DNase I hypersensitive sites flank the mouse class II major histocompatibility complex during B cell development

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ABSTRACT

The mouse class II major histocompatibility complex (MHC) encodes a polymorphic, multigene family important in the immune response, and is expressed mainly on mature B cells, on certain types of dendritic cells and is also inducible by gamma-interferon on antigen presenting cells. To study the regulatory elements which control this expression pattern, we have examined the chromatin structure flanking the class II MHC region, in particular during B cell differentiation. Using a panel of well-characterised mouse cell lines specific for different stages of B cell development (pre-B, B, plasma cell) as well as non-B cell lines, we have mapped the DNase I hypersensitive (DHS) sites adjacent to the mouse MHC class II region. The results presented show, for the first time that there are specific hypersensitive sites flanking the class II MHC locus during pre B cell, B cell and plasma cell stages of B cell differentiation, irrespective of the status of class II MHC expression. These hypersensitive sites are not found in T cell, fibroblast or uninduced myelomonocytic cell lines. This suggests that these DHS sites define a developmentally stable, chromatin structure, which can be used as a marker of B cell lineage commitment and may indicate that a combination of these hypersensitive sites reflect regulatory proteins involved in the immediate expression of a particular class II MHC gene or possibly control of the entire locus.

INTRODUCTION

The major histocompatibility complex (MHC) cell surface glycoproteins, class I and class II, are members of the immunoglobulin supergene family and function as key markers in the recognition of self versus non-self by the immune system. The MHC proteins have been extensively studied in the mouse (H-2) and human (HLA) systems and share both structural and functional homology within classes, as well as between species. Class I MHC antigens are expressed on most somatic cells and represent the classically defined major transplantation antigens, while class II MHC gene products are found on B cells, dendritic

cells, certain types of accessory cells and can be induced by gamma-interferon on antigen presenting cells (for reviews and references therein: 1–5).

We have chosen the mouse class II MHC locus as a model system for studying the regulation of expression of a multigene family, in particular during B cell differentiation, for the following reasons: i) the class II region is relatively compact, spanning 120 kb on chromosome 17; it consists of six genes (Ob-Ab-Aa-Eb1-Eb2-Ea) (Fig. 1) encoding two expressed class II MHC antigens, A (A alpha, A beta heterodimer) and E (E alpha, E beta heterodimer) (6); ii) sequence information for most of the genes, as well as overlapping cosmids for the region are available (7, 8); iii) a wide variety of inbred mouse strains and transformed mouse cell lines specific for different stages of B cell development exist; and iv) the cell surface expression of the MHC class II alpha-beta heterodimers is coordinately regulated (9, 10), and is found on B cells but not on pre B cells and plasma cells. In spite of extensive information concerning the functional role of class II MHC in the recognition of foreign antigen by T cells, relatively little is known concerning the molecular mechanisms controlling the expression of class II MHC during development of the B cell lineage.

Before a gene is transcribed, the surrounding chromatin region becomes sensitive to various nucleases, in particular DNase I. As such, most 'active genes' are preferentially sensitive to mild digestion by DNase I. These areas of increased sensitivity have been mapped by using techniques, such as indirect end labelling (11), and have been shown to be located 5' or 3' of genes, which are actively transcribed or about to be, and can be tissue specific for genes so regulated (for review see 12, 13). This has been well-documented for gene families as diverse as *Drosophila* heat shock proteins (12), *Xenopus* ribosomal genes (14) and beta-globin genes from a variety of species including chicken (12) and human (15–17). These 'hypersensitive sites' are thought to reflect discrete regions of 'open' chromatin which are accessible to regulatory proteins. Such factors would initially include those affecting DNA supercoiling (such as topoisomerases) which would then allow additional or new trans-acting factors important in the control and regulation of development to interact with the relaxed and open chromatin structure (18). A combination of both cis- and trans-acting elements are probably necessary for the

establishment of an active chromatin domain which would permit expression of developmentally regulated, tissue specific genes. Interestingly, the presence of DNase I hypersensitive sites, defined for the developmentally regulated, human beta-globin gene family, has been shown to correlate with the formation of an active chromatin domain for genes linked in cis, conferring position-independent, copy dependent expression (locus control region; 17,19,20).

Of particular relevance to our studies on B cell ontogeny is the suggestion that the establishment of tissue specific patterns of DNase I hypersensitive (DHS) sites is a prerequisite and/or early step to cell type or lineage commitment (12). Except for the mouse class II Aa (21) and human class II DRa (22, 23) genes, as well as certain of the class I MHC genes (24), the chromatin flanking the major histocompatibility region has not been previously studied in detail. In order to ascertain the developmentally important control elements in the regulation of class II MHC genes, we have mapped the DNase I hypersensitive sites flanking the class II region present in a panel of mouse cell lines and discuss their possible role during B cell lineage commitment.

