Epigenetic Control of Transgene Expression and Imprinting by Genotype-Specific Modifiers

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Summary

Expression and DNA methylation of the transgene locus TKZ751 are controlled by genotype-specific modifier genes. The DBA/2 and 129 genetic backgrounds enhanced expression, while the BALB/c background suppressed expression, but only following maternal inheritance of the BALB/c modifier. Epigenetic modification of the transgene locus was cumulative over successive generations, which in BALB/c mice resulted in an irreversible methylation after three consecutive germline passages. Therefore, at the TKZ751 locus the germline fails to reverse previously acquired epigenetic modifications, a process that is usually essential to restore the genomic totipotency. Hence the genotype-specific modifier genes regulate penetrance and expressivity as well as parental imprinting of the TKZ751 locus through epigenetic modification.

Introduction

We have recently developed a method to investigate the control of transgene expression by means of a readily detectable *lacZ* reporter gene linked to an HSVtk cryptic promoter (Allen et al., 1988). Expression of each of these randomly integrating transgenes is highly prone to a variety of position effects imposed by the *cis*-acting factors of the chromosomal domains in which they reside and by *trans*-acting factors that may induce epigenetic modifications on such integration loci (Allen et al., 1988; Kothary et al., 1989). Both the *cis*- and *trans*-acting factors may combine to produce variations in penetrance and expressivity as well as germline-dependent imprinting of transgenes (Surani et al., 1990). In particular, the importance of genetic determinants in modulating transgene expression has not yet been fully appraised. For example, *trans*-acting genotype-specific modifier gene products are likely to interact with specific transgene loci to modulate their expression. Modifier genes were originally postulated to influence phenotypic variations by controlling penetrance and expressivity of responding loci (Fisher, 1931; Haldane, 1941). Modifiers may repress or derepress expression by direct or indirect influences on transcription and chromatin structure, resulting in subsequent epigenetic changes, such as DNA methylation, which maintain and propagate the newly established state of the responding locus (Wein-

traub, 1985; Holliday, 1987; Pillus and Rine, 1989; Surani et al., 1990).

One of the primary objectives of our investigations is to understand the mechanisms of genomic imprinting, a reversible germline-specific epigenetic marking process that confers functional differences on maternal and paternal genomes. Such differences have been demonstrated unequivocally in a variety of genetic and embryological studies (Cattanach, 1986; Solter, 1988; Surani et al., 1990). Furthermore, it has been shown that DNA methylation and expression of transgene loci can be influenced by their parental origin (Reik et al., 1987; Sapienza et al., 1987; Swain et al., 1987; Hadchouel et al., 1987). However, the imprinting data of transgenes have presented a number of paradigms and paradoxes that are discussed in detail elsewhere (Surani et al., 1988. 1990). The precise mechanism of imprinting of transgenes and its relationship to the imprinting of any endogenous genes and their role in development, if any, remain to be established.

Some transgene inserts, like some dominant mutations in mice and humans, display both parental origin effects as well as variable penetrance and expressivity (Sapienza, 1989; Reik, 1989; Surani et al., 1990), which in mice can be observed as the influence of the products of modifier genes present in different inbred backgrounds. For example, the penetrance of the gene *fused* decreases after maternal transmission to 12%, while that of paternal *fused* is affected only to 70%, in response to a suppressor modifier in some inbred strains including C57BL/6 (Agulnik and Ruvinsky, 1988). Variations in penetrance and expressivity of the mutation *disorganized* are similarly influenced by genetic background (Hummel, 1958). In the case of Huntington's disease in humans, there is also variable expressivity, which is influenced by parental origin as well as by genetic factors (Folstein et al., 1985; Ridley et al., 1988). These observations suggest that the penetrance and expressivity of these alleles are intimately linked with their parental origin as well as by their response to different modifying proteins.

In this study, we have carried out a systematic investigation of the influence of the germline and genetic determinants on the epigenetic modification and expression of a transgene locus. The locus was detected in mice with independent inserts of a transgene that is highly susceptible to chromosomal position effects (Allen et al., 1988; Kothary et al., 1989). We show that the transgene locus TKZ751 is subject to regulation by genotype-specific modifiers and in some instances to parental origin effects. This study also provides important insights into the role of the germline, which normally reprograms the genome by erasing all epigenetic modifications to restore totipotency. However, this does not occur at the TKZ751 locus. This fallibility of the germline results in cumulative epigenetic modification of the transgene locus through successive generations, which provides a basis for the analysis of grandparental effects. The transgenic locus TKZ751 is significant
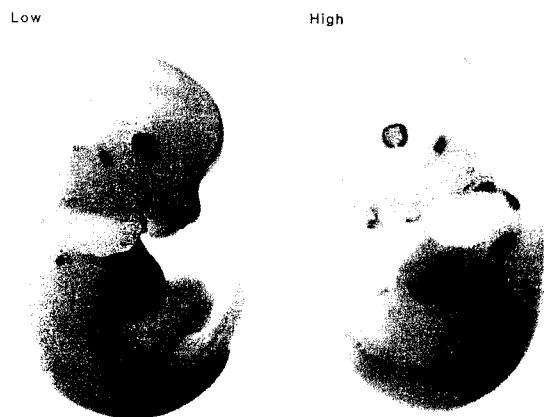
Figure 1. High and Low Expression of *lacZ* in Transgenic Fetuses

