genes depend on both long range chromosomal influences and on sequences in the transgene array.

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GERMLINE transformation of mice has been invaluable in understanding processes that control gene expression and has led to the identification of tissue-specific control elements (Grosveld and Kollias 1992). However, a common feature of transgenic mice is the wide variation in expression levels among individuals carrying the same transgene (Grosveld and Kollias 1992). In addition, quite often DNA constructs express in a mosaic or variegated fashion, by which transgene expression occurs in some, but not all, the cells within a population of equivalent transgene-bearing cells (Troyer et al. 1993; Porter and Meyer 1994; Ramírez et al. 1994; Elliott et al. 1995; Dobie et al. 1996; Festenstein et al. 1996; Milot et al. 1996; Morley et al. 1996; Graubert et al. 1998). Thus, the transcriptional potential of a given construct is controlled by more than just its cis-control elements. Recent work by Whitelaw, Martin, and colleagues suggest that the variability of expression levels among transgenic lines results from the differences in the proportion of cells that express the transgene (Robertson et al. 1995). The variegated expression also occurs in Drosophila (Henikoff 1990), yeasts (Gottschling et al. 1990; Allshire et al. 1994), and plants (Meyer et al. 1993), although the mechanisms responsible for such silencing events are still poorly understood.

Chromosomal context is important for variegation, shown by the mosaic expression patterns of genes located near or at pericentromeric heterochromatin in Drosophila (Henikoff 1990). No systematic analysis of the integration site of DNA constructs has been carried out in transgenic mice, although integration at pericentromeric regions has been reported with unexpected frequency (Dobie et al. 1996; Festenstein et al. 1996; Milot et al. 1996). As observed before in Drosophila (Dorer and Henikoff 1994) and in plants (Assaad et al. 1993), variegation of DNA constructs in mice is related to the repeated nature of multicopy transgene arrays (Garrick et al. 1998), although a direct relation between copy number and proportion of expressing cells is not always observed (Festenstein et al. 1996; Zhuma et al. 1999). Also, variegation correlates with chromatin accessibility but not with DNA methylation (Garrick et al. 1996). However, the presence in transgenic constructs of intact locus control regions (LCRs), which have a dominant activity, usually results in homogeneous, nonmosaic expression patterns irrespective of the integration site (Milot et al. 1996; Kioussis and Festenstein 1997).

The above features of variegating transgenes in mice derive mostly from constructs expressed in hematopoietic tissues (Elliott et al. 1995; Robertson et al. 1995;
In this report we have studied transgene variegation in stratified epithelia, such as those that line the surface of the skin and parts of the digestive tract (FUCHS and WEBER 1994). We have used the lacZ gene of Escherichia coli fused to sequences from the keratin K5 gene, which encodes one of the intermediate filament polypeptides specific to the basal layer of stratified epithelia. The remarkable tendency of these K5-lacZ (K5Z) constructs to express in a mosaic fashion allowed us to ask whether copy number and chromosomal integration site are major determinants of variegation and how such variegation can be affected by the presence of transcriptionally active sequences. The results show that copy-number reductions do not affect variegation of K5Z constructs and that position effects are imposed by telomeric regions. We also find a variability in β-galactosidase (β-gal) activity levels per cell between different variegating lines and that the presence of strong activating sequences turns variegation into a homogeneous and efficient expression of lacZ in almost all cells.

MATERIALS AND METHODS

Transgenic mice: The K5Z construct was obtained by ligation of sequences from the bovine K5 locus (~5300 bp to +140 bp) to a lacZ gene, as previously described (RAMÍREZ et al. 1994). To construct K5ZMAR the 5′A element of the chicken lysozyme gene in plasmid pB-1X1 (STIEF et al. 1989) was ligated to the 5′ end of K5Z. The K5Zlox construct was obtained by insertion of an oligonucleotide containing a single copy of the 34-bp lox site at the 3′ end of K5Z. The K5ZHK10 construct contains sequences from the bovine K5 locus (~5300 bp to +50 bp) fused to a genomic fragment spanning the entire coding region of the human K10 (HK10) locus (RIEGER and FRANKE 1988). Transgenic mice were generated and genotyped as described (RAMÍREZ et al. 1994). For Cre recombination, circular Cre-expression vector pCAGGS-Cre (ARAKI et al. 1995) was injected into mouse eggs at 5 ng/μl.

