The EMBO Journal vol.9 no.9 pp.2843–2848, 1990

Tissue specific and position independent expression of the complete gene domain for chicken lysozyme in transgenic mice

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Communicated by A.E.Sippel

A 21.5 kb DNA fragment carrying the entire chicken lysozyme gene locus was introduced into the germ line of mice. The fragment contains the transcribed region plus 11.5 kb 5′-flanking and 5.5 kb 3′-flanking sequences including all known *cis*-regulatory elements and the 5′ and 3′ attachment elements (A-elements) which define the borders of the DNase I sensitive chromatin domain. All sequences which adopt a DNase I hypersensitive chromatin conformation *in vivo* are present on the construct. Seven founder mice were analysed. All of these expressed chicken lysozyme RNA at high levels specifically in macrophages, as is the case in the donor species. Expression levels are dependent on the copy number of integrated genes indicating that a complete gene locus, as defined by its chromatin structure, functions as an independent regulatory unit when introduced into a heterologous genome.

Key words: chicken lysozyme locus/chromatin domain/macrophage specific gene activity/position independent expression/transgenic mice

Introduction

The chicken lysozyme gene locus is an extensively studied model for the influence of flanking DNA regions on the regulation of tissue specific gene activity. Experiments aimed at elucidating the mechanisms of differential gene activation have not been limited to the investigation of *cis*-acting elements close to the promotor region of the gene. We expected larger flanking regions to be involved in regulation since we have observed that different functional states of the gene are characterized by a different structural organization of a larger chromatin domain (for review see Sippel *et al.*, 1989).

The chicken lysozyme gene is expressed in the tubular gland cells of the oviduct under the control of steroid hormones (Schütz *et al.*, 1978). Expression of the same gene in macrophages is constitutive and is initiated at the same promotor as in oviduct cells (Hauser *et al.*, 1981; Theisen *et al.*, 1986). The lysozyme gene serves as a marker for myeloid differentiation in the haematopoietic system. The gene is progressively and very selectively activated only in late stages of macrophage differentiation (Sippel *et al.*, 1987; Cross *et al.*, 1988). The oviduct and the macrophage modes of transcriptional regulation can be correlated to alternative states of chromatin structure which develop in oviduct and myeloid cells (Fritton *et al.*, 1984, 1987). Two different sets out of a total of nine DNase I hypersensitive sites (DHS) cluster around the active transcriptional unit of the gene. It was shown that five of the seven DHS in the 5′-flanking chromatin of the gene, mark positions of tissue specific enhancer and silencer elements (Thiesen *et al.*, 1986; Steiner *et al.*, 1987; Baniahmad *et al.*, 1987; Hecht *et al.*, 1988; Sippel *et al.*, 1988). When expressed or committed for expression the transcribed sequences plus the entire cluster of DHS are embedded in a region of general DNase sensitivity spanning ~20 kb (Jantzen *et al.*, 1986; Strätling *et al.*, 1986). The 5′ and the 3′ borders of the DNase sensitive chromatin domain coincide with DNA regions which are attached to nuclear matrix material (Phi-Van and Strätling, 1988) and may be anchorage points for a looped chromatin domain. Recently we have shown that DNA elements which overlap these attachment regions (A-elements) confer *cis*-acting functions important for the transcriptional activity of the gene (Stief *et al.*, 1989). A-elements stimulate transcription of stably reinserted reporter gene 'mini-domains' and protect them from chromosomal position effects. Taken together the results of our structural and functional studies point to a model in which the lysozyme gene is organized in a looped chromatin domain, which comprises an independent regulatory unit for the control of gene activity.

Little is known about the precise role of the various *cis*-acting DNA elements in the developmentally controlled activation of the gene locus during macrophage differentiation. Expression analysis by DNA transfection into transformed haematopoietic culture cells is unable to reveal hierarchical functions of individual elements during the differentiation pathway. Due to the lack of a transgenic chicken model we decided to generate transgenic mice carrying the entire wild type chicken lysozyme gene domain including 5′ and 3′ A-elements. Here we demonstrate that cross-species gene transfer results in high level macrophage specific gene expression in the recipient mammal. We also show that the entire transferred gene locus behaves as a regulatory unit independent of its chromosomal position.