Two day 13 transgenic embryos from the same litter show high and low levels of expression. These embryos are from a cross between the male transgenic founder mouse 751 and (C57BL/6 × CBA)F1 females.

as a model system to study the control of penetrance and expressivity as well as imprinting by genetic factors.

Results

TKZ751 is one of five lines of transgenic mice (TKZ) that express a HSVtk-*lacZ* transgene. The unique expression patterns seen in each of the TKZ transgenic lines reflects the strong influence of chromosomal position effects on this transgene (Allen et al., 1988). The complex pattern of *lacZ* expression of TKZ751 (which will be described in detail elsewhere) is first detected on day 11 of gestation, associated with development of the forelimbs and later seen in a number of disparate tissues on days 13–14 of gestation (see Figure 1).

Segregation of High and Low Expressing Phenotypes; Correlation with DNA Methylation

The founder transgenic male, 751, with a mixed ([C57BL/6 ♀ × CBA ♂]F1 ♀ × CFLP ♂) genetic background, when backcrossed onto the (C57BL/6 ♀ × CBA ♂)F1 parental hybrid strain gave rise to fetuses in which either high or low expression was observed in equal proportions (Figure 1).

Since variations in the levels of CpG methylation are often correlated with variations in gene expression, methylation of the transgenomes in high and low expressing em-
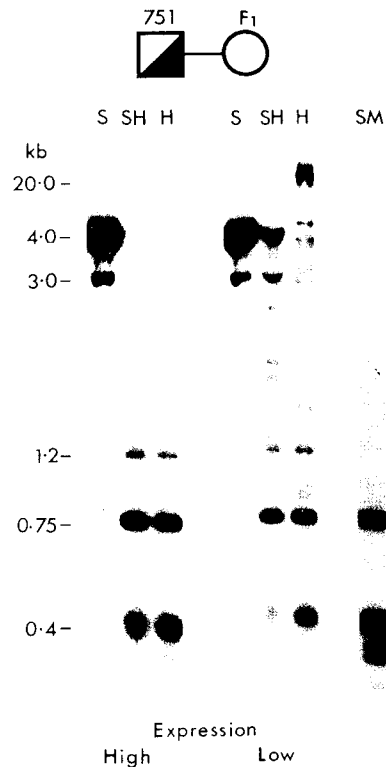

Figure 3. High and Low Levels of Expression Are Inversely Correlated with DNA Methylation at the Transgene Locus

The transgene locus in high expressing embryos was undermethylated compared with that of low expressing siblings (Figure 1). Restriction digests are S, SacI; SH, SacI plus HpaII; H, HpaII; SM, SacI plus MspI. The 20 kb transgene flanking band is relatively hypomethylated compared with the 3 kb flanking band.

bryos was determined. The DNA methylation analysis was carried out by restricting embryo DNA with SacI, which cuts once within the transgene, and by comparing this with DNA restricted with both SacI and the methylation-sensitive enzyme HpaII. A restriction map of the transgene illustrating the potentially methylatable HpaII sites and the SacI site is shown in Figure 2. The restriction analysis indeed showed an inverse correlation between the levels of expression and DNA methylation of the transgenome (Figure 3). Furthermore, the 20 kb flanking band was relatively more hypomethylated compared with the 3 kb flanking band. The transgene and flanking bands were digested completely with MspI, the non-methylation-sen-
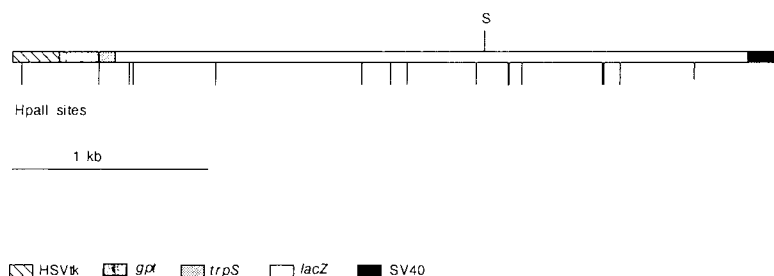

