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Cell lineage specificity of chromatin configuration around the immunoglobulin heavy chain enhancer

A.M.Ford, S.M.Watt[1], A.J.W.Furley[2], H.V.Molgaard and M.F.Greaves

Leukaemia Research Fund Centre, Institute of Cancer Research, London SW3 6JB, UK

[1]Present address: Imperial Cancer Research Fund, Medical Oncology Unit, St Bartholomew's Hospital, West Smithfield, London EC1A 7BE, UK

[2]Present address: Department of Biochemistry and Biophysics, College of Physicians and Surgeons, Columbia University, NY 10032, USA

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As tested by DNase I hypersensitivity, the chromatin structure of the IgH enhancer region in human B cell precursor cell lines is in an open or accessible conformation. All T cell lines, with either germline or rearranged IgH genes, were also hypersensitive to DNase I but in contrast to B cell precursors showed no detectable Cμ expression. Normal thymocytes similarly had a hypersensitive IgH enhancer site. In contrast to lymphoid cells, all myeloid cell lines tested, as well as normal granulocytes, were not DNase I hypersensitive and did not express Cμ. A putative lymphomyeloid progenitor cell line KG1, although having a germline configuration of Ig genes, produced Cμ transcripts and was hypersensitive to DNase I in the IgH enhancer region. After induction of myeloid differentiation the Ig enhancer region of KG1 cells is no longer hypersensitive or transcriptionally active. Two *Hha*I restriction sites on either side of the IgH enhancer were not methylated in all Cμ-expressing lines but methylated in non-expressing cell lines. These results show that an open chromatin structure around the heavy chain enhancer is necessary but insufficient for initiating transcription from unrearranged IgH genes and further suggests this region may be in an open or accessible configuration prior to lineage commitment and closed following adoption of the myeloid lineage.

Key words: cell lineage/chromatin structure/differentiation/enhancer/immunoglobulin gene

Introduction

The tissue specific expression of immunoglobulin heavy chain genes is now known to be controlled by at least three DNA sequence elements: the promoter region of the variable gene (Mason *et al.*, 1985; Dreyfus *et al.*, 1987; Grosschedl and Baltimore, 1985), intragenic sequences (Grosschedl and Baltimore, 1985) and an enhancer sequence in the major intron of the heavy chain gene (Banerji *et al.*, 1983; Gillies *et al.*, 1983; Neuberger, 1983; Mercola *et al.*, 1985). The heavy chain gene enhancer has been shown to be active even in early B cells which have not yet completed Ig μ gene rearrangement (Gerster *et al.*, 1986) suggesting that the enhancer is important not only in initiating Ig gene expression in immature B cells but also the rearrangement process. Spontaneous deletions of the enhancer during the heavy chain class switch in mature B cells do not affect levels of Ig gene expression, indicating that the enhancer may only be required for the initial activation of the IgH gene and not for the continued maintenance of its expression (Wabl and Burrows, 1984; Klein *et al.*, 1984).

IgH and T cell receptor (TCR) germline transcripts can both be found in the 'inappropriate' lineage of normal and leukaemic lymphoid cells (Kemp *et al.*, 1980a,b; Calman and Peterlin, 1986), suggesting that the region of origin of the IgH germline transcripts, the $J_H-C\mu$ intron, can be accessible to regulatory proteins in both lineages prior to detectable partial (DJ) or full (VDJ) rearrangements. Partial rearrangements of IgH or TCRβ gene segments are also common in the 'inappropriate' lymphoid lineage (reviewed in Greaves *et al.*, 1987; Minden and Mak, 1986; Waldmann, 1987) and, more rarely, in myeloid cells (Rovigatti *et al.*, 1984; Cheng *et al.*, 1986). Since the IgH enhancer is relatively active in T as well as B cells (Grosschedl and Baltimore, 1985; Scholer and Gruss, 1985; see also Mason *et al.*, 1985) and the recombinases required for the assembly of a functional IgH gene are shared by both T and B cells (Yancopoulos *et al.*, 1986), it follows that some other parameter(s) normally restricts complete VDJ functional rearrangement and expression of the IgH gene to the B cell lineage.

Changes in gene transcription are now frequently associated with local alterations in chromatin structure and the association of tissue- or stage-specific transcription with DNase I sensitivity or hypersensitivity is well documented (Weintraub and Groudine, 1976; for review, see Elgin, 1981; Thomas *et al.*, 1985). The IgH gene enhancer has been shown to be hypersensitive to DNase I in B cells (Mills *et al.*, 1983) implying that it is in a region of open chromatin structure, and also shown to contain several binding sites for proteins that specifically regulate Ig gene expression (Ephrussi *et al.*, 1985; Church *et al.*, 1985; Schlokat *et al.*, 1986; Weinberger *et al.*, 1986; Sen and Baltimore, 1986). It seems probable therefore that in order to assemble and transcribe Ig genes, they are maintained in a conformation that is at least partially open and accessible. The stage in early haemopoietic differentiation at which this characteristic becomes detectable is not immediately obvious. Commitment to the B cell lineage could involve a selective induction of accessibility (cf. Alt *et al.*, 1986). Conversely, multipotential haemopoietic cells (and earlier embryonic cells) could have an open or accessible configuration with all lineages but B cells (and perhaps some T cells) subsequently reverting to a closed inaccessible configuration, similar to the model of selective gene expression proposed by Caplan and Ordahl (1978). This issue is of general biological significance since accessibility of genes encoding lineage-

