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Chromosomal position effects in chicken lysozyme gene transgenic mice are correlated with supression of DNase I hypersensitive site formation

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ABSTRACT

The complete chicken lysozyme gene locus is expressed copy number dependently and at a high level in macrophages of transgenic mice. Gene expression independent of genomic position can only be achieved by the concerted action of all cis regulatory elements located on the lysozyme gene domain. Position independency of expression is lost if one essential cis regulatory region is deleted. Here we compared the DNase I hypersensitive site (DHS) pattern formed on the chromatin of position independently and position dependently expressed transgenes in order to assess the influence of deletions within the gene domain on active chromatin formation. We demonstrate, that in position independently expressed transgene all DHSs are formed with the authentic relative frequency on all genes. This is not the case for position dependently expressed transgenes. Our results show that the formation of a DHS during cellular differentiation does not occur autonomously. In case essential regulatory elements of the chicken lysozyme gene domain are lacking, the efficiency of DHS formation on remaining cis regulatory elements during myeloid differentiation is reduced and influenced by the chromosomal position. Hence, no individual regulatory element on the lysozyme domain is capable of organizing the chromatin structure of the whole locus in a dominant fashion.

INTRODUCTION

Transcriptional activation of transgenes is frequently impaired by chromosomal position effects. Irrespective of the copy number of integrated DNA fragments at one specific chromosomal site highly variable expression levels per transgene copy are often observed (1). In addition, the correct temporal and spatial pattern of expression can be disturbed. An incorrect expression pattern is a heritable feature of one transgenic line which varies with the chromosomal integration site of the injected DNA (1,2). Ectopic transgene expression is most likely caused by the influence of juxtaposed cis regulatory elements active in other tissues than the one the transgene is normally expressed (3,4). Chromatin mediated position effects on integrated transgenes are mostly suppressive and are caused by neighbouring chromatin configurations unfavorable for transgene expression (1). A special case, observed both in Drosophila and Yeast, is the cell autonomous transgene suppression after insertion into the vicinity of telomeric or centromeric sequences. Integration of a transgene or a translocated endogenous gene into these chromosomal regions leads to a variegated expression phenotype. Here, depending on the extent of heterochromatin spreading into euchromatic regions, transgene expression levels vary from cell to cell (5,6).

Unpredictably variable, position dependent transgene expression is one of the major obstacles of gene regulation studies in transgenic mice. A considerable effort has therefore been made to identify and characterize cis regulatory elements leading to tissue specific, high level and position independent expression of transgenes. Experiments along this line have first led to the discovery of the so called locus control regions (LCRs) which mediate these properties (7–10). Later on it became clear that the functional unit of gene expression is most likely the entire chromatin domain of a gene locus, which by definition contains the complete set of cis regulatory elements. Each cis regulatory DNA element including subelements of LCRs is responsible for a distinct subaspect of gene regulation, whereby tissue specificity of expression is only one aspect of the global control. We and others have shown, that deletion of essential cis regulatory elements from a gene locus can lead to changes in the expression pattern during development, to loss of copy number dependency of expression or both (11,12).

The chicken lysozyme gene is an example for a differentiation dependently expressed gene in the myeloid lineage of the hematopoietic system. Expression gradually increases during macrophage differentiation and reaches its highest level in the mature activated macrophage (13–15). Along with increasing transcription the chromatin in the lysozyme locus is extensively reorganized (14,16). All tissue specific DHSs present in the 5′ flanking region of the gene locus collocate with cis regulatory

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elements responsible for certain regulatory features of the gene (17–19). At the multipotent myeloid progenitor stage and in other nonexpressing tissues only a DHS at a silencer element located −2.4 kb upstream of the transcriptional start site (20) is present. At the myeloblast stage a low level of lysozyme mRNA is detected and DHSs are formed at the distal enhancer located at −6.1 kb and at the promoter. Later in differentiation at the promonocytic stage a second DHS appears at the medial enhancer located at −2.7 kb. Parallel with the formation of transcription factor complexes over the medial enhancer and with the transcriptional level increasing, the DHS at the silencer element disappears (14,16).

In expressing cells of the oviduct and macrophages the gene is located in a domain of general DNase I sensitivity (21,22). The complete lysozyme locus spanning the entire DNase I sensitive domain is expressed copy number dependently and at a high level in macrophages of transgenic mice (23). Deletion of one enhancer region within the whole lysozyme locus does in most cases not impair tissue specificity of expression (11). However, constructs which carry only one enhancer region show highly variable expression levels per gene copy.

