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A Stage-Specific Enhancer of Immunoglobulin J Chain Gene Is Induced by Interleukin-2 in a Presecretor B Cell Stage

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Summary

Interleukin-2 (IL-2)–induced transcription of the J chain gene was used as a model for analyzing cytokine regulation during B cell development. To determine whether IL-2 signals are targeted to a J chain gene enhancer as well as to its promoter, the sequences flanking the J chain gene were first examined for DNase I hypersensitivity. Of six sites identified, two strong ones, 7.5 kb upstream of the J chain gene, were found to be associated with an enhancer that is active only during the antigen-driven stages of B cell development. Further analyses of the enhancer in the IL-2–responsive presecretor BCL_1 cells showed that the enhancer is activated at this stage by an IL-2 signal that functions by opening the enhancer chromatin and stimulating STAT5 to bind to a STAT5 element critical for the enhancer induction. Moreover, after this early induction stage, the enhancer was shown to be constitutively open and active in terminally differentiated plasma cells.

Introduction

Expression of the J chain gene has provided a useful model to analyze cytokine regulation of B cell antibody responses. The J chain gene is activated late in a primary immune response by the binding of interleukin 2 (IL-2) and/or IL-5 to high-affinity receptors expressed on antigen-stimulated B lymphoblasts (Nakanishi et al., 1984; Matsui et al., 1989). The cytokine signals result in a rapid accumulation of J chain mRNA transcripts (Lamson and Koshland, 1984) and subsequent synthesis of the J chain protein required for the assembly of pentamer immunoglobulin M (IgM) antibody (Niles et al., 1995). Moreover, these events can be reproduced in vitro with presecretor lymphoid cell lines such as B cell leukemia 1 (BCL_1) that retain the ability to express high-affinity IL-2 and/or IL-5 receptors (Brooks et al., 1983; Blackman et al., 1986).

Analyses of the BCL_1 model have shown that IL-2 induces two changes in the J chain gene promoter. One is an opening of the chromatin structure that extends approximately 170 bp upstream of the transcriptional start site (Minie and Koshland, 1986). A second is the decreased binding of the transcription factor BSAP (B cell–specific activator protein, also known as Pax5) to a repressor element located between base pairs −126 and −113 in the promoter (McFadden and Koshland, 1991; Rinkenberger et al., 1996). The interaction of BSAP with this element mediates the silencing of the J chain gene during the early antigen-independent stages of B cell development, and the repression is relieved during the antigen-driven stages by an IL-2–induced down-regulation of BSAP RNA expression (Rinkenberger et al., 1996). The BCL_1 system has also been used to identify a second target of IL-2/IL-5 regulation, the zinc finger Blimp-1 nuclear protein (Turner et al., 1994). Transcripts of the *Blimp-1* gene are rapidly induced following IL-2 or IL-5 treatment, and their expression leads to the induction of J chain message and a high level of IgM secretion. As yet, however, no direct interaction has been demonstrated between Blimp-1 and J chain gene sequences.

In the present work, the analyses of this model were extended to determine whether the IL-2 signal is targeted to a J chain gene enhancer as well as to its promoter. Transient transfection assays had shown the J chain promoter itself had little or no detectable ability to drive expression of a reporter gene (Lansford et al., 1992). It seemed likely, therefore, that J chain gene activity was strongly dependent on an enhancer element, as is the case for the genes encoding other immunoglobulin chains (Hagman et al., 1990; Henderson and Calame, 1995). In this report, we describe the identification of an enhancer sequence by examining the flanking sequences of the J chain gene for DNase I hypersensitive sites (HSSs) associated with J chain gene transcription in plasma cell lines. We show that two strongly hypersensitive sites 7.5 kb upstream of the transcriptional start site are induced by IL-2 treatment of presecretor BCL_1 cells and that the sequence containing these sites functions as a B cell–specific and developmentally regulated enhancer. Furthermore, we identify STAT5 (signal transducer and activator of transcription 5) as one of the IL-2 targets regulating the induction of the J chain gene enhancer. Finally, we show that in terminally differentiated plasma B cells such as S194 and MOPC315, the enhancer of the J chain gene is constitutively active in the absence of IL-2 and STAT5 stimulation.

Results

Identification of Six DNase I Hypersensitive Sites in the Upstream Region of the Expressed J Chain Gene

As a first approach in locating the putative J chain enhancer, the flanking sequences of the active J chain gene were examined for DNase I hypersensitive sites (HSSs). These sites provide indices of open chromatin structures that are known to be associated with *cis*-acting control elements, such as enhancers, silencers, insulators, locus control regions (LCRs), and matrix attachment regions (MARs). Nuclei from plasma cell lines expressing the J chain were subjected to time course

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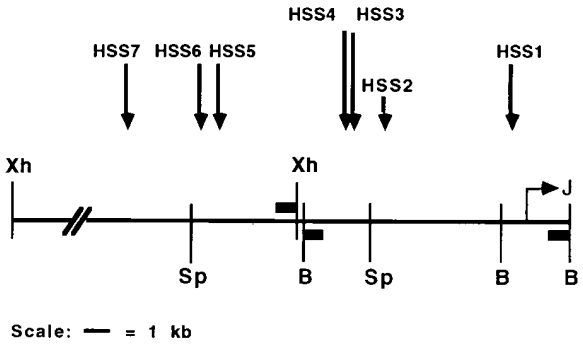

Figure 1. Identification of DNase I HSSs 5′ of the Active J Chain Gene

Nuclei from J chain–expressing S194 cells were subjected to time-dependent digestion with DNase I. The DNA was then extracted, secondarily digested with appropriate restriction enzymes, and electrophoresed on 0.8% agarose gels. HSS 1 was mapped by probing BamHI-digested DNA with a 600 bp EcoRI–BamHI sequence from the 3′ end of the 2.85 kb BamHI fragment; HSSs 2, 3, and 4 were identified by probing BamHI-digested DNA with a 165 bp BamHI–DraI sequence from the 5′ terminus of the 8.2 kb BamHI fragment; and HSSs 5 and 6 and HSS 7 were mapped by probing SpeI–XhoI–digested and XhoI-digested DNAs, respectively, with a 310 bp sequence from the 3′ terminus of the 23 kb XhoI fragment. The thick bars on the map represent the respective probes; the vertical arrows indicate the location of the HSSs, and their length is a rough measure of the degree of sensitivity; and the horizontal arrow indicates the transcriptional start site of the J chain gene. B, BamHI; Sp, SpeI; and Xh, XhoI.

digestion with DNase I. The DNA was then extracted, treated with appropriate restriction enzymes, and analyzed in Southern blots with probes derived from the termini of the relevant restriction fragments. By this strategy, six DNase I HSSs were found to span a 16 kb sequence immediately 5′ of the J chain gene promoter (Figure 1). The sites were designated HSSs 2–7; HSS 1 was reserved for the nuclease hypersensitive site induced in the J chain gene promoter (Minie and Koshland, 1986). No additional sites were detected in another 16 kb of sequence upstream nor in 14 kb of sequence 3′ to the J chain gene.