β-Galactosidase activity and immunofluorescence: Tissues were dissected and frozen in OCT medium. Histochemistry and immunodetection were done on 7-μm cryosections loaded on poly-L-lysine-coated slides as described (RAMÍREZ et al. 1994). The mouse K5 was detected using a rabbit antipeptide antiseraum (BAbCo), HK10 with monoclonal antibody AE2 (ICN Biochemicals), and β-gal with a rabbit antiseraum (5Prime-3Prime, Boulder, CO). Human epidermal growth factor receptor (HEGFR) was detected with monoclonal antibody EGFR528 (Santa Cruz Biotechnology). Secondary antibodies were Texas-red-conjugated donkey IgG anti-rabbit IgG and fluorescein-conjugated goat IgG anti-mouse IgG from Jackson ImmunoResearch Labs (West Grove, PA).

Southern and Northern blot analyses: These were performed as described (CASANOVA et al. 1995). To normalize for loading differences in Southern blots, we used a Thy1 probe. Known amounts of plasmids were used to help determine copy numbers of the various transgenes. HK10 and mouse K5 (mk5) mRNAs were detected in Northern blots using genomic fragments spanning the last exon of the HK10 and of the mk5 genes, respectively. Hybridization signals were quantitated using a Phosphorimager and Imagequant software (Molecular Dynamics, Sunnyvale, CA).

RESULTS

Variegated expression of K5Z constructs in transgenic mice: The basic K5Z construct used consists of 5.3 kb of 5′ flanking and promoter sequences of the bovine K5 gene fused to a lacZ gene of E. coli (Figure 1). Only three lines (B4, B7, and B38) showed a detectable expression and were used to derive stable lines. Analysis of sections of adult tissues (4- to 8-week-old animals) showed restricted expression of the transgene to stratified epithelia and their appendages, like that of the endogenous K5 gene. A discrepancy, however, was the mosaic expression of the transgene in all epithelia of every animal of lines B4, B38 (Figure 2A), and B7 (not shown). Detection of β-gal activity was done using various conditions that allowed us to rule out that mosaic expression was due to trivial reasons, such as differential accessibility of substrate or others. In addition, the proportion of expressing cells varied among the three lines. Thus, line B38 showed β-gal activity in a larger number of cells in each epithelium than lines B4 or B7 did. There were exceptions, such as the esophagus, where the proportion of expressing cells was consistently higher in line B4 than in B38. Moreover, β-gal activity per cell was also higher in line B38 than in the other two lines, as indicated by the intensity of X-gal staining of β-gal-positive cells after simultaneous, timed incubation with the substrate. Figure 2B shows the results for palate epithelium, but similar results were observed in the tongue and tail skin of these lines and in other lines described below (not shown).

Such a variegated expression of K5Z transgenes contrasts with the usually nonmosaic expression from similar constructs in which a number of cDNAs are driven by the same K5 regulatory sequences used in the K5Z construct. Figure 3 shows examples of the expression of both wild-type and truncated cDNAs encoding HEGFR. In the three lines analyzed, the protein product of the transgenes is detected in an even, nonmosaic fashion in the basal layer of stratified epithelia. Similar results have been obtained by other researchers who have used the K5 regulatory sequences present in the K5Z construct to drive expression of a variety of cDNAs. We conclude that the variegated expression of K5Z mice is neither due to lack of cis-acting sequences nor to a consequence of a heterogeneity among epithelial cells. Instead, it appears most likely that it is the consequence of a process in which transgene activity occurs only in a subset of cells and in their descendants.

DNA fluorescence in situ hybridization (FISH) analysis: DNA FISH was performed as described (MILOT et al. 1996).