Table I. Summary of the DNase I and methylation status of cell lines used in this study

Cell type	Cell line	IgH rearranged	μ expression		Enhancer H.S.	*Hha*I sites methylated
			Cyt	mRNA		
B	RPMI 1788	2+	+	+	+	−
Pre B	NALM 1	2+	+	+	+	−
	NALM 6	2+	+	+	+	−
	NALM 16	2+	−	+	+	−
	REH	2+	−	+	+	−
Pre-pro B	FLEB 14	gl	−	+	+	−
Pre T	CEM	gl	−	−	+	+
	MOLT 4	gl	−	−	+	+
	HPBALL	1°1+	−	−	+	+
	JM	1°1+	−	−	+	+
	HUT 78	gl	−	−	+	+
	Thymocytes	gl	−	+	+	−
Myeloid	HL60	gl	−	−	−	+
	ML1	1°1+	−	−	−	+
	EM2	gl	−	−	−	+
	Granulocytes	gl	−	−	−	−
'Lymphomyeloid'	KG1a	gl	−	+	+	−
	KG1	gl	−	+	+	−
	ind KG1	gl	−	−	−	−
Non-haemopoietic	A431	gl	−	−	−	+

gl = germline; 1°1+ = 1 germline, 1 rearranged; 2+ = both rearranged; H.S. = hypersensitive site; pre-pro B = very immature B cell precursor (see Katamine *et al.*, 1984); ind = induced, for myeloid differentiation (see text).

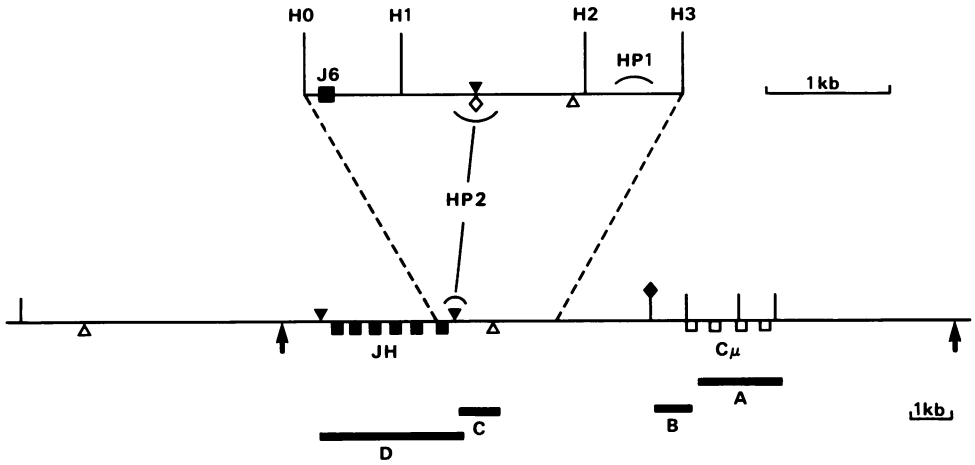

Fig. 1. Restriction enzyme map of the human μ gene. The map is based on previously published sites and sequence data (Ravetch *et al.*, 1981; Ford *et al.*, 1983; Mills *et al.*, 1983) and only the relevant enzyme sites are shown. Details of probes A−D are given in Materials and methods. *Eco*RI, |; *Bam*HI, ↑; *Bgl*II, ▼; *Hin*dIII, △; *Sac*I, ◆; H0-H3, *Hha*I; IgH enhancer core region ◇; HP = hypersensitive site.

restricted functions can be presumed to be linked to the genetic mechanisms underlying cell lineage commitment (Elgin, 1981). We have investigated the cell lineage specificity of accessibility and methylation in the IgH enhancer region by analysis of normal and leukaemic human blood cells.

Results

Ig rearrangement and expression

We have previously reported the Ig gene organization and expression in a range of cell lines established from patients with lymphoid or myeloid leukaemias (Ford *et al.*, 1983;

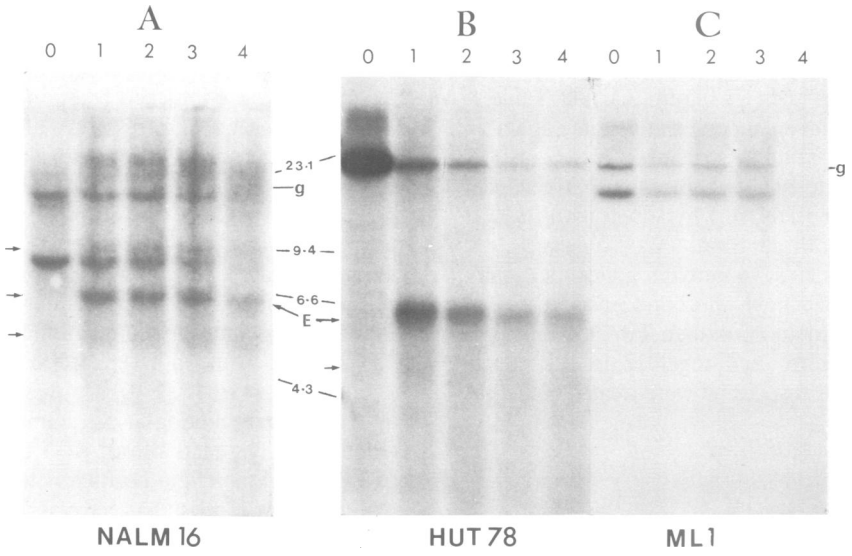

Fig. 2. DNase I-hypersensitive site analysis of the μ gene in human B, T and myeloid cell lines. (**A**) NALM 16−pre-B cell line; (**B**) HUT 78−T cell line; (**C**) ML1−myeloid cell line. **Lane 0** nuclei incubated without added DNase I. **Lanes 1−4** nuclei incubated with 1.2, 1.8, 2.7 and 4 μg/ml DNase I respectively. Each lane contains 10 μg DNA restricted with *Eco*RI and hybridized to probe B. Arrows indicate hypersensitive sites. E = enhancer hypersensitive site; g = germline. Mol. wt markers are indicated in kb.

