Chromatin insulation by a transcriptional activator

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In eukaryotic genomes, transcriptionally active regions are interspersed with silent chromatin that may repress genes in its vicinity. Chromatin insulators are elements that can shield a locus from repressive effects of flanking chromatin. Few such elements have been characterized in higher eukaryotes, but transcriptional activating elements are an invariant feature of active loci and have been shown to suppress transgene silencing. Hence, we have assessed the ability of a transcriptional activator to cause chromatin insulation, i.e., to relieve position effects at transgene integration sites in cultured cells. The transgene contained a series of binding sites for the metal-inducible transcriptional activator MTF, linked to a GFP reporter. Clones carrying single integrated transgenes were derived without selection for expression, and in most clones the transgene was silent. Induction of MTF resulted in transition of the transgene from the silent to the active state, prolongation of the active state, and a marked narrowing of the range of expression levels at different genomic sites. At one genomic site, prolonged induction of MTF resulted in suppression of transgene silencing that persisted after withdrawal of the induction stimulus. These results are consistent with MTF acting as a chromatin insulator and imply that transcriptional activating elements can insulate active loci against chromatin repression.

In higher eukaryotes, differentiated cell types arise through transcription of a subset of the full complement of genes shared by all cells, with each cell type transcribing a characteristic set of genes. Genes with distinct patterns of cell type-specific transcription are found in close proximity to one another; their regulatory elements are widely distributed, and elements regulating different genes may be interspersed. Individual genes must establish and maintain their patterns of expression without being subject to (or subjecting other genes to) interference from regulatory elements that may act over very large distances. Furthermore, much of the genome, including nonexpressed genes, is maintained in a transcriptionally repressed state resembling heterochromatin, and expressed genes must be shielded against repressive effects of this chromatin. These conditions suggest that some elements must mediate the formation of independently regulated loci, preventing inappropriate repression or activation.

The influence of chromatin on gene expression is manifested in position effects on translocated or randomly integrated genes. Position effect may be divided into stable and variegating types (1). In variegating position effect, the integration site influences a stochastic choice between mitotically heritable on and off states, and the gene in question is either active or silent in a given cell, resulting in a mosaic population of expressing and nonexpressing cells (2–4). Stable position effect involves an influence of the integration site on the rate of transcription; the same transgene is typically found to be expressing at different levels in different integration sites (5, 6). A gene may be subject to both types of position effect.

Chromatin insulators, also known as boundary elements, are DNA elements capable of suppressing position effects and/or blocking the action of distant enhancers (7, 8). Several examples of chromatin insulation have been studied in detail, including *scs*/*scs′*, *Gypsy*/*Su*(*hw*), *Fab-7*, the chicken β-globin 5′HS4 element (9–12), and elements bordering the mating type loci in yeast (13–15). Chromatin insulators have usually been assayed by their ability to block suppressive position effects in randomly selected integration sites. There are, however, few clear examples in which a discrete element has been shown to block repressive chromatin effects in its native context, and these examples occur in yeast (14, 15). Only a few higher eukaryotic loci are known to contain elements that have insulator activity in ectopic contexts, and so the extent to which insulator elements are responsible for the formation of expression domains is unclear.

Whether insulator elements are common or uncommon, all expressed loci do contain transcriptional activating elements, which some experiments have demonstrated can counteract repressive position effects. Transgenes in mice and cultured cells are commonly subject to mosaic silencing that varies widely with integration position and can be suppressed by transcriptional enhancers (16–20). Transcriptional activators also suppress centromeric and telomeric position effects (21, 22). The ability to counteract repressive chromatin effects may be an integral part of transcriptional activation in situations, such as cell differentiation, where silencing is used as a means of gene regulation (20, 23). This function in some ways resembles chromatin insulation.

With this resemblance in mind, we have tested the ability of a transcriptional activator to function as a chromatin insulator in cultured cells. The GFP reporter construct carries multiple binding sites for the metal-inducible activator MTF. Cell lines carrying the transgene construct were derived without selection for expression of the reporter or any linked transcription unit and without induction of the activator. On induction of the activator, sites with low expression levels increase expression, whereas sites with high expression are only slightly affected. Induction makes silent transgenes more likely to become active and active transgenes less likely to become silent. Thus, the transcriptional activator suppresses both stable and variegating position effects in a manner that resembles chromatin insulation. This property may be essential to the formation of transcription domains.