HSSs 2–7 were located by use of the BamHI and XhoI restriction enzymes and probes as illustrated in Figure 1. Mapping revealed that the sites were distributed in two groups and varied in susceptibility to DNase I digestion. One group was located between 6 and 7.5 kb upstream of the transcriptional start site and consisted of two very strong sites (HSSs 3 and 4), approximately 200 bp apart, flanked by a third weak site (HSS 2) 1.5 kb away. The second group was located 12–16 kb 5′ of the transcriptional start site and consisted of three sites (HSSs 5–7) of medium DNase I sensitivity, roughly equivalent to that displayed by the promoter HSS 1 site.

Development of Four of the Six HSSs (3–6) Correlates with J Chain Gene Expression

To assess the relationship between the hypersensitive sites and J chain gene transcription, the assays were extended to J chain–negative cell lines representative of immature and mature B cells and helper T cells. The resulting Southern blots showed a strong association betweeen HSSs 3, 4, 5, and 6 and J chain gene transcription. The chromatin at these sites was completely resistant to DNase I digestion in non-B cells; it became very slightly DNase I sensitive in the WEHI231 and K46R cell lines, representative of immature and mature B cells, respectively; and it was readily accessible to DNase I cleavage in the MPC11 and S194 cell lines, representative of plasma cells expressing high levels of J chain (Figure 2A). Moreover, the pattern of chromatin changes in the upstream HSSs 3, 4, 5, and 6 during B cell development was identical to that previously shown to occur at the HSS 1 site in the J chain gene promoter (Figure 2A) (Minie and Koshland, 1986). In contrast, sites 2 and 7 were found to be independent of J chain gene expression. The weak HSS 2 was confined to B cells, but appeared in DNA from J chain–negative as well as J chain–positive cells (data not shown). The relatively strong HSS 7 was present in all lymphoid DNA examined (Figure 2B).

Regulation of HSSs 3 and 4 by IL-2

In previous studies, advantage was taken of the IL-2–inducible BCL_1 cell line to demonstrate that treatment with the cytokine induces chromatin changes in the J chain promoter. The changes were manifest by the development of a DNase I HSS (HSS 1) (Minie and Koshland, 1986). The same system was used in the present study to assess a potential IL-2 responsiveness of the HSSs. BCL_1 cells were cultured for 2 days in the presence or absence of IL-2. Nuclei were harvested and subjected to time-dependent digestion with DNase I. Southern blots of the DNase I–digested samples showed that HSSs 3 and 4 were just barely detectable in the DNA from untreated BCL_1 cells (Figure 2C). The finding that the chromatin in these cells was slightly accessible to DNase I attack was consistent with their presecretor stage of development. In the DNA from IL-2–treated cells, however, HSSs 3 and 4 were very accessible to DNase I cleavage (Figure 2C), indicating that the cytokine induced a complete opening of the chromatin.

Enhancer Activity of the Sequences Containing HSSs 3 and 4

The function of the J chain–associated hypersensitive sequences was evaluated by transient transfection analyses using a luciferase reporter construct containing 1.2 kb of J chain gene promoter sequence (Lansford et al., 1992). The luciferase assay system was chosen because of its sensitivity; with the conventional chloramphenicol acetyl transferase (CAT) reporter assay, no J chain promoter activity could be detected with a promoter-alone construct, whereas with the luciferase assay, values of 1000 and 10,000 relative light units (RLU) were obtained in J chain–negative and J chain–expressing B cells, respectively. As these baseline values were reliably greater than those (200–500 RLU) of mock or basic vector transfections, it was possible to quantitate any enhancer-induced increase in promoter activity.

For the analyses of the HSSs 3 and 4 sequences, a 4 kb Sau3AI–SpeI fragment containing the two sites was subjected to systematic 5′ and 3′ deletions (Figure 3).

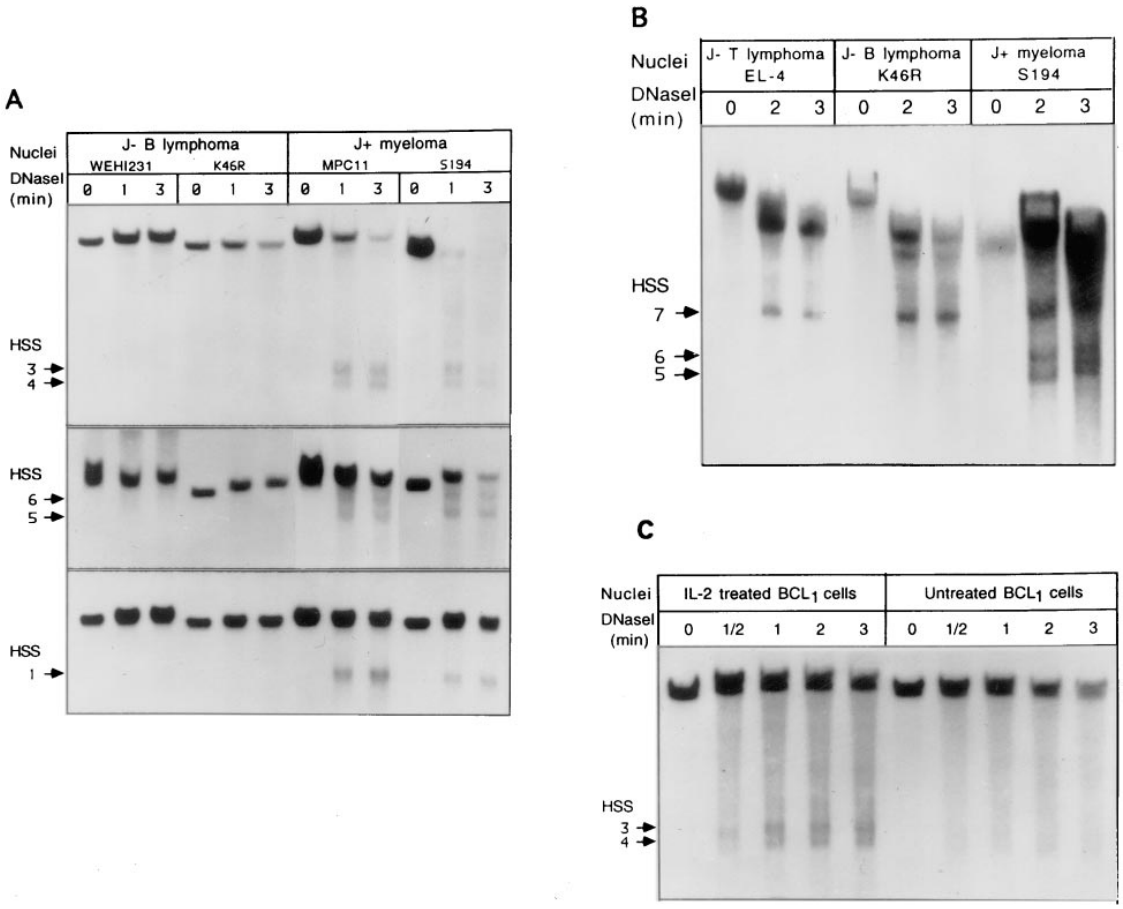

Figure 2. Characterization of DNase I HSSs of the J Chain Gene

(A and B) Development of DNase I HSSs 3, 4, 5, and 6 correlates with J chain gene expression. Nuclei from J chain–negative B and T cell lymphomas and from J chain–positive myelomas were digested with DNase I for the times indicated. The DNA was extracted and assayed on Southern blots for the presence of (A) HSSs 1–6 and (B) HSS 7 by the procedures described in the legend to Figure 1.