Results

Generation of transgenic mice carrying the chicken lysozyme locus

The 21.5 kb DNA fragment injected into fertilized mouse eggs contained the sequence information for the entire chicken lysozyme locus. The borders of the construct coincide with the boundaries of the domain of general DNase

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sensitivity in active chromatin (Jantzen *et al.*, 1986) including the 5′ and 3′ A-elements. Except for a 700 bp *Xba*I deletion in the middle of the second intron, no sequence changes were introduced (Figure 1). Seven founder mice were generated with copy numbers of the integrated gene ranging from two to ~70 copies per cell (Table I). Copy numbers of the transgene were calculated from quantitation of Southern blots as shown in Figure 2A. The blot was hybridized with a probe which recognizes a single 6.3 kb *Eco*RI fragment containing the 5′ part of the chicken lysozyme gene (Figure 1). To control for loading differences of mouse genomic DNA the blot was stripped and rehybridized with a *Thy-1* gene probe (Figure 2A, lower panel). Additionally, a concentration series of *Eco*RI digested chicken genomic DNA was loaded onto the gel for copy number standardization. A second blot with *Eco*RI digested transgenic mouse DNA was hybridized with the radioactively labelled entire polyIIIilys plasmid to determine the integrity of the integrated DNA (Figure 2B). All *Eco*RI fragments are present in the genome of each founder mouse (Figure 1), indicating that no major rearrangements had occurred during integeration. For all transgenic mice a novel band of 2.2 kb appears, while a 1.75 kb end fragment gives rise to a weaker signal. This new *Eco*RI fragment is most likely due to a small deletion at the very end of some of the DNA fragments before integration. The 5′ *Eco*RI site affected by this deletion is directly adjacent to one of the *Xho*I sites used to excise the insert from the plasmid vector. Thus, the 2.2 kb fragment is most likely derived from a fusion with the next tandemly inserted locus. Control digests with *Bam*HI, which has a cleavage site marking the 5′ end of the 5′ A-element located 56 bp downstream of the *Eco*RI site showed that this site was not affected by the short terminal deletion (data not shown).

Table I. Expression levels of chicken lysozyme transcripts in transgenic mice

Founder mouse no.	Copy no.[a]	RNA from (tissue)	Lysozyme signal (arb. units) 14 days exp.	Lysozyme signal (arb. units) 4 days exp.	β-actin signal (arb. units)	Normalized values[b]
1	4	Mϕ	4214	1212	2294	2437
2	2	Mϕ	3218	653	2227	1352
3	20	Mϕ	20 230	8698	2893	13 872
4	70	Mϕ	29 316	17 386	2317	34 621
5	20	Mϕ	17 075	7344	4614	7344
6	6	Mϕ	9079	2030	2454	3816
7	4	Mϕ	5246	1021	(546)	(8628)
1	4	Brain	4793	3184	(744)	(26 832)
2	2	Brain	3550	2053	2393	5379
3	20	Brain	7377	4996	2326	13 467
4	70	Brain	21 898	20 265	2606	48 757
5	20	Brain	11 853	9197	6270	9197
6	6	Brain	6262	3790	5552	4280
7	4	Brain	3546	2050	2733	4703

[a]Copy numbers were determined by densitometric scanning of different exposures of two blots performed as shown in Figure 2A. Differences in the two experiments were 10–20% for the low copy number mice and up to 30% for the high copy number mice.
[b]The lysozyme signal of the 4 day exposure of the gel shown in Figure 5 was normalized against the β-actin signal. The values in brackets indicate lanes with a β-actin signal that does not correspond to the precalibration value.

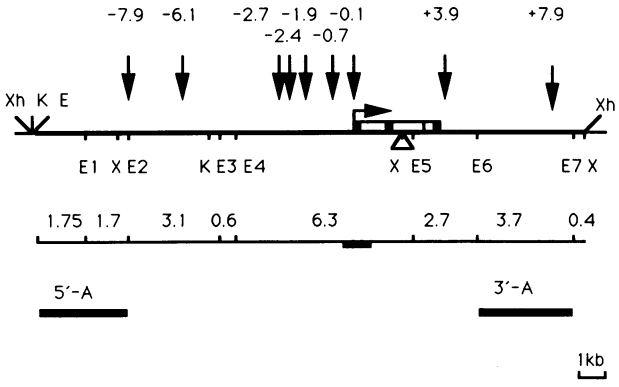

Fig. 1. Map of the construct carrying the chicken lysozyme locus. The arrows mark the position of the nine DNase I hypersensitive chromatin sites mapped previously (Fritton *et al.*, 1983, 1984). The bars below indicate the position of the 5′ and 3′ A-elements. The coding region with its intron–exon structure and the direction of transcription is indicated above the upper line. The triangle indicates the position of a 700 bp deletion. The bold line represents lysozyme sequences. The single line represents vector sequences. Restriction sites drawn above the upper line come from the polylinker, sites below the line are present on the lysozyme fragment. The lower line shows the position of *Eco*RI fragments on the lysozyme domain fragment plus the position of the p0.8 probe (stippled bar). E, *Eco*RI; K, *Kpn*I; X, *Xba*I; Xh, *Xho*I.