Furley *et al.*, 1986b, 1987). The rearrangement data are summarized in Table I. Earlier results of cytoplasmic staining for μ chains (Minowada *et al.*, 1979; Hurwitz *et al.*, 1979) were confirmed in the present study by Northern blot analysis and hybridization to probe A (Figure 1). However, we were able to detect Cμ transcripts in two pre-B cell lines, REH and NALM 16, both of which had no cytoplasmic μ chain protein, detectable by immunofluorescent staining with anti-μ antibody (data not shown).

DNase I hypersensitive site analysis

Using probe B (Figure 1), we observed the location of hypersensitive sites by the appearance of sub bands created by the partial cutting of the rearranged or germline μ genes by DNase I. Figure 1 shows the germline configuration of the human Ig μ constant region locus with the relevant restriction sites. The diagram also shows the position of the two previously described intron hypersensitive sites corresponding to the switch region site (HP1) and the enhancer region site (HP2), respectively (Mills *et al.*, 1983). (The 5′ J_H sites are not shown.)

Hypersensitive sites found in our cell lines were compared with those seen in the mature B cell line RPMI 1788 (Mills *et al.*, 1983). The results show that cells of the pre-B lineage including a cell line with unrearranged IgH genes—FLEB 14 (Katamine *et al.*, 1984) produce a similar DNase I pattern to that of RPMI 1788 (i.e. an open chromatin structure around the enhancer, switch and 5′ J_H regions). The result obtained for the pre-B cell line Nalm 16 is shown in Figure 2A. The DNA from untreated nuclei (lane 0) shows two rearranged *Eco*RI fragments of 16 kb and 8 kb hybridizing to the *Sac*I−*Eco*RI fragment, probe B. DNA from nuclei digested with increasing amounts of DNase I (lanes 1−5) shows progressive disappearance of these *Eco*RI fragments and the appearance of nuclease generated sub-fragments corresponding to the DNase I-hypersensitive sites within the gene.

Figure 2B shows the DNase I pattern of the T (Sezary) cell line HUT 78 with a 17-kb germline *Eco*RI fragment.

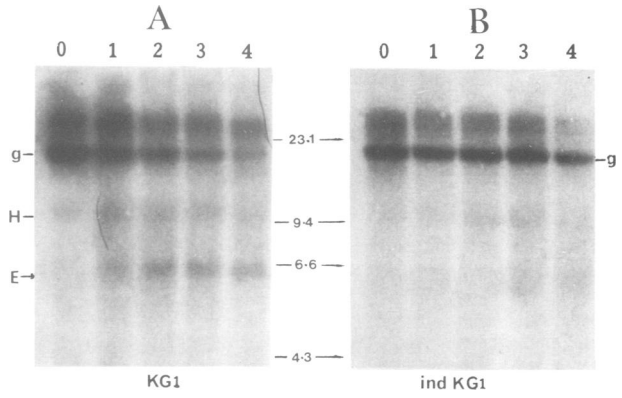

Fig. 3. DNAse I-hypersensitive site analysis of the μ gene in induced and non-induced KG1 cells. (**A**) Non-induced KG1; (**B**) myeloid-induced KG1 (see text); **lane 0** nuclei incubated without added DNase I; **lanes 1−4** nuclei incubated with 1.2, 1.8, 2.7 and 4 μg/ml DNase I respectively. Each lane contains 10 μg DNA restricted with *Eco*RI and hybridized to probe B. The bands above germ line are partial digest products. H = hypersensitive site; E = enhancer hypersensitive site; g = germline. The position of the mol. wt markers are given (kb).

A strongly hybridizing sub-fragment is clearly visible at 6 kb and corresponds to the enhancer region hypersensitive site, showing this region to be in an open conformation even in non-Cμ-expressing T cells (see Table I). A weakly hybridizing switch region hypersensitive site is also visible. In contrast, none of the established myeloid cell lines show hypersensitivity to DNase I including the line ML1 (Figure 2C) which has a rearranged IgH allele (Palumbo *et al.*, 1984; Furley *et al.*, 1987).

Recent studies in our laboratory have suggested that the cell line KG1 may correspond to a lymphomyeloid progenitor cell since the parent line undergoes myeloid differentiation in response to GM-CSF or phorbol ester (Koeffler *et al.*, 1981) and its subline KG1a expresses some immature T cell characteristics (Furley *et al.*, 1986b). The apparent T lineage affiliation of KG1a is reinforced by a recent obser-

vation that a further subline has undergone rearrangement of its TCRβ genes (G.Kansas, personal communication). Both KG1 and KG1a express sterile Cμ transcripts from germline Ig genes and as seen in Figure 3A and Table I, both show a strong enhancer hypersensitive site, again an indication of an open chromatin structure.