Figure 2. Restriction Map of HpaII and SacI Sites within the HSVtk-*lacZ* Transgene
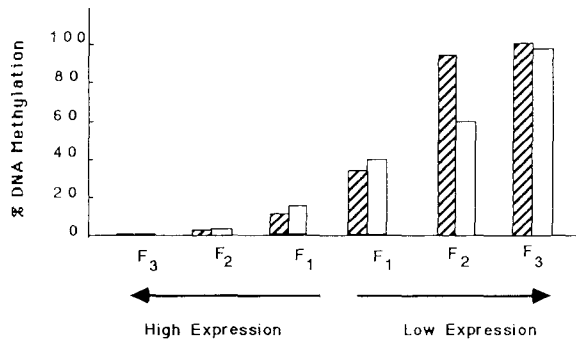
Figure 4. Cumulative Changes in DNA Methylation with the Segregation of High and Low Expression in TKZ751 Mice

Transgenic sublines with either high or low levels of expression could be segregated by selective breeding with (C57BL/6 × CBA)F1 mice. The methylation differences shown in Figure 3 became exaggerated with each generation. The percentage of methylation was determined by densitometric scanning of Southern blots and is best illustrated as a bar graph. The methylation changes that are seen in the soma (spleen; open bars) with each generation are also seen in the germline (testis; hatched bars).

sitive isoschizomer of HpaII, to give major restriction products of 0.75 and 0.4 kb.

Analysis of transgene methylation with DNA from tail biopsies from adult mice was subsequently used to provide an accurate guide for the levels of transgene expression in their progeny. Embryos analyzed in the F2 generation also showed segregation to high and low expressing phenotypes. However, these expression differences were exaggerated when compared with F1 embryos. Embryos de-

rived from F1 mice with low expression exhibited low or even lower levels of expression, while embryos derived from F1 mice with high expression exhibited high or even higher levels of expression. Changes in the levels of expression in the F2 embryos were also reflected in the methylation status of the transgenome. By selective breeding, the phenotypes could be segregated further in the F3 generation. In the subline with low expression, there was a complete loss of expression associated with complete methylation of the transgenome, while in the subline with high expression there was complete demethylation of the transgenome (Figure 4).

Variable Expression in Individual Cells

The variations in expression seen in the embryo whole mounts (Figure 1) could be due either to quantitative differences in the levels of expression in all cells or to variations in the total number of cells with similar levels of expression, resulting in a mosaic pattern of transgene expression. Similarly, the partial methylation patterns observed, for example, in Figure 3 could be due to mosaicism with cell to cell variations in methylation or to differential methylation of the individual transgene copies within the tandem repeat.

To determine the nature of the changes in expression, stained embryos with high or low levels of transgene expression were sectioned, and similar sections were compared (Figure 5). This analysis showed that in individual embryos with high and low expression, lacZ expression varied in every cell more or less equally and with very little cell to cell variation in the intensity of β-galactosidase
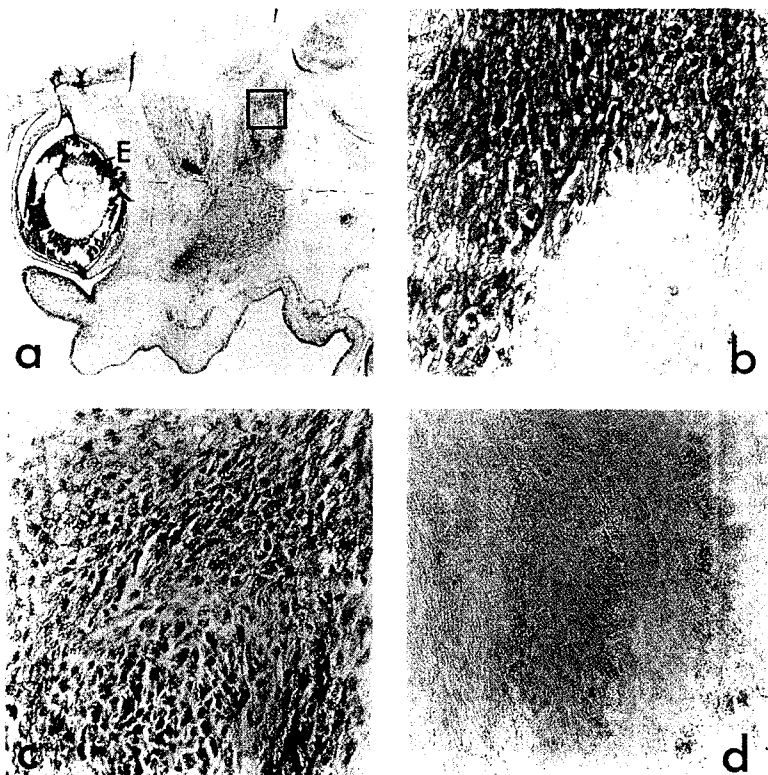