(C) An IL-2 signal opens the chromatin structure of the J chain gene. Nuclei were isolated from BCL_1 cells cultured for 2 days in the presence and absence of IL-2 and digested with DNase I as indicated; DNA was extracted and then digested with BamHI. The Southern blot was hybridized with the BamHI–DraI probe from the 5′ end of the BamHI 8.2 kb fragment.

The fragments generated were inserted in both orientations 5′ to the J chain promoter sequence, and the resulting constructs were assessed for enhancer activity by transient transfection into J chain–expressing S194 myeloma cells. Assays of the inserts in 5′-to-3′ orientation showed that HSSs 3 and 4 were associated with a sequence that amplified the activity of the J chain promoter 16- to 23-fold (Figure 3). This amplification was one-tenth that provided by a 1 kb insert containing the full μ heavy-chain intron enhancer (Figure 3) and was roughly equivalent to that provided by the κ chain intron enhancer (data not shown). The 1.1 kb NdeI–NheI fragment was found to be the smallest sequence that provided full enhancement. Any further reduction in size, either 5′ or 3′, resulted in a significant loss in activity (Figure 3). Assays of the inserts in 3′-to-5′ orientation gave a similar pattern of activities, except that the amplification in each case was 20%–30% less (data not shown).

For analyses of the function of HSSs 5 and 6, a 4.5 kb SpeI–XhoI fragment containing the two sites (Figure 1) was inserted 5′ to the J chain promoter and its activity was assessed following transient transfection of the construct into S194 cells. In contrast to the HSSs 3 and 4 sequence, that associated with HSSs 5 and 6 displayed no enhancer activity (data not shown). The average luciferase RLU was 1.4 times the RLU value obtained for the J chain promoter alone. This finding indicated that the correlation between the development of HSSs 5 and 6 and J chain gene expression is likely to represent some other transcriptional control, such as a boundary element or LCR.

Characterization of the J Chain Gene Enhancer

The properties of the J chain gene enhancer sequences containing HSSs 3 and 4 were defined by comparing their activities in J chain–silent and J chain–expressing lymphoid cell lines (Table 1). Transient transfection assays showed that none of the enhancer plasmid constructs was capable of activating the J chain promoter in T cell, immature B cell, or mature B cell lines, in which the genomic J chain enhancer retains a closed chromatin structure. On the other hand, the enhancer constructs were functional in all plasmacytomas, such

Table 1. Specificity of J Chain Gene Enhancer Activity

Test Construct		Lymphoid Cell Lines[a]					
		J Chain negative			J Chain Positive		
Enhancer	Promoter	EL4	WEHI231	K46R	S194	MPC11	MOPC315
JE4.0kb	J	1.0[b]	1.1	1.1	23	7	83
JE1.8kb	J	1.0	1.4	1.6	16	8	51
JE1.1kb	J	1.8	1.2	1.3	19	10	51
JE0.5kb	J	1.7	1.5	1.4	5.0	4	16
JE4.0kb	Fos	2.2	ND	ND	9.1	ND	ND
JE1.8kb	Fos	2.1	ND	ND	8.6	ND	ND
Eμ1.0kb	J	1.0	9.5	16	203	98	110

ND, not determined.

[a] EL-4 is a T cell line; WEHI231 and K46R are lymphoma lines representative of immature and mature B cells, respectively; S194, MPC11, and MOPC315 are plasmacytoma lines.

[b] Fold increase over the activity of the J chain or the Fos promoter alone. Each value represents the average of at least two independent determinations.

as S194 (Table 1), in which the genomic J chain enhancer and promoter have an open chromatin conformation and the J chain is expressed. These results might be explained in several ways. The test plasmids could have assumed the chromatin structure of the endogenous enhancer and promoter and behaved accordingly in the various cell types. Alternatively, the test plasmids could have retained an open conformation but have been unable to function in J chain–negative lymphoid cells because a critical transcription factor(s) was lacking. The latter explanation is consistent with the variation in enhancer activity observed among the plasmacytoma cell lines. The enhancer construct was four times as active in MOPC315 and one half as active in MPC11 cells as in the transfection standard S194 cells (Table 1). Since control transfections showed that the differences in activity could not be attributed to differences in transfection efficiency, it seemed likely that they reflected the relative availability of transcription factors contributing to enhancer activity. In any case, the data clearly indicate that the J chain enhancer associated with HSSs 3 and 4 is B cell specific and stage specific in its activity.

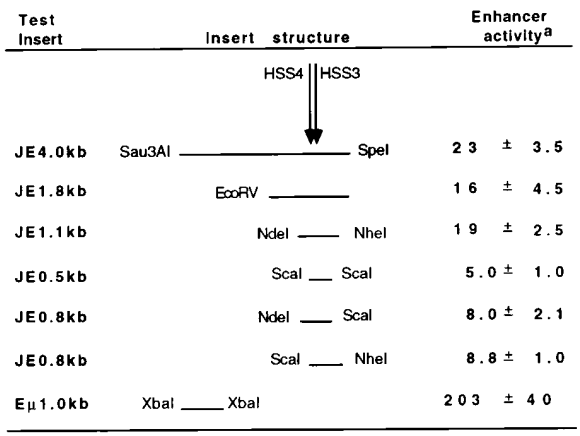

Figure 3. Sequences Containing HSSs 3 and 4 Function as Enhancers of J Chain Gene Expression

A 4 kb Sau3AI–SpeI fragment containing HSSs 3 and 4 was subjected to successive 5′ and 3′ deletions. The fragments generated were examined for their ability to activate the J chain gene promoter by transient transfection–luciferase assays in J chain–expressing S194 myeloma cells. The mean enhancer activities (± SEM) were calculated from the average values of at least two independent, duplicate determinations of luciferase activity.