It was of interest to determine whether the IgH enhancer region remained open and DNase I hypersensitive in KG1 following myeloid differentiation. We observed, in preliminary experiments, that ionomycin added in combination with 12-*O*-tetra decanoyl phorbol-13-acetate (TPA) induced more complete maturation than TPA alone and so these two substances were used together in subsequent experiments. KG1 cells in suspension were treated with TPA and ionomycin and after 4 days the adherent cells were scraped off and analysed for DNase-I hypersensitive sites and expression of Cμ transcripts. Myeloid (monocytic) maturation was confirmed by staining for esterase activity which was strongly expressed in all cells. As illustrated in Figure 3B, the enhancer hypersensitive site appears to close up as the induced cells undergo myeloid maturation, presumably making the chromatin inaccessible to transcription factors. This is reflected by the sharp fall seen in the steady-state level of Cμ transcription in the induced KG1 cells (both adherent and non-adherent fractions) shown in Figure 4. As controls, the putative lymphoid cell precursor subline KG1a and a B cell precursor cell line Nalm 1 (Minowada *et al.*, 1979) are resistant to TPA-induced myeloid differentiation and continue to produce μ mRNA (Figure 4). The response of KG1 cells to the combination of TPA and ionomycin is unlikely to be due to the selection of a subset of cells that have already acquired myeloid characteristics. In the induction experiment, viability is maintained at >95% over the 4-day period with no increase in cell number; TPA plus ionomycin therefore inhibits cell proliferation of KG1 cells. Additionally, >95% of KG1 cells attach to plastic following treatment with TPA and ionomycin and acquire macrophage morphology and cytochemical reactivity (esterase). Many of these cells subsequently detach easily but as indicated in Figure 4 lanes 1 and 5, both attached and detached cells showed a similar response in terms of a reduction in μ mRNA levels. Further cell sorting and subcloning of KG1 cells to see if all cells have the characteristics described would nevertheless be of interest.

It is interesting to note that the weakly hybridizing subband at 9.6 kb was only apparent in the KG1 lines (see Figure 3A) and in the less mature T cell lines (and maps to a region around the DQ52 gene segment and its promoter). Furthermore, unlike the enhancer hypersensitive site it does not appear to close on myeloid induction of KG1 (Figure 3B). We have recently found that although KG1 cells do not produce T cell receptor (TCR)β transcripts, they do have appreciable levels of TCRγ mRNA. When KG1 cells are induced to undergo myeloid differentiation, TCRγ mRNA,

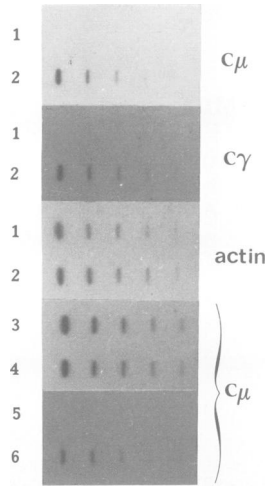

Fig. 4. RNA slot blot analysis of cytoplasmic RNA extracted from TPA plus ionomycin-induced and non-induced cell lines (see text). **Lane 1**, TPA plus ionomycin treated adherent KG1; **lane 2**, non-treated KG1; **lane 3**, non-treated KG1a; **lane 4**, TPA plus ionomycin-treated non-adherent KG1a; **lane 5**, TPA plus ionomycin-treated non-adherent KG1; **lane 6**, TPA plus ionomycin-treated non-adherent NALM 1. Each lane contains samples of 2, 1.0, 0.5, 0.25 and 0.125 μg total cellular RNA respectively, hybridized to the DNA probes shown in the figure.

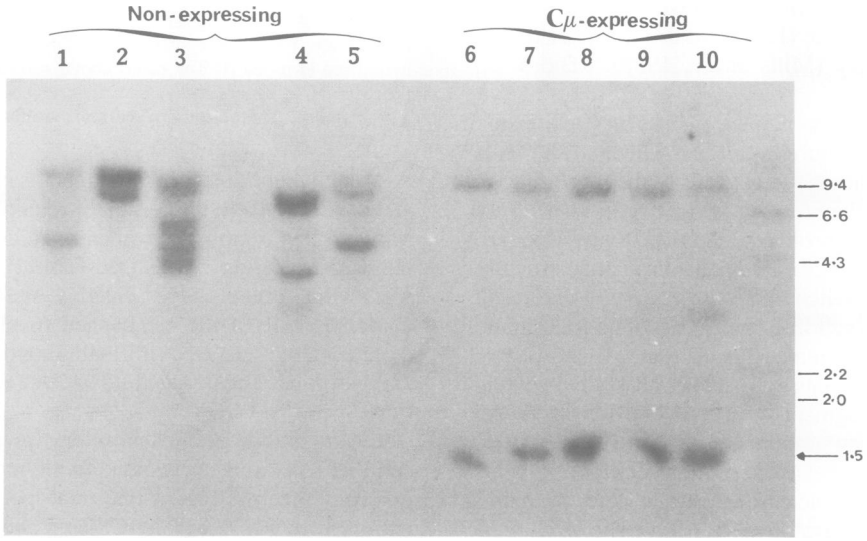

Fig. 5. Methylation status of Cμ-expressing and non-expressing cell lines. Cell line DNA (10 μg) was analysed by a *Bam*HI and *Hha*I double digest and hybridized with probe C (see Figure 1). **(1)** JM; **(2)** HPBALL; **(3)** CEM; **(4)** ML1; **(5)** HL60; **(6)** KG1; **(7)** KG1a; **(8)** REH; **(9)** NALM 1; **(10)** RPMI 1788. Mol. wt markers to the right of blot (in kb).

in common with μ mRNA, is no longer detectable (Figure 4) indicating that this lymphoid lineage-associated gene may also become inaccessible to transcription factors.