To gain insight into the molecular basis of chromosomal position effects we investigated the chromatin of position independently and position dependently expressed lysozyme gene clusters in macrophages of transgenic mice. We find that DHS formation on cis regulatory elements is directly correlated to transcription. We therefore do not find an enhancer element on the lysozyme gene domain which by itself is capable of organizing the chromatin structure of the gene locus in a dominant fashion, thus acting independently of the genomic position of the transgene. We conclude, that the efficiency of the cooperative formation of transcription factor complexes during development and their combined stability are the rate limiting steps in locus activation.

MATERIAL AND METHODS

Cell culture and transgenic mice

HD11 cells were grown in standard Iscoves medium containing 8% fetal calf serum (FCS) and 2% chicken serum (CS). Transgenic mice carrying various chicken lysozyme domain constructs (11) were kept as homozygous lines in our own mouse colony. Primary macrophages were prepared from the peritoneal cavity of transgenic mice four days after Thioglycolate injection as described (23). For each construct cells from 15 to 20 mice were taken in culture in standard Iscove's medium supplemented with 10% FCS and 10% L-cell conditioned medium for 16 hrs (23). Embryonic fibroblasts were prepared from mouse embryos 12 days after fertilization by removing head, internal organs like the fetal liver and blood containing tissue. The remaining tissue was digested with 0.25% collagenase (Sigma), 20% FCS in PBS for 1.5 hrs, single cells were plated directly on cell culture plates in standard Iscove's medium, 10% FCS and left in the incubator for 16 hrs.

DHS analysis

Mapping of DNase I hypersensitive chromatin sites was performed as described with some variations (24). Nuclei were prepared by homogenizing cultured cells in buffer 1 (0.15 mM Spermin, 0.5 mM Spermidin, 15 mM Tris−HCl pH 7.5, 60 mM KCl, 15 mM NaCl, 2 mM EDTA, 0.5 mM EGTA and 500 mM Sucrose), followed by a wash with buffer 2 (buffer B, 0.5% Triton X-100) and buffer 3 (like buffer 1 but with 350 mM Sucrose). Aliquots of 2×10^7 nuclei in buffer D were frozen in liquid nitrogen and stored at −80°C. After thawing, nuclei were centrifuged and resuspended in buffer 4 (0.15 mM Spermin, 0.5 mM Spermidin, 15 mM Tris−HCl pH7.5, 60 mM KCl, 15 mM NaCl, 0.2 mM EDTA, 0.2 mM EGTA). Buffer 4 was added to a final volume of 500µl, DNase I (Boehringer) was added and the digestions were started by addition of 4 mM $MgCl_2$ and 2 mM $CaCl_2$. Incubations (15 min, 4°C) were stopped by the addition of 10 µl 0.5M EDTA. HD11 nuclei were digested with DNase I concentrations of 0, 3, 6, 12 and 24 units/ml, nuclei of mouse macrophages were digested with 0, 2, 4, 5, 7 and 13 units/ml if not otherwise indicated. Genomic DNA was isolated as described (24) and restricted with EcoRI. The amount of DNA loaded was adjusted with respect to the copy numbers of the individual mouse lines, so usually 30 µg of fragmented HD11 DNA and between 3 µg and 37 µg of fragmented mouse DNA per slot were analysed on 1% agarose gels. DNA was transferred to Biodyne B (Pall) membranes and hybridized with the probes 1 (0.29kb HindIII fragment), 2 (1.65kb HindIII fragment) and 3 (0.8kb HindIII−XbaI fragment). A PstI−BstNI fragment covering exon 2 of the endogenous mouse lysozyme gene was used as a probe recognizing a 8.8 kb EcoRI fragment of the mouse lysozyme M gene and a 3.3 kb fragment of the mouse lysozyme P gene (not shown in our analysis). The hypersensitive site seen in our experiments most likely corresponds to DHS 11 of the mouse M gene (25) which generates a fragment of approximately 6.7 kb in length.

