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PROSPECTS

Dynamic Chromatin: The Regulatory Domain Organization of Eukaryotic Gene Loci

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Abstract It is hypothesized that nuclear DNA is organized in topologically constrained loop domains defining basic units of higher order chromatin structure. Our studies are performed in order to investigate the functional relevance of this structural subdivision of eukaryotic chromatin for the control of gene expression. We used the chicken lysozyme gene locus as a model to examine the relation between chromatin structure and gene function. Several structural features of the lysozyme locus are known: the extension of the region of general DNAaseI sensitivity of the active gene, the location of DNA-sequences with high affinity for the nuclear matrix in vitro, and the position of DNAaseI hypersensitive chromatin sites (DHSs). The pattern of DHSs changes depending on the transcriptional status of the gene. Functional studies demonstrated that DHSs mark the position of cis-acting regulatory elements. Additionally, we discovered a novel cis-activity of the border regions of the DNAaseI sensitive domain (A-elements). By eliminating the position effect on gene expression usually observed when genes are randomly integrated into the genome after transfection, A-elements possibly serve as punctuation marks for a regulatory chromatin domain. Experiments using transgenic mice confirmed that the complete structurally defined lysozyme gene domain behaves as an independent regulatory unit, expressing the gene in a tissue specific and position independent manner. These expression features were lost in transgenic mice carrying a construct, in which the A-elements as well as an upstream enhancer region were deleted, indicating the lack of a locus activation function on this construct. Experiments are designed in order to uncover possible hierarchical relationships between the different cis-acting regulatory elements for stepwise gene activation during cell differentiation. We are aiming at the definition of the basic structural and functional requirements for position independent and high level gene expression. The result of these experiments will have important consequences for random gene transfer with predictable and reproducible expression of transgenes.

Key words: chicken lysozyme gene, loop organization of chromatin, locus control region, locus boundary element, position independent expression

In recent years an increasing number of studies were performed in order to elucidate the molecular mechanisms of cell type and cell stage specific gene activation. The general aim of the analysis was to identify and characterize cis-active DNA-sequences and trans-acting protein factors involved in gene regulation. In most of the experiments transient DNA transfer into cells in culture or in vitro transcription systems were used and therefore the chromosomal context in which transcriptional activation normally takes place in the cell was not considered. However, with increasing evidence for the influence of chromatin structure on gene expression it becomes necessary to study the activity of genes in their natural nuclear environment. The role of chromatin structure in gene regulation has then to be examined by stable chromosomal reinsertion of gene constructs followed by functional and structural analysis. The chicken lysozyme gene offers attractive features to study the role of chromatin in eukaryotic gene regulation. The lysozyme gene is one of the major egg-white protein genes and is expressed in the tubular gland cells of the chicken oviduct under the control of steroid hormones [Schütz et al., 1978]. Transcription from the very same gene in macrophages is initiated at the same promoter as in oviduct cells [Hauser et al., 1981; Theisen et al., 1986] but does not respond to steroid hormones. The gene is progressively and selectively activated only in late stages of macrophage differentiation. Thus, it can serve as a model for developmentally controlled gene activation in the myeloid branch of the hematopoietic system [Sippel et al., 1987]. In search for the

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basis of the differential regulation of the gene in the various cell types, it was found that alternative chromatin structures develop in oviduct, myeloid, and non-expressing cells. In this overview we summarize recent results of studies about the function of a chromatin domain in cell-stage and cell-type specific gene activation.

LYSOZYME GENE CONFINED IN A STRUCTURAL CHROMATIN DOMAIN

The overall organization of eukaryotic chromatin is the result of the packaging of the genetic material in several levels of higher order structures. The DNA is first wrapped around histone octamers connected by histone H1-covered linker DNA regions [Richmond et al., 1983]. This 10 nm "beads on a string" fiber is again wound up into a 30 nm solenoid filament, which represents a more compact structure with six nucleosomes per turn [McGhee et al., 1980]. Pictures of lampbrush chromosomes as well as of metaphase and interphase chromatin spreads of histone depleted nuclei suggest consecutive loops as the next level of chromatin organization [Gall, 1956; Paulson and Laemmli, 1977; Vogelstein, 1980]. At their base such loops appear to be attached to either chromosomal protein scaffolds or nuclear matrix material, respectively. Besides facilitating the high compaction of DNA in the eucaryotic nucleus, functional aspects of the loop organization might be even more important. DNA in eucaryotic nuclei must have a dynamic conformation allowing local processes like DNA-repair and transcription to occur. For theoretical reasons it was predicted that DNA must be topographically compartimentalized into independent units to permit local diversity of function [Cook, 1973; Gasser and Laemmli, 1987; Nelson et al., 1986; Jackson, 1986]. Such functional units might be congruent with a loop structure. Questions arose: how are genes organized with respect to these loops, and what is the functional relevance of the loop structure of chromatin for the expression of genes?