MATERIALS AND METHODS

Cell Lines

The cell lines used in the present study (described in table 1) were obtained from the following sources: BII FL4-2.1, (formerly GCG; C.Paige unpublished), 18-81 (25), Wehi-231 (26), J558, Hopc I (27), Wehi-3 (28) were all obtained from C.Paige; Ltk- (29) from A.Tunnacliffe; EL4 (30) from U.Staertz; A20 (31) from F.Melchers. Cells were grown in

Iscoves modified dulbeccos medium (Gibco), monothioglycerol (80 μ M), Pen/strep antibiotics, 5–10% Fetal Calf Serum (Flow), following established tissue culture methods. All cell lines (except as noted) were subcloned before analysis by serial dilution, and checked for lineage specific characteristics as summarized in Table 1.

DNase I Hypersensitivity Assay

Protocols from a variety of published methods were combined and optimized for reproducibility, ease of manipulation, etc. (32–34). DNase I hypersensitivity assays were performed on freshly isolated cells, using ice cold buffers. Briefly: $1-2 \times 10^8$ cells were washed twice in PBS, with a final wash in RSB + 1mM PMSF (RSB: 10mM Tris Buffer, pH 7.4, 10mM NaCl, 5mM $MgCl_2$). Cells were resuspended in 10 mls RSB buffer/50% glycerol/0.2% NP40, 1mM PMSF and lysed by pipetting approximately 10 times in the above solution, then left on ice for 5–10 minutes. Nuclei were visualised by staining with Trypan Blue and spun down at 4000 rpm, 10 minutes at 4°C in a Heraeus Minifuge RF. Cells were resuspended in RSB buffer + 0.1mM PMSF (50 mls) and respun 2500 rpm 10 minutes. Final pellet was resuspended in RSB buffer only, at 10^8 nuclei/ml. Generally, increasing concentrations of DNase I (Boehringer-Mannheim DNase I, RNase-free) were added to 100 μ l/ 10^7 nuclei in prepared tubes on ice. A zero minute control was included, as well as a 10 minute control without DNase I at 37°C to control for endogenous nuclease. DNase I was diluted freshly into RSB buffer and added to the nuclei to obtain a final concentration per ml of: 0.1 U/ml, 1.0 U/ml, 2.0 U/ml, 5.0 U/ml, 10 U/ml, 20 U/ml, 40 U/ml and 100 U/ml. Nuclei were incubated for 10 minutes at 37°C and the reaction

B CELL STAGE SPECIFICITY

Table 1

Cell Lines	Source	Ig						CLASS II		
		Rearrangement			mRNA			λ 5	mRNA	Cell Surface
		μ	κ	λ	μ	κ	λ			
<u>Pre B</u>										
*BII FL 4-2.1 (bx d)	Fetal Liver ⁺	R	G	G	+			+		
*18-81 (d)	Adult bone marrow ⁺	R	R	G	+			+		
<u>B cell</u>										
*WEHI 231 (d)	Lymphoma (IgM, κ)	R	R	G	+	+			+	+
A20 (d)	Lymphoma (IgG2a, κ)	R	R	G		NT			+	+
<u>Plasma cell</u>										
*J558 (d)	Myeloma (IgA, λ 1)	R	R	R	NT	+	+			
*HOPCI (d)	Myeloma (IgG2a, λ 1)	R	R	R	NT	+	+			
<u>Non-B cell lines</u>										
*EL4 (b)	T cell lymphoma	G	G	G						
*WEHI-3 (d)	Myelomonocytic tumour	G	G	G					γ IFN inducible	
LTK ⁺ (k)	Fibroblast cell line	G	G	G						

* Subcloned by serial dilution; R = rearranged; G = germline; NT = not tested; + = Abelson-MuLV transformed.

was stopped by removing tubes to an ice bath and adding EDTA to final concentration of 20mM. Some assays were performed at a fixed concentration (previously determined for optimal sensitivity) of DNase I (10 U/ml) and incubated at 37°C for increasing time: 0, 12 (without DNase I) 2,4,8,16 minutes (Wehi 3 shown in figure 4).

To prepare large molecular weight DNA, nuclei were set in agarose blocks (35). Briefly: Following treatment with DNase I, nuclei (still intact) were diluted in TE, then diluted 1:1 v/v with 1.5% low melting point agarose to give a final concentration of 2×10^6 nuclei /60 μ l block, and set in block formers cooled on ice. Ten-twenty nuclei-containing blocks were then lysed in 5 mls of 1% Sarcosyl, 0.5 M EDTA, 0.5 mg/ml Proteinase K, and incubated for 36 hrs at 50°C. Blocks were rinsed in a large excess (generally 2–3 times initial lysis volume) of TE plus PMSF (40 μ g/ml), 3 $\times$ 20 minutes at 50°C, finally rinsed in TE and either used immediately or stored in 0.5M EDTA at 4°C. DNA was stable under these conditions for up to 1–2 years at 4°C. Restriction enzyme digests were performed according to manufacturer's recommendation, in a final volume of 400 μ l overnight (> 16 hrs) in a 5 fold excess of enzyme. (Each block contains approximately 20 μ g of DNA).