The reduction of transgene copies has no effect on variegation of K5/lacZ constructs: To investigate the effect of copy number on K5Z variegation we used the lox/Cre system of specific recombination that allows the generation of transgenic mice with different copies.
Figure 1.—Structure of K5 constructs used to generate transgenic mice. Keratin K5 denotes the 5′ flanking and promoter sequences of the bovine K5 locus. The boxes labeled lacZ represent the protein coding region of the trpS-lacZ gene and pA, a SV40 polyadenylation signal. White boxes indicate exon 1 sequences of the K5 gene, whereas black boxes denote the exons of the HK10 gene. The box labeled MAR corresponds to the 5′A element from the chicken lysozyme locus, and the box labeled loxP represents the site recognized by the Cre recombinase of bacteriophage P1. Arrows denote the transcription initiation site and the direction of transcription.

of the construct at the same chromosomal location (Araki et al. 1995). We used K5Zlox, a construct identical to K5Z except for the addition of a single copy of the 34-bp recognition site (loxP) of the Cre recombinase of bacteriophage P1 (Figure 1). Since transgenes usually integrate in multimeric arrays, whenever a head-to-tail arrangement occurs Cre-mediated recombination between loxP sites in individual K5Zlox transgenes will reduce copies in the array. We generated six stable K5Zlox transgenic lines. Two of them showed no β-gal activity (or only in a few cells) whereas the other four showed variegated expression similar to that of K5Z constructs (Table 1). Cre-mediated recombination was carried out by microinjection of a Cre expression vector in zygotes obtained from mating wild-type female mice with hemizygous K5Zlox males. We used line 4734 (7 copies) with a reduced proportion of nonexpressing cells and line 4712 (14 copies) with many nonexpressing cells. The offspring from microinjected oocytes that showed a reduced transgene copy number were mated with wild-type animals to establish lines hemizygous for reduced-copy progeny founders, 4734rec.a and 4734rec.b (Figure 4A, lanes 1 and 2). Similarly, from line 4712 we obtained three lines with a reduced number of copies, 4712rec.a, 4712rec.b, and 4712rec.c (Figure 4A, lanes 4–6). Cre-mediated recombination was carried out also by crossing hemizygous K5Zlox mice (lines 4712, 4715, 4734, and 4736) with a mouse that expressed the Cre recombinase in all stratified epithelia. Examples of copy-number reduction specific for stratified epithelial cells in double K5Cre/K5lox transgenic mice derived from lines 4715 (mouse 6603) and 4736 (mice 6483 and 6484), compared with the absence of recombination in single K5Zlox transgenic mice (mice 6607 and 5419), are shown in Figure 4B.

Figure 2.—Expression properties of K5Z mice. (A) Comparison of lacZ expression and endogenous K5 localization in adult tissues of K5Z mice. Tissue sections of transgenic lines B4 and B38 were incubated overnight at 37°C with X-gal to detect the β-gal activity and counterstained briefly with eosin. The mouse K5 was detected by indirect immunofluorescence with an anti-K5 serum. (B) Comparison of β-gal activity per cell. Palate tissue sections of lines B4 and B38 stained at 37°C with X-gal for the indicated periods of time.

Tissue sections of the parental line 4712 and of its reduced-copy progeny line 4712rec.c, which retained one to two copies, showed no significant alteration of the β-gal activity after copy-number reduction (see Figure 4C, I and II). A similar result was obtained for mice 4712rec.a and 4712rec.b (not shown), which retained two copies and one copy, respectively. Figure 4C, III–VIII, shows tissue sections from the 4734 parent line and from line 4734rec.a, in which the only perceived alteration was the reduced intensity in β-gal staining, but not in the proportion of expressing cells. Also, double K5Cre-K5lox mice showed no alterations in their expression patterns upon copy-number reduction (not shown).
Taken together, these results indicate that copy-number reduction has little effect on the variegation of K5Z transgenes.