To answer these questions, the structural features of one specific gene locus had to be investigated and had to be set into correlation with its transcriptional status. One characteristic structural feature of active genes is their preferential sensitivity towards digestion with DNAaseI compared to their relative insensitivity in the inactive, nontranscribed state [Weintraub and Groudine, 1976; Garel and Axel, 1976]. A support for the chromatin domain model was the finding that DNAaseI sensitivity is not restricted to the coding region of active genes but extends into their flanking chromatin and gradually drops towards the very 5'- and 3'-end of each locus. This phenomenon has been described for the chicken ovalbumin gene cluster [Alevy et al., 1984], the chicken lysozyme gene [Jantzen et al., 1986], and the human apolipoprotein B gene [Levy-Wilson and Fortier, 1989]. The size of the DNAaseI sensitive domain is specific for every gene investigated so far and may contain several, coordinately regulated genes as exemplified for the chicken ovalbumin and the human β-globin gene cluster [Lawson et al., 1980; Forrester et al., 1986].

The extension of the DNAaseI sensitive domain of the active chicken lysozyme gene was mapped to be 20 to 24 kb [Jantzen et al., 1986] gradually terminating around −10 kb and +10 kb relative to the transcription start as schematically outlined in the top half of Figure 1. Distal to both transition regions in oviduct nuclei "condensed" chromatin of more than 10 kb in length extended in either direction. When vertebrate genes are located inside dynamic chromatin domains, analogous to the puffed appearance of highly active chromosomal regions of giant polytene chromosomes of Dipterian flies, several questions arise immediately:

1. Which mechanisms initiate the "opening" of a specific chromatin domain? Is the process of chromatin decondensation a separate process prior to the initiation of transcription?
2. Which specific cis-regulatory signals function as punctuation marks to specify the sizes of individual chromatin domains?
3. Once an open chromatin domain is generated, which boundary mechanism prevents the spreading of the chromatin decondensation into neighbouring regions?

S/MARS, A-ELEMENTS, AND BOUNDARY FUNCTIONS

It has previously been noticed that looped DNA in metaphase chromosomes and interphase nuclei when microscopically analyzed after histone extraction is attached to nuclear matrix material [Paulson and Laemmli, 1977; Vogelstein, 1980]. In the chicken lysozyme locus the 5'- and 3'-border regions of the DNAaseI sensitive domain collocate with DNA-sequences having an increased binding affinity to nuclear matrix material in vitro [Phi-Van and Strätling,