Figure 5. lacZ Expression in TKZ751 Is Non-mosaic

Shown are expression differences between high and low expressing embryos analyzed in tissue sections. Expression is suppressed in every cell in low expressing mice compared with high expressing mice. In (a) a patch of expression in the cheek of a TKZ751 embryo is shown. E, eye. Expression in the same region is shown from embryos with high (b), intermediate (c), and low (d) levels of expression. The expression differences seen in Figure 1 are therefore not due to variations in the absolute numbers of cells expressing the transgene and constituting a mosaic expression pattern.
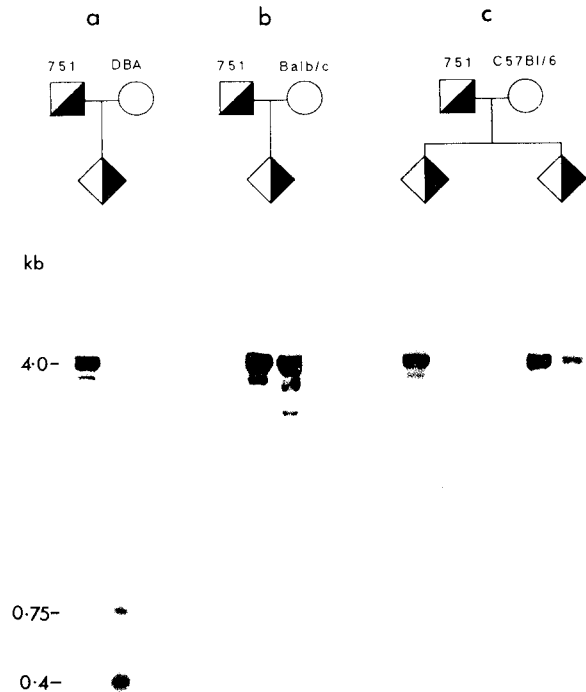
Figure 6. DNA Methylation of the Transgenome Varies with Different Genetic Backgrounds

All the progeny from crosses between the founder male (751) and the wild-type DBA/2 females were uniformly hypomethylated (a), while all the progeny obtained from crosses with wild-type BALB/c females were uniformly hypermethylated (b). Progeny from the 751 × C57BL/6 cross were either hypo- or hypermethylated in equal proportions (c). For each cross, the restriction digests shown are SacI and SacI plus HpaII, as in Figure 3.
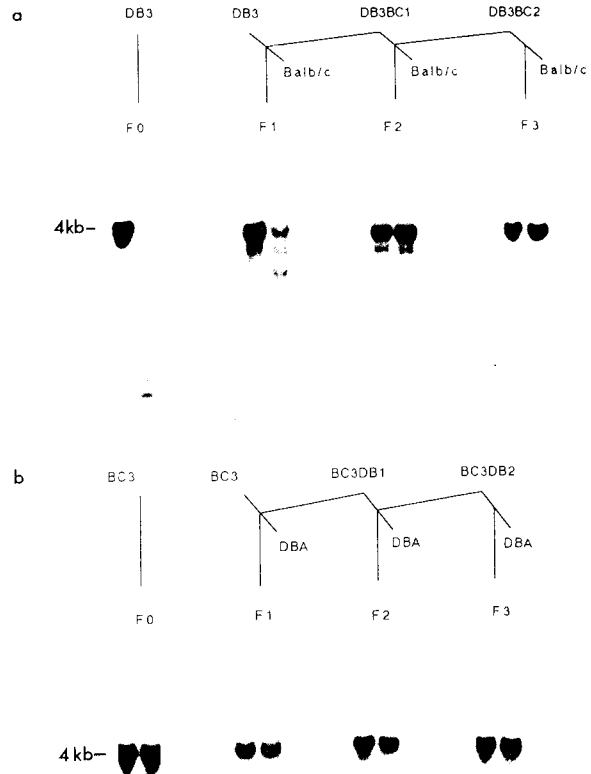

Figure 7. Methylation by BALB/c Modifier Genes Shows Dominance over Demethylation by DBA/2 Modifiers

The hypomethylated transgene locus in DB3 mice (F3 generation backcross of the transgene onto DBA/2) becomes increasingly methylated with each germline passage into BALB/c (a). By contrast, the fully methylated transgene locus in BC3 mice (F3 generation backcross of the transgene onto BALB/c) is irreversible even after three consecutive backcrosses onto the DBA/2 background (b). Methylation analysis is with SacI and SacI plus HpaII.

staining. Hence, the variations in expression observed in whole embryos are not due to variations in the absolute numbers of positive and negative cells.