The presence of an LCR-type sequence in K5Z transgenic arrays overcomes variegation: The tendency of K5Z transgenes to express in a variegated fashion did not change when the 5′A element of the chicken lysozyme gene, a piece of DNA that reportedly increases efficiency of expression of heterologous genes in transgenic mice (McKnight et al. 1992), was included in the K5Z construct (Table 1). In contrast, a construct consisting of a genomic fragment spanning all exons of the human keratin K10 gene fused to the same K5 sequences that drive lacZ expression in K5Z mice (K5HK10; see Figure 1) showed homogeneous expression of HK10 in four stable lines (665, 821, 1213, and 1227). Figure 5A shows tissue sections from line 821 analyzed with an antibody that recognizes human K10. Note that although K10 is specific to suprabasal cells, it is expressed in the basal cells of K5HK10 mice, suggesting that the HK10 moiety of the construct does not alter the tissue-specific expression properties of the K5 moiety. A fifth line (825) showed limited but significant variegation (not shown). Quantitative expression analysis of these lines showed a direct correlation between HK10 mRNA levels and copy number, a feature commonly associated with LCRs (see Figure 5B).

To investigate the interaction between a variegating and a nonvariegating transgene at the same integration site we coinjected K5Z and K5HK10 constructs in mouse embryos. Eight stable transgenic lines were obtained bearing both constructs, and their lacZ expression patterns contrasted with that of mice bearing the K5Z construct alone. Thus, the β-gal expression pattern in six of the lines (3578a, 3578b, 3668, 4460, 4472, and 4483) was almost indistinguishable from that of the endogenous K5 gene (Figure 6A), except for very small clusters of cells lacking β-gal activity (see below). Of the other two lines, one (4290) expressed β-gal in most epithelial cells, although not as extensively as in the precedent lines, whereas the other (4464) showed few β-gal-expressing cells. The expression of HK10 was almost identical to that of lacZ (not shown). Table 1 summarizes these data. We conclude that the presence of sequences with LCR-type properties (K5HK10) enhances significantly the expression of variegating constructs (K5Z) in the same transgene array.

Heterogeneity of expression of individual copies within a transgene array: The detailed study of the few cases of mosaic expression in K5Z + K5ZHK10 lines, which was done by double immunofluorescence with rabbit anti-β-gal antibodies and with a mouse anti-HK10 monoclonal antibody, led to some unexpected observations. Thus, although line 3668 expressed in a nonvariegated fashion in most epithelia, patches of cells in the epithelium of the ventral tongue did not express lacZ but expressed HK10 (Figure 6B, III and IV). Conversely, in areas of the tongue and tail skin epithelia of line 3578b some cells expressed lacZ but not HK10 (not shown). The most perplexing pattern was seen in small regions of various epithelia of line 4290. Here, some cells expressed β-gal only, while other cells expressed HK10 only and yet other cells expressed both or neither of the two genes (Figure 6B, V and VI). In contrast, a more conventional scenario was observed in the line with more extensive variegation (4464), in which cells expressed either both or neither of the genes (Figure 6B, VII and VIII). These data indicate a differential expression of some of the copies within the transgene array.

Chromosomal position of transgenes: To determine whether the variegated expression of K5Z constructs correlates with chromosomal position, we performed FISH on metaphase chromosomes. Only a subset of transgenic lines was available when we did this analysis. The insertion site of the transgene was localized by hybridization to the K5Z construct. Nine lines had the transgenes inserted at several positions throughout the long chromosomal arm, of which five showed peritelomeric insertions. Another two lines had pericentromeric transgenes. Additional FISH analyses were done for seven of the lines using the K5Z construct together with either mouse γ-satellite sequences, which recognize centromeric regions, or with a probe for telomeric repeats. A representative transgene-bearing chromosome from each transgenic line is shown in Figure 7 and the localization data are summarized in Table 1. In two variegating K5Z lines, B4 and B38, the transgene was integrated into middle positions in the long chromosomal arm. Similar integration sites were found for the nonexpress-
Variegation of K5 Transgenes in Stratified Epithelia