In situ hybridization

Peritoneal macrophages from transgenic mice were plated directly on 3-aminopropyl-triethoxysilane coated slides and left in the incubator with cell culture medium (see above) for four hours. Slides were fixed in 4% paraformaldehyde, 5 mM $MgCl_2$ in PBS and washed subsequently with 70% ethanol and 100% ethanol. In situ hybridization with ^{35}S labelled antisense riboprobes was performed essentially as described (26). Riboprobes specific for chicken lysozyme were prepared from linearized plasmid pBS lys1 carrying the chicken lysozyme cDNA in the Bluescript KS (M13−) vector (27). A probe specific for transcripts of the mouse lysozyme M gene was prepared from linearized plasmid pMLC1 carrying the mouse lysozyme M cDNA in the Bluescribe (M13+) vector (28).

RESULTS

Mouse lines used for chromatin analysis

We analyzed the DHS pattern in the 5'untranslated region of the lysozyme gene in ten mouse lines carrying different constructs in which various parts of the chicken lysozyme locus were deleted (Figure 1 A and B). Mice carrying construct XS which contains the full complement of cis regulatory elements without the 5'and 3'flanking matrix attachment regions express the lysozyme gene at a high level and in a copy number dependent manner in macrophages, exactly as it has been observed with the complete chicken lysozyme gene locus (11,23). Construct dXK carries a deletion of the upstream −6.1 kb enhancer region, in construct dSS the region around the −2.7 kb medial enhancer has been removed. Construct XSdSS carries a deletion of the −2.7 kb enhancer region, in addition the 5'and the 3'matrix attachment regions (29,30) were removed. All three latter constructs are subject to suppressive chromosomal position effects leading to

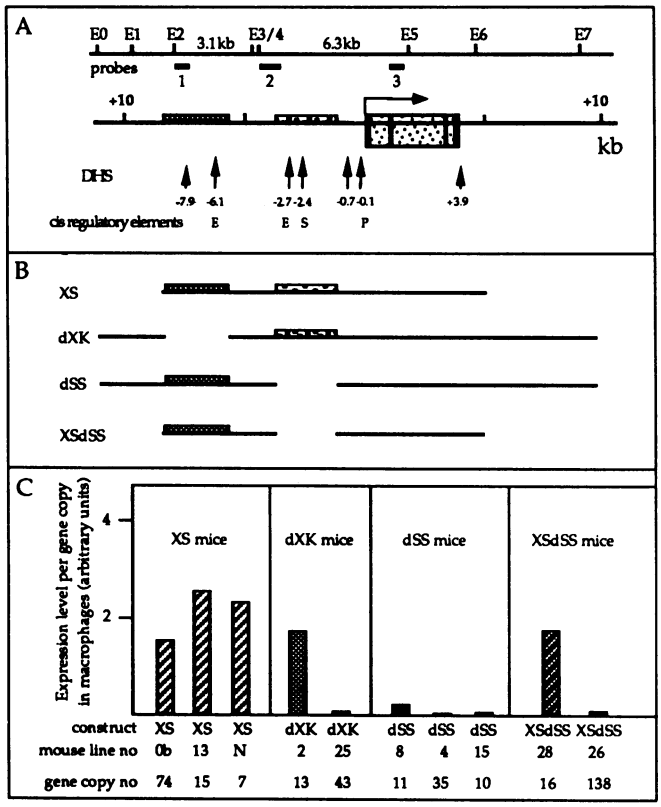

Figure 1. Mouse lines carrying deletion mutants of the chicken lysozyme gene domain used for chromatin analysis. (**A**) Map of the lysozyme locus showing the location of the EcoRI sites (E0 to E7) and the location of the probes used for DHS mapping. The middle panel shows the coding region indicated by the stippled box with the exon sequences drawn as black bars and the transcriptional start site as horizontal arrrow. The positions of the DHSs mapped in macrophages are shown as vertical arrows, constitutive DHSs are indicated as smaller arrows. The position of the upstram enhancer region and the medial enhancer region are indicated as stippled boxes. The nature of the cis regulatory elements and their position relative to the transcriptional start site are shown in the lowest panel. E: enhancer element; S: silencer element; P: promoter elements. (**B**) Constructs used to generate transgenic mice. The position of the upstream and the medial enhancer are indicated as stippled boxes, which were left out in case of a deletion. (**C**): Expression levels per gene copy in macrophages for 10 different mouse lines. Only transcripts originating from authentic lysozyme promoter sequences as defined by S1 nuclease protection analysis with a probe spanning the transcriptional start site were considered (11,31).