Tissue specific expression of the lysozyme gene in transgenic mice

The pattern of chicken lysozyme gene expression in various tissues of transgenic mice was examined using a highly

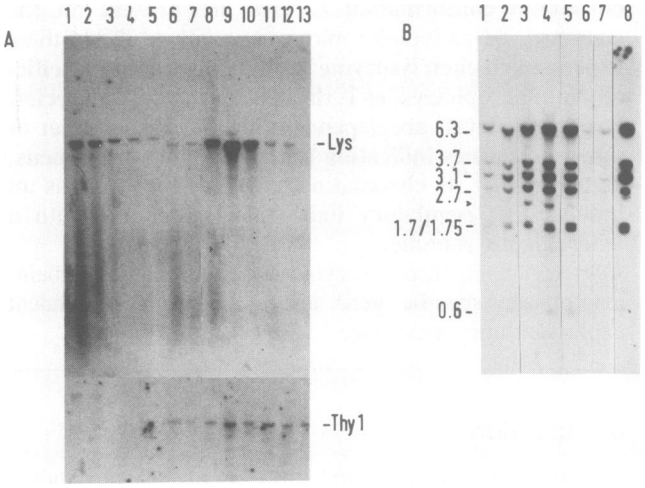

Fig. 2. Southern blot analysis of chicken lysozyme sequences integrated into the mouse genome. (**A**) 1.5 μg of mouse tail DNA was digested with *Eco*RI, subjected to electrophoresis on a 0.8% agarose gel and blotted. Different amounts of *Eco*RI restricted chicken genomic DNA were run on the same gel. The blot was probed with plasmid pUCp.0.8, which, due to the deletion, recognizes a 6.3 kb *Eco*RI band in the transgenic DNA and a 7 kb band in the chicken DNA. The filter was stripped and rehybridized with a *Thy-1* probe (lower panel). Lanes 1–5: 3.2, 1.6, 0.8, 0.4 and 0.2 μg of chicken DNA respectively. Lanes 6–12: mice nos 7, 6, 5, 4, 3, 2, 1 respectively. Lane 13: non-transgenic control. (**B**) 3–4 μg of mouse tail DNA was cut with *Eco*RI, loaded on a 0.8% agarose gel and blotted. The blot was probed with radioactively labelled plasmid pIIIilys. Lanes 1–7: mice nos 7, 6, 5, 4, 3, 2, 1 respectively. Lane 8: 10 ng of *Eco*RI digested pIIIiLys plasmid. The additional vector band runs together with the 3.1 kb band. The arrow marks the position of the novel 2.2 kb band The samples were originally loaded in a different order.

sensitive RNase protection assay (Melton *et al.*, 1984; Steiner *et al.*, 1987). The RNAs of different tissues from four transgenic founder mice plus one offspring from mouse no. 3 were analysed. All of these showed the same tissue specific expression pattern of lysozyme transcripts. The analysis of two founder mice (mouse no. 2 with four copies of the transgene, mouse no. 4 with 70 copies of the transgene) as well as the analysis of a non-transgenic control mouse are shown in Figure 3A and B. As a positive control for lysozyme expression levels RNA from the lysozyme expressing promyelocytic chicken cell line HD11/HBC1 was examined. Chicken lysozyme transcripts can only be detected in peritoneal macrophages, brain and at very low levels in the bone marrow. The presence of RNA in each sample was monitored by performing an S1 analysis with a β-actin probe (data not shown). Several observations can be made from this experiment. First, the same transcriptional start sites as in chicken cells are used in the mouse, including the minor lysozyme upstream start sites (Grez *et al.*, 1981). However, these seem to be used to a lesser extent in the mouse brain as compared with utilization in mouse and chicken macrophages. Secondly, the level of lysozyme gene transcripts per cell in mouse peritoneal macrophages, which can be isolated as a homogeneous cell population, is comparable with the level in chicken macrophage like cells (compare Figure 3A lanes 2 and 11). Thirdly, despite the high expression of chicken lysozyme under steroid control in the chicken oviduct, no RNA product of the transgene could be detected in the female sex organs of the mouse (uterus and ovary plus oviduct, Figure 3B lanes 10 and 11). This indicates that the presence of sex hormone receptors alone might not be sufficient to trigger steroid controlled expression of the gene.