Electrophoresis gels were 0.8% agarose in TEA buffer and run at 40V for 20 hours.

Probes and Hybridisation Conditions

Mouse cosmid 32.1 and 1518 (8) were obtained from M.Steinmetz. DNA probes used in this work were isolated and subcloned from the appropriate cosmid or plasmid following established techniques (36). The DNA probes used for analysis of the cell lines described in Table 1 were obtained from the following sources: For Immunoglobulin heavy chain rearrangement and expression, pJl (from A.Trauncker) and p/mu 1.2 HindIII containing exons 3–4 of the mu heavy chain (from R.Scheuermann); for kappa light chain rearrangement and expression: pHX12 (from A.Trauncker) and pEck (from L.D'Hoostelaere); for lambda light chain rearrangement and expression, pV lambda 2 (37), for lambda 5 expression, 7pB 12-1 (38) and for class II MHC expression: Ea (39), Eb (40), Aa (41), Ab (42). Probes used for indirect end labelling were as follows: BS/ Ea 695 bp is a Pst I/Kpn I 695 bp fragment covering the second exon of Ea, isolated from cosmid 32.1 and subcloned into Bluescript (BS) vector (Stratagene). BS/5'Ea 1.1 kb is a Sac I/ Sal I 1.1 kb fragment, isolated from cosmid 32.1. p/Ob 6.6 kb is a BamHI fragment isolated from cosmid 1518 and covering exons 1–4 of Ob and subcloned into puc 18, the probe is a 1.7 kb AccI fragment covering exon 2. The beta major globin probe (pBM 700) was obtained from M.V.Wiles (43).

Southern blots were performed using Zeta Probe Nylon membranes (BioRad) according to manufacturer's recommendations. Briefly: Alkaline blotting was performed in 0.4 M NaOH for 4–6 hours, the filters were neutralised and dried before use. Filters were hybridized to randomly primed 32 P-labelled probes ($1-2 \times 10^9$ cpm/ μ g DNA probe) (44). Hybridisation was at 65°C, $1.5 \times$ SSPE ($20 \times$ SSPE=3.6 M NaCl, 0.2 M Na phosphate, 0.2 M EDTA), 10% (w/v) dextran sulfate, 1% SDS, 0.5% (w/v) BLOTTO (Carnation nonfat dry milk), 0.5 mg/ml denatured salmon sperm DNA for at least 16 hours. Filters were washed to $0.25 \times$ SSC ($20 \times$ SSC= 3M NaCl, 0.3M citrate), 0.5% SDS for 2 $\times$ 30 minutes at 65°C. Filters were exposed to preflashed XAR Film (Kodak) between intensifying screens at -70°C for 2–7 days.

RESULTS

Mouse Cell lines

Pure populations of cells specific for different stages of B cell ontogeny (pre-B, B and plasma cell stages) are difficult to isolate in numbers necessary for molecular analysis, as specific cell surface markers are not easily available, particularly for early progenitors. To circumvent this problem we have used a number of well characterised cell lines (Table 1). For each differentiation stage more than one cell line has been used and each one (except A20 and Ltk-) was subcloned and then verified for specific characteristic lineage markers (see Table 1). All B cell lines gave the expected B cell stage specificity: Immunoglobulin gene DNA rearrangement and expression, at both message and protein levels is as published (45,46); mRNA for lambda 5 is detected specifically in pre-B cell lines (47); class II MHC is expressed only at the mature B cell stage, and is inducible by gamma-interferon in the Wehi-3 myelomonocytic cell line. These cell lines were therefore regarded as representative of the different stages of B cell differentiation and were used in the experiments described in this paper.

The rationale for these experiments is straightforward. As the class II MHC multigene family is coordinately expressed and regulated throughout B cell ontogeny, we assume that we can analyse the locus as a single developmental unit. We therefore examined the flanking regions 5' of Ob and 5' of Ea for DNase I hypersensitive sites (Fig.1). We first studied the chromatin region flanking the Ea gene as its promoter and enhancer elements have been extensively characterised and sequenced (5,48).