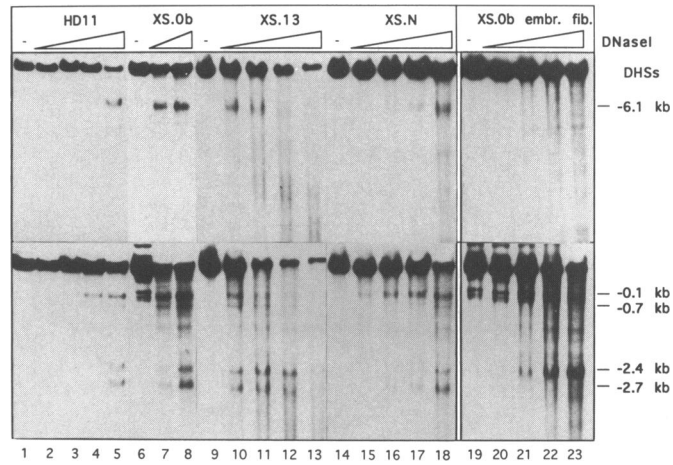

Figure 2. Chromatin analysis of three mouse lines expressing the lysozyme gene in a position independent manner. (**A**):Nuclei were prepared from chicken HD11 promacrophage cells (lanes 1–5, 50 μg DNA/slot) and from macrophage cells of mouse lines XS 0.b (lanes 6–8, 10 μg DNA/slot), XS 13 (lanes 9–13, 25 μg DNA/slot) and XS N (lanes 14–18, 30 μg DNA/slot) and digested with increasing amounts of DNase I. HD11, XS 13 and XS N nuclei were DNase I digested with the concentrations described in Methods. XS 0.b nuclei were digested with 0, 5 and 10 units/ml. Genomic DNA was prepared, restricted with EcoRI and transferred to a nylon membrane. The filter was hybridized with probe 1 (upper panel) and subsequently with probe 3 (lower panel). (**B**) The same analysis as described in (A) was performed for embryonic fibroblasts prepared from day 12 embryos from mouse line XS 0.b.

a highly variable expression level per gene copy in macrophages of different transgenic mouse strains (11). For chromatin analysis, we selected three XS mouse lines which express similar lysozyme mRNA levels per gene copy (Figure 1 C, XS mice no 0b, 13, N), two dXK lines one of which expresses a high (mouse line no 2) and one which expresses a very low mRNA level per gene copy (mouse line no 25). In addition we investigated the chromatin of three dSS lines which in average show a very low expression level per gene copy. One mouse line expressed the transgene at a low but significant level (mouse line no 8), whereas two mouse lines express the gene at a very low level per gene copy (mice no 4 and 15). XSdSS mouse lines carry the corresponding construct without flanking regions, one of them (mouse line no 28) expresses a high level of lysozyme mRNA per gene copy whereas the other one does not (mouse line no 26). All transcripts measured originate from the lysozyme promoter as we have shown by S1 nuclease protection assays with a probe spanning the transcriptional start sites (31), thus excluding readthrough transcription from a promoter located in flanking host sequences (11).

In macrophages of all transgenic mice DHS formation correlates with transcription

The chromatin analysis of three XS mouse lines is shown in Figure 2. Nuclei were prepared from peritoneal macrophages of each mouse line and digested with increasing amounts of DNase I, followed by Southern blot analysis with EcoRI restricted genomic DNA (Figure 2, lanes 6 to 18). Probe 1 hybridized to a 3.1 kb EcoRI fragment carrying the −6.1 kb enhancer (upper panel), probe 3 recognizing a 6.3 kb fragment which carries the −2.7 kb enhancer region and the promoter (lower panel) as indicated in Figure 1 A. For comparison, the DHS pattern formed in lysozyme expressing HD11 chicken promacrophage cells is shown (Figure 2, lanes 1 to 5). As control for a nonexpressing tissue (data not shown) we determined the DHS pattern from embryonic fibroblasts prepared from day 12 embryos of mouse line XS 0.b and (Figure 2, lanes 19–23). The comparison of the DHS pattern between the XS mouse lines demonstrates that in each of them the same DHS pattern as in HD11 cells is formed. All DHSs are formed at the authentic position and with the correct relative intensity. In nonexpressing cells only the DHS at the silencer element is present, exactly as in nonexpressing cells of the chicken (24,32). A different picture emerges in case of those mouse lines in which transgene expression is subject to suppressive position effects. In mouse line dXK 2 the expression level per transgene copy is high (Figure 1 C) and the DHS pattern is undistinguishable from the pattern seen in XS mice with probe