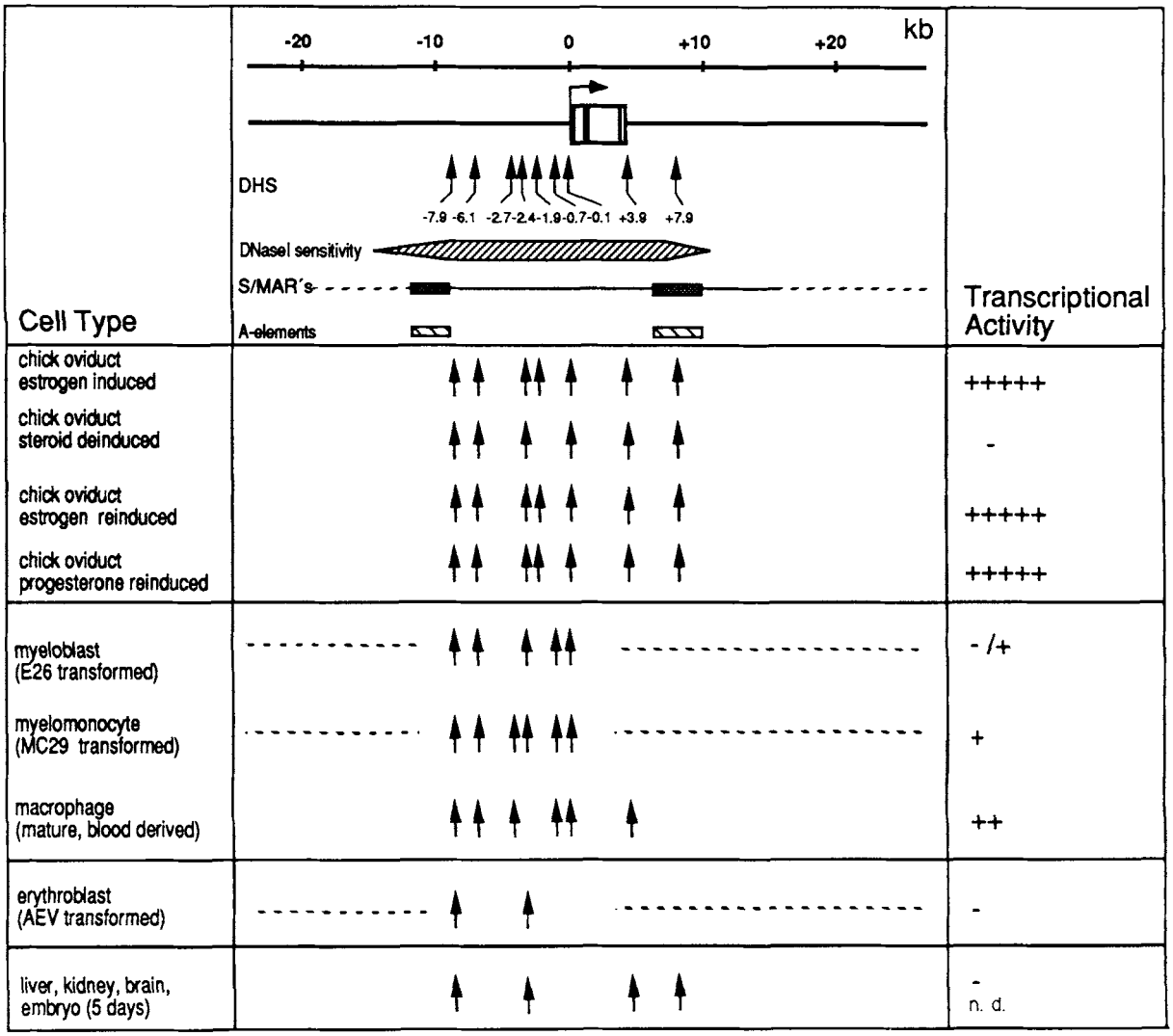

Fig. 1. The chromatin domain of the chicken lysozyme gene. The actively transcribed chicken lysozyme gene with its four exons and three introns (top panel; filled and open bars, respectively) resides in a 22 kb chromatin domain of elevated DNAaseI sensitivity (hatched double arrow). The only scaffold associated or matrix attachment regions (S/MARs; stippled bars) around the gene are found at the edges of the DNAaseI sensitive domain and collocate with the A-elements (widely hatched bars). Cell-type specific transcriptional activity of the gene and its mode of regulation (lower panels) correlate with distinct patterns of DNAaseI hypersensitive sites (DHS; vertical arrows). The positions of the DHSs relative to the transcriptional start site are given in the top panel of the figure. Dashed lines indicate chromatin regions not analyzed for the presence of DHSs or S/MARs.

1988; Fig. 1, top panel]. DNA-elements having this activity are called scaffold- or matrix-attachment regions (S/MARs). The same collocation of S/MARs with the border regions of the DNAaseI sensitive domain has been described for the human apolipoprotein B gene [Levy-Wilson and Fourier, 1989]. S/MARs which flank transcriptional units have been mapped in other genes [for review: Gasser and Laemmli, 1987; Bode and Maass, 1988], albeit only in the case of the two genes mentioned above the correlation to DNAaseI sensitive domains is hereto made. It is tempting to speculate that the S/MARs found at the end of DNAaseI sensitive domains serve as structural elements defining the base of a chromosomal loop and in addition may be involved in the functional separation of adjacent domains. To test this, an assay had to be developed, which linked the presumed structural features of the border elements to the function as boundaries of regulatory units. In respect to this we made use of the fact that transgenes

randomly inserted into the chromosome frequently underly the so-called "position effect," a phenomenon operationally defined as the influence of neighbouring genomic regions on the level and specificity of transgene expression. The position effect was repeatedly employed in so-called "enhancer-trap" experiments, in which reporter genes containing only a weak promotor were integrated randomly into the genome in the hope of getting close to a regulatory element. Developmentally regulated genes were identified by screening a large number of transgene insertions for a desired phenotype of expression [O'Kane and Gehring, 1987]. We speculated that boundary elements would insulate an enclosed transcriptional unit from either positive or negative position effects, thereby causing each unit to have the same level of activity, regardless of its position in the chromosome. This in turn means that the expression level should be directly correlated to the copy number of randomly inserted transgenes.