DNase I hypersensitive (DHS) sites 5' of Ea are present at all stages of B cell development

Figure 2 shows the results from a DNase I hypersensitive assay performed on nuclei isolated from pre B (18-81), B (A20) and plasma (J558) cell lines. After mild digestion with DNase I, the nuclei were cast into agarose blocks, and all subsequent steps performed in agarose blocks. Indirect end labelling (11) was used to map the position of the DNase I hypersensitive (DHS) sites immediately 5' of the Ea gene. The probe (P-K) maps close to one end of the 8.3 kb EcoRI fragment as represented in figure 2. The appearance of sub-fragments indicates the presence of DHS sites, the largest fragment size being the furthest away from the probe used. Hypersensitive sites are clearly seen at 3.8 kb and 2.6 kb in pre B, B cell and plasma cell lines, and are most strongly detected in the B cell line A20. The 3.8 kb fragment corresponds to the region of the W box (B cell specific enhancer) (49,50) and the 2.6 kb fragment maps to the promoter proximal region containing the X and Y boxes (5). Additional hypersensitive sites are seen in A20 and J558 cell lines at 5.8 kb, and in A20 alone

Figure 1. Mouse H-2 I Region. Arrows show direction of transcription, White boxes represent pseudogenes, shaded boxes genes without known translation products, and black boxes are expressed genes. Class II MHC region is between 300–500 kb here; K is a class I gene.

at 3.4 kb and 2.0 kb (these are indicated by shortened arrows in figure 2). The presence of the major hypersensitive sites at 5.8 kb, 3.8 kb and 2.6 kb was confirmed in the other cell lines at the same B cell stage as listed in Table 1 (BII FL 4-2.1, Wehi 231, Hopc I respectively, data not shown). The apparent difference in signal intensity, particularly for the 3.8 and 2.6 kb

sub-fragments is probably due to qualitative differences such as length of exposure and cell line used.

The results in figure 2 show that there are DHS sites immediately flanking the Ea gene. To rule out the possibility that these sites are ubiquitous to all cell lines we examined DHS sites in non-B cell lines.

Figure 2. DNase I Hypersensitive (DHS) sites during B cell development. Examples of pre B, B, and Plasma cell lines are shown (see Table 1). DNase I was added in increasing concentrations, as indicated by the direction of the arrow, C refers to controls (see methods for details), sizes in Kb refers to the approximate sizes of the DHS sites in each cell line. Probe (shaded box) covering the first domain of Ea (695 bp Pst/Kpn fragment) was used in this assay. This probe detects a 8.3 kb EcoR I fragment. Sub-bands indicate the presence of DHS sites and arrows indicate the approximate position of the site. The Ea map is compiled from sequence information (accession number M18158, genebank), filled boxes refer to exons, and KB (-1700), W (-1200) and XY (-100, -80 respectively) refer to DNA binding sequences upstream of the Ea gene. (K00971, embl accession number).

Figure 3. DNase I Hypersensitive sites in non-B cell lines. The same probe and conditions as in figure 2 were used. The entire autorad is shown to indicate the absence of DHS sites specific to the Ea gene in non-B cell lines.

DNase I hypersensitive sites 5' of Ea are not present in non-B cell lines

Figure 3 shows the results of DNase I hypersensitive assays performed on nuclei isolated from three representative non-B cell lines: Wehi-3 (myelomonocytic tumour), EL4 (T cell lymphoma) and Ltk- (fibroblast cell line). The assays were performed as for figure 2, and no DHS sites are seen over the whole range of DNase I concentrations used (0.1–100 U/ml). There remains the formal possibility that no hypersensitive sites are detected in the non-B cells due to inefficient DNase I digestion or some other artefact. However, this is not the case, since for example, the fibroblast cell line Ltk- showed some reduction in signal intensity of the 7.4 kb fragment at the higher concentrations of DNase I, and the Wehi-3 cell line revealed the appearance of DHS sites associated with the expression of class II MHC (see discussion), upon induction by gamma-interferon (data not shown). These results indicate that the DHS sites shown in Figure 2 are independent of the expression of class II MHC only in cell lines of the B cell lineage.

We next confirmed the location of these hypersensitive sites and examined the chromatin further upstream for additional DHS sites.

B cell lineage hypersensitive sites are also present further upstream of Ea

A DNase I hypersensitivity assay was performed on nuclei isolated from the pre B cell line 18-81 and the myelomonocytic cell line Wehi 3 using a single copy DNA probe (Sc-S), which indirectly end-labels a 9.4 kb KpnI fragment (figure 4). Three hypersensitive sites are found in the 18.81 cell line (Panel A) generating sub-fragments of 6.8 kb, 3.7 kb and 2.0 (and 1.8) kb. These fragments sizes were also detected in the pre-B cell