The 4.0 and 1.8 kb enhancer sequences were also tested in the context of a heterologous promoter expressed in many cell types. For these experiments, plasmids were constructed with the enhancer fragments located 5′ to a minimal c-*fos* promoter and the luciferase gene. The c-*fos* construct contained only a TATA box and a single CREB (cAMP response element binding protein) element (Gilman et al., 1986) and gave baseline values comparable to those of the J chain promoter construct in B cell transient transfections. Although the transfection assays showed that the J chain enhancer was capable of transactivating the c-*fos* promoter in plasmacytomas, the amplification was only one half that seen in the context of the J chain promoter. This difference suggested that cooperative interactions may occur between factors binding to the enhancer and the J chain promoter to effect a higher level of activation.

Sequence and In Vivo Footprinting Analyses

Sequence analyses of the 1.1 kb NdeI–NheI enhancer fragment revealed that the potential consensus sequences for factor binding were concentrated within a 150 bp region surrounding the PstI site. This region included a STAT5, an Ets (a family of Ets domain proteins related to avian erythroblastosis virus E26 transforming specific gene), a C/EBP (CCAAT-enhancer binding protein), and two CBF (core binding factor) consensus binding sites; two consensus E2 boxes; and an 8/9 match to the nuclear factor κB (NF-κB) consensus sequence (Figure 4A). One additional CBF consensus site and a STAT3 consensus site were found near the 5′ end and 3′ end of the 1.1 kb NdeI–NheI fragment, respectively.

Although both the 5′ and 3′ sides of the 1.1 kb enhancer fragment were necessary for full enhancer activity, the core 0.5 kb ScaI fragment containing the concentrated consensus sites was explored first to analyze the relationship of the various potential regulatory elements to enhancer activity by dimethyl sulfate (DMS) in vivo footprinting assays. Using the gene-specific primers indicated in Figure 4A, more than 0.5 kb of DNA sequence

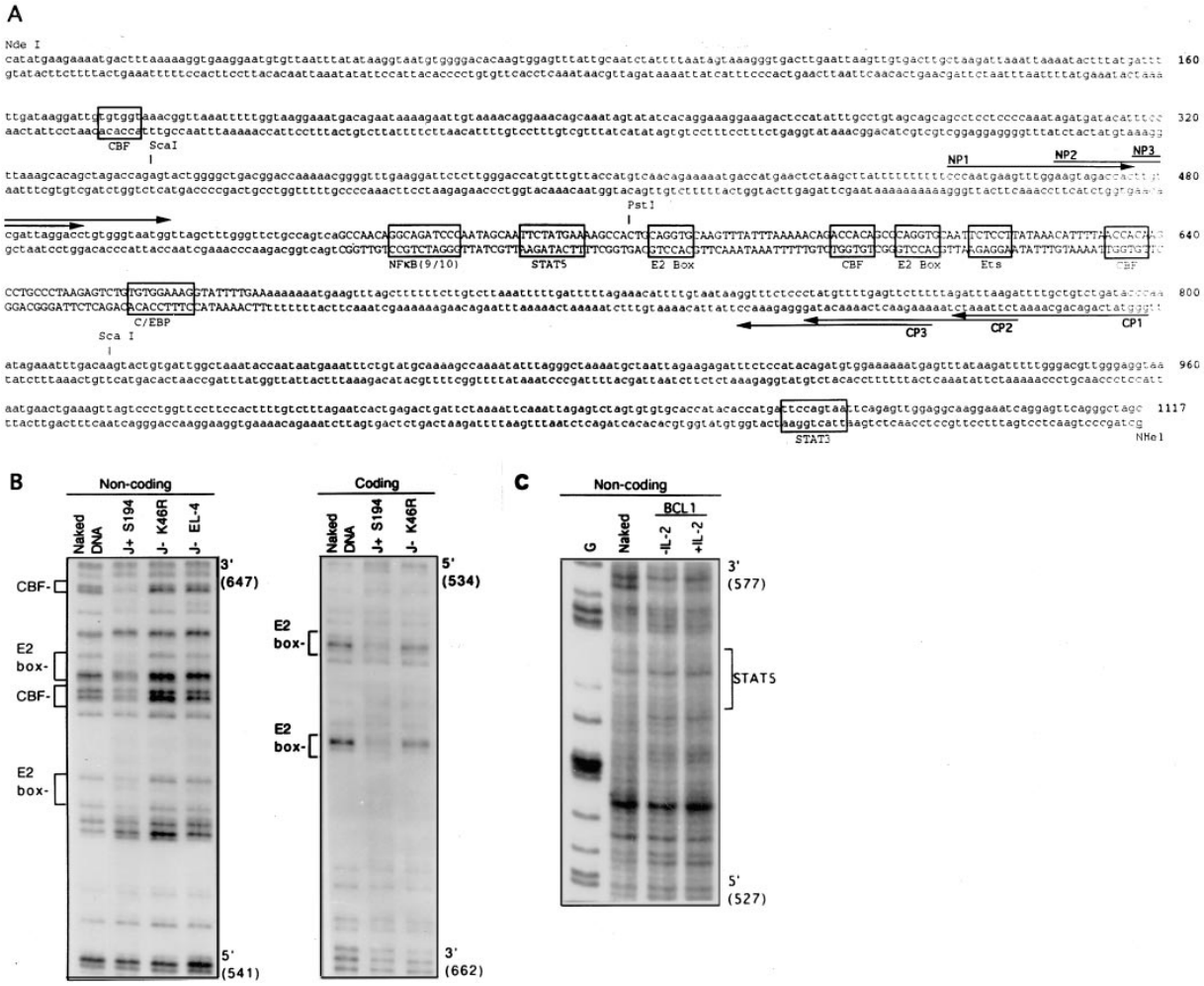

Figure 4. Sequence and In Vivo Footprinting Analyses for the J Chain Gene Enhancer

(A) The sequence of a 1.1 kb NdeI–NheI J chain gene enhancer. The core 150 bp sequence containing a concentration of potential consensus factor binding sites was indicated by capital letters, with flanking sequences by lower-case letters. The potential consensus sequences are boxed. Primers used for in vivo footprinting experiments are indicated by arrows (NPs 1–3 for the noncoding strand and CPs 1–3 for the coding strand).

(B) DMS in vivo footprinting of the noncoding and coding strands of the core enhancer sequence shown in (A). Footprints were detected only in the DNA from J chain–positive S194 cells; the G residues in and around the E2 boxes were protected on both strands, those in the CBF site on the noncoding strand.