We therefore transfected so-called "minidomains" containing the lysozyme promoter, the −6.1 kb lysozyme enhancer (see Table I), and a CAT reporter gene flanked by two 5′-border elements into chicken promacrophage cells in culture [Stief et al., 1989]. In transient transfection assays no difference between constructs with or without border elements could be seen. A different picture emerged when these constructs were stably integrated into the genome and gene expression was studied in single cell derived clones each representing an independent genomic integration event. Here, the presence of border elements had a twofold effect on gene expression: on the one hand, an over tenfold stimulation of expression was observed; on the other hand, expression levels became directly dependent on the copy number of integrated transgenes characteristic for each cell clone. Cell clones carrying constructs lacking border elements show no correlation between copy number of integrated DNA-constructs and reporter gene activity, demonstrating the deregulating influence of neighbouring chromatin at random sites of genomic insertion. As could be seen in our clonal position effect assay, border elements, which due to their attachment function were named A-elements, insulated enclosed transcription units from the influence of random neighbouring chromatin. By buffering the position effect A-elements represent a new type of cis-regulatory DNA sequence. Their stimulatory activity is only apparent when analyzed in chromosomal context and not in transient transfection assays, which discriminates them from classical enhancers. However, A-elements exert their full activity only when they are part of a complete transcriptional unit. In cell clones containing DNA-constructs in which only the lysozyme promoter was flanked by A-elements and the −6.1 enhancer was not present, trans-

TABLE I. Characteristics of Some Regulatory Switches in the Chicken Lysozyme Gene Domain

Regulatory element	DHS in cell type	Preferentially active in tested cell type	Identified trans-factors	
			Family	Ref.[a]
−6.1 kb enhancer (early)	Oviduct All myeloid cells	All myeloid cells	NF-1 AP-1 (jun/fos)	1
−2.7 kb enhancer (late)	Monocytes Macrophages	Monocytes	Pu (ets) AP-1 (jun/fos)	2
−2.4 kb silencer	Oviduct Myeloblasts Monocytes Non-expressing cells	Myeloblasts Monocytes	TR (c-erbA)	3
−1.9 kb HRE	Oviduct		Steroid receptors PR, GR, (ER)	4
−0.1 kb promoter	Oviduct All myeloid cells	All myeloid cells	PR, GR	5

[a]References: 1. Grewal et al. [1991], submitted; 2. Grussenmeyer et al., in preparation; 3. Baniahmad et al. [1990]; 4. Hecht et al. [1988]; 5. Renkawitz et al. [1984].

genes were not expressed in a copy number dependent manner [Stief et al., 1989].

A stimulatory activity has recently also been found for S/MARs flanking the human β-interferon gene [Mielke et al., 1990]. Bordering DNA elements flanking the two *Drosophila* melanogaster heat shock genes (Hsp70) at the cytogenetic locus 87A7 have recently been shown to mediate position independent regulation of randomly integrated reporter gene constructs [Kellum and Schedl, 1991]. These elements, however, show no affinity for nuclear matrices in vitro [Udvardy et al., 1985]. These examples indicate that elements with a boundary function blocking long distance regulatory effects indeed exist. It is, however, not clear yet how the observed boundary function of the various identified elements relates to chromosome structure. It is possible that A-elements which confine regulatory units for transcription are only a subgroup of a broader family of genomic elements which subdivide chromosomes into functional units of higher order chromatin organization.

DNAaseI HYPERSENSITIVE SITES ARE CONFINED WITHIN DNAaseI SENSITIVE DOMAIN AND INDICATE PRESENCE OF REGULATORY ELEMENTS

Nucleosomal arrays are occasionally interrupted by DNAaseI hypersensitive sites (DHSs), preferably in the chromatin of active gene loci. DHSs mark the position of short 50 to 400 bp nucleosome free chromatin regions in which sequence specific non-histone DNA binding proteins have access to their recognition motifs [McGhee et al., 1981; Emerson and Felsenfeld, 1984]. Within an area of 50 kb analyzed up to nine DNAaseI hypersensitive sites cluster around the coding region of the chicken lysozyme gene. Irrespective of the cell type analyzed, all are located within the limits set by the active chromatin domain of general DNAaseI sensitivity (Fig. 1, top panel). The region in which DHSs occur seems to be constant in size, independent of the transcriptional status of the gene, adding another structural feature in support of the domain organization of eukaryotic gene loci.