### TABLE 1

Characteristics of K5 transgenic lines

<table>
<thead>
<tr>
<th>Constructs</th>
<th>Lines</th>
<th>Copies</th>
<th>Expression</th>
<th>Integration site</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>K5Z</strong></td>
<td>B4</td>
<td>21</td>
<td>Variegated (+++)</td>
<td>Middle arm</td>
</tr>
<tr>
<td></td>
<td>B7</td>
<td>15</td>
<td>Variegated (+++)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B15</td>
<td>36</td>
<td>–ve</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>B22</td>
<td>18</td>
<td>–ve</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>B38</td>
<td>4</td>
<td>Variegated (+)</td>
<td>Middle arm</td>
</tr>
<tr>
<td></td>
<td>B41</td>
<td>18</td>
<td>–ve</td>
<td>ND</td>
</tr>
<tr>
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<td>–ve</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>1727</td>
<td>5</td>
<td>Variegated (+)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>1736</td>
<td>9</td>
<td>Variegated (+++)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>1739</td>
<td>35</td>
<td>–ve</td>
<td>Middle arm</td>
</tr>
<tr>
<td></td>
<td>1740</td>
<td>30</td>
<td>–ve</td>
<td>ND</td>
</tr>
<tr>
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<td>4</td>
<td>Nonmosaic</td>
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</tr>
<tr>
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<td>821</td>
<td>10</td>
<td>Nonmosaic</td>
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</tr>
<tr>
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<td>20</td>
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<td>4716</td>
<td>40</td>
<td>Variegated (+)</td>
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<td>Telomeric</td>
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<tr>
<td></td>
<td>4736</td>
<td>45</td>
<td>–ve</td>
<td>ND</td>
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<tr>
<td></td>
<td>4751</td>
<td>3</td>
<td>Variegated (+)</td>
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<td><strong>K5 + K5HK10</strong></td>
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<td>ND</td>
</tr>
<tr>
<td></td>
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<td>Nonmosaic</td>
<td>ND</td>
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<tr>
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<td>Variegated (+)</td>
<td>Telomeric</td>
</tr>
<tr>
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<td>3, 1-2</td>
<td>Nonmosaic</td>
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<td></td>
<td>4464</td>
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<td>Telomeric</td>
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<tr>
<td></td>
<td>4483</td>
<td>1, 1</td>
<td>Nonmosaic</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> Expression in adult tissues as detected by X-gal staining. Lack of expression, or expression in very few cells, is indicated as –ve. The degree of variegation is indicated from few nonexpressing cells (+) to a high proportion of nonexpressing cells (++++).

<sup>b</sup> Middle arm indicates lack of co-localization of transgene and centromeric or telomeric signals; telomeric indicates co-localization of transgene and telomeric signals on the long chromosomal arm; pericentromeric indicates that the signal of the transgene is on the centromere or very close to the telomere of the short arm. ND, not done.

<sup>c</sup> Copies of K5Z and K5HK10, respectively.

The integration site is centromeric or telomeric on the short arm in these two lines, because in our hands both signals do overlap (not shown). Among the lines with peritelomeric insertions were K5Z + K5HK10 with a high (4290) and low (4464) proportion of expressing cells. Similarly, K5Zlox lines with a reduced (4734) or extensive (4712 and 4716) variegation showed insertions at peritelomeric regions. Most interestingly, 4290 and 4464 are the two variegating K5Z + K5ZHK10 lines, suggesting that lack of expression in them could be related, at least in part, to position effects imposed by telomere proximity.

### DISCUSSION

The wide occurrence of variegated expression among transgenic lines has been noted recently. The mechanisms of appearance of variegation are likely multiple. Some insight into these mechanisms has been obtained from analysis of transgene expression in erythroid and lymphoid tissues. In this report we study transgene variegation in stratified epithelia.
DNA sequences and silencing: Some sequences can act as loci for gene silencing, as has been proposed to explain the poor expression of ovine β-lactoglobulin genes in transgenic mice (Clark et al. 1997). Here, we describe 17 K5Z mouse lines that either express in a variegated fashion or do not express at all. This adds to the cumulative evidence of unreliable expression of many lacZ-containing constructs, particularly in adult tissues (Paldi et al. 1993; Thorey et al. 1993; Cui et al. 1994; Guy et al. 1996). Interestingly, we show that when cDNA fragments are exchanged by the lacZ gene, in equivalent K5 constructs, they express more efficiently, with little or no variegation. Other researchers have used our K5 promoter sequences to drive expression of a number of cDNAs, and in most cases they report nonmosaic expression (Robles et al. 1996; Pierce et al. 1998; Wang et al. 2000). This suggests that variegation (or even lack of expression) of K5Z constructs in mice can be due, at least in part, to lacZ sequences, rather than to the absence of cis-acting sequences required for appropriate expression in the basal layer of stratified epithelia. Nevertheless, the efficient, nonmosaic (almost) expression of the K5HK10 construct cannot be interpreted as an indication of the negative effects of the lacZ gene, because it could be argued that it is due, instead, to the LCR-like properties of HK10 alone or with K5 sequences. In any case, when the transgene arrays consisted of a mixture of K5Z and K5HK10 sequences most of the lines showed a nonmosaic expression pattern. This is reminiscent of the lack of variegation often associated with transgenes harboring LCRs (Kiousis and Festenstein 1997).