line BII FL 4-2.1 (data not shown), but are not found in the Wehi-3 cell line (Panel B). This KpnI fragment overlaps the EcoRI fragment shown in figure 2 by 4.3 kb and a composite map of both restriction enzymes is shown in the lower panel of figure 4. The apparent doublet, around 2.0 kb (KpnI digest), therefore corresponds to the 5.8 kb fragment (EcoRI digest) seen in the B cell and plasma cell lines in figure 2. The absence of the 5.8 kb EcoRI sub-fragment in the 18-81 cell line in Figure 2 is probably due to the sensitivity of hybridisation, as it is clearly seen using a different DNA probe and restriction enzyme digest. Panel C shows the same 18.81 blot used in figure 2, but now hybridised to a non-MHC gene probe. As shown in figure 4, the mouse beta-globin probe reveals the expected EcoRI genomic DNA fragments of 6.8 kb (beta-major) and 14 kb (cross-hybridising beta-minor gene). No other sub-bands are seen, which confirms the appearance of sub-bands in the B cell lineage cell lines, as being representative of the chromatin structure of the class II MHC locus, rather than a possible artefact of the cell lines used. These results indicate that there are specific DHS sites 5' of Ea present at all stages of B cell development tested, even in pre B and plasma cell lines which show no detectable expression of class II MHC. If the class II MHC locus represents a coordinately regulated unit during development, then the 5' flanking region of Ob should also be in an active chromatin domain as indicated by an increased sensitivity to DNase I digestion.

DNase I hypersensitive sites are also present 5' of Ob during B cell ontogeny

The results of DNase I hypersensitive assays performed on nuclei isolated from pre-B (18-81), B (Wehi 231) and non-B (Ltk-) cell lines is shown in figure 5. The Ob probe used (Ac-Ac), indirectly end labels a 14 kb KpnI fragment. Sub-fragments are

Figure 4. DNase I Hypersensitive sites present further upstream of Ea. The probe used was 5' Ea probe (SacI/SalI 1.1 kb) black box shows its location on the map. This probe indirectly endlabeled a 9.4 kb Kpn I fragment, Wehi-3 is the non-B cell line control and the beta-major globin probe was included as a control to confirm the specificity of the DHS sites. Arrows indicate the location of hypersensitive sites in the KpnI digest and in the EcoRI digest.

Figure 5. DHS sites are present 5' of Ob. Symbols as per figure 2. The smearing of bands is due to the presence of low level repeats detected by the probe. A probe covering the first domain of Ob was used (1.7 kb *AccI* fragment) and indirectly end labels a 14 kb fragment. The arrows show the approximate location of the DHS sites. Ltk⁻ is the non-B cell line control.

seen at 6.8 kb, 3.7 kb, 3.0 kb and 2.6 kb, in the pre-B and B cell lines but not in the Ltk⁻ cell line. The 3.7 kb sub-band corresponds to the proximal promoter region as indicated by the X,Y boxes at approximately -200 bp of the start site. The results show that there are specific DHS sites flanking the 5' region of Ob, that are not present in non-B cell lines. Plasma cell lines showed similar patterns (data not shown). See figure 6 and Discussion.

DISCUSSION

In this paper we have looked at the mouse class II MHC as a developmentally regulated unit during B cell differentiation and have shown that there are specific DNase I hypersensitive (DHS) sites flanking the class II MHC region within 10kb 5' Ob and 10kb 5' Ea. These DHS sites are specific to the class II MHC locus as they occur only in those cell lines which are either developmentally programmed to express class II MHC or which actually do express class II MHC at the cell surface. To do this, we have used a variety of transformed cell lines (Table 1), specific for different stages of B cell development, as shown by their immunoglobulin DNA rearrangement and expression or by their ability to express the MHC class II E and A heterodimer. Figure 6 is a summary of the results presented in this paper and shows all specific hypersensitive sites as defined by their presence in pre B, B, and plasma cell lines but not in class II negative non-B cell lines.

The upstream region 5' of Ea contains the following defined groups of DNase I hypersensitive sites (Figure 6). Groups I (-50 bp from the cap site) and II (-1.2 kb) reflect regions of chromatin which are open for the binding of regulatory proteins important in the expression of class II MHC Ea (and it is these groups which are clearly present upon gamma-interferon induction of Wehi-3, see later). These map closely to (DHS sites are

Figure 6. Summary of specific DHS sites mapped 5' Ob and 5' Ea. Dotted lines indicate portion of class II region that has been mapped, note different scales in class II region, Ob map and Ea map. DHS sites have been called groups to indicate their mapping to a region and incorporates the possibility of more than one DHS site for each group. Numbering starts with I being closest to the start site of the gene in question, rather than any statement about intensity of signal. Also the gene should be specified, when referring to DHS sites, as only the XY boxes found 5' of all class II MHC genes have the possibility of sharing similar trans-acting factors involved in their expression. Therefore, Ob DHS I-V and Ea groups I-V.

approximate within 200–500 bp), but are not necessarily identical with, the promoter (X,Y boxes) and enhancer (W box) regions defined for Ea (51,52). The striking difference in signal intensities for group I and group II DHS sites (18-81, J558 vs A20 in figure 2) should be treated with caution as other cell lines at the same B cell stage (data not shown) do not show these differences. The

DNase I hypersensitive assay is qualitative and thus any speculation concerning the concentration of factors important in expression of class II MHC gene products is premature.