Different subsets of DHSs in the chromatin of the chicken lysozyme gene locus (Fig. 1, lower panel) are found in the oviduct, myeloid cells, or non-expressing cells [Fritton et al., 1984, 1987]. Transient transfection studies were performed, in which various sections of the 5′-flanking region of the lysozyme gene fused to reporter genes were analyzed. These experiments showed for five DHSs a functional role in the control of lysozyme gene regulation [Sippel and Renkawitz, 1989; Sippel et al., 1989]. The features of the different cis-regulatory elements are summarized in Table I. For example, in the oviduct a DHS 1.9 kb upstream of the transcription start appears after primary induction of the organ with estrogen; it disappears when the hormone is removed and reappears when one member of the four different classes of steroids is readministered [Fritton et al., 1984]. Parallely, in transient transfection studies it could be demonstrated that DNA sequences of the −1.9 kb DHS harbour a steroid response element [Hecht et al., 1988].

Other examples are the DHSs at −2.4 kb and −2.7 kb. These DHSs respond to signals appearing in the progressive differentiation of myeloblast cells towards the mature macrophage. In early monocytic cells, where lysozyme transcription is low, the −2.4 kb DHS is predominant, while in mature macrophages only the −2.7 kb DHS is present [Sippel et al., 1988]. By transient transfection experiments it could be shown that the −2.4 kb DHS harbours a silencer element while the −2.7 kb DHS harbours a macrophage specific enhancer [Baniahmad et al., 1987, 1990; Müller et al., 1990]. From the switch in hypersensitivity from −2.4 kb to −2.7 kb during macrophage differentiation it is suggested that the −2.4 kb silencer is responsible for suppression of the promotor in early myeloid-, oviduct-, and non-expressing cells, whereas in more mature stages of macrophages the silencer is inactive and transcription of the gene gets activated by the enhancer at −2.7kb.

Generally it can be stated from these results that:

1. the activity of the different cis-regulatory elements is reflected in the chromatin structure;
2. the structural status of the chromatin changes with the differential expression of the gene; and
3. cell-type and cell-stage specific expression results from the combined function of different regulatory elements.

DEVELOPMENTALLY CONTROLLED ACTIVATION OF CHICKEN LYSOZYME LOCUS: THE CONCEPT OF LCR-FUNCTION

The full regulatory potential of cis-active elements during cell differentiation cannot be studied in cells in culture. Due to the lack of true in vitro cell differentiation systems transgene con-

structs are best studied in transgenic organisms. This most rigorous test makes it possible to follow transgene behaviour through the entire ontogeny of the organism in every possible cell type at every stage of cell differentiation. To examine whether the chicken lysozyme locus contains the full complement of all cis-active sequence information we generated mice carrying the entire, structurally defined lysozyme chromosomal domain in their germ line [Bonifer et al., 1990]. The injected 21.5 kb construct carried sequences starting upstream of the 5'-A-element and ending downstream of the 3'-A-element without any significant sequence changes compared to the wild type locus. Seven founder mice were generated which carried two to seventy copies of the lysozyme locus per cell. Analysis of lysozyme transcript distribution and level in different mouse tissues shows that the chicken transgene behaved as an independent regulatory unit in each of the seven mice regardless of its random position in the host genome. Chicken lysozyme mRNAs in mouse hematopoietic cells are restricted to macrophage cells as in the donor animal. The transcription level is comparable to the transcript level in chicken macrophages. Transcription in the mouse proceeds from the same promotor used in chicken cells and transcription levels show a direct correlation to the copy number of integrated transgenes. The consistently correct behaviour of the transgene with respect to cell type specificity and level of expression is most likely the result of the transfer of the gene with its entire regulatory unit ("regulon"). It is obvious that such a unit must contain not only the coding sequences, but also sequences controlling the transcriptional activation of the locus in a temporarily correct way as well as elements suppressing the deregulating influence of neighbouring chromatin. That this is indeed the case was demonstrated by the analysis of transgenic mice carrying a construct in which the A-elements and the upstream −6.1 enhancer region were deleted [Bonifer et al., 1991]. In transgenic mice containing the transgene locus from −5.4 kb to +5.1 kb chicken lysozyme expression levels in macrophages were generally very low and copy number dependent expression was lost (Fig. 2). Some mice did not express lysozyme mRNA in macrophages at all.