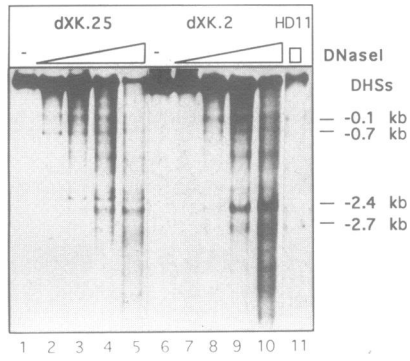

Figure 3. Chromatin analysis of dXK mouse macrophages which express the lysozyme gene at a low level per gene copy (dXK 25, lanes 1–5, 8 μg DNA/slot) or at a high level per gene copy (dXK 2, lanes 6–10, 27 μg DNA/slot). Lane 11: HD11 cells (20 μg DNA/slot). The experiments were performed as described in Figure 2 except that the filter was hybridized with probe 3 only. dXK 2 and dXK 25 nuclei were digested with 0, 3, 6, 9 and 16 units/ml and HD11 nuclei with 24 units/ml.

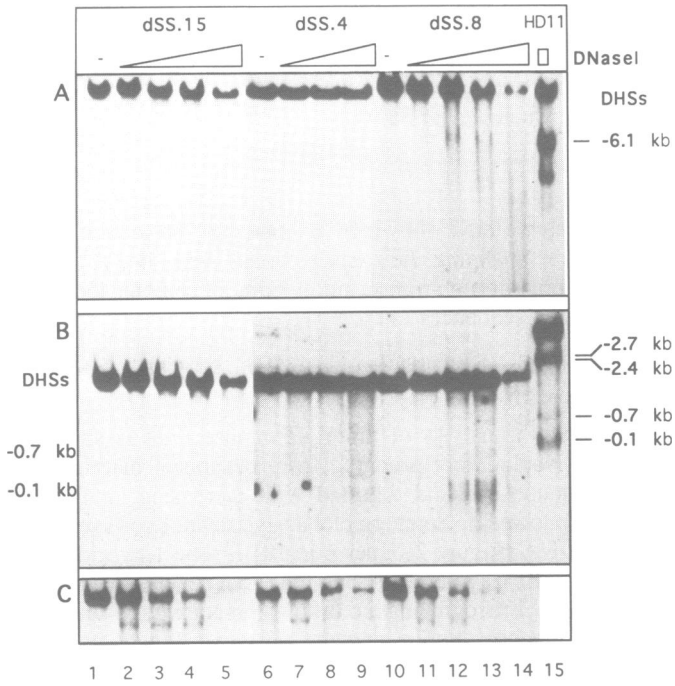

Figure 4. Chromatin analysis of dSS mouse macrophages expressing the lysozyme gene at a low level (dSS 15, lanes 1–5, 37 μg DNA/slot; dSS 4, lanes 6–9, 10 μg DNA/slot) or an intermediate level (dSS 8, lanes 10–14, 30 μg DNA/slot). Lane 15: HD11 cells (20 μg DNA/slot). The experiment was performed as described in Figure 2, the filters were hybridized with probe 1 (**A**) probe 2 (**B**) or a probe recognizing exon 2 of the endogenous mouse lysozyme gene (**C**). DNase I digestions of dSS 15, 4 and 8 nuclei were performed with the same DNase I concentrations as described in Methods, only for the dSS 4 digestion the second step (2 units/ml) was omitted. HD11 nuclei were digested with 24 units/ml. For the detection of mouse lysozyme DHSs the filters with dSS 15 and dSS 8 DNA were stripped and reprobed with the exon 2 probe of the endogenous mouse lysozyme gene. For the dSS 4 mouse line a new filter with 30μg DNA/slot of the same DNase I digestion series as used for chicken lysozyme DHSs detection was prepared and hybridized with the mouse lysozyme exon 2 probe.