(C) An IL-2–induced in vivo DNase I footprinting over the STAT5 site in BCL_1 cells. Lane G is a DMS in vivo footprinting reaction of a naked S194 DNA.

in the middle of the 1.1 kb J chain enhancer was investigated. Analyses of the DNA from two cell lines with an inactive J chain gene enhancer, the EL-4 T cell lymphoma and the K46R mature B cell lymphoma, did not reveal any detectable footprints over the sequence tested. The pattern of G reactivity was essentially identical to that of naked DNA (Figure 4B). In contrast, four clear footprints were observed within the consensus-clustered 150 bp region from S194 myeloma cells where the J chain gene enhancer is active: two over the E2 boxes on both the coding and noncoding strands and two over the CBF sites on the noncoding strand (Figure 4B). No in vivo footprint other than those seen within the 150 bp core region was detected over the more than 500 bp region. Although the putative G-rich NF-κB, Ets, and C/EBP sites are consensus-like, no protein binding was observed in our in vivo footprinting experiments.

IL-2 Regulation of STAT5 Binding to the J Chain Enhancer

Because IL-2 was shown to induce expression of the J chain gene by opening of the chromatin structure of the J chain enhancer in BCL_1 cells (Figure 2C) and because a STAT5 consensus site was identified within the J chain enhancer (Figure 4A), a possible role of IL-2–inducible STAT5 (Hou et al., 1995) in the process was investigated. To do so, the in vivo footprinting experiment was performed in IL-2–induced BCL_1 cells. DNase I in vivo footprinting was used because it was not possible to obtain

definitive evidence for factor binding by the DMS method, with only one G residue present on either strand in the STAT5 sequence (TTCTATGAA). An induced footprint over this STAT5 sequence was detected in the IL-2–treated BCL_1 cells (Figure 4C), although one strong band in the middle of a footprinted STAT5 sequence remained. This pattern of footprint might be a characteristic of STAT5 binding. The E2 and CBF sites were also footprinted in IL-2–treated BCL_1 cells, although the footprints were weak and not as prominent as in S194 cells (data not shown). However, E2 and CBF turned out to be not the IL-2–induced factors in our electrophoretic mobility shift assay (EMSA) analyses (discussed below).

EMSA was employed to verify factor binding to the footprinted motifs and to examine the effect of IL-2 stimulation on factor content and its binding. For these experiments, nuclear extracts were prepared from unstimulated J chain–negative BCL_1 cells and IL-2–stimulated J chain–positive BCL_1 cells and assayed for factor content and binding, using as probes the E2, CBF, STAT3, and STAT5 motifs from the enhancer sequences. No IL-2–related differences were observed in the binding of E12/E47 (Murre et al., 1989), CBF (Speck and Stacy, 1995), or STAT3 to their respective probes (Figures 5A–5C). In each case, the complexes formed with extracts from untreated or IL-2–treated cells were equivalent in intensity and mobility. Interestingly, the STAT3 motif (TTCCA GTAA) found near the 3′ end of the 1.1 kb enhancer fragment was shown not to have cross-binding activity with the STAT5 motif (TTCTATGAA) of the core region (data not shown). Furthermore, to our surprise, the STAT3 binding activity was present constitutively in the BCL_1 cells regardless of IL-2 treatment (Figure 5C).

A clear difference was observed, however, in the binding pattern of the STAT5 element present within the 150 bp core region (Figure 5D). Two slowly migrating complexes were generated with extracts from IL-2–stimulated cells but not from unstimulated cells (Figure 5D, lanes a and b). The factors responsible for the new complexes were identified as STAT5 proteins by several criteria. Both complexes were specifically inhibited by a 100 molar excess of the classic GAS (interferon-γ–activated sequence) element from the Fcγ receptor I (FcγRI) gene (Hou et al., 1995), but not by a 100 molar excess of an irrelevant oligonucleotide sequence (Figure 5D, lanes c and d). Both complexes were supershifted by the addition of a rabbit antiserum specific for the murine STAT5a and STAT5b proteins, but not by preimmune rabbit serum (Figure 5D, lanes e and f).

Finally, the presence of a phosphorylated and active STAT5 protein was further assessed by Western blot analysis of nuclear extracts. A phosphorylated form of STAT5 was detected in the IL-2–treated BCL_1 cells (Figure 5E, lanes 2 and 5), but not in untreated BCL_1 cells (Figure 5E, lane 1), well correlating with their binding activities as shown by EMSA analysis (Figure 5D). Surprisingly, however, the phosphorylated STAT5 was not detected in the J chain–expressing S194 and MOPC315 plasma cells (Figure 5E, lanes 3 and 4). EMSA analysis also confirmed the absence of STAT5 binding activity in these plasma cells (data not shown). There are numerous possible explanations of these results. One possibility is that the plasma cells have passed the IL-2 required stage for induction of J chain gene expression during terminal B cell development and then maintain its expression constitutively in the absence of IL-2 and STAT5 stimulation.

Role of STAT5 in IL-2–Induced Enhancer Activity

A regulatory role for STAT5 was confirmed by mutating the STAT5 motif of the JE1.1kb plasmid and measuring the effect of the mutation on enhancer activity in the presence or absence of IL-2. The substitutions (TTCTAT GAA to caCTATGtc), previously shown to abrogate binding of STAT5 to the FcγRI GAS motif (Hou et al., 1995), were also demonstrated to abrogate its binding to the STAT5 motif of the J chain enhancer in our EMSA analyses (data not shown). The STAT5 mutant construct was transfected into BCL_1 cells and compared with the wild-type counterpart for their ability to induce the enhancer with or without IL-2 treatment. The STAT5 mutant construct resulted in an about 80% loss in its ability to induce the enhancer in the presence of IL-2, compared with that of wild-type control (Figure 6). This STAT5 mutant effect is believed to be caused by a failure of STAT5 to bind to the mutated sequence, since STAT5 was shown to be the only IL-2–induced binding activity on the site in EMSA analyses (Figure 5D).

Discussion

In the present study, the long-suspected J chain gene enhancer was identified as a 1.1 kb sequence located 7–8 kb upstream of the J chain gene start site. The identification was made by correlating the development of hypersensitive sites in the flanking sequences with J chain gene transcription, and assaying the hypersensitive sequences for enhancer function following transient transfection. Of the five strong hypersensitive sites observed, only a 1.1 kb sequence containing two of the sites, HSSs 3 and 4, met the criteria of an enhancer by producing, independently of its orientation, a 10- to 50-fold increase in J chain promoter activity. Structural analyses suggested that the 1.1 kb J chain enhancer is organized similarly to the heavy-chain intron enhancer (Forrester et al., 1994). It contains a 150–200 bp core element with multiple binding sites for transcription factors and AT-rich flanking sequences that may represent MARs. However, transgenic experiments will be required to characterize the flanking sequences and to determine whether they provide control regions, MARs, and/or LCRs, as their counterparts in the heavy-chain intron enhancer do (Forrester et al., 1994; Jenuwein et al., 1997). Alternatively, the more distant DNase I hypersensitive sequences, HSSs 5, 6, and 7, may serve to mark an LCR, as is the case for the heavy-chain 3′ enhancer (Madisen and Groudine, 1994).