These results are reminiscent of experiments where the expression of transgenes that lacked a so-called "Locus Control Region" (LCR) was analyzed in transgenic mice. DNA elements with a dominant control function in gene expression have first been described in the 5'-end of the human β-globin gene cluster. The presence of these sequences is necessary for high level, cell-type specific and position independent expression as well as for correct temporal activation of the globin genes in transgenic mice [Grosveld et

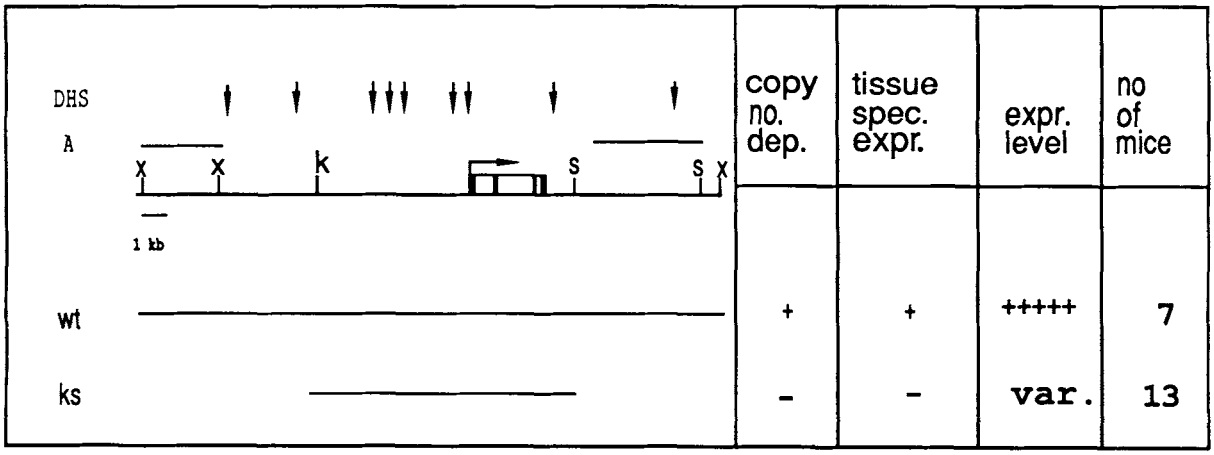

Fig. 2. Expression of chicken lysozyme gene constructs in transgenic mice. The left panel shows a schematic map of the chicken lysozyme locus indicating the gene with its intron-exon structure and the transcription start (horizontal arrow). The vertical arrows mark the position of the nine DHSs. The position of the A-elements is indicated by a horizontal line above the map (A). The lines below the map indicate the two constructs analyzed in transgenic mice. Restriction sites: k = KpnI; S = SmaI; X = XbaI. Results of the analysis of transgene expression are shown in the right panel. Expression of the entire gene locus (wt) is copy number dependent, occurs consistently at high level, and is macrophage specific independent of its position in the genome, while in the deletion mutant (ks) macrophage expression levels are either very low or absent, are not copy number dependent, and tissue specific expression depends on the position of chromosomal integration.

al., 1987; Enver et al., 1990]. The β-globin LCR collocates with a set of four developmentally stable DNAaseI hypersensitive sites [Tuan et al., 1985; Forrester et al., 1986]. The DNA elements around two of those sites have enhancer activity in transfection assays [Collis et al., 1990]. Similar to the lysozyme locus, also the β-globin cluster, including active and inactive genes as well as nontranscribed flanking sequences, comprises a continuous domain of DNAaseI sensitive chromatin. The "open" chromatin structure appears to be dependent on the presence of at least parts of the LCR, as can be deduced from chromatin analysis of mutant globin genes [Dhar et al., 1989; Forrester et al., 1990]. For this reason it is possible that LCRs are a new type of cis-active DNA element necessary for the cell-type specific activation of an entire locus. LCRs will have to function in the course of cell differentiation prior to later regulatory events and therefore they are distinctly different and superordinate elements compared to mere transcriptional enhancers. However, no experiments have up to now been performed to demonstrate a chromatin organizing activity of the β-globin LCR directly in order to decide whether or not LCR elements have a cause and effect relationship with the overall chromatin structure.