Using model (simplified) constructs, it has been shown that variegation varies depending on whether an enhancer is present or not. Thus, it has been proposed that enhancers increase the probability of expression by preventing variegation alone, without affecting the transcription rate of the promoter (Walters et al. 1996; Sutherland et al. 1997) or by affecting both (Bouhas-sira et al. 1997). Our data on K5Z, a construct with a complex set of regulatory sequences, support the latter model because the differences in β-gal activity per cell among our K5Z lines, which are higher in the lines with a higher proportion of expressing cells, could indicate a variable transcription rate. An alternative possibility, however, takes into account possible differences in active copies in the array, as indicated by the intriguing observation in some of the K5Z + K5HK10 lines that

![Figure 4](image-url)
Variegation of K5 Transgenes in Stratified Epithelia

Figure 5.—Expression of K5HK10 transgenes. (A) Immunofluorescence analysis of HK10 in tissue sections of a representative K5HK10 line. (B) Copy-number-dependent expression of K5HK10 in transgenic mice. Ten micrograms of total tailskin RNA isolated from a nontransgenic mouse (lane 1) and from K5HK10 lines (lanes 2–6) was analyzed by Northern blot using a HK10 probe. To correct for loading differences, a K5 probe was used. The normalized values of K10 mRNA are plotted against gene copy number.

not all copies in the transgene array are active within the same cell. This is consistent with previous evidence about chromatin heterogeneity within multicopy transgene arrays in mouse lines that showed either variegated/position-dependent or efficient expression (Huber et al. 1996; Whitelaw and Webster 1998).

Variegation and chromosomal position: The influence of the integration site for variegated expression of transgenes is well established for constitutively heterochromatic regions at or near centromeres (Dobie et al. 1996; Festenstein et al. 1996; Milot et al. 1996). Indeed, the two lines we find having pericentromeric insertions correspond to variegated (K5HK10 825 line) or nonexpressing (K5Zlox 4715 line) conditions. However, in this study we provide evidence that transgene variegation does not require that the transgenes are located in or close to the centromere, in agreement with a previous report (Graubert et al. 1998). In particular, we show that the transgenes in a number of variegating lines are located at peritelomeric sites. While mosaic silencing of genes inserted at telomeric positions has been observed for both yeast (Gottschling et al. 1990) and Drosophila (Cryderman et al. 1999), well-documented evidence of such silencing was lacking in mammals. Because of the potential silencing effects of lacZ sequences it could be argued that variegation of transgenes inserted into peritelomeric sites is not necessarily only a reflection of a telomeric position effect. However, the observation that, among the lines in which the effects due to lacZ sequences can be overcome (by K5HK10 sequences), the two variegating ones, but not the one that expresses in a nonmosaic fashion, bear peritelomeric transgenes supports telomeric position effects in transgenic mice. This is consistent with recent observations of telomeric position effects on replication timing of human loci (Ofr et al. 1999) and indicates that both gene expression and DNA replication can be affected by telomeric sequences in mammals.