Mosaicism with respect to DNA methylation can only be detected by analyzing a single methylation-sensitive site. However, such an analysis could not be carried out here because the TKZ751 insert consists of about five to six copies of the transgenes in a head to tail tandem repeat. Hence, while transgene expression is nonmosaic, subtle variations in DNA methylation between cells cannot be ruled out.

High and Low Expressing Phenotypes Are Determined by Genetic Background

It is likely that the initial segregation into high and low expressing phenotypes was due to the segregation of a single allele encoding a trans-acting factor that regulates the transgene locus with effects on DNA methylation and expression. This is possible because of the heterogeneous genetic background of the founder animal. The genetic control of expression and methylation was examined further by introducing the transgenome into mice of defined genetic backgrounds.

The analysis demonstrated that BALB/c mice had a dominant effect that resulted in a marked increase in the level of methylation of the transgenome with a consequent suppression of transgene expression in all of the progeny (Figure 6a). The DBA/2 (Figure 6b) and 129 backgrounds (data not shown) demethylated the transgenome. By the F3 generation, the transgenome could be considered to be completely unmethylated on the DBA/2 background (DB3), but by contrast, was fully methylated on the BALB/c background (BC3).

On the C57BL/6 background, both high and low transgene expression was observed in equal numbers of embryos, similar to that observed with (C57BL/6 ♀ × CBA ♂)F1 mice (Figure 6c). A similar segregation of phenotypes was apparent on the CBA background; however, this has not yet been studied extensively. The origin of the methylating influence in the founder mouse, 751, is unknown. However, since the C57BL/6 and CBA backgrounds do not modify the phenotype in any one direction exclusively, they may be neutral with respect to modification of
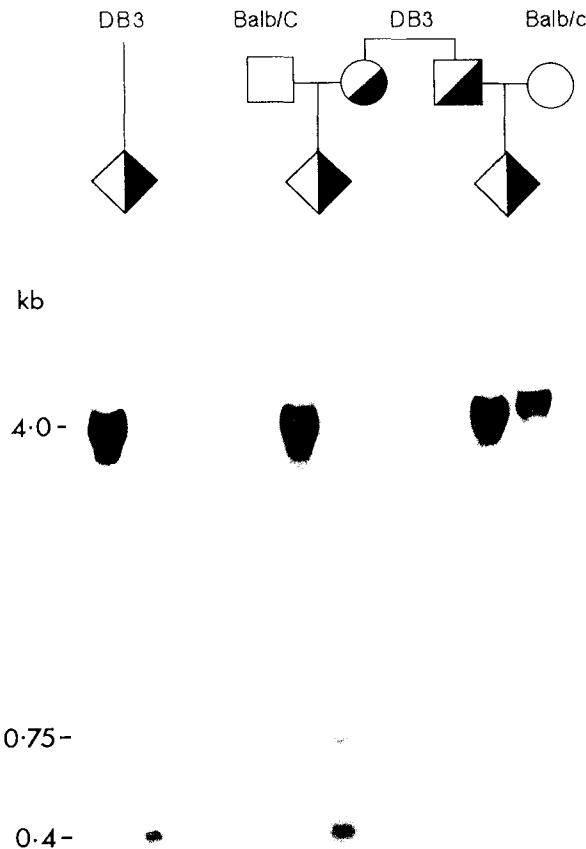
Figure 8. Transgene Methylation Controlled by the BALB/c Modifier Gene Is Dependent on Its Parental Origin

The DB3 transgene locus becomes methylated only when it is introduced into BALB/c females, but it remains unchanged when introduced into BALB/c males. Methylation analysis is with SacI and SacI plus HpaII.

the transgene, and therefore the methylating influence may have originated from the outbred CFLP mice. Backcrossing experiments with CFLP mice were not performed because it is an outbred strain. The differences in expression observed on the C57BL/6 background could therefore be explained by segregation of a modifier gene originating from the CFLP mouse stock.

Reversibility of High and Low Phenotypes
Expressivity of the transgene is apparently under the control of strain-specific modifier genes. These modifier genes could have a dominant role either as activators or suppressors of the transgene locus. To distinguish between these two modes of regulation, it was necessary to investigate the extent to which the phenotypes could be reversed. This was tested by crossing the completely hypomethylated transgene capable of high expression from DB3 males onto a BALB/c background and crossing completely hypermethylated, nonexpressing transgenes from BC3 males onto a DBA/2 background.