Several observations indicate that LCR elements are found not only in the human β-globin locus, but are present also in other differentially expressed genes. LCRs may be masterswitches being the first DNA elements that respond to differentiation signals appearing during development, thereby playing an essential role in the commitment of a gene towards expression. Sequences with LCR-activity were also found downstream of the human CD2 gene [Greaves et al., 1989], far upstream of the human α-globin gene cluster [Higgs et al., 1990], and downstream of the chicken β-globin gene [Reitman et al., 1990]. In the case of the chicken lysozyme locus the following observations, besides the above mentioned experiments with transgenic mice, point to the −6.1 kb enhancer region having the possible LCR-function. The activity of this enhancer is indicated by the appearance of a strong DHS at −6.1 kb, which is the sole lysozyme specific DHS besides the −0.1 kb site at the promoter, that is strictly present in all potentially active cell types (oviduct and myeloid cells), regardless of whether the gene is transcribed or not [Sippel et al., 1988; see also Fig. 1]. In addition, this DHS is present in all transformed myeloid cell lines representing various differentiation stages of myeloid precursor cells even when the transcriptional activity of the lysozyme gene is still very low (Fig. 1). This is in contrast to the DHS at the −2.7 kb late enhancer, which appears only late in macrophage differentiation and is not present in the oviduct. Future experiments with transgenic mice will have to show whether or not the −6.1 kb enhancer region on its own or in cooperation with other cis-regulatory elements is capable of directing the correct temporal and spatial expression pattern of injected chicken lysozyme constructs.

IMPLICATIONS FOR STABLE GENE TRANSFER

Currently the true influence of chromatin organization on eukaryotic gene regulation appears to be difficult to study because structure and expression of any DNA construct integrated randomly into the cellular genome is unpredictably influenced by the position effect. One possibility to overcome this experimental problem is the replacement of gene sequences at their natural location by homologous recombination [Capecci, 1989]. Gene targeting by homologous recombination is, however, not possible in cases for which no cellular selection system is available. In this case vector systems have to be designed, which overcome the position effect by other means. It is obvious that a more detailed understanding of the basis of the position effect is needed to define the basic requirements for the position independent and repeatedly correct expression of transgenes.

From our studies of the chicken lysozyme gene a congruence between a chromatin domain, most likely a chromosomal loop, and a regulatory unit for gene activity is suggested. A regulatory unit would need certain cis-regulatory elements like boundary elements (A-elements), a locus control region (LCR), as well as promoter and enhancer sequences to allow transgene function to be independent of the respective random chromosomal environment.

Figure 3A shows in a schematic way our current model of transcriptionally active and inactive chromatin organization as it is viewed from the experimental evidence described in this article. According to this model inactive chromatin is in a compact, DNAaseI insensitive state and is not accessible to transcription factors. In active chromatin the anchoring points of the loop have not changed, but inside the boundaries, as a result of LCR action, the compact structure has