Methylation status

To determine the pattern of methylation around the Ig enhancer, we have used, in particular, the methylation-sensitive enzyme *Hha*I. Although the enzyme has no isoschizomer (cf. *Msp*I and *Hpa*II), it allows a comparison of methylation status to be made between cell lines. The sequence 5′GCGC3′ is not cut by *Hha*I if either of the cytosine residues are methylated. DNA from each cell line was digested initially with *Bam*HI and then secondarily with *Hha*I. Fragments were separated on 1.5% agarose gels and filters hybridized to the *Bgl*II−*Hin*dIII probe (C). If both *Hha*I sites H1 and H2 are not methylated (Figure 1), hybridization to probe C will result in the appearance of a 1.5-kb enhancer-spanning fragment. Figure 5 shows the analysis of a number of Cμ-expressing and non-expressing cell lines. All Cμ-expressing cell lines show a 1.5-kb hybridizing fragment confirming that both *Hha*I sites are not methylated. A similar result was obtained with normal thymocytes. No such hybridizing fragment was seen in any of the non-Cμ-expressing T or myeloid cell lines. Subsequent hybridization of the blots to probes B and D (Figure 1) suggests that both sites may be methylated in non-transcribing cell lines (data not shown). This striking correlation of methylation with cell type-specific transcription would suggest that these results are not likely to be due to a fortuitous distribution of polymorphic *Hha*I sites in the different human cell lines used. The only discrepancy in the reciprocal correlation between presence of Cμ mRNA and methylation at the *Hha*I sites around the enhancer in the J−Cμ intron is observed in normal granulocytes where this region unexpectedly appears to be demethylated despite the lack of detectable Cμ mRNA expression.

Interestingly, the *Hha*I site 3′ to the enhancer maps to a cryptic splice site in a region of the J_H−Cμ intron which is highly conserved between murine and human Ig genes. This region also includes the enhancer core sequence and the octanucleotide recognition sequence. It has been shown that initiation of sterile transcription begins heterogeneously downstream from the octanucleotide sequence and that the cryptic splice site (735 bp downstream from the most 5′ start site) creates a large non-translatable exon (or nontron) which is fused to the Cμ domain (Lennon and Perry, 1985). In order to determine whether hypermethylation in this region could limit transcription to incomplete 'intron transcripts' not detectable by the constant region probe (A), we hybridized the intron probe (probe C, Figure 1) to myeloid and T cell Northern blots but were unable to detect any such transcripts (data not shown). Table I shows a summary of the DNase I and methylation status of the lymphoid and myeloid cell lines in this study.

Discussion

Our results show that T and B lineage lymphocytes have an open, DNase I-hypersensitive IgH enhancer region irrespective of the rearrangement status and transcriptional activity of their Ig genes. In contrast, normal granulocytes and all *bona fide* myeloid cell lines, including the cell line ML1, which has nevertheless undergone a rearrangement of one Cμ allele (Palumbo *et al.*, 1984; Furley *et al.*, 1987), showed no DNase I hypersensitivity in the IgH enhancer region.

Although the cell lines used in this study are all aneuploid leukaemic derivatives, they show an overall fidelity of cell lineage-associated gene expression and are not therefore grossly aberrant (Greaves, 1986). Furthermore, our finding that a normal polyclonal population of immature T cells (thymocytes) also has a DNase I-hypersensitive IgH enhancer region indicates that the chromatin configuration in leukaemic clones is unlikely to be an aberrant feature. DNase I hypersensitivity in the IgH gene intron has also previously been observed in mouse thymocytes and T cell lines (Storb *et al.*, 1981).

It seems likely that the role of the IgH enhancer in B cell progenitors is to make IgH joining regions accessible to the recombinases involved in IgH gene rearrangement. There are several lines of evidence which suggest that not only accessibility but also transcription of the germline IgH gene segments precedes and is essential to the gene rearrangement process (Blackwell *et al.*, 1986; Yancopoulos and Alt, 1985). The observations of IgH enhancer DNase I hypersensitivity and Cμ transcription in the cell lines KG1 and KG1a is consistent with this model. The immature haemopoietic cell line KG1 and its descendant subline KG1a both have a DNase I-hypersensitive IgH enhancer and both produce germline Cμ transcripts. Since KG1a has some T cell characteristics, we believe that KG1 may represent a lymphomyeloid progenitor cell or to be of sufficiently immature myeloid phenotype to retain some features of the common progenitor cell (Furley *et al.*, 1986b).

Significantly, the DNase I hypersensitivity is lost when KG1 cells are induced to differentiate into myeloid cells and Cμ mRNA as well as TCRγ mRNA, both present in the parent cells, are no longer detectable. These data suggest the intriguing possibility that a lineage-specific gene (IgH or TCRγ) may be available for expression and rearrangement before lineage commitment and adopt a closed chromatin configuration only after commitment and some degree of maturation into an alternative (i.e. myeloid) lineage.