Transgene repeat arrays and silencing: Examination of Table 1 suggests that very-high-copy-number lines tend to either express in a highly variegated manner or

Figure 6.—Expression of K5Z + K5HK10 transgenes. (A) Nonvariegated lacZ expression in sections of tongue (I), tailskin (II), esophagus (III), palate (IV), and footsole (V) of a representative transgenic line. (B) Double immunofluorescence of HK10 (green) and β-gal (red) in sections of dorsal (B, I and II and V–VIII) and ventral tongue (B, III and IV) from the indicated lines.
not at all. However, our analysis of the effect of gene repetition on variegation shows that a reduction in copy number of K5Z transgenes does not increase the proportion of expressing cells, which suggests that repeated induced gene silencing is unnecessary for transgene repression. This is in contrast to results by GARRICK et al. (1998) who studied the expression properties of an α-globin/lacZ construct. The discrepancy could be explained by several differences between the two transgenic systems, such as the distinct cell types involved or the possibility that subsets of chromosomal sites are more favorable to repression than others. Thus, it is conceivable that in three of the K5Zlox lines we used for copy-number reduction, the peritelomeric (two) or pericentromeric (one) position of their transgenes may impose a silencing effect regardless of copy number. An alternative explanation lies in the compartmentalization model of gene silencing. This model, put forward to explain the silencing of transgenes located far away from constitutive heterochromatin, proposes that the variegating locus, after local heterochromatinization, is mislocalized to a constitutive heterochromatic compartment within the nucleus (SABL and HENIKOFF 1996). Recently, a similar correlation has been observed between variegation and transgene localization to heterochromatic regions of tissue culture cells (FRANCASTEL et al. 1999). In transgenic mice, it has been reported that a silent transgene integrated into a peritelomeric region co-localizes with centromeric satellite DNA on the nucleolus (MANUELIDIS 1991). It seems, however, that the size of the transgenic insert is critical for such a nuclear relocalization (MANUELIDIS 1991). If this were the case, the α-globin/lacZ transgene arrays, which seem larger than the K5Zlox transgenes we have studied, may find themselves in singular heterochromatic compartments within the interphasic nuclei from which they can be mobilized upon copy-number reduction. Anyhow, our conclusion that K5Z variegation does not depend on the copies of the transgene is consistent with other reports of no apparent correlation between transgene copy number and degree of variegation (ZHUMA et al. 1999).

In summary, we have shown that variegation of K5Z transgenes in stratified epithelia of transgenic mice is not due to the lack of regulatory sequences. Instead, our data suggest that variegation can be related to prokaryotic lacZ sequences that may cooperate with other repressor influences such as chromosomal position ef-

**Figure 7.**—FISH of transgenic lines. (A–N) Bone marrow metaphase chromosomes were hybridized to a mixture of K5Z (pink) and mouse γ-satellite probes (green) or to K5Z DNA (yellow) and a probe specific for mouse telomeric repeats (pink; HANISH et al. 1994). Chromosomes were also stained with 4′-diamidino-2-phenylindole (blue). (O–R) Bone marrow metaphase chromosomes hybridized to the K5Z construct alone (pale green). For the K5ZMAR 1739 line, the transgene signal is yellow and chromosomes were stained with propidium iodide. Transgenic lines are indicated.
fects. This silencing effect, however, can be efficiently overcome by strong activating sequences. Also, we have shown that insertion of transgenes in pericentromeric regions is not required for variegation and have provided evidence for telomeric position effects in transgenic mice. Finally, although we cannot rule out entirely the effect on variegation of the number of copies in a transgenic array, we have shown that, at least in some cases, both variegation and lack of expression are independent of copy number.

We thank P. Vassalli for pCAGGS-Cre plasmid, A. Sippel for pB1-X1 plasmid, T. De Lange for pSXneo-1.6-T2AAG3 plasmid, A. Lengveld for technical assistance, J. Martinez, R. Lázaro, and E. Sole for animal care, and C. Calés for critical reading of the manuscript. This work was supported by Comisión Interministerial de Ciencia y Tecnología (CICYT) grants PB94-1230 (J.J.), PB94-0089, and PB97-1238 (M.V.). L.P. was supported by a fellowship from the Universitat Autònoma de Barcelona.

LITERATURE CITED


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Communicating editor: S. Henikoff