The activity of the J chain gene enhancer was found to be B cell–specific and strictly confined to the antigen-driven stage of B cell differentiation. The evidence was obtained from a combination of DNase I hypersensitivity, in vivo footprinting, and in vivo functional assays. Such analyses showed that the enhancer was in an inactive state in J chain–negative B cell lines, representative of early antigen-independent stages. The DNA was packaged in a closed conformation that did not allow detectable factor interaction. In contrast, the enhancer was

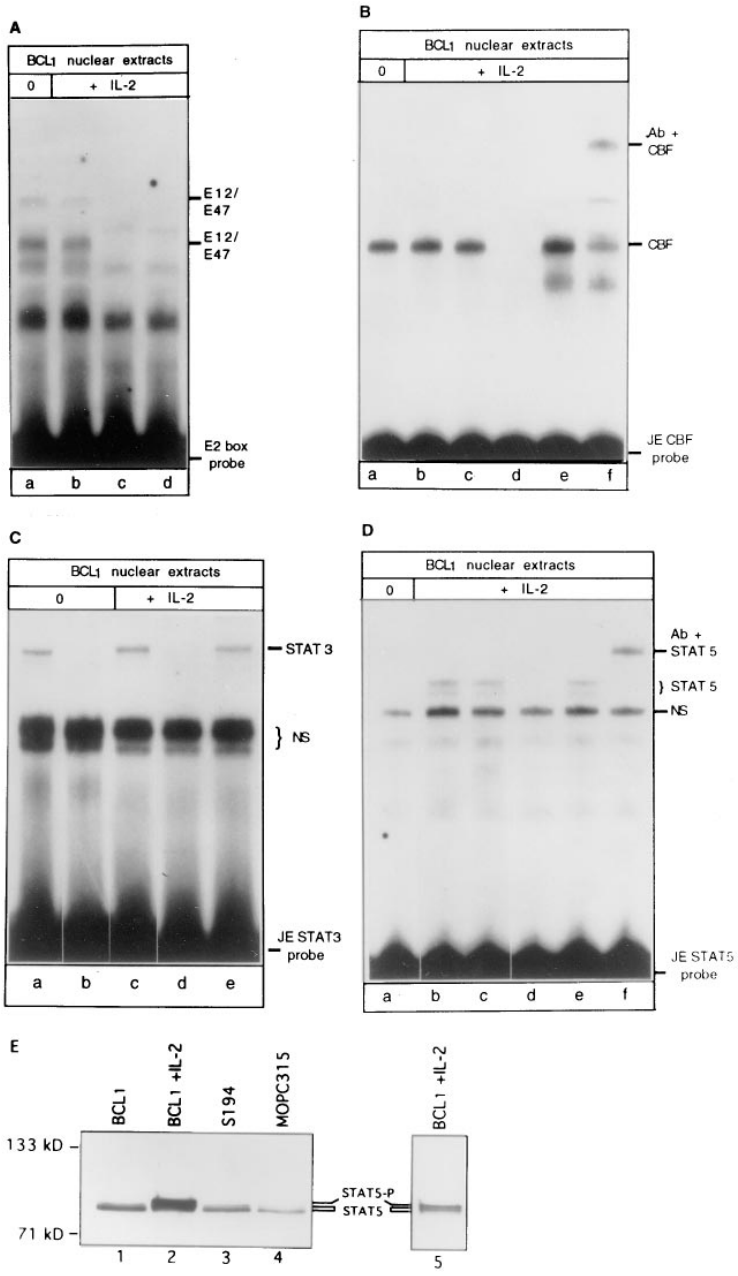

Figure 5. IL-2 Regulation of Factor Content and Binding to the J Chain Gene Enhancer

(A) IL-2–independent binding of E12/E47. EMSA analysis of the complexes formed with BCL_1 nuclear extracts and an E2 oligonucleotide from the J chain gene enhancer. Lanes: (a) extract from untreated cells; (b–d) extracts from IL-2–treated cells (b) in the absence of antibody and in the presence of (c) anti-E12 antibody and (d) anti-E47 antibody.

(B) IL-2–independent binding of CBF. EMSA analysis of the complexes formed with BCL_1 nuclear extracts and a CBF oligonucleotide from the J chain gene enhancer. Lanes: (a) extract from untreated cells; (b–f) extracts from IL-2–treated cells (b) alone, (c–f) in the presence of (c) a 100 molar excess of irrelevant oligonucleotide, (d) a 100 molar excess of the CBF oligonucleotide from the T cell receptor β enhancer (Wotton et al., 1994), (e) preimmune rabbit serum, and (f) anti-CBFβ2 antibody (Lu et al., 1995).

(C) IL-2–independent binding of STAT3. EMSA analysis of the complexes formed with BCL_1 nuclear extracts and a STAT3 oligonucleotide from the J chain gene enhancer. Lanes: (a and b) extract from untreated cells (a) in the absence of antibody and (b) in the presence of anti-STAT3 antibody; (c–e) extracts from IL-2–treated cells (c) in the absence of antibody and in the presence of (d) anti-STAT3 antibody and (e) anti-STAT5 antibody. NS, nonspecific binding complex.

(D) IL-2–dependent binding of STAT5. EMSA analysis of the complexes formed with BCL_1 nuclear extracts and a STAT5 oligonucleotide from the J chain gene enhancer. Lanes: (a) extract from untreated cells; (b–f) extracts from IL-2 treated cells (b) alone, (c–f) in the presence of (c) a 100 molar excess of an irrelevant oligonucleotide, (d) a 100 molar excess of the GAS oligonucleotide from the FcγRI promoter (Hou et al., 1995), (e) preimmune rabbit serum, and (f) anti-STAT5b antibody that is specific for both STAT5a and STAT5b. NS, nonspecific binding complex.

(E) Western blot analysis for STAT5 in the nuclear extracts of BCL_1, S194, and MOPC315. Two bands indicative of unphosphorylated a and b isoforms were detected in IL-2–untreated BCL_1, S194, and MOPC315 cells, whereas a new band indicative of a phosphorylated form of STAT5a and/or STAT5b was detected on top of those two bands only in IL-2–treated BCL_1 cells. Lane 5 is from another blot with a short chromogenic reaction time.

switched to an active state in cell lines representative of various J chain–positive plasma cell stages. The chromatin structure was opened to allow factor binding and consequent J chain gene transcription. To our knowledge, the J chain gene enhancer is the only known lymphoid enhancer to exhibit such a limited pattern of activity. This pattern means that the enhancer may provide a useful tool for limiting the expression of transfected genes to the antigen-driven stage of B cell differentiation and thus for analyzing the contribution of such genes to the B cell immune response.