The DNase I hypersensitivity of the IgH enhancer in thymocytes and T cells and the observation of Cμ transcripts in human thymocytes (see Table I) and frequently in mouse T cell lines and thymocytes (Kemp *et al.*, 1980a,b) presumably reflects the very similar mechanisms of rearrangement in the T cell receptor and IgH genes. Inappropriate and incomplete IgH gene rearrangements are also frequently observed in T cells (Davey *et al.*, 1986; Furley *et al.*, 1987; Greaves *et al.*, 1987). We do not observe Cμ transcripts in the T cell lines we have analysed and the absence of sterile transcripts in these cell lines seems to correlate with a higher level of methylation near the enhancer in the IgH gene. Methylation may play a role in controlling germline transcription or increased levels of methylation may follow the cessation of transcription and help to further repress it in T cell lines. The latter interpretation is suggested by our data on KG1 which on being induced to differentiate ceases to transcribe Cμ without cell division occurring and without any change in methylation.

It is still far from clear what determines the accessibility and activity of the IgH enhancer in B and T cells. The recently identified specific binding sequences in the IgH

enhancer region (Church *et al.*, 1985; Ephrussi *et al.*, 1985; Weinberger *et al.*, 1986; Sen and Baltimore, 1986; Schlokat *et al.*, 1986) probably bind at least three different proteins (Weinberger *et al.*, 1986; Schlokat *et al.*, 1986) but these proteins appear to be present in a wide variety of cell types. Nevertheless, DMS protection experiments carried out on living cells show that the protein-binding sites in B cells are specifically altered in their accessibilty to DMS-induced methylation (Ephrussi *et al.*, 1985; Church *et al.*, 1985). It seems that more subtle changes in the currently identified binding proteins, or other as yet unidentified proteins, determine the specific structure of the IgH enhancer in B cells and its accessibility and relative activity in B and T cells.

Clearly the accessibility and activation of a functional immunoglobulin gene involves a complex hierarchical sequence of developmentally regulated events, the full complement of which are lineage specific. Initiation of this sequence is presumably linked to the process of cell lineage restriction and in this respect our suggestive evidence for accessibility of the IgH enhancer region prior to B, T and possibly myeloid lineage commitment is of considerable interest. Work is currently underway to confirm this with the more clearly defined haemopoietic progenitor cell lines of murine origin. Preliminary results confirm the data and suggestion presented here, in so far as normal, IL-3 dependent multipotential progenitor cells (Spooncer *et al.*, 1984), close a previously open μ enhancer region following physiological induction of myeloid differentiation on bone marrow stromal cell monolayers *in vitro* (A.Ford *et al.*, unpublished observations).

Materials and methods

Cell lines and culture conditions

KG1 and KG1a cell lines kindly provided by Dr D.Golde (Koeffler and Golde, 1978; Koeffler, 1983), were maintained in RPMI 1640 medium supplemented with 20% foetal calf serum (FCS). KG1 has the cytochemical, functional and immunological characteristics of myeloblasts but has been shown to express $C\mu$ transcripts from germline Ig genes (Furley *et al.*, 1986b). The subline KG1a expresses some markers associated with immature T cells including T3(CD3) proteins, produces low level transcripts from germline TCRβ receptor genes and also $C\mu$ transcripts from germline Ig genes (Furley *et al.*, 1986b; Watt *et al.*, 1987).

The origin and phenotypic characteristics of other established cell lines used in this study are listed in Table I. They were provided by Drs J.Minowada, R.C.Gallo, A.Keating and T.Honjo. These cells were grown in RPMI 1640 medium containing 10% FCS at 37°C in a 5% CO_2-gassed incubator at concentrations between 2×10^5 and 1×10^6 per ml.

Human juvenile thymocytes were obtained from patients undergoing cardiac surgery with the consent of the Ethics Committee of the Institute of Cancer Research and Royal Marsden Hospital. Each thymus was washed in ice-cold RPMI 1640 medium containing 10% FCS to remove extraneous blood and teased out to obtain single lymphocyte suspensions. The low density cells (density <1.077 g/ml) were separated on Ficoll–Hypaque and immunophenotyped using immunofluorescence techniques with a series of monoclonal antibodies. The isolated cells were 92–98% positive for CD2 and 35–37% positive for cell surface T cell receptor Cα/β CD3 (Furley *et al.*, 1986a).

Mature neutrophils were obtained from the peripheral blood of healthy volunteers by selecting the high density cells after Ficoll–Hypaque fractionation (density >1.077 g/ml). Erythrocytes also sedimented with the neutrophils and the majority of these were removed by lysis of the cell pellet with 0.147 M NH_4Cl (pH 6.8). Morphologically, 100% of the nucleated cells obtained were mature neutrophils as assessed by May–Grunwald–Giemsa staining.

KG1 induction

10^8 KG1, KG1a and NALM 1 cells in suspension were treated with 10^{-7} M TPA (Sigma) and 1.6 μg/ml ionomycin (Calbiochem) for 4 days. The non-adherent KG1, KG1a and NALM 1 cells were collected. Most of the induced KG1 cells were however adherent and these were collected by scraping the flask with a rubber policeman. Cells were analysed for DNase I hypersensitivity, methylation of the enhancer region and for $C\mu$, $C\gamma$ and actin expression.

Purification of nuclei

Nuclei were purified from $\sim 1 \times 10^8$ cells essentially as described previously (Siebenlist *et al.*, 1984).