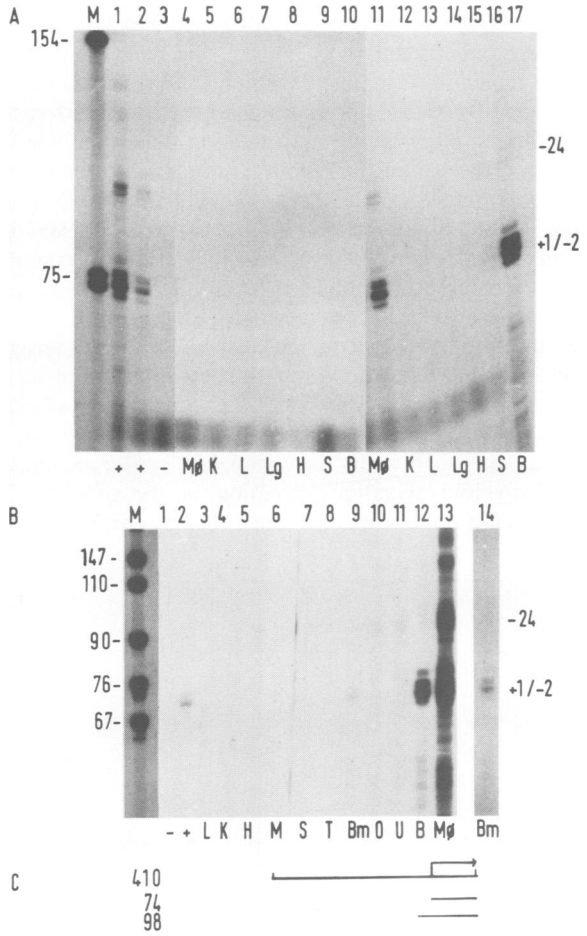

Fig. 3. Tissue specific expression of the lysozyme gene in transgenic mice. 4.5 μg RNA of various tissues of a non-transgenic mouse (**A**, lanes 4–10), mouse no. 1 (**A**, lanes 11–17) and mouse no. 4 (**B**, lanes 3–14 were subjected to an RNase protection assay with a 410 bp anti-sense probe from the lysozyme promotor region. The samples were loaded on a 7% sequencing gel. The gel was exposed to Kodak XAR 70 film for 3 weeks (**A**) or overnight (**B**). (**A**) lanes 1 and 2: 16 μg, 6 μg HD11/HBC1 RNA, lane 3: no RNA; (**B**) lanes 1 and 2: no RNA, 12 μg HD11/HBC1 RNA. M (upper panel), marker; K, kidney; L, liver; Lg, lung; H, heart; M, skeletal muscle; S, spleen; T, thymus; Bm, bone marrow O, ovary and oviduct; U, uterus; B, brain; Mϕ, peritoneal macrophages. Lane 14: longer (3 days) exposure of lane 9. (**C**) schematic drawing of the length of the probe and the expected protected fragments.

Expression of the chicken lysozyme gene in mouse macrophages differentiated in vitro

To investigate further the cell type specific expression of the chicken lysozyme gene in transgenic mice, an *in vitro* differentiation experiment using cultured primary spleen cells was performed. One spleen from a mouse belonging to a homozygous line generated from the high copy number founder mouse no. 4 (Table I) containing ~140 transgenes per cell was divided into two parts. One part was used to prepare RNA directly. The second part was dissected to prepare a cell suspension which was then cultured for 2 weeks in mouse L-cell conditioned medium. L-cells are known to secrete CSF-1, a cytokine which induces macrophage growth and differentiation (Metcalf, 1985). After 2 weeks the culture contained almost exclusively adherent macrophage-like cells. RNA was prepared from these cells and, together with the spleen RNA, was analysed for chicken lysozyme transcripts (Figure 4). A β-actin S1 analysis from aliquots of the same RNA samples was used to standardize the assay for RNA amounts (Figure 4, lower panel). It can be seen that the level of lysozyme transcripts in macrophages differentiated *in vitro* is strongly increased relative to RNA from total spleen, whose haematopoietic cells belong almost exclusively to the B-lymphoid and the erythroid lineage. The weak signal observed in total spleen RNA most likely originates from the small amount of