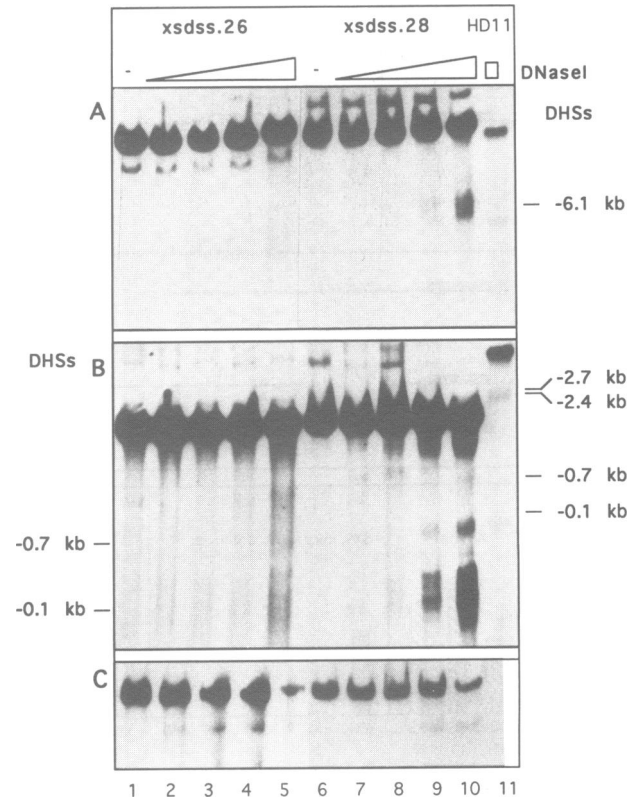

Figure 5. Chromatin analysis of XSdSS mouse macrophages expressing the lysozyme gene at a low level (XSdSS 26, lanes 1–5, 3 μg DNA/slot) or at a high level (XSdSS 28, lanes 6–10, 20 μg DNA/slot). Lane 11: HD11 cells (30 μg DNA/slot). The experiment was performed as described in Figure 2, the filters were hybridized with probe 1 (**A**) probe 2 (**B**) or a probe recognizing exon 2 of the endogenous mouse lysozyme gene (**C**). DNase I digestions of XSdSS 26 and XSdSS 28 nuclei were performed with the same amounts of enzyme as described in Methods. HD11 nuclei were digested with 16 units/ml. For the detection of mouse lysozyme DHSs the filter with the XSdSS 28 DNA was reprobed with the exon 2 probe. For the XSdSS 26 mouse line a new filter with approximately 25μg DNA/slot of the same DNase I series as used before was prepared and hybridized with the mouse lysozyme exon 2 probe.

3 (Figure 3, lanes 6–10). In contrast, in the inactive mouse line dXK 25 containing the same DNA construct the DHS pattern is hardly visible (Figure 3, lanes 1–5). Mouse line dSS 8 which expresses the gene at low but significant level per gene copy, do form DHSs over the −6.1 kb enhancer and the promoter (Figure 4, lanes 10–14). Mouse lines 4 and 15 do not show any (Figure 4, lanes 1 to 5) or only very weak (Figure 4, lane 6–9) DHS formation. The same correlation holds true for XSdSS mice. In the highly expressing mouse line DHSs are formed, whereas in the low expressing mouse line DHS formation is very weak (Figure 5). To control DNase I digestion of nuclear DNA, we analysed the same DNase I digestion series with a probe hybridizing to a fragment of the endogenous mouse lysozyme gene which is expressed at a high level in macrophages and which carries several DHS (25). Figure 4 C and Figure 5 C demonstrate, that a mouse lysozyme DHS can be detected in all five DNase I digestion series, independent of the intensity of DHS signals from the transgene.