DNase I-hypersensitive site analysis

Nuclei were resuspended at $\sim 3 \times 10^7$ per ml and digested for 3 min at room temperature with the amounts of DNase I shown in the figure legends. After termination of reactions (Siebenlist *et al.*, 1984), DNA was purified, limit restricted, electrophoresed and hybridized as described (Mills *et al.*, 1983). This strategy allows hypersensitive sites to be mapped with reference to the *Eco*RI restriction site 5′ to the constant region by the method of indirect end labelling (Wu, 1980). See Figure 1.

Analysis of methylation status

DNA was prepared from cell lines as described previously (Ford *et al.*, 1983). After pre-digestion with *Bam*HI, DNA was restricted with the methylation-sensitive enzyme *Hha*I. This enzyme recognizes the sequence 5′ G-C-G-C 3′. However, G^{m}CGC methylation and GCG^{m}C methylation protect DNA from *Hha*I digestion. Electrophoresis through 1.5% agarose and subsequent hybridization were as described above.

Isolation of RNA

Total cellular RNA was isolated from cell lines using the guanidinium isothiocyanate method of Chirgwin *et al.* (1979). 10 μg total RNA was prepared for electrophoresis by denaturation in 50% formamide, 2.2 M formaldehyde, 20 mM sodium phosphate pH 7.6 run on 1.1% formamide gels (Pritchard and Holland, 1985) and hybridized as before (Furley *et al.*, 1986b). RNA slot blots were performed essentially as described by White and Bancroft (1982), using a BRL hybridot system.

Description of probes (see Figure 1)

(A) 1.6-kb constant region cDNA probe isolated from the B cell line RPMI 1788 (H.V.Molgaard and A.M.Ford, unpublished). This probe contains only constant region and 3′-untranslated sequences and was used to detect $C\mu$ expression.

(B) Analysis of hypersensitive sites was performed using the 900-bp *Sac*I–*Eco*RI genomic fragment (kindly provided by Dr F.Mills) to indirectly end-label the J_H–$C\mu$ intron (Mills *et al.*, 1983).

(C)(D) Methylation studies were performed using the *Bgl*II–*Hin*dIII probe (C) or the J_H-containing *Bgl*II–*Bgl*II probe (D). These probes were isolated from CH28-6, a kind gift of Dr P.Leder (Ravetch *et al.*, 1981).

The TCRγ gene probe was isolated from a γ cDNA clone, a kind gift from Dr T.Mak (Toyonaga and Mak, 1987). A murine genomic actin probe (Willison *et al.*, 1986) that efficiently cross-hybridizes with human RNA/DNA was used as a positive control in all Northern and slot blot experiments. All DNA probes were radio-labelled by the random primer method (Feinberg and Vogelstein, 1986).

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References

Alt,F.W., Blackwell,T.K., De Pinho,R.D., Reth,M.G. and Yancopoulos,G.D. (1986) *Immunol. Rev.*, **89**, 5–30.
Banerji,J., Olson,L. and Schaffner,W. (1983) *Cell*, **33**, 729–740.
Blackwell,T.K., Moore,M.W., Yancopoulos,G.D., Suh,H., Lutzker,S., Selsing,E. and Alt,F.W. (1986) *Nature*, **324**, 585–589.
Calman,A.F. and Peterlin,B.M. (1986) *J. Exp. Med.*, **164**, 1940–1957.
Caplan,A.I. and Ordahl,C.P. (1978) *Science*, **201**, 120–130.
Cheng,G.Y., Minden,M.D., Toyonaga,B., Mak,T.W. and McCulloch,E.A. (1986) *J. Exp. Med.*, **163**, 414–424.
Chirgwin,J.M., Przybyla,A.E., Macdonald,R.J. and Rutter,W.J. (1979) *Biochemistry*, **18**, 5294–5299.
Church,G.M., Ephrussi,A., Gilbert,W. and Tonegawa,S. (1985) *Nature*, **313**, 798–801.
Davey,M.P., Bongiovanni,K.F., Kaulfersch,W., Quertermous,T., Seidman,