The hypersensitive sites described in this paper were not only limited to those regions of probable importance in the immediate expression of class II MHC genes, such as the promoter and enhancer regions. Other groups mapped further upstream (as yet unsequenced) are: Group III (−3.4 kb), Group IV (−5.4 kb) and Group V (−8.4 kb) and were present in all B cell lineage cell lines examined. These sites could represent the binding of regulatory proteins important in class II MHC expression and/or other proteins important in formation of stable open chromatin structures. Sal I restriction enzyme digests confirmed that there were apparently no additional hypersensitive sites other than Groups I–V, within a total of 20 kb 5' of Ea (data not shown),

The upper part of Figure 6 shows the DHS sites 5' of Ob (Groups I and II). These were mapped to the vicinity of the X,Y boxes (−200bp) which form part of the promoter of all class II MHC genes (53) and to a region approximately 3.6 kb upstream of the promoter region respectively. The presence of specific DHS sites 5' of Ob indicates that this region is part of the class II MHC developmentally regulated domain. There remains the formal possibility that since Ob does not form an alpha, beta heterodimer that can be recognised by an antibody, or that a candidate alpha chain gene has not yet been found, that a more active chromatin region is located further upstream of Ob (greater than the −10 kb mapped here) or further within the class II locus towards the genes encoding the expressed A alpha, A beta heterodimer (see figure 1), or within a cell type not tested.

The presence of DHS sites 5' of Ob (groups I, II) and 5' of Ea (groups I–V) suggests that the class II MHC locus is in an active or open chromatin structure throughout B cell development (as defined by the cell lines used). These hypersensitive sites can therefore be used as a marker of B cell lineage commitment as the DHS sites are present even before detectable class II MHC message only in cell lines of the B cell lineage, but not in uninduced myelomonocytic cell lines or other class II negative non-B cell lines. Early work with multigene families in other species led to the suggestion that the appearance of tissue specific patterns of DNase I hypersensitive sites correlated with an early step in cell type commitment (12). When viewed in this light, the presence of such 'patterns' of DHS sites as described here throughout B cell development, would apparently define the formation of a transcription complex which remains stable during multiple rounds of replication, until the appropriate signal for further differentiation is received (14). This leads to the subsequent expression of the class II MHC gene products at the cell surface (the mature B cell phenotype), and then further differentiation to the end stage of B cell development (plasma cell stage and antibody secretion). Therefore, the absence of class II MHC expression during the pre B cell stage and plasma cell stage of development would indicate the need for additional or new factors to bind to appropriate regulatory regions before stable expression of the differentiated phenotype occurs. Perhaps only the concentration of transcription factors (occurring as a gradual accumulation during the pre B cell stage and then as a gradual loss during the plasma cell stage) or even the presence/absence of a repressor factor is important in the regulation of class II MHC expression (54). The DHS sites described here are present from the pre B cell stage, are they present even earlier, at a pro B cell stage? The possibility of using more primary systems such as Dexter and Whitlock-Witte type cultures (reviewed in 55) could

perhaps overcome the present dependence on cell lines, until more specific markers for early B cell progenitor cells are available.

Gene commitment in a particular lineage or cell type is thought to be irreversible (56). In the case of the class II MHC locus during B cell ontogeny, this would mean that the establishment of particular groups of hypersensitive sites reflects an open chromatin structure that is stable (as the sites are present from the pre B cell stage) until further differentiation factors interact with the locus. Which of the various hypersensitive sites defined here reflect developmentally regulated proteins which affect the formation of an open chromatin domain and which sites reflect the immediate expression of a particular class II MHC gene is not firmly established. We would expect that all class II MHC genes would have specific DHS sites corresponding to the promoter/enhancer regions during B cell development (Groups I and II in this paper) or upon induction of expression, by gamma-interferon for example. Indeed the induction of cell surface class II MHC in the myeloid cell line Wehi-3 is accompanied by the appearance of Ea DHS groups I and II (at least) and further experiments are in progress to determine whether all of the DHS groups defined in this paper are capable of being induced or whether some are formally B cell lineage specific. In any case, it is only during B cell ontogeny, that detectable expression of class II MHC is not required for the establishment of these groups of hypersensitive sites. Certainly the absence of Ea DHS Group I in inbred mouse strains, which have a deletion in the promoter region of Ea (57) does not lead to an absence of Eb transcription or the expression of A complex at the cell surface. This reflects the absence of cis sequences important for Ea transcription rather than the absence of sequences critical for the formation of an open chromatin structure. There remains, however, the very interesting possibility that the important regulatory elements for class II MHC expression bind to regions far upstream of the defined promoter/enhancer regions of Ea (Groups III–V and beyond?). Absence of these regulatory elements would result in an inactive chromatin structure correlating with the absence of specific hypersensitive sites, and effectively silencing an entire locus, or leading to an absence of expression as would occur in non-B cell lines.