Materials and Methods

Plasmid Construction. pγ/GFP/3MRE was derived from pγ/β-geo/3MRE (17). β-geo was replaced with GFP coding sequence amplified from pPGK/GFP/lox. The 3MRE in pγ/GFP/3MRE consists of three copies of the sequence −32 to −220 (relative to the transcription start site) from the mouse metallothionein-I promoter. pCMV/CD20 was a gift from Jim Roberts (Fred Hutchinson Cancer Research Center).

Culture Conditions. Cells were cultured in RPMI medium 1640 with 100 units/ml penicillin G sodium, 100 μg/ml streptomycin sulfate, 2 mM L-glutamine (all GIBCO/BRL), and 10% bovine calf serum (HyClone). Zinc sulfate stock (30 mM) was prepared in water and sterile filtered. Cells were maintained in log-phase growth for all experiments.

Abbreviations: MRE, metal response element; MTF, metallothionein transcription factor; FACS, fluorescence-activated cell sorter; LCR, locus control region.

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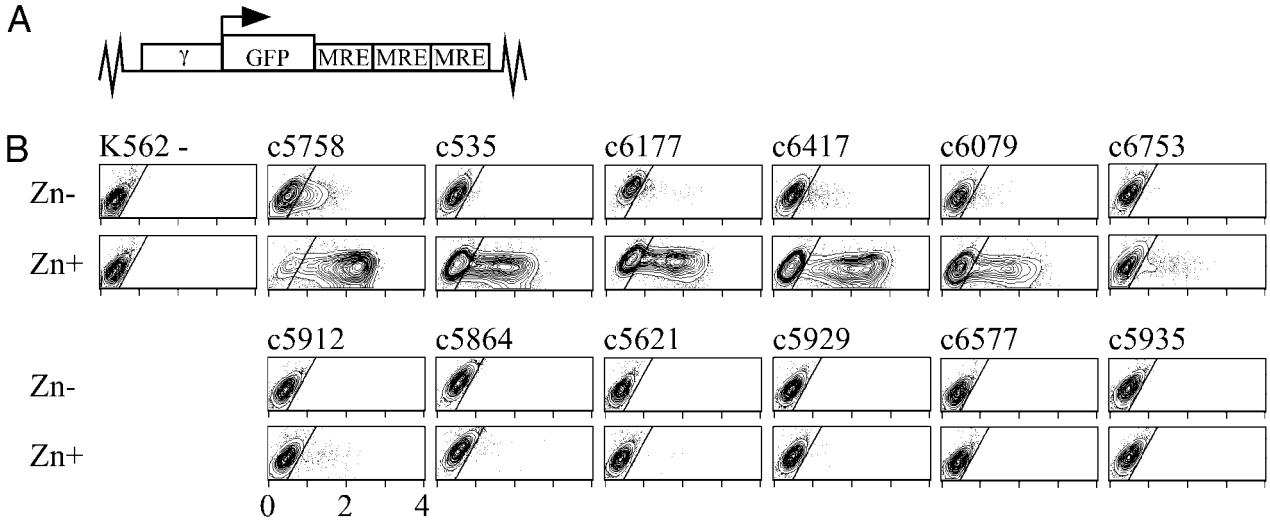

Fig. 1. The transcriptional activator MTF suppresses position-dependent silencing. (*A*) The γ/GFP/3MRE construct. γ is the human ^{A}γ-globin promoter. Downstream of GFP are three copies of the upstream region of the mouse metallothionein-I promoter, each of which carries six metal response elements, binding sites for the transcriptional activator MTF. This construct was linearized and transfected into K562 erythroid cells, and clones carrying single integrated copies were derived without selection for GFP expression. (*B*) Flow cytometric analysis of GFP expression in K562 clones carrying γ/GFP/3MRE. The FACS-analysis plots show propidium iodide level on the *y* axis and green intensity (GFP expression level) on the *x* axis. The axis labels 0, 2, and 4 denote, respectively, 10^0, 10^2, and 10^4 arbitrary fluorescence intensity units. In 12 of the 17 clones (those shown here), γ/GFP/3MRE is silent in all or nearly all cells maintained in standard RPMI medium 1640. MTF is induced by adding zinc sulfate to 80 μM. Subclones of each clone were split into medium with (Zn+) or without (Zn−) zinc, incubated 24 h, and FACS-scanned. As shown here, in 10 of 12 clones, induction of MTF results in establishment of GFP expression in some cells. Experiments were done at least three times, and representative scans are shown.