J.G., Hershfield,M.S., Kurtzberg,J., Haynes,B.F., Davis,M.M. and Waldmann,T.A. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 8759–8763.
Dreyfus,M., Doyen,N. and Rougeon,F. (1987) *EMBO J.*, **6**, 1685–1690.
Elgin,S.C.R. (1981) *Cell*, **27**, 414–415.
Ephrussi,A., Church,G.M., Tonegawa,S. and Gilbert,W. (1985) *Science*, **227**, 134–140.
Feinberg,A.P. and Vogelstein,B. (1984) *Anal. Biochem.*, **137**, 266–267.
Ford,A.M., Molgaard,H.V., Greaves,M.F. and Gould,H.J. (1983) *EMBO J.*, **2**, 997–1001.
Furley,A.J.W., Mizutani,S., Weilbaecher,K., Dhaliwal,H.S., Ford,A.M., Chan,L.C., Molgaard,H.V., Toyonaga,B., Mak,T., van den Elsen,P., Gold,D., Terhorst,C. and Greaves,M.F. (1986a) *Cell*, **46**, 75–87.
Furley,A.J., Reeves,B.R., Mizutani,S., Altass,L.J., Watt,S.M., Jacob,M.C., van den Elsen,P., Terhorst,C. and Greaves,M.F. (1986b) *Blood*, **68**, 1101–1107.
Furley,A.J.W., Chan,L.C., Mizutani,S., Ford,A.M., Weilbaecher,K., Pegram,S.M. and Greaves,M.F. (1987) *Leukemia*, **1**, 644–652.
Gerster,T., Picard,D. and Schaffner,W. (1986) *Cell*, **45**, 45–52.
Gillies,S.D., Morrison,S.L., Oi,V.T. and Tonegawa,S. (1983) *Cell*, **33**, 717–728.
Greaves,M.F. (1986) *Science*, **234**, 697–704.
Greaves,M.F., Furley,A.J.W., Chan,L.C., Ford,A.M .and Molgaard,H.V. (1987) *Immunol. Today*, **8**, 115–116.
Grosschedl,R. and Baltimore,D. (1985) *Cell*, **41**, 885–897.
Hurwitz,R., Holier,J., Le Bien,T., Minowada,J., Gajl-Peczalska,K., Kubonishi,I. and Kersey,J. (1979) *Int. J. Cancer*, **23**, 174–180.
Katamine,S., Otsu,M., Tada,K., Tsuchiya,S., Sato,T., Ishida,N., Honjo,T. and Ono,Y. (1984) *Nature*, **309**, 369–372.
Kemp,D.J., Harris,A.W., Cory,S. and Adams,J.M. (1980a) *Proc. Natl. Acad. Sci. USA*, **77**, 2876–2880.
Kemp,D.J., Wilson,A., Harris,A.W. and Shortman,K. (1980b) *Nature*, **286**, 168–170.
Klein,S., Sablitzky,F. and Radbruch,A. (1984) *EMBO J.*, **3**, 2473–2476.
Koeffler,H.P. (1983) *Blood*, **62**, 709–721.
Koeffler,H.P. and Golde,D.W. (1978) *Science*, **200**, 1153–1154.
Koeffler,H.P., Bar-Eli,M. and Territo,M.C. (1981) *Cancer Res.*, **41**, 919–926.
Lenon,G.G. and Perry,R.P. (1985) *Nature*, **318**, 475–478.
Mason,J.O., Williams,G.T. and Neuberger,M.S. (1985) *Cell*, **41**, 479–487.
Mercola,M., Goverman,J., Mirell,C. and Calame,K. (1985) *Science*, **227**, 266–270.
Mills,F.C., Fisher,L.M., Kuroda,R., Ford,A.M. and Gould,H.J. (1983) *Nature*, **306**, 809–812.
Minden,M.D. and Mak,T.W. (1986) *Blood*, **68**, 327–336.
Minowada,J., Koshiba,H., Janossy,G., Greaves,M.F., and Bolum,F.J. (1979) *Leukemia Res.*, **3**, 261–266.
Neuberger,M.S. (1983) *EMBO J.*, **2**, 1373–1378.
Palumbo,A., Minowada,J., Erikson,J., Croce,C.M. and Rovera,G. (1984) *Blood*, **64**, 1059–1063.
Pritchard,R.H. and Holland,I.B. (eds) (1985) *Basic Cloning Techniques*. Blackwell Scientific, Oxford, pp. 95–97.
Ravetch,J.V., Siebenlist,U., Korsmeyer,S., Waldmann,T. and Leder,P. (1981) *Cell*, **27**, 583–591.
Rovigatti,U., Mirro,J., Kitchingman,G., Dahl,G., Ochs,J., Murphy,S. and Stass,S. (1984) *Blood*, **63**, 1023–1027.
Schlokat,U., Bohmann,D., Scholer,H. and Gruss,P. (1986) *EMBO J.*, **5**, 3251–3258.
Scholer,H.R. and Gruss,P. (1985) *EMBO J.*, **4**, 3005–3013.
Sen,R. and Baltimore,D. (1986) *Cell*, **46**, 705–716.
Siebenlist,U., Henninghausen,L., Battey,J. and Leder,P. (1984) *Cell*, **37**, 381–391.
Spooncer,E., Boettiger,D. and Dexter,T.M. (1984) *Nature*, **310**, 228–230.
Storb,U., Arp,B. and Wilson,R. (1981) *Nature*, **294**, 90–92.
Thomas,G.H., Siegfried,E.L. and Elgin,S.C.R. (1985) In Reeck,G., Goodwin,G. and Pridgiminech,P. (eds), *Chromosomal Proteins and Gene Expression*. Plenum, NY, pp. 77–103.
Toyonaga,B. and Mak,T. (1987) *Annu. Rev. Immunol.*, **5**, 585–620.
Wabl,M.R. and Burrows,P.D. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 2452–2455.
Waldmann,T.A. (1987) *Adv. Immunol.*, **40**, 247–321.
Watt,S.M., Karhi,K., Gatter,K., Furley,A.J.W., Katz,F.E., Healy,L.E., Altass,L.J., Bradley,N.J., Sutherland,D.R., Levinsky,R. and Greaves,M.F. (1987) *Leukemia*, **1**, 417–426.
Weinberger,J., Baltimore,D. and Sharp,P.A. (1986) *Nature*, **322**, 846–848.
Weintraub,H. and Groudine,M. (1976) *Science*, **193**, 848–856.
White,B.A. and Bancroft,F.C. (1982) *J. Biol. Chem.*, **257**, 8569–8572.
Willison,K.R., Dudley,K. and Potter,J. (1986) *Cell*, **44**, 727–738.
Wu,C. (1980) *Nature*, **286**, 854–860.
Yancopoulos,G.D. and Alt,F.W. (1985) *Cell*, **40**, 271–281.
Yancopoulos,G.D,. Blackwell,T.K., Suh,H., Hood,L. and Alt,F.W. (1986) *Cell*, **44**, 251–259.

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