An immediate methylation of the hypomethylated transgene from DB3 males was seen when it was introduced

onto the BALB/c background (Figure 7a). By contrast, no reversal in the phenotype was achieved by placing the transgene from nonexpressing BC3 males onto a DBA/2 background, even after four consecutive backcrosses to DBA/2 (93.75% DBA/2; Figure 7b). However, reversal could be achieved if the transgenome was initially partially methylated (data not shown). Therefore, the modifying gene(s) present in BALB/c mice is a dominant suppressor of gene expression at this locus. Furthermore, once the locus became fully methylated, there was no further requirement for the BALB/c modifier to maintain and propagate the transgenome in a stable, nonexpressing, methylated form.

It is possible that reversal of the partially methylated phenotype by DBA/2 and the 129 inbred strains may be achieved, at least in part, through segregation away from the suppressor modifier. However, it is more likely that the DBA/2 and 129 strains possess enhancing modifier genes that activate the transgene locus, because compared with the results using C57BL/6 mice, no segregation to high and low phenotypes was observed. Furthermore, the enhancing activity of DBA/2 mice appears recessive with respect to the suppressing activity of the modifier in BALB/c.

Modification by BALB/c Shows a Parental Origin Effect
As described above, the transgene became methylated when transgenic DB3 males were mated with wild-type BALB/c females. Strikingly, in the reciprocal cross between DB3 transgenic female siblings and BALB/c males, there was no detectable methylation of the transgenome. Therefore, the influence of the BALB/c modifier shows a parental origin effect, being active only when maternally derived (Figure 8). Such a maternal effect could occur because of a direct interaction of the transgenome with an oocyte cytoplasmic factor. Alternatively, the influence on the transgenome could be indirect, requiring an interaction with a product of a maternal allele expressed after fertilization.

To distinguish between the nuclear and cytoplasmic effects discussed above, DB3 transgenic male mice were crossed with (DBA/2 × BALB/c)F1 female mice. In such a cross, egg cytoplasm will be similar in all oocytes but their genotypes will vary; half of the eggs will carry the BALB/c modifying locus while the rest will carry the DBA/2 modifying locus. The results of this cross showed a clear split into embryonic phenotypes with either high or low expression at a frequency of 1:1 (similar to Figure 1). Therefore, the suppression of transgene activity is associated with the segregation of the BALB/c modifier gene in half of the F1 eggs. Nevertheless, this result does not exclude an additional requirement for a BALB/c oocyte cytoplasmic factor. In the reciprocal cross between DB3 transgenic female mice and wild-type (DBA/2 × BALB/c)F1 males, all progeny were high expressing, with no methylation of the transgene locus.

Discussion

In the transgenic mouse line TKZ751, expression of the

transgene is dependent on a chromosomal position effect. In addition to a complex spatial and temporal pattern of expression, the TKZ751 transgene locus is differentially regulated on different genetic backgrounds, resulting in variable penetrance and expressivity. Differences in expression are correlated with different levels of DNA methylation of the transgenome, which are controlled in the first instance by genotype-specific modifiers.

Changes in Expression with Cumulative Epigenetic Modification (DNA Methylation)

Progressive and cumulative epigenetic modifications (DNA methylation) through successive generations in (C57BL/6 ♀ × CBA ♂)F1 mice resulted in the segregation of mice with either high or low expression phenotypes. The cumulative epigenetic inheritance was probably detectable because of the multicopy nature of the transgene locus. Furthermore, the transgene locus is likely to be modified as early as the blastocyst stage before the germ cell lineage is established (Soriano and Jaenisch, 1986). As the methylation pattern is the same in all tissues assayed, the transgene modification must have occurred by the time cells are allocated to the definitive germ layers: the ectoderm, endoderm, and mesoderm. The epigenetic control of transgene expression in TKZ751 differs from that of the previously described germline-dependent imprinting of transgenes (Surani et al., 1988). In previous studies, there was reprogramming by demethylation of the transgenes in the testis (Reik et al., 1987; Sapienza et al., 1987; Swain et al., 1987). By contrast, in TKZ751, the changes observed in the soma were also seen in the germ cells, at least in sperm (and testis). It is expected that the germline must reverse all previously acquired epigenetic changes to restore nuclear totipotency and reset the genome for further germline-specific imprinting (Monk, 1988; Solter, 1988; Surani et al., 1990). Clearly, such reprogramming is not infallible, as witnessed in the case of TKZ751 in which cumulative epigenetic inheritance was observed. Such progressive epigenetic changes have important implications for understanding grandparental effects manifested in some human genetic disorders (Surani et al., 1990; Reik, 1989).