Derivation of Stable Clones Without Selection. Log-phase K562 cells (2×10^7) were electroporated (260 V, 960 μF; Bio-Rad Gene Pulser with capacitance extender) with 50 μg of *Sca*I linearized pγ/GFP/3MRE and 5 μg of supercoiled pCMV/CD20 plasmid in 0.4 ml of RPMI 1640 medium. Thirty-six hours later, cells were stained with AbCD20–phycoerythrin conjugate (Sigma); 10^5 healthy $CD20^+$ cells were fluorescence-activated cell sorter (FACS)-sorted in bulk, cultured 2 days, and then FACS-cloned into 96-well plates. GFP expression was ignored in both cell sorts. A total of 2,160 clones were expanded, and aliquots were prepared for PCR. Cells were washed with PBS, resuspended in 100 μl of 1.0 mg/ml proteinase K in PCR lysis buffer (50 mM KCl/10 mM Tris, pH 8.3/2.5 mM $MgCl_2$/0.45% Nonidet P-40/0.45% Tween 20), incubated at 55°C for 4 h, then at 95°C for 10 min, and then stored at −20°C.

To screen for the presence of GFP in each clone, PCR was performed using 2 μl of genomic DNA in 20-μl reactions. Thirty-five amplification cycles were performed with annealing at 60°C, using primers within the GFP coding sequence (GFP05, 5′-ACATGAAGCAGCACGACTTC-3′; GFP06, 5′-TGCTCAGGTAGTGGTTGTC-3′). The product was detected on agarose gel (261 of the 2,160 clones tested positive). Copy number of integrated transgenes in positive clones was determined by Southern blot analysis (24). Clones with a single transgene were used.

Subcloning and Experimental Assays. Each line was subcloned by FACS-sorting single cells into 96-well plates. GFP expression was ignored, and no clone was exposed to zinc sulfate before or during subcloning. Subclones were tested by PCR for the presence of the transgene, and five from each clone were used in the assay for establishment of expression. Each was split in triplicate into medium with or without 80 μM zinc sulfate, incubated for 24 h, and subjected to FACS analysis. For the expression level assay, subclones were pooled and plated into medium with 0, 20, 40, 60, and 80 μM zinc sulfate added, incubated for 6 days, and FACS-scanned. For the silencing and stabilization assays, the original clones were plated into medium with 80 μM zinc sulfate for 24 h, then 100,000 GFP^+ cells were double FACS-sorted and split into five aliquots in medium alone and five in medium with 80 μM zinc sulfate. These were passaged up to 50 days and FACS-scanned at intervals.

FACS Analysis. Cells were pelleted and resuspended at ≈10^6 cells per ml in PBS with 100 ng/ml propidium iodide. FACS analysis and sorting were carried out on FACSCalibur and Vantage cytometers with the standard fluorescein filter set (Becton Dickinson). Data were analyzed with the FLOWJO software (Tree Star, San Carlos, CA). Cells were determined to be GFP^+ or GFP^- on the basis of a gate on K562-negative cells set such that <0.1% of the K562 population passed the GFP^+ gate.

Northern blots on cells assayed to be GFP^+ and GFP^- by FACS show them to be + and −, respectively, for GFP mRNA.

Results

Experimental Strategy. We designed a strategy to obtain cell clones carrying integrated transgenes without selecting for transgene expression, because requiring expression likely eliminates clones in which the transgene is integrated in repressive sites. In addition, we used an inducible activator so that the effect of the activator could be analyzed without changing integration position. Cell clones were derived in the absence of the inducing stimulus. The transgene construct, γ/GFP/3MRE, contains the GFP reporter (25), with expression driven by the human γ-globin promoter (Fig. 1*A*). Downstream of GFP, we placed three copies of the upstream region of the mouse metallothionein-I promoter. Each copy contains six metal response elements (MREs), which are binding sites for the ubiquitous metallothionein transcription factor (MTF) (26–29). Heavy metals (such as zinc) cause release of MTF from inhibition, after which MTF binds to MREs and activates transcription (30).