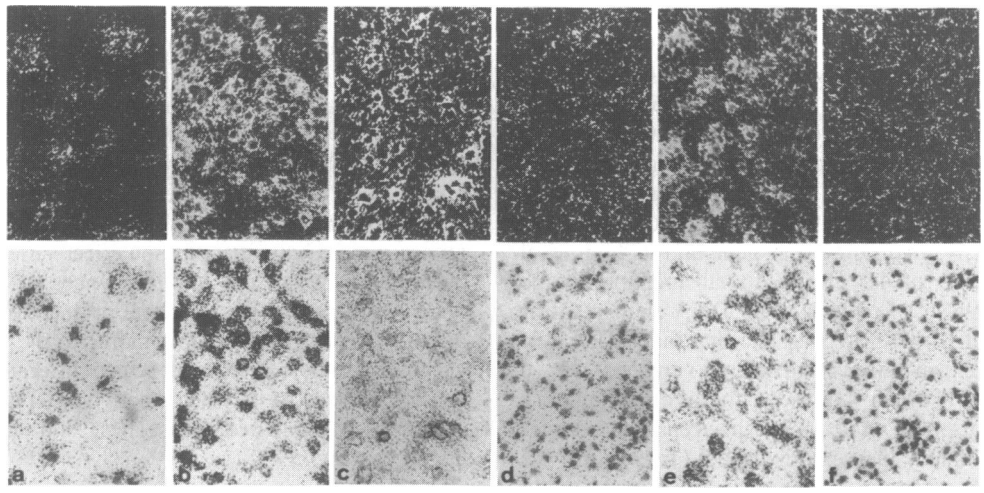

Figure 6. mRNA expression analysis by *in situ* hybridization to macrophage populations from mouse strains XS 0.b (**a**, **b**), dXK 2 (**c**), dXK 25 (**d**), XSdss 28 (**e**) and XSdSS 26 (**f**). The cells were hybridized with the mouse lysozyme antisense probe in (a) and with the chicken lysozyme antisense probe in (b−f). The upper panel shows the dark field photographs, the lower panel to the corresponding bright field photographs. Note that there is some experimental variation in the signal intensities in both the expression of the endogenous gene (a) as well as the transgene (c, e), which is most likely due to cell clustering.

All cells within a given macrophage population express lysozyme transgenes at similar levels

All lysozyme transgenes in the individual mouse lines are arranged in clusters at one chromosomal site (data not shown) as it is frequently observed. Two possibilities exist to explain the low level of lysozyme mRNA in some of the position dependently expressing mouse lines. One possibility would be a homogenously low transcriptional frequency in all cells of one given macrophage population. The other possibility would be a different transcriptional level in each individual cell as it is observed with transgenes being subject to position effect variegation. A low but detectable mRNA level would in this case be the result of a mixture of non expressing cells, cells expressing every gene in a given cluster and cells with intermediate stages. To distinguish between these two possibilities we analyzed transgene expression on the cellular level by *in situ* hybridization. The results of these experiments are shown in Figure 6. As control for a gene expressed in every cell we hybridized the same macrophage populations with a probe complementary to the endogenous mouse lysozyme mRNA (28). *In situ* hybridization with a chicken lysozyme antisense probe to a population of peritoneal macrophages of XS 0.b mice revealed, that every cell expressed the transgene (Figure 6 b) as it has been observed for mice carrying the entire wild type chicken lysozyme gene construct (33). The expression pattern is similar than that observed with the mouse lysozyme antisense probe (Figure 6 a). The comparison between dXK 2 mouse macrophages and dXK 25 mouse macrophages revealed, that in the dXK 2 mouse line the lysozyme gene is expressed in the same fashion as the XS 0.b construct with no significant differences in cellular mRNA distribution as compared to the mouse lysozyme control (Figure 6 c, d, respectively). The variation in signal intensities observed for both the expression of the endogenous as well as the transgene with highly expressing mouse lines is most likely due to a higher local RNA concentration at the position of cell clusters. In every individual cell of the dXK 25 macrophage population the hybridization signal intensities approach the background level determined with the chicken lysozyme sense probe (data not shown). No heterogeneities of expression are observed. The same result was found with all other mouse lines (XSdSS and dSS) investigated in this analysis (Figure 6 e, f, data not shown). Within the observed experimental variation each transgenic macrophage population expressed the transgene at similar levels in every cell.