At the level of individual cells, variable penetrance and expressivity of transgene inserts can manifest themselves in at least two different ways. Variable expression could affect all cells more or less equally, or such changes could occur due to fluctuations in the total number of expressing cells, which are otherwise identical. These cells would be invariant with respect to the absolute levels of expression. The latter case would constitute true mosaicism with respect to the transgene expression. In the case of the TKZ751 locus, expression differences affected all cells equally. By contrast, another transgene locus apparently showed a mosaic expression pattern (McGowan et al., 1989). It has been postulated that mosaic patterns of epigenetic modification may form the basis for subsequent specification of cell lineages during development (Sapienza et al., 1989a). Selection of cells from a mosaic population apparently occurs in the chick by recruitment of the cells positive for the HNK-1 marker from the epiblast into the primitive streak (Stern and Canning, 1990). A somewhat similar phenomenon is observed in Saccharomyces cerevisiae, in which genetically identical cells can be phenotypically distinct because of differences in epigenetic modifications at the mating-type locus (Pillus and Rine, 1989). At present there is no compelling evidence to show that the mosaic expression pattern of any transgene correlates with cell allocation during development, although mosaicism with respect to transgene expression has been observed in a number of instances in differentiated tissues with apparently identical cells (Katsuki et al., 1988; Sweetser et al., 1988a, 1988b; McGowan et al., 1989).

The different methylation states of the TKZ751 locus show remarkable similarities to the developmental regulation of the suppressor mutator transposable (Spm) element in maize. The Spm element can exist in three states: the active form is hypomethylated, while the inactive or cryptic form is completely methylated, and the third form, which is programmable, is reversibly inactive and is partially methylated (Banks et al., 1988). As in the case of the nonexpressing TKZ751 transgenome, the cryptic form of the Spm element is stable and cannot be demethylated and reactivated. The overall similarity between the regulation of the Spm elements and the TKZ751 transgene activities is most intriguing considering the extreme evolutionary divergence of mice and maize. It will be of great interest to determine if the modifiers of gene expression form part of a highly conserved group of regulatory proteins. If that is the case, then a primary role for cytosine methylation in this regulation of gene expression comes into question, since it has been argued that methylation of CpG islands evolved independently in the animal and plant kingdoms long after they diverged (Antequera and Bird, 1988).

If such a family of modifier genes exists, it may include regulatory genes involved in position effect variegation in Drosophila, although, significantly, there is no role for CpG methylation in this instance. The phenotype observed is due to gene inactivation by the variable spread of heterochromatin into a normally active gene. The gradual loss of expression in the case of the TKZ751 locus may be similar, as it could result from the sequential inactivation of individual copies of the transgene present in the tandem repeat. This is evident from the fact that the 3 kb flank becomes methylated prior to the 20 kb flank when breeding the transgene onto a genetic background that results in methylation of the locus. The gradual cumulative effect of epigenetic modification suggests that the spread of inactivation is confined to about 6–10 kb with each generation. Such a fine genetic control may mean that the modification occurs over a very short period of time, or that the modifiers of this locus are limiting.

Both suppressors and enhancers of position effect variegation have been identified in Drosophila (Locke et al., 1988). For example, a mutation in the gene for the heterochromatin-specific nonhistone chromosomal protein C1A9 causes a dominant suppression of position effect variegation (James and Elgin, 1986). Furthermore, modifers of variegation also show parental origin effects (Spofford,

1961). Position effect variegation associated with a spread of heterochromatization is also seen in mammals at X chromosome–autosome translocation breakpoints (Russell, 1963). It is generally accepted that methylation of the inactive X chromosome helps to maintain its inactive state rather than play a causative role in establishing it as heterochromatic (Kaslow and Migeon, 1987; Lock et al., 1987). It is likely that modifiers of the TKZ751 locus and other similarly responding transgene loci (Sapienza et al., 1989b) first influence the activity of the transgene, while DNA methylation is a subsequent epigenetic modification that is employed to propagate the locus in a stable form.

Influence of Genotype-Specific Modifiers on Transgene Expression

The detection of high and low expressing phenotypes in equal proportions can be explained by simple cosegregation of a single modifying allele with the transgene from the founder mouse, 751. When different inbred mice were examined for the presence of modifying alleles, we found that expression was enhanced on DBA/2 and 129 backgrounds but suppressed on BALB/c, while C57BL/6 had no decisive effect on modulating transgene expression in either direction. It is important to stress that the variations in expression seen in the line TKZ751 are dictated by a position effect and are unlikely to be due to the transgene insertion or the transgene sequences themselves, because other TKZ transgenic lines when tested did not show any changes in expression with different genotypes (N. D. A., unpublished data). However, in a similar study, McGowan and colleagues (1989) found that for a transgene insert with an hsp70 promoter linked to lacZ (Kothary et al., 1988), expression was diminished in BALB/c mice, while with another transgene with a quail troponin I gene (379-TN1), suppression was observed on a C57BL/6 background (Sapienza et al., 1989b). Hence it appears that there are likely to be strain-specific modifier suppressors and enhancers that can act on transgene inserts. Their effect is likely to be dependent on the site of transgene integration and may possibly occur at different and specific stages of development.