Linearized γ/GFP/3MRE was electroporated into K562 erythroleukemia cells with a supercoiled CD20 expression plasmid. Two days later, cells expressing CD20 were sorted by FACS,

and at 4 days, single cells were sorted into wells and expanded as clones without selection. The clones were assayed by PCR for the presence of γ/GFP/3MRE; 261 of 2,160 clones were positive, indicating that only ≈12% of transiently transfected clones actually incorporated the reporter gene. Positive clones were Southern blotted to establish copy number, and the 16 clones with single unrearranged copies were studied further. We also analyzed c6219, which carries the full γ promoter and GFP coding sequence but only part of the MRE region. None of these clones carried the CD20 expression vector, and the transgene was integrated in a different site in each clone. At no time during the process of derivation was GFP expression required.

Once the clones were derived, we assessed the expression characteristics of the transgene in the 17 different integration sites in both induced and uninduced states. FACS permits rapid assessment of both the proportion of cells that express GFP within a population and the level of GFP expression in individual cells (25). We find that induction of the transcriptional activator suppresses both stable and variegating position effects on the transgene.

A Transcriptional Activator Drives Reversion of Position-Dependent Silencing. Because the clones are derived without selection for reporter expression, comparison of the induced and uninduced states will reveal any tendency of the activator to drive establishment of expression from the silent state. Approximately 10 weeks after transfection, we analyzed expression of GFP by FACS and assayed the response to induction with zinc. Within the set of 17 clones, there was considerable heterogeneity of transgene expression in the uninduced state (Fig. 1*B*, Zn−). In five clones, GFP was expressed by all of the cells (not shown); in others, there was a mixture of expressing and silent cells, and in six clones there were no expressing cells. This indicates that a variegating position effect acts on the transgene, with a strength that differs widely among integration sites. The result implies that our derivation strategy yields a broader sample of integration sites than a drug-selection strategy, which would never yield clones that did not express the transgene. Northern blot analysis confirms the variation in proportions of expressing cells noted on FACS (data not shown).

We asked whether induction of MTF increases the probability that a transgene will successfully establish GFP expression. The 12 clones that do not constitutively express GFP were induced with 80 μM zinc sulfate (Zn) for 24 h; this concentration of zinc is not toxic to K562 cells. The cells were then FACS-scanned to assay the proportion of GFP^+ cells (Fig. 1*B*). Only two clones (c6577 and c5935) failed to produce GFP^+ cells when induced with zinc. In the other 10 clones, induction of MTF activity results in some proportion of GFP^+ cells; this proportion is highly variable from clone to clone (compare c5758 and c5621). Establishment of expression is a stochastic process; cells that fail to establish expression initially are as likely to establish expression in a second induction as was the originally induced population (data not shown). Thus, although the extent of transgene silencing is highly dependent on integration position, induction of MTF reduces transgene silencing at most integration sites.

The Transcriptional Activator Prevents Silencing of Active Transgenes. Because the position-dependent silencing discussed in the preceding section (Fig. 1) was observed after derivation of clones over a period of 10 weeks, silencing of the transgene might have occurred in a progressive fashion during the derivation. Twelve clones were split into medium with 80 μM zinc for 24 h to establish expression in a subset of cells. GFP^+ cells were sorted from this mixed population, resorted to achieve >99.5% purity, and split into medium alone or medium with 80 μM Zn. At time 0, both populations uniformly express GFP. They were then passaged for up to 50 days and FACS-scanned at regular intervals to assay the proportion of GFP^+ cells in each population (Fig. 2).