The B cell lineage hypersensitive sites described spanning both promoter regions of Ob and Ea as well as sequences far upstream, in particular groups III–V, are reminiscent of upstream sites described for other multigene families in particular the human beta-globin gene locus. These erythroid specific hypersensitive sites confer position-independent, copy-dependent expression of genes linked in cis in transgenic mice, the so-called locus control region (17, 20). Similar DHS sites have been described for the human CD2 gene (58), the chicken lysosyme gene (59) and the human alpha globin gene family (60). Functional experiments in transgenic mice are necessary (and in progress) to resolve the question as to whether these apparently developmentally programmed patterns of hypersensitive sites reflect the action of a putative locus control region.

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REFERENCES

- Klein, J. (1982) *Immunology: The Science of Self-Non Self Discrimination*. John Wiley and Sons, New York.
- Mellor, A.L. (1986) In Maclean, N. (ed), *Oxford Surveys on Eukaryotic Genes*, Oxford University Press, Oxford, England, Vol. 3, pp. 95–140.
- Stroynowski, I. (1990) *Ann. Rev. Immunol.* 8, 501–530.
- Carson, S. and Trowsdale, J. (1986) In Maclean, N. (ed), *Oxford Surveys on Eukaryotic Genes*, Oxford University Press, Oxford, England, Vol. 3, pp. 63–94.
- Benoist, C. and Mathis, D. (1990) *Ann. Rev. Immunol.* 8, 681–715.
- Klein, J. (1990) *Immunogenetics* 32, 147–149.
- Flavell, R.A., Allen, H., Huber, B., Wake, C. and Wiedera, G. (1985) *Immunol. Rev.* 84, 29–50.
- Steinmetz, M., Stephan, D. and Fisher-Lindahl, K. (1986) *Cell* 44, 895–904.
- Long, E.O., Mach, B. and Accolla, R.S. (1984) *Immunogenetics* 19, 349–352.
- Paulnock-King, D., Sizer, K.C., Freund, Y.R., Jones, P.P. and Parnes, J.R. (1985) *J. Immunol.* 135, 632–636.
- Wu, C. (1980) *Nature* 286, 854–860.
- Elgin, S.C.R. (1981) *Cell* 27, 413–415.
- Weisbrod, S. (1982) *Nature* 297, 289–295.
- Brown, D.D. (1984) *Cell* 37, 359–365.
- Tuan, D., Solomon, W., Li, Q. and London, I.M. (1985) *Proc. Natl. Acad. Sci. USA* 82, 6384–6388.
- Forrester, W.C., Thompson, C., Elder, J.T. and Groudine, M. (1986) *Proc. Natl. Acad. Sci. USA* 83, 1358–1363.
- Grosveld, F., van Assendelft, G.B., Greaves, D.R. and Kollias, G. (1987) *Cell* 51, 975–985.
- Weintraub, H. (1985) *Cell* 42, 705–711.
- Forrester, W.C., Takegawa, S., Papayannopoulou, T., Stamatoyannopoulos, G. and Groudine, M. (1987) *Nucleic Acids Res.* 15, 10159–10177.
- Ryan, T.M., Behringer, R.R., Martin, N.C., Townes, T.M., Palmiter, R.D. and Brinster, R.L. (1989) *Genes Dev.* 3, 314–323.
- Liou, H.-C., Polla, B.S., Aragnol, D., Leserman, L.D., Griffith, I.J. and Glimcher, L.H. (1988) *Proc. Natl. Acad. Sci. USA* 85, 2738–2742.
- Ting, J.P.-Y., Carrington, M.N., Salter, R.D., DeMars, R. and Cresswell, P. (1985) *Immunogenetics* 22, 571–583.
- Gonczy, P., Reith, W., Barras, E., Lisowska-Grospierre, B., Griscelli, C., Hadam, M.R. and Mach, B. (1989) *Mol. Cell. Biol.* 9, 296–302.
- Maschek, U., Pulm, W. and Hammerling, G.J. (1989) *EMBO J.* 8, 2297–2304.
- Siden, E.J., Baltimore, D., Clark, D. and Rosenberg, N.E. (1979) *Cell* 16, 389–396.
- Warner, N.L., Harris, A.W. and Gutman, G. (1975) In Seligmann, M., Preud'Homme, J.L. and Kourilsky, F. (eds.), *Membrane Receptors of Lymphocytes*, Elsevier, North Holland, pp. 203–216.