Influence of Parental Origin of Modifier Genes on Transgene Methylation and Expression

The dominant influence of the BALB/c modifier gene showed a parental origin effect since the transgene became methylated (and expression was suppressed) only when it was introduced into female BALB/c mice. Furthermore, genetic studies involving crosses between DB3 males and (BALB/c × DBA/2)F1 females result in both high and low phenotypes, which clearly indicate that the influence is due to a maternally derived genomic component. The failure of the paternally derived BALB/c allele to modify the transgene locus may mean that the modifier itself is down-regulated by a germline-specific imprint. Alternatively, the maternally and paternally derived alleles may be equivalent, but require an interaction with a BALB/c oocyte cytoplasmic factor.

Since no parental origin effect was seen in the initial breeding studies with (C57BL/6 ♀ × CBA ♂)F1 mice, it is possible that the BALB/c modifier and the modifier inherited from the founder mouse are in fact different, although they act on the same locus. If this is the case, we can postulate that the parental origin effect of the modifier present in BALB/c is indeed dependent on an interaction with other BALB/c genes or a BALB/c specific oocyte cytoplasmic factor; this would explain the apparent lack of parental origin effect in the F1 mice. The possible involvement of specific nuclear–cytoplasmic interactions that modify the transgene locus is currently being resolved by nuclear transplantation studies.

The influence of strain-specific modifier genes on the imprinting of transgenes may be a general phenomenon (Hadchouel et al., 1987; Sapienza et al., 1989b; W. Reik, personal communication). The extinction of expression of a transgene for the hepatitis viral antigen when crossed into C57BL/6 female mice (Hadchouel et al., 1987) appears analogous to the extinction of lacZ expression in TKZ751 mice by BALB/c females reported here. Collectively, these results suggest that different genic loci may be imprinted on different genetic backgrounds. Consequently, in an outbred population the number and variety of imprinted genes may be quite diverse. Furthermore, genotype-specific variations in imprinting affecting certain genetic traits would be reflected in phenotypic differences resulting from variable penetrance and expressivity on different backgrounds. It is interesting that variations in the penetrance and expressivity of some genetic diseases in humans, such as Huntington's disease and myotonic dystrophy, show parental origin effects (reviewed in Reik, 1989). Similar associations among penetrance, expressivity, and the parental origin of different genotypes are seen for some mutations in the mouse, for example, fused and disorganized (Agulnik and Ruvinsky, 1988; Hummel, 1958). In this regard, genomic imprinting can be considered as an extreme example of modifier control of gene expression. Although this argues that a diverse number of genes are differentially imprinted depending on their parental origin and genetic background, it does not exclude the possibility that there are some key regulatory genes that are always subject to germline-specific imprinting, which account for the similar failure of parthenogenetic or gynogenetic embryos of different genotypes (Surani and Barton, 1983; Solter, 1988; Surani et al., 1990; R. Fundele, personal communication).

Experimental Procedures

Mice

The transgenic mouse line TKZ751 was derived as described previously (Allen et al., 1988). The genetic analysis was carried out by backcrossing the transgene into DBA/2J, C57BL/6J, and CBA mice (Bantam & Kingman) and BALB/cJ and 129 mice (Olac), starting from the founder male, 751. The F1 hybrids used were derived from inbred mice of the same stock, (C57BL/6 × CBA) and (BALB/c × DBA/2).

Preparation and Analysis of DNA

High molecular weight DNA was prepared from tail biopsies, testis, and day 13 embryos (Allen et al., 1987). For the methylation analysis, DNA was restricted with SacI, the reaction mixture was then split into two, and one half was restricted further with HpaII. Restricted DNA was electrophoresed, Southern blotted, and hybridized to the HSVtk-lacZ probe derived from pHFBG2 (Allen et al., 1988). The degree of methyl-

ation was assessed by the disappearance of the SacI restriction products upon double digestion with HpaII and could be quantitated by densitometric scanning.

β-Galactosidase Expression Assay

Embryos were recovered, fixed, and stained for β-galactosidase activity as described previously (Allen et al., 1988). Embryos with different expression levels could be sorted by eye. A more detailed comparison of expression levels was made by analyzing 2 μm sections of prestained embryos, which were counterstained with neutral red.

Acknowledgments

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