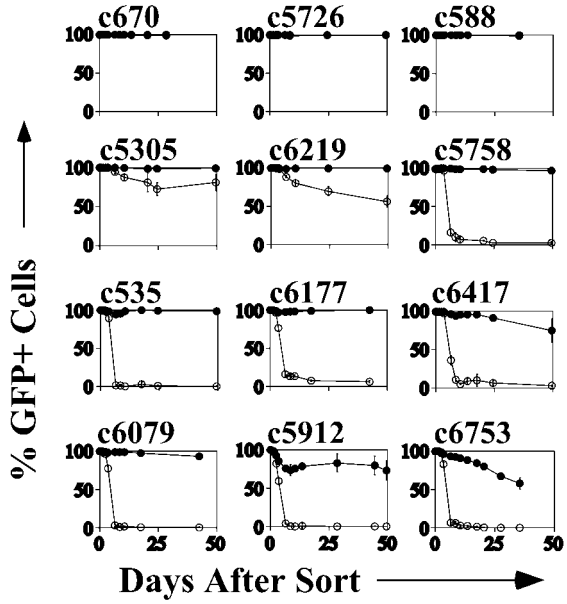

Fig. 2. MTF maintains the active transcriptional state. Clones were induced with 80 μM zinc sulfate for 24 h, and GFP^+ cells were double-sorted to achieve a >99.5% GFP^+ population. These were split into five aliquots without zinc sulfate (○) and five aliquots with 80 μM zinc sulfate (●). These cultures were passaged for 50 days and FACS-scanned at intervals to assay the proportion of GFP^+ cells. Silencing was assayed in the five constitutively expressing clones (670, 5726, 588, 5305, and 6219) and in the seven other clones from which sufficient GFP^+ cells could be FACS-sorted. Induction of MTF retards silencing of GFP in every clone in which silencing occurs and abolishes silencing in six of those nine clones. Error bars represent ±2 standard deviations.

In those clones displaying progressive silencing of GFP expression, zinc induction either slows or abolishes silencing. Four clones (c670, c5726, c588, and c5305) display very stable GFP expression over time (Fig. 2) whether or not MTF activity is induced, and one other clone (6219) undergoes little silencing. At the remaining seven integration sites, expression of the transgene is critically dependent on induction of MTF activation; the transgene is immediately silenced if cells are plated into medium without added zinc. Induction maintains expression in each of these clones, although to variable extents. In clones 5758, 6079, 535, and 6177, for example, there is no silencing of GFP expression so long as induction is maintained. In contrast, in c6753, GFP expression is lost at an appreciable rate despite MTF induction (although even in this clone, induction significantly slows the rate of silencing). Clones that maintain stable expression also have the highest incidence of reversion from silencing on induction (compare Figs. 1*B* and 2). This result is similar to previous results we have obtained (17) and is consistent with the transgene being subject to a variegating position effect, which drives and maintains a silent transcriptional state but is antagonized by the transcriptional activator.

Induction of the Activator Suppresses Position Effects on Expression Level. Stable position effect causes integration site-dependent differences in expression level (1), which likely reflect differences in transcription rate. Transcriptional activators are generally thought to regulate transcription rate. However, analysis of transgenes in cell lines derived by selection for expression of a drug-resistance marker revealed that some such activators, when bound at a distance from the promoter, function primarily to regulate transcription state (17, 31). Because we found that a