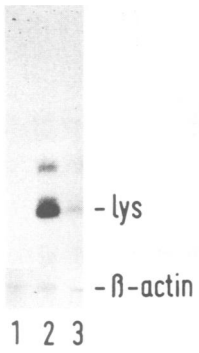

Fig. 4. Expression of the lysozyme gene in macrophages differentiated *in vitro*. 2.5 μg RNA from spleen derived *in vitro* differentiated macrophages (lane 2), spleen (lane 3) and a spleen from a non-transgenic mouse were analysed in a RNase protection assay with the lysozyme promotor probe as described. To calibrate the assay for RNA amounts an S1 analysis with a human β-actin probe was performed (lower panel). The samples were loaded onto a 7% sequencing gel, which was exposed to a Fuji RX film for 24 h.

macrophages originally present in the organ. This low level can only be measured because this homozygous mouse strain carries >100 copies of the active transgene locus per cell. Taken together with the finding that no detectable transgene expression occurs in cells of the T-lymphoid lineage in the thymus, this experiment demonstrates that chicken lysozyme gene expression in the haematopoietic system of the mouse is confined to macrophages.

Expression of the chicken lysozyme locus in the mouse is position independent

We next analysed whether the construct containing the entire lysozyme locus carries enough information to be expressed in a position independent manner, thus being independently regulated regardless of its accidental chromosomal position. We therefore compared the levels of lysozyme specific mRNA in peritoneal macrophages and in the brain of the seven founder mice carrying different copy numbers of the chicken lysozyme locus (Figure 5A and B). Since in this case specific RNA levels were compared in the same cell type of different animals, RNA concentrations were precalibrated by an S1 analysis of β-actin specific RNA as described in Materials and methods. An additional β-actin S1 assay was performed with an aliquot of the precalibrated RNA sample used for the lysozyme RNA determination (Figure 5, lower panels). Lysozyme specific RNA levels were determined by an RNase protection assay on the same precalibrated RNA samples. To ensure that excess lysozyme specific probe was used for quantitation two different amounts of HD11/HBC1 RNA were used as positive controls. The lysozyme and β-actin specific bands were scanned densitometrically and the resulting values for β-actin RNA were used to normalize the values for lysozyme RNA. The results are depicted in Table I. For two out of 14 samples (mouse no. 7, macrophages and mouse no. 1, brain) the β-actin signals do not correspond to their precalibration values, probably for trivial reasons. The resulting normalized lysozyme specific values were plotted versus the gene copy number of the transgene for each animal. It can be seen in Figure 6, that normalized RNA levels in six out of seven cases in mouse macrophages as well as in six out of seven cases in mouse brain correlate with the copy number of the transgene. For two mice in two different tissues the normalized lysozyme expression levels appear to be higher. Note however, that independent of the β-actin control, lysozyme RNA levels for all seven founder mice in macrophages parallel those in brain (Figure 5, upper panel). It can also be seen in Table I, that directly measured lysozyme expression levels for all 14 samples depend on the gene copy number, including those two cases in which titration of β-actin transcripts failed. This would mean that position independent expression of the chicken lysozyme gene in transgenic mice holds true for all seven founder mice analysed.

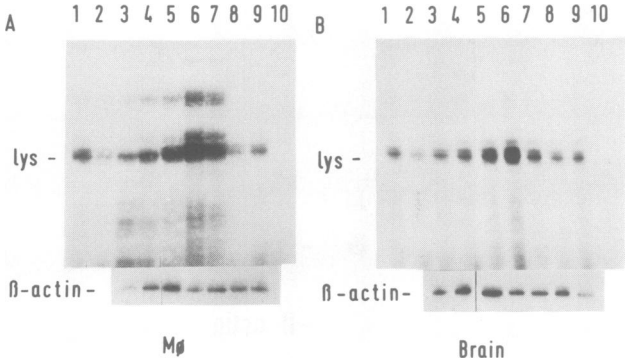

Fig. 5. Copy number dependent expression of chicken lysozyme in transgenic mice. Aliquots of peritoneal macrophage RNA or brain RNA of mouse nos 1–7 were twice precalibrated by β-actin S1 analysis. In a second round ~2 μg peritoneal macrophage RNA (**A**) or brain RNA (**B**) from mice nos 1–7 were analysed for lysozyme RNA content by an RNase protection assay. A β-actin S1 analysis performed from an aliquot of the precalibrated sample is shown in the lower panel. Lanes 1 and 2: 6 μg and 3 μg HD1/HBC1 RNA, lanes 3–9: mice nos 7–1 respectively. Lane 10: no RNA.