- Weigert, M., Cesari, I.M., Yonkovich, S.J. and Cohn, M. (1970) *Nature* 228, 1045–1047.
- Ralph, P., Moore, M.A.S., and Nilsson, K. (1976) *J. Exptl. Med.* 143, 1528–1533.
- Kit, S., Dubbs, D.R., Piekarski, L.J. and Hsu, T.C. (1963) *Exptl. Cell Res.* 31, 297–312.
- Shevach, E.M., Stobo, J.D., and Green, I. (1972) *J. Immunol.* 108, 1146–1151.
- Kim, K.J., Kanellopoulos-Langevin, C., Merwin, R.M., Sachs, D.H. and Asofsky, R. (1979) *J. Immunol.* 122, 549–554.
- Fritton, H.P., Sippel, A.E. and Igo-Kemenes, T. (1983) *Nucleic Acids Res.* 11, 3467–3485.
- Enver, T., Brewer, A.C. and Patient, R.K. (1985) *Nature* 318, 680–683.
- Wolf, S.F. and Migeon, B.R. (1985) *Nature* 314, 467–469.
- Hermann, B.G., Barlow, D.P. and Lehrach, H. (1987) *Cell* 48, 813–825.
- Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) *Molecular Cloning, A Laboratory Manual*, Vol. 1–3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
- Carson, S. and Wu, G.E. (1989). *Immunogenetics* 29, 173–179.
- Kudo, A., Sakaguchi, N. and Melchers, F. (1987) *EMBO J.* 6, 103–107.
- Mathis, D.J., Benoist, C., Williams, V.E., Kanter, M. and McDevitt, H.O. (1983) *Cell* 32, 745–754.
- Mengle-Gaw, L. and McDevitt, H.O. (1983) *Proc. Natl. Acad. Sci. USA* 80, 7621–7625.
- Benoist, C.O., Mathis, D.J., Kanter, M.R., Williams, V.E. and McDevitt, H.O. (1983) *Proc. Natl. Acad. Sci. USA* 80, 534–538.
- Estess, P., Begovich, A.B., Koo, M., Jones, P.P. and McDevitt, H.O. (1986) *Proc. Natl. Acad. Sci. USA* 83, 3594–3598.
- Wiles, M.V. and Keller, G. (1991) *Development* 111, 259–267.
- Feinberg, A.P. and Vogelstein, B. (1984) *Anal. Biochem.* 137, 266–267.
- Alt, F., Rosenberg, N., Lewis, S., Thomas E. and Baltimore D. (1981) *Cell* 27, 381–390.
- Alt, F.W., Yancopoulos, G.D., Blackwell, T.K., Wood, C., Thomas, E., Boss, M., Coffman, R., Rosenberg, N., Tonegawa, S. and Baltimore, D. (1984) *EMBO J.* 3, 1209–1219.
- Sakaguchi, N. and Melchers, F. (1986) *Nature* 324, 579–582.
- Blonar, M.A., Burkly, L.C. and Flavell, R.A. (1989) *Mol. Cell. Biol.* 9, 844–846.
- Dorn, A., Benoist, C. and Mathis, D. (1989) *Mol. Cell. Biol.* 9, 312–320.
- Koch, W., Benoist, C. and Mathis, D. (1989) *Mol. Cell. Biol.* 9, 303–311.
- Dorn, A., Bollekens, J., Staub, A., Benoist, C. and Mathis, D. (1987) *Cell* 50, 863–872.
- Dorn, A., Fehling, H.J., Koch, W., Le Meur, M., Gerlinger, P., Benoist, C. and Mathis, D. (1988) *Mol. Cell. Biol.* 8, 3975–3987.
- Larhammar, D., Hammerling, U., Rask, L. and Peterson, P.A. (1985) *J. Biol. Chem.* 260, 14111–14119.
- Latron, F., Jottrand-Bellomo, M., Maffei, A., Scarpellino, L., Bernard, M., Strominger, J.L. and Accolla, R.S. (1988) *Proc. Natl. Acad. Sci. USA* 85, 2229–2233.
- Whitlock, C., Denis, K., Robertson, D. and Witte, O. (1985) *Ann. Rev. Immunol.* 3, 213–235.
- Metcalf, D. (1989) *Nature* 339, 27–30.
- Mathis, D.J., Benoist, C., Williams, V.E., Kanter, M. and McDevitt, H.O. (1983) *Proc. Natl. Acad. Sci. USA* 80, 273–277.
- Greaves, D.R., Wilson, F.D., Lang, G. and Kioussis, D. (1989) *Cell* 56, 979–986.
- Bonifer, C., Vidal, M., Grosveld, F. and Sippel, A.E. (1990) *EMBO J.* 9, 2843–2848.
- Higgs, D.R., Wood, W.G., Jarman, A.P., Sharpe, J., Lida, J., Pretonious, I.-M. and Ayyub, H. (1990) *Genes Dev.* 4, 1588–1601.