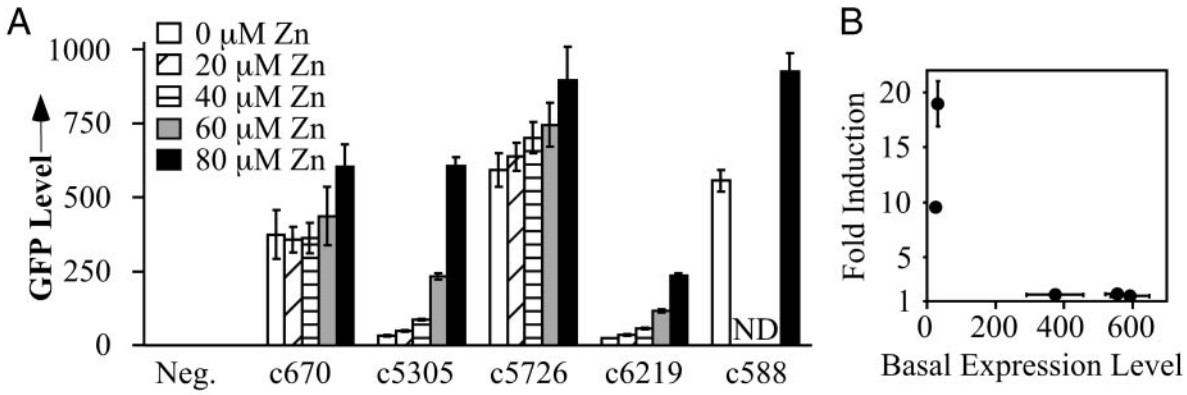

Fig. 3. Induction of MTF reduces position effects on GFP expression levels. (*A*) GFP$^+$ cells were sorted from each clone that constitutively produces GFP$^+$ cells and split into aliquots with 0, 20, 40, 60, or 80 μM zinc sulfate added to the medium. After 6 days, cells were FACS-scanned to assay the level of expression. The experiment was repeated five times. The bar graph shows the average mean GFP expression level in each clone as measured by FACS scan. (*B*) Fold induction, defined as (expression level in 80 μM Zn)/(expression level in 0 μM Zn), is inversely proportional to basal (uninduced) expression level. Induction of MTF has variable effects on the level of expression. At three integration sites with high basal expression levels, the enhancer changes the level of expression little. But at two sites with low basal expression levels, the enhancer has a large effect, increasing the level 20-fold in c5305. A similar effect was seen in the clones that did not constitutively express GFP (not shown).

nonselective derivation procedure recovered a broader range of integration sites, we asked whether MTF could increase the level of transgene expression in these cell clones.

In the majority of clones, it is difficult to assess the activator's relative effect on the level of expression, because without induction of MTF the GFP reporter is silent. Thus, we assayed the five clones that exhibit GFP expression that is stable enough to allow determination of expression level, without a confounding contribution from rapid transgene silencing or activation. GFP$^+$ cells were sorted by FACS, plated into medium with 0, 20, 40, 60, or 80 μM zinc, grown for 6 days, and then FACS-scanned to determine the mean level of expression (Fig. 3*A*). All five clones show an increase in the mean level of GFP expression with zinc induction. The increase varies, however, from barely measurable in clones 670 and 5726 to 20-fold in c5305. Fold induction is strongly inversely proportional to the uninduced expression level; clones with a high basal level of expression show the least induction and vice versa (Fig. 3*B*). A 30-fold difference among the five clones, in the mean level of expression without zinc induction, reduces to a 4-fold difference in level among clones when MTF is induced. This result is consistent with the suppression of stable position effect by MTF.

Suppression of Variegating, but Not Stable, Position Effect Can Continue After Withdrawal of Prolonged MTF Induction. The results discussed above indicate that the transcriptional activator suppresses position effects when it is bound to the transgene. We next asked whether the transgene continuously depends on MTF activity to suppress the variegating position effect, i.e., whether withdrawal of zinc results in resumption of silencing at the rate seen in uninduced cells. Four clones were selected and maintained continuously in 80 μM zinc; at intervals, we removed aliquots and continued growth in medium without supplemental zinc. If the transgene depends on continuous MTF activity to maintain expression, then GFP expression should silence promptly when zinc induction is withdrawn, and this should occur no matter how long the cells have been maintained in zinc.

Although immediate silencing on withdrawal from Zn was observed in three of the clones, the fourth (c6177) displayed a very different pattern (Fig. 4*A*). In c6177, maintenance in Zn resulted in a continuous increase in the stability of expression after withdrawal of Zn (Fig. 4*A*). c6177 cells rapidly silence transgene expression if removed from 80 μM Zn shortly after induction is initiated. After several weeks of culture in Zn, however, c6177 cells no longer required Zn induction to maintain GFP expression. At intermediate time points, the c6177 cells are a mixture of two populations, one that silences promptly on withdrawal of zinc and another that maintains GFP expression for a longer period (not shown). In this genomic site, therefore, continuous binding of the transcriptional activator seems to result in an epigenetic modification that persists when the activator is no longer present.

Induction of MTF both suppresses transgene silencing and increases the level of transgene expression (see above). Thus, we asked whether the long-term effects of MTF induction on silencing in c6177 were accompanied by a stable increase in expression level, as measured by the level of GFP in individual cells. Prolonged induction did not result in a change in the induced level of GFP expression. When zinc induction was withdrawn, GFP expression (in cells FACS-gated as GFP$^+$) fell to a level that was the same whether the cells had been maintained in zinc for a brief period or up to 6 weeks (Fig. 4*B*). Thus, prolonged binding of a transcriptional activator can create an epigenetic change that suppresses a variegating (silencing) position effect; this change may persist when the activator is no longer present, but suppression of a stable position effect (on transcription rate) does not persist. This finding implies that the two position effects are not directly linked.