Discussion

Cross-species gene transfer

Here we report the generation of transgenic mice carrying the chicken lysozyme gene in their germ line. The rationale for these experiments was, on the one hand, to create a mouse model in which the developmental regulation of the gene could be studied during haematopoietic cell differentiation. On the other hand, we wanted to determine whether our previous studies of both chromatin structure and functional elements of the locus (Theisen *et al.*, 1986; Hecht *et al.*, 1988; Sippel *et al.*, 1989; Stief *et al.*, 1989) had provided sufficient information to define a complete regulatory chromosomal locus ('regulon') rather than a gene with a subset of *cis*-active regulatory elements.

Synthesis of mRNA from the chicken lysozyme transgene locus in the haematopoietic system of the mouse is restricted to macrophage cells. This result shows that the molecular mechanisms which govern lysozyme expression in the chicken, are sufficiently evolutionarily conserved to also direct myeloid specific expression in the mouse. This conservation might reflect the fact that macrophages from

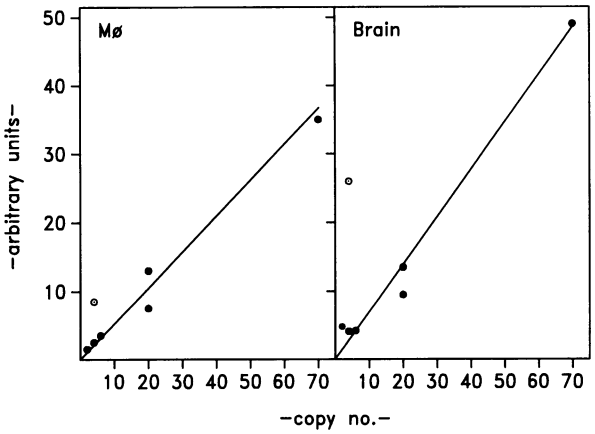

Fig. 6. Linear relationship between lysozyme gene expression and the copy number of the integrated transgene. Lysozyme and β-actin specific signals in different exposures of the experiment shown in Figure 5 were subjected to densitometric scanning. The resulting OD values for the lysozyme specific signals were then normalized against the OD values for the β-actin specific signals as shown in Table I and plotted against gene copy number. Normalized lysozyme RNA levels in macrophage RNA of mouse no. 7 and in brain RNA of mouse no. 1 are indicated by open symbols because the hybridization signals for β-actin RNA in this experiment do not correspond to their precalibration values.

all vertebrates express lysozyme as part of an ancient antibacterial defence mechanism (Ossermann, 1976). Comparative studies between cloned lysozyme genes from vertebrates show a high degree of sequence similarity for the coding region. Sequence comparisons of the immediate 5′-flanking regions of human, mouse and chicken lysozyme, however, do not show striking sequence similarities (Peters *et al.*, 1989; Cross *et al.*, 1988). Nothing is yet known about whether this observation holds true also for the more distal regions of the lysozyme loci. As has been demonstrated for the chicken gene it is to be expected that the expression of the respective mammalian genes will also be controlled by *cis*-regulatory elements distributed over an extensive chromatin domain. Most likely the combinatorial action of several conserved *trans*-acting factors directs tissue specific expression, a concept which does not necessarily lead to easily recognizable elements on the level of DNA sequence. However, all *cis*-acting regulatory elements of the chicken lysozyme gene are marked by DNase I hypersensitive sites (DHS) in chromatin (Sippel *et al.*, 1988). A specific set of enhancers and silencers, their *in vivo* activity indicated by the presence of DHS, are responsible for the cell specific expression of the gene in chicken myeloid culture cells (Theisen *et al.*, 1986; Steiner *et al.*, 1987; Baniahmad *et al.*, 1987; Sippel *et al.*, 1988). An important question for the future will be, whether the same or a subset of DHS are formed in mouse chromatin and whether the same or a subset of the *cis*-acting elements function to activate the chicken gene during differentiation of the myeloid branch of the mouse haematopoietic system. The surprising expression of the transgene in mouse brain, a tissue in which the expression of the endogenous gene cannot be detected (Cross *et al.*, 1988) and the observation that no transgene products can be detected in female reproductive tissue demonstrates that not all aspects of the complex regulation of the donor gene might function in the recipient mammal. Future *in situ* hybridization studies should reveal whether transgene expression in mouse brain is restricted to macrophages and microglia, which are cells that may be recruited to the central nervous system from circulating monocytes of haematopoietic origin (Perry and Gordon, 1987).