DISCUSSION

No individual regulatory element of the chicken lysozyme locus has a dominant chromatin organizing activity

In its natural chromosomal location every gene is packaged into nucleosomal structures, whereby nucleosomes are not randomly distributed. Many transcription factors recognize chromatin templates differently than naked DNA. Some of them bind with reduced affinity or are even unable to bind to DNA organized in a nucleosome in vitro (34). Also differences in nucleosome biochemistry (for instance the presence of acetylated histones) can influence transcription factor accessibility in vivo and in vitro (35,36). It is, however, by no means clear which interactions between chromatin components and transacting factors drive the formation of active chromatin in a chromosomal environment. An attractive hypothesis is the idea of the existence of dominant cis regulatory elements responsible for the initial structural activation of a gene locus. Histone acetylation could be such a structural activation step (37). In a second step along with ongoing cellular differentiation, this structural reorganization would allow further transcription factor binding leading to full transcriptional activation (38,39). In such a model chromatin organizing activity and transcriptional stimulation would be a successive and hierarchically organized process. However, the data presented in this paper as well as other recently published experiments indicate a more complex mechanism of active chromatin formation during cellular differentiation (40). In mice carrying a copy number dependently expressed construct expression of every gene copy correlates with the presence of the correct DHS pattern. In case of the lysozyme locus the presence of only one enhancer and the promoter on a transgenic construct is not

sufficient to create an active chromatin structure at every chromosomal position. Moreover, either all DHSs are present with the correct relative frequency or they are all absent or weak, indicating, that the cis regulatory elements cooperate. Thus, instead of a hierarchical model of chromatin activation by specialized regulatory elements, chromatin reorganization is accomplished by the cooperative action of a variety of cis regulatory elements. We propose that the prerequisite for the formation of active chromatin on a transgene and its high level expression irrespective of its chromosomal location is the presence of a complete set of cis regulatory DNA elements. For one particular gene it is therefore possible that the presence of a promoter and a single enhancer is sufficient to create an active chromatin domain, in which DHSs at these cis regulatory elements (40,41) are formed, while for another gene only a larger collection of cis regulatory elements is capable to perform the same function. The structural changes in chromatin might accompany the activation of gene expression by transcription factors. They might facilitate a strong interaction between transcription factor complexes bound to upstream sequences with the promoter protein complex and in such a way assure a high constant transcription frequency. We only find strong DHS formation with simultaneously high levels of transgene mRNA in macrophage cells. However, at the present state we are unable to decide, whether chromatin reorganization at the upstream cis regulatory elements is a prerequisite for transcription or whether the onset of transcription is inseparatebly connected to DHS formation. Future transgenic mouse experiments in which promoter sequences of the lysozyme locus are altered have to show, whether DHS formation and mRNA synthesis can be uncoupled.

Suppression of lysozyme gene expression by chromosomal position effects correlates with the suppression of DNase I hypersensitive site formation

The chromosomal position effect on transgene expression is the most convincing argument for a direct influence of chromatin structure on gene expression. In Drosophila position effect variegation as a result of transgene integration into the vicinity of telomeric or centromeric regions has been used to identify chromatin components involved in transcriptional silencing by spreading heterochromatin (1). The nature of positional influences on gene expression in other chromosomal regions is less clear (42). In mouse lines that carry the full complement of lysozyme cis regulatory sequences DHSs are formed with the right relative frequency. In case of a low expression level per gene copy in mouse lines carrying constructs where one essential cis regulatory element is lacking, the DHS formation on the remaining regulatory elements is impaired. This indicates an impediment in transcription factor binding to their recognition sites. By *in situ* hybridization experiments we were able to show that the same degree of transgene repression is found in every individual cell. Hence, position dependently expressed lysozyme transgenes are not subject to position effect variegation and are most likely not repressed by telomeric or centromeric heterochromatin. All transgenic mouse lines used in this analysis carry multiple copies of the lysozyme locus and transgene expression as well as DNase I hypersensitivity is not completely absent (11). We therefore suggest, that only some gene repeats within a cluster are transcribed at a given timepoint. Although the dynamic nature of DNase I digestion makes a strictly quantitative analysis difficult, our results indicate that on some of the genes within the cluster hypersensitive sites are formed. This indicates that in a chromatin environment unfavorable for gene expression and in case one essential cis regulatory element on a transgene construct is lacking the formation of active chromatin occurs with reduced efficiency. This in turn could mean that the rate limiting step of locus activation during development is the strength of transcription factor complex assembly and the stability of assembled factor complexes. Support for this idea comes from reports which show, that position effect variegation of a transgene carrying upstream activating sequences (UAS) integrated at the yeast telomere can be overcome by increasing the intracellular dose of transcription factors binding to the UAS (43). It will be very interesting to determine, which differences in the chromatin fine structure exist between position independently and position dependently expressed transgenes, both in macrophages and nonexpressing cells.

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