Discussion

Position effects reflect the long-range influence of chromatin structure and may silence transcription or alter its efficiency. All higher eukaryotic genes are flanked by and interspersed with other genes and repetitive sequences including retroelements, whose chromatin structure may exert position effects (32, 33). Thus, it has been widely postulated that transcription units must possess elements capable of insulating them from repressive effects of chromatin in their vicinity (7, 8). We have shown here that position effects can be suppressed by the metallothionein transcription factor MTF; similar transcriptional activators bind to all active genes, and so this finding implies that chromatin insulation may be accomplished by the regulatory sequences considered as enhancers and upstream promoter elements. Significantly, at one of the genomic sites we studied, the activator was required only transiently; silencing continued to be suppressed after withdrawal of the activator.

The effect of the transcriptional activator is essentially to make each integration site more like the others. MTF makes the

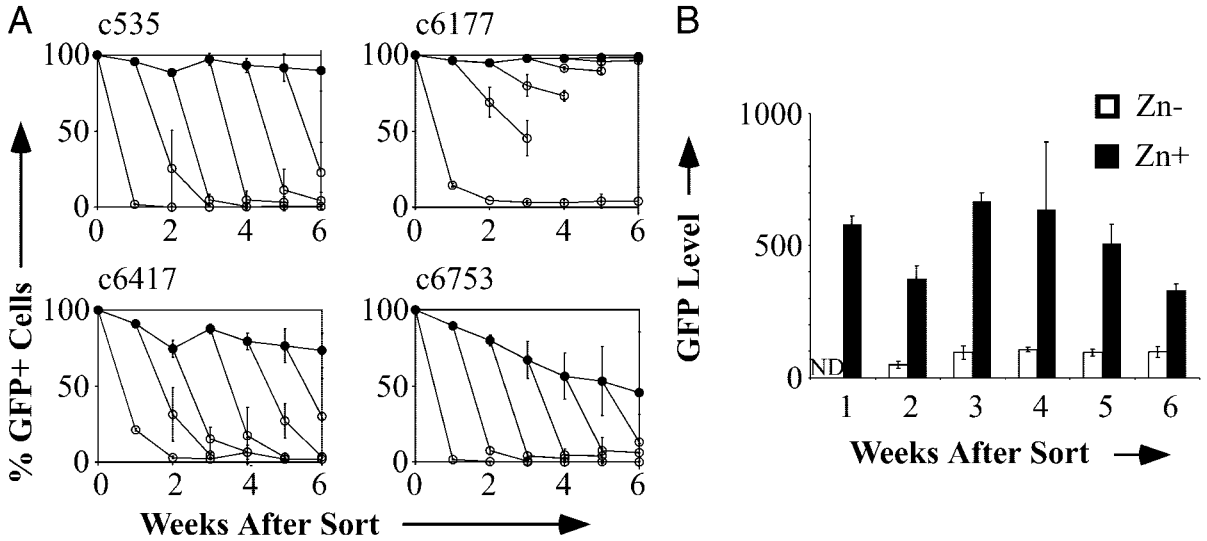

Fig. 4. Prolonged induction of MTF can result in persistent suppression of variegating position effect after induction is withdrawn. (*A*) Clones were induced with 80 μM zinc for 24 h, and GFP$^+$ cells were sorted twice and split into five aliquots without (○) and five aliquots with (●) 80 μM zinc. These cultures were passaged for 6 weeks and FACS-scanned at weekly intervals to assay the proportion of GFP$^+$ cells. Every week, the induced aliquots were split into medium with and without zinc sulfate. The aliquots in medium alone were passaged for 2 weeks and FACS-scanned twice to measure GFP$^+$ cells. Clone 6177 cells initially require constant MTF to maintain GFP expression. However, over time this clone progressively loses its dependence on zinc induction. In contrast, three other clones never lose their requirement for induction to maintain expression. (*B*) The level of GFP expression in GFP$^+$ c6177 cells does not increase as cells acquire an increasingly stable GFP expression phenotype. Filled bars show the average level of GFP expression among five aliquots in medium with 80 μM Zn; these cultures correspond to those assayed in the filled circles in *A*. Open bars show the average level of GFP expression in gated GFP$^+$ cells from the five aliquots that have been cultured 1 week in medium without zinc, after the indicated number of weeks in medium with 80 μM zinc. Although prolonged induction with zinc results in persistent suppression of silencing (see *A*), it does not have an equivalent effect on the GFP expression level; the level always falls when zinc is withdrawn.

transgene more likely to express (and continue expressing) and also makes expression level more similar from site to site. This is consistent with MTF contributing to establishment of a domain that is relatively independent of the influence of flanking chromatin. The effect is only relative, but the transgene is small and contains only a single group of MREs. Presumably, a gene in its native context is adapted to have as many transcription factor binding sites as necessary for it to be expressed appropriately.