The transgenic locus behaves as a position independent unit

The copy number dependent expression of the chicken lysozyme gene in mouse macrophage and brain tissue indicates that our transgene construct contains all the necessary *cis*-active information to function as an independent genomic locus. The high level of expression independent of the position in the host genome is most likely the result of the combined action of functional enhancers and the 5′ and 3′ A-elements. Recently we have shown that reporter gene−lysozyme 'mini-domain' constructs, when stably integrated into the genome of chicken promacrophages in culture, expressed reporter transcripts copy number dependently and on a high level in a number of cell clones which represent individual random integration events (Stief *et al.*, 1989). The position independent expression in cultured cells depended on the simultaneous presence of flanking A-elements and an enhancer originally located at −6.1 kb (Theisen *et al.*, 1986) in the lysozyme gene region. The 'mini-domain' constructs transfected into cells produced an exponential rather than a linear relationship between copy number of integrated genes and total expression levels (Stief *et al.*, 1989). The apparent cooperativity of clustered 'mini-domains' which is not observed with the entire lysozyme locus in mice might be due to the significant difference in domain size. Additionally, it is possible that the balanced expression of re-integrated new chromosomal loops needs a combination of *cis*-acting elements not present on the 'mini-domain' construct.

High copy number dependent expression of gene constructs in transgenic mice has recently been found for constructs carrying a human β-globin 'mini-locus' and for a construct containing the human CD2 gene (Grosveld *et al.*, 1987; Greaves *et al.*, 1989). It was shown that this activity was conferred by sequences adopting a super DNase I hypersensitive conformation at 5′ and 3′ distal sites of the loci respectively. It could be demonstrated that these elements are responsible for the full activation of these two loci during development. It will also be interesting to investigate whether in these systems position independent function of the constructs depends on two types of DNA elements similar to those in the lysozyme locus or whether position independent expression can be mediated by a number of different mechanisms.

Structural studies have led to the conclusion that large parts of the eukaryotic genome are organized in looped chromatin domains which are anchored at their base to nuclear matrix or scaffold material (for review see Gasser and Laemmli, 1987). The chicken lysozyme locus belongs to one of the first vertebrate gene regions for which 5′ and 3′ attachment regions were mapped (Phi-Van and Strätling, 1988). The results of structural studies indicate that the attachment regions are superimposed on the ends of the domain of general DNase I sensitivity of active chromatin. Furthermore, gene transfer experiments show that the attachment regions function as *cis*-elements necessary for the suppression of the position effect (Stief *et al.*, 1989). These data are consistent with a model in which chromosomal loops harbour confined regulatory units for gene activity. The use of constructs capable of creating independent regulatory domains in the genome of transgenic mice should enable us to study the full impact of all DNA elements on the differentiation dependent chromatin changes which in turn are necessary for lineage specific gene activation during haematopoiesis.

Materials and methods

Construction of polylllilys

Plasmid pUCB-1X1 carrying the 5′ A-element (Phi-Van and Strätling, 1988) was digested with *Xba*I and ligated to a 10.5 *Xba*I fragment (X1−X2) from λlys31 (Lindenmaier *et al.*, 1979) carrying the 5′-flanking region of the lysozyme gene and the transcribed reigon to the middle of the second intron. The resulting construct was named pUCB-1X2. A 7 kb *Asp*718−*Xba*I (X2) fragment from λlys31 was cloned into the polyIIIi vector (Lathe *et al.*, 1987). The resulting construct pIIIiA1X2 was cleaved with *Xba*I and ligated to a 6.9 kb *Xba*I fragment (X3−X4) from λlys30 (Lindenmaier *et al.*, 1979) carrying the 3′ end of the locus including the 3′ A-element (Phi-Van and Strätling, 1988). The resulting construct was named pIIIiA1X4. The construct pUCB-1X2 was cleaved with *Asp*718, the 7 kb *Asp*718 fragment carrying the 5′ A-element plus 5′-flanking sequences up to the *Asp*718 site was isolated and ligated into pIIIiA1X4 restricted with *Asp*718. The resulting plasmid pIIIilys contains the lysozyme gene locus from −11.7 kb to +9.0 kb which can be excised by cleavage with *Xho*I, *Not*I or *Sfi*I.