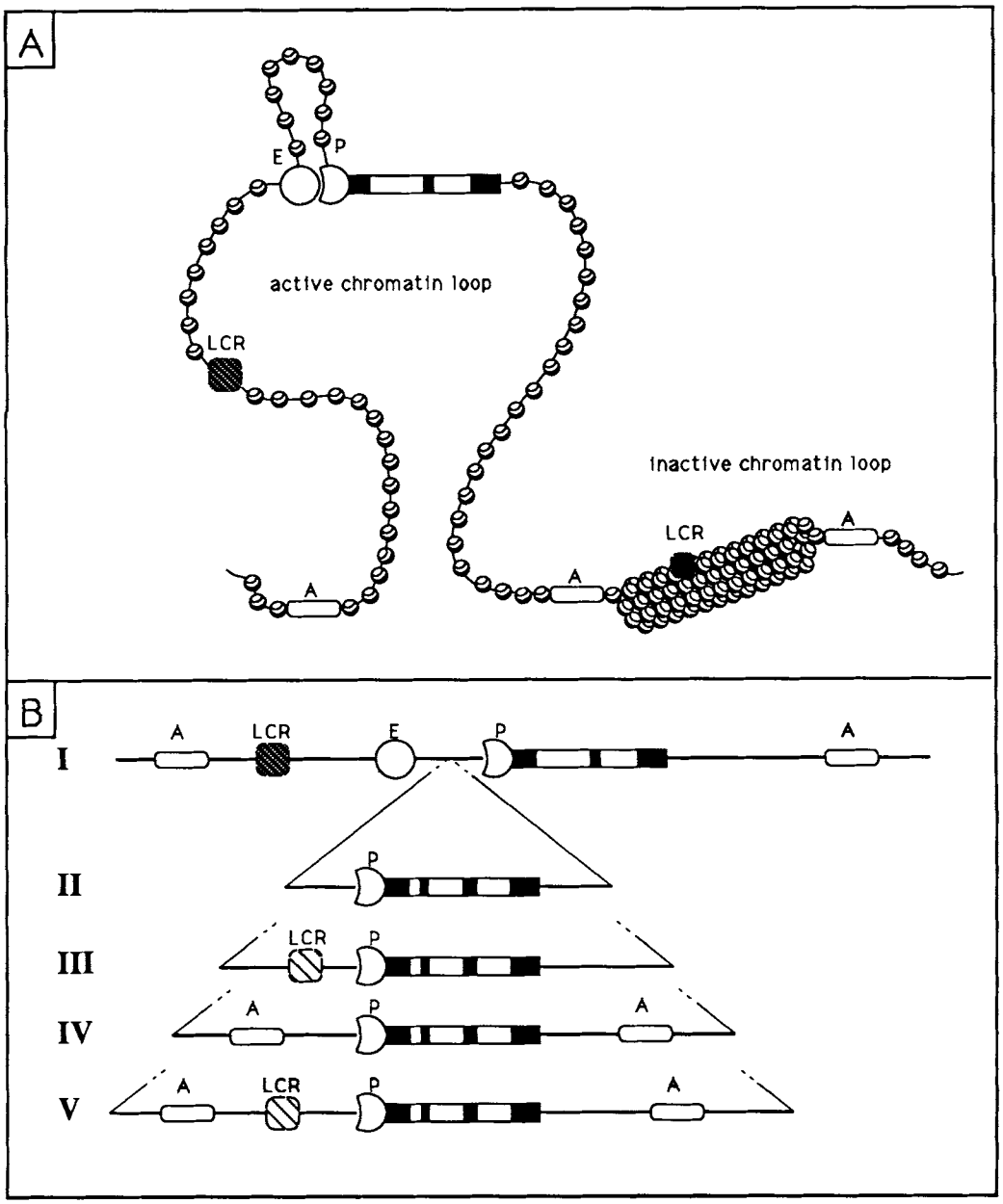

Fig. 3. Chromatin domains, possibly chromosomal loops, as regulatory units for gene expression. **A:** Schematic representation of hypothesized chromatin loops in which the DNA is wrapped around nucleosomes either in an "open," active (left) or a condensed, inactive (right) conformation. Chromatin conformation correlates with the activity of a gene (filled and open bar) and its associated cis-regulatory elements (LCR: locus control region; A: attachment or A-elements; E: enhancer; P: promoter). **B:** A potential target domain for transgene insertion is shown at the top of the panel (I; symbols and abbreviations as in A). Integration of transgenes with various sets of cis-regulatory elements (II–V) may result in drastically different expression specificities (see text for details).

changed into the open, DNAaseI sensitive state, allowing also enhancers without LCR-function to interact with the promotor. Figure 3B depicts in a schematic way what would happen in the event of random genomic integration of a transgene. The entire genome can be imagined as a chain of regulatory domains consisting of a gene or genes with LCR, promotor(s), and enhancer(s) confined by two A-elements (I). Transgenes will nearly always enter such a regulatory domain resulting in most cases in unpredictable deregulation of the host domain and the trans-

gene (position effect). Transgenes without their own regulatory elements will come under the influence of the regulatory elements of the host domain or stay transcriptionally silent (II). A construct with LCR (III) will, due to its dominant enhancer activity, override the influences of the host domain resulting in position independent transgene expression. Occasionally it might occur that two LCRs compete for the expression of the same gene. A different situation arises when transgenes are framed by A-elements. Depending on the absence or presence of an LCR they should either be inactive (IV) or cell-type specifically active (V) and their expression should be uninfluenced by the cis-regulatory elements present in the host domain.

CONCLUSIONS

The sum of the results described in this article indicates that we begin to understand the relationship between higher order chromatin organization and the regulation of eukaryotic gene expression in its natural chromosomal environment. Despite the fact that we will have to await the development of new biophysical techniques to directly visualize the chromatin structure of a single copy gene, functional studies with stably integrated transgenes have given us enough results for a new model of eukaryotic chromosome organization. The regulatory domain organization of eukaryotic genomes has a number of important implications for stable gene transfer. The construction of improved transgenic vector systems, with which position effects on gene expression can be eliminated, will not only leave valuable prospects for practical medical or agricultural approaches but will also finally enable basic scientists to study the functional influence of chromatin organization on gene regulation uncoupled from effects of neighbouring chromosomal structures by molecular genetic approaches.

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