Because it does not rely on expression of the transgene, the method we used to derive these cell lines reveals a spectrum of position effects that includes extremely repressive integration sites; we find that in most integration sites the GFP reporter is silent unless MTF is induced. The common strategy of deriving clones by selection for drug resistance may require relatively stable and high-level expression for growth of a cell clone; this may affect results even if the selectable marker is not on the same plasmid as the reporter, because separate plasmids are typically cointegrated when transfected together and so will be subject to the same position effects (34, 35). In this regard, it is interesting that clones with high basal levels of expression display little increase with induction. These clones are the most likely to survive drug selection, which would explain why previous studies have found that expression level in similar clones is not increased by the presence of enhancer elements (17, 31). The same correlation between HIV basal promoter activity and Tat induction has been observed in another cell culture model (6).

The similarity between the insulation accomplished by MTF in this system and that noted with elements such as *scs/scs′*, *Gypsy/Su(hw)*, *Fab-7*, and the chicken β-globin 5′HS4 element (9–12) may reflect common mechanisms based on alterations of chromatin structure. MTF and *Su(hw)* are transcriptional activators and can be expected to drive histone acetylation. *Scs* and *scs′* are promoters (36). The chicken β-globin 5′HS4 element was identified through its disruption of chromatin structure, and is characterized by high-level histone acetylation (7). It has also been observed that synthetic transcriptional activators can suppress centromeric position effect variegation (21).

Perhaps the most remarkable finding in this study was that silencing continued to be suppressed after withdrawal of prolonged induction in c6177, whereas expression level promptly fell to the lower uninduced level no matter how long induction had been maintained. This implies that the effects of MTF on silencing and transcription rate are mediated by different mechanisms and that silencing can be suppressed by some persistent epigenetic modification. A similar phenomenon has been shown to be mediated by Polycomb and trithorax group proteins binding at the *Fab-7* "cellular memory module" in the *Drosophila* bithorax complex (37). Hyperacetylation of histone H4 is an epigenetic mark for the activated *Fab-7* state, which persists through mitosis.

Our results imply that chromatin insulation, as assayed by suppression of position effects, is a property of transcriptional activators. Higher eukaryotic genomes contain very large amounts of constitutively silent chromatin, and activity of any locus may require that the repressive effects of such structures be counteracted. We have postulated previously that the large numbers of activating elements in many higher eukaryotic loci may contribute to this function (20, 23, 38). This duality of activator function is particularly evident in the analyses of the β-globin locus control region (LCR), which consists of binding sites for erythroid-specific and ubiquitous transcription factors (39). Deletion of the LCR from the native β-globin locus results in a dramatic reduction in transcription rate but does not result in silencing of the locus (38, 40). Presumably, the presence of multiple transcription-factor binding sites throughout the native locus contributes to the insulation of the locus in the absence of the LCR, thus revealing the LCR effect on rate. Similarly, addition of LCR elements to genes integrated at ectopic genomic sites that reside in an open chromatin structure increases transcription rate (41). In contrast, at repressive genomic sites, elements of the LCR are required to suppress transgene silenc-

ing (17, 18), thus revealing an insulator role of the LCR in the establishment and maintenance of the active state.

The concept of insulator elements arose as an explanation for the existence of independently regulated domains. To date, very few elements that seem to act as insulators have been identified, and of those only the yeast elements clearly function as insulator elements in their native contexts (14, 15). On the other hand, transcriptional activating elements are an invariant feature of active genes. Thus, elements that behave solely as chromatin insulators may be less common than previously thought and may have evolved in highly specialized contexts. Such elements exhibit only the ''barrier'' or insulator activity that is also a part of enhancer function. Enhancers/activators, in contrast, also exert a more complex activity that includes insulation. Their ability to create and maintain an independent expression domain implies that such elements play an important role in shielding active loci from repression.

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