Transgenic mice

The 21.5 kb *Xho*I fragment containing the entire lysozyme gene locus was purified from vector sequences as described (Kollias *et al.*, 1986). Injection into fertilized oocytes from CBA × C57bl mice and the identification of

transgenic animals by analysis of tail DNA were performed as described (Brinster *et al.*, 1985; Southern, 1975). In order to eliminate mice chimeric for the injected gene, DNA from liver, brain, spleen and lung was tested for the presence of the lysozyme gene by Southern blotting. Copy numbers of the transgene were determined by densitometric scanning of different exposures of Southern blots from *Eco*RI digested genomic DNA of transgenic mice. The filters were successively hybridized with promotor-containing plasmid pUCp0.8 (Jung *et al.*, 1980) which recognizes a single 6.3 kb *Eco*RI fragment on the transgenic lysozyme locus and with a *Thy-1* probe (Grosveld *et al.*, 1987) to normalize for loading differences. *Eco*RI digested chicken genomic DNA served as standard for single copy gene intensity. The difference in genome size between mouse and chicken was taken into account for the calculation. Founder mice no. 3 and no. 4 carrying 20 and 70 copies, respectively, of the transgene were bred before expression analysis in order to generate mouse strains homozygous for the chicken lysozyme locus.

RNA preparation and precalibration

RNA from various tissues were prepared with LiCl/urea (Auffray and Rougeon, 1979). RNAs from cultured cells and from primary macrophages were prepared by lysing the cells in 6 M guanidinium/HCl, 10 mM Tris, pH 7.5. After three phenol/chloroform extractions and ethanol precipitation, nucleic acids were analysed for the presence of intact RNA by gel electrophoresis and used for analysis. Peritoneal macrophages from founder mice (usually $2-5 \times 10^6$ cells per mouse) yielded only $\sim 15-20$ μg nucleic acids. For the exact comparison of lysozyme specific transcript levels in macrophages and brains of different founder mice by RNase protection assay as shown in Figure 5, samples were twice precalibrated with an S1 analysis of β-actin and diluted accordingly. The level of β-actin transcripts was assumed to be the same in the same cell type of different animals. S1 protection assays were performed as described (Weaver and Weissmann, 1979) using as a probe an excess of an end-labelled 320 bp *Ava*I fragment from the plasmid pHFβA1 (Gunning *et al.*, 1983) carrying the human β-actin cDNA. Hybridization was carried out overnight at 55°C, digestion with 100 U S1 nuclease per assay was performed for 2 h at 30°C. Digestion yielded a protected fragment of $\sim$100 bp.

Titration of lysozyme specific transcripts

RNase protection assays using a 410 bp anti-sense transcript from a *Nco*I cleaved pBS plasmid carrying a 530 bp *Bst*NI fragment from the lysozyme promotor region (-461 bp to $+68$ bp) were performed as described (Melton *et al.*, 1984; Steiner *et al.*, 1987). A third S1 analysis of β-actin from one tenth of the precalibrated RNA solution was performed to verify the RNA concentrations.

Cell cultures

HD11/HBC1 cells (Beug *et al.*, 1979) were grown in standard Iscove's medium (Gibco) supplemented with 8% fetal calf serum and 2% chicken serum. Mouse L-cells were grown in standard RPMI 1640 medium supplemented with 10% fetal calf serum. Primary macrophages from mice were harvested by peritoneal flush with PBS. They were cultured in RPMI 1640 plus 20% L-cell conditioned medium for 2–3 days. Spleen derived macrophages were obtained by dissecting the spleen tissue, culturing the cells in RPMI 1640 plus 20% L-cell conditioned medium for 2 days and removing the non-adherent cells by washing with PBS. Macrophage colonies were obtained after 2 weeks in the above described culture media.

Acknowledgements

We thank L.Runkel, K.-H.Klempnauer, A.Hecht and A.Müller for helpful comments. C.B. would like to thank Georgina Lang. This work was supported by grants of the Deutsche Forschungsgemeinschaft (SFB 229, A2), by Fonds der Chemischen Industrie and by the Medical Research Council. During part of the time C.B. was a fellow of the Deutsche Forschungsgemeinschaft.

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Received on February 13, 1990; revised on June 11, 1990