In addition to characterizing the J chain gene enhancer, the present study demonstrated that IL-2 delivers the "on" signal to the enhancer. The evidence was obtained from analyses of the IL-2 responsive presecretor line, BCL_1. IL-2 stimulation of these cells induced two enhancer-related changes: opening of the chromatin structure and phosphorylation of the STAT5 transcription factor and its binding to the enhancer motif. The importance of the STAT5 interaction was established by the 80% loss in enhancer inducibility that followed a mutation of the STAT5 element. These data provide the first example of IL-2–dependent STAT5 gene regulation in B cells. The related regulation of the IL-2 receptor α chain (IL-2Rα) is likely to provide another such example. Recent studies have shown that transcription of the IL-2Rα gene in T cells is initiated by IL-1 or antigen stimulus and subsequently up-regulated and

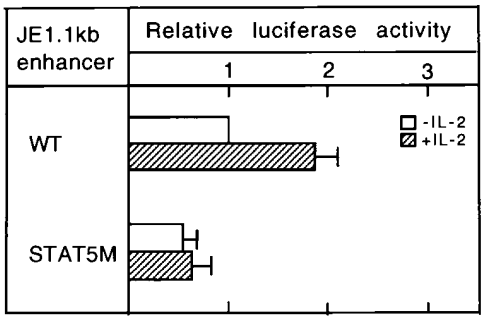

Figure 6. STAT5 Element Is Critical for the IL-2 Induction of J Chain Gene Enhancer

The 1.1 kb enhancer plasmid containing clustered mutations in the STAT5 element (STAT5M) was electrotransfected into BCL_1 cells and compared with the wild-type plasmid (WT) for their ability to induce the luciferase expression in the presence or absence of IL-2. The luciferase activities obtained are expressed relative to the value obtained with the wild-type enhancer in the absence of IL-2, which was set to 1. The values given represent the average of six independent determinations, and the calculated SEMs are represented by error bars.

maintained by an IL-2 stimulus (Sperisen et al., 1995). IL-2 responsiveness maps to an upstream enhancer that contains a STAT element and depends on STAT5 binding for its activity (Sperisen et al., 1995; Lecine et al., 1996). Since the α chain is required for the formation of the high-affinity receptor that mediates IL-2 signaling in B cells as well as T cells, the mechanisms regulating α chain expression would be expected to be the same in the two cell types. Moreover, the requirement of a high-affinity receptor for effective IL-2 signaling explains why the activity of the J chain gene enhancer is limited to the antigen-driven stages of B cell development.

Studies of the mechanism of IL-2 regulation of B cell immune responses have identified several nuclear outcomes of the IL-2 signal. They include the activation of the J chain gene enhancer and the likely activation of the IL-2Rα chain enhancer (discussed above), as well as the activation of the J chain gene promoter (Rinkenberger et al., 1996) and the heavy-chain 3′ enhancer (J. J. Wallin and M. E. K., unpublished data). In each case, the IL-2 signal has been shown to induce a change in transcription factor expression: the activation of STAT5 for the J chain and IL-2Rα chain enhancer functions (this report and Lecine et al., 1996) and the decrease in BSAP expression to relieve the inhibition of the J chain promoter (McFadden and Koshland, 1991; Rinkenberger et al., 1996) and 3′ heavy-chain enhancer functions (Neurath et al., 1995). In each case, the IL-2 signal has also been shown to induce an opening in the chromatin structure of the target promoter or enhancer (Minie and Koshland, 1986; Giannini et al., 1993; Soldaini et al., 1995).

The question then arises as to whether the binding of STAT5 and the loss in BSAP binding are responsible for the chromatin changes, or alternatively, whether a second, as yet unidentified, target of the IL-2 signal generates the open DNA structure that allows regulation by STAT5 and BSAP. Such a second signal could function by activating any one of several processes known to lead to a transcriptionally permissive chromatin structure, such as histone acetylation (Brownell and Allis, 1996; Ogryzko et al., 1996) or release of a topologically restrained chromatin domain (Cai and Levine, 1995; Corces, 1995). These possibilities need to be explored further. However, recent reports that STAT1 and STAT2 recruit CBP/p300 (Bhattacharya et al., 1996; Zhang et al., 1996; Horvai et al., 1997; Montminy, 1997) might be interesting because CBP/p300 was shown to have a histone acetyltransferase (HAT) activity (Ogryzko et al., 1996), which is involved in the regulation of chromatin structure (Grunstein, 1997). In these regards, we speculate that IL-2 signaling might be transduced through the binding of a phosphorylated STAT5 to the J chain enhancer and result in the opening of chromatin by recruiting a HAT activity–containing CBP/p300-like protein. Moreover, a chromatin-directed signal could help to explain the regulatory mechanisms of other cytokines, such as IL-4, that are known to induce chromatin changes in their gene targets (Berton and Vitetta, 1990).

Experimental Procedures

Cell Culture

The cell lines were maintained at 37°C in Dulbecco's modified Eagle's medium or RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 0.1 mM MEM nonessential amino acids, 1 mM MEM sodium pyruvate, 50 μM 2-mercaptoethanol, 100 U/ml penicillin G, and 100 μg/ml streptomycin in an atmosphere of 7% CO_2 saturated with water. For the BCL_1 chromatin experiments, 35 μl of recombinant IL-2 (28 Takeda U/μl = 1 mg/ml [Takeda Chemical Industries]) were added to a 70 ml culture of early logarithmic-phase cells on day 1 and an additional 35 μl of IL-2 on the next day. Cells were harvested for DNase I hypersensitivity assay on day 3, when the concentration of cells was approximately 5×10^5/ml.

DNase I Hypersensitivity Assay

Cells in logarithmic-phase growth ($0.5–1.0 \times 10^6$/ml, 85 ml) were harvested by centrifugation and then allowed to swell in 10 ml of hypotonic solution (10 mM Tris-HCl [pH 7.9], 10 mM KCl, 3 mM dithiothreitol, 0.1 mM EDTA, 0.1 mM EGTA, 0.75 mM spermidine, 0.15 mM spermine) for 20 min on ice. Triton X-100 was added to a final concentration of 0.1%, and the cell membranes were broken by 20 strokes with a B pestle in a Dounce homogenizer. After the homogenate was centrifuged at 4°C for 7 min at 1500 rpm, the pelleted nuclei were resuspended in 2.5 ml of DNase I digestion buffer (0.25 M sucrose, 60 mM KCl, 15 mM NaCl, 15 mM Tris-HCl [pH 7.5], 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5 mM dithiothreitol, 0.1 mM EGTA, 5% glycerol). For the zero time point in the assay (the DNase I untreated control), 500 μl of the nuclear suspension was removed and added to 50 μl of quenching solution (200 mM EDTA, 10% SDS). Five units of DNase I (1 U/μl, 0.5 μg/μl) were then added to the remaining 2 ml; the suspension was mixed well at room temperature; and the digestion was stopped at time points of 0.5, 1, 2, and 3 min by transferring 500 μl samples to 50 μl of quenching solution. The quenched viscous DNA samples were treated with 20 μl of RNase A (10 mg/ml) for 30 min at 37°C and then with 18 μl of proteinase K (20 mg/ml) overnight at 55°C. The DNA was purified by phenol-chloroform extraction and ethanol precipitation, digested with appropriate restriction enzymes, and analyzed in Southern blots. Southern blots (Zeta-Probe, Bio-Rad) were performed with random primer-labeled probes as described (Sambrook et al., 1989).

Isolation of J Chain Gene 5′ Flanking Sequences

Two overlapping DNA sequences containing the six upstream HSSs described in this report were isolated by screening mouse genomic libraries. One was obtained by probing the T cell hybridoma library 1.9.2 (a generous gift from A. Winoto) with J chain cDNA. The second was obtained by probing a mouse BALB/c liver library (ML1030j,

Clontech) with a 165 bp BamHI–DraI fragment, described in the legend to Figure 1.

Plasmid Constructions for Enhancer Analyses

The plasmids for testing J chain enhancer activity were constructed by inserting either a J chain promoter DNA fragment (−1153 to +33) or a minimal c-*fos* promoter (−71 to +109) into the BamHI and/or HindIII sites at the 3′ end of the polylinker in the pGL2-Basic luciferase reporter vector (Promega). Restriction enzyme fragments containing the DNase I hypersensitive sites, and thus potential enhancers, were inserted at the 5′ end of the plasmid polylinker as follows: the Sau3AI–SpeI (4 kb), EcoRV–SpeI (1.8 kb), and ScaI (0.5 kb) fragments were cloned in the SmaI site in both orientations; the NdeI–NheI (1.1 kb) and the ScaI–NheI (0.8 kb) fragments were cloned into the vector SmaI and NheI sites in 5′-to-3′ orientation; and the NdeI–ScaI insert (0.8 kb) was made by deleting the 297 bp ScaI–NheI fragment from the NdeI–NheI insert. As a positive control, the 1 kb XbaI fragment of the immunoglobulin heavy-chain intron enhancer was cloned into the unique BamHI site 3′ of the luciferase reporter. The structures of all constructs were confirmed by restriction digests and/or sequencing.

Mutagenesis of the pGL2 plasmid containing the 1.1 kb NdeI–NheI enhancer insert was performed with a site-directed mutagenesis kit (Transformer, Clontech). A gene-specific primer, 5′-CCCAATAGCAA CACTATGTCAAGCCACTGCAGG-3′, was used to introduce the indicated 4 bp changes into the STAT5 element of the J chain enhancer. A selection primer, 5′-CTTATCATGTCTAGATCTGTCGACCGATG-3′, was used to introduce the indicated 2 bp changes that converted the unique BamHI site 3′ of the luciferase reporter to a BglII site. The generated mutant was verified by sequencing.

Transient Transfections and Luciferase Assays

Transient transfections of all cell lines except BCL_1 were performed by the DEAE-dextran technique (Grosschedl and Baltimore, 1985). In each case, 0.5–1.0 × 10^7 cells in logarithmic growth phase were transfected with 0.5 pmol (2.2–3.5 μg) of supercoiled test plasmid. After 2 days of incubation at 30°C, the cells were harvested and analyzed for luciferase activity using Analytical Luminescence Laboratory equipment and reagents (Enhanced Luciferase Assay Kit). The protein content of the cell extracts was determined by Bradford assay (Bio-Rad).

Transient transfection of BCL_1 cells was performed by electroporation as follows. Cells were harvested from two 150 mm confluent plates by pipetting up and down during two phosphate-buffered saline (PBS) washes and one final RPMI 1640 wash, and resuspended in 1.2 ml RPMI 1640. A 300 μl aliquot of the cell suspension was used for each transfection with 2 pmol (10 μg) of the test plasmid. The electroporation was performed at room temperature using a Bio-Rad Gene Pulse Apparatus with a 0.4 cm cuvette. After pulsing at settings of 960 μF and 300 V, 1 ml of RPMI 1640 supplemented with serum and additives as noted above was added immediately to the cuvette and then transferred to 100 mm plate with or without IL-2 (14 Takeda U/ml). The transferred cells were initially cultured at 37°C for 1–3 hr and then at 30°C for 19–22 hr for luciferase expression.

In Vivo Footprinting via Ligation-Mediated PCR

DMS in vivo footprint analysis by ligation-mediated (LM) PCR was performed as described (Ausubel et al., 1994) with the following modifications. For the preparation of the DNA, 70 ml of cells in logarithmic-phase growth (0.5–1.0 × 10^6 cells/ml) were pelleted and resuspended in 2 ml of the same media used for cell culture. The resuspended cells were mixed with 20 μl of 10% DMS (in ethanol) and incubated at room temperature for 2–5 min. The reaction was stopped by adding 3 ml of 2-mercaptoethanol (1 M) and then 20 ml cold PBS. After the cells were collected by centrifugation in the cold, they were washed once with 40 ml of cold PBS, resuspended in 2 ml of cold PBS, and lysed by adding 250 μl of quenching solution (200 mM EDTA, 10% SDS, 50 mM EGTA). The lysed cells were treated with 100 μl of RNase A (10 mg/ml) for 30 min at 37°C and then with 90 μl of proteinase K (20 mg/ml) overnight at 55°C. The DNA was purified and treated with 1 M piperidine as described by Ausubel et al. (1994).

For IL-2–induced in vivo DNase I footprinting in BCL_1 cells, cells grown on a 150 mm plate were treated for 3 hr with IL-2 (28 Takeda U/ml). The cells were swelled by replacing media with 10 ml of the described hypotonic solution supplemented with Na_3VO_4 (1 mM) and okadaic acid (0.25 μM) for 7 min, and the cell membranes were broken by 15 strokes with a B pestle in a Dounce homogenizer. After the homogenate was centrifuged at 4°C for 3 min at 1800 rpm (Sorvall RT6000B), the pelleted nuclei were resuspended in 2 ml of the described DNase I digestion buffer and treated with 50 U of DNase I for 30 s. The digestion was stopped by adding 250 μl of the quenching solution. The DNA was prepared as in the DNase I hypersensitivity assay and modified at the 3′-OH ends for LM-PCR as described (Tanguay et al., 1990).

LM-PCR was performed using 2.5–5 μg samples of the purified DNA. The primers for the noncoding strand were as follows: NP1 for first-strand synthesis, CCCAATGAAGTTTGGAAGTAGACCAC; NP2 for amplification of ligated DNA, AAGTAGACCACTTGTCGATTAGG ACC; and NP3 for labeling, TTGTCGATTAGGACCTGTGGGTAATGG. The equivalent primers for the coding strand were: CP1, GGGTATC AGACAGCAAAATCTTAAATC; CP2, TCTTAAATCTAAAAAGAACTC AAAACATAG; and CP3, AAAGAACTCAAAACATAGGGAGAAACC. Vent(exo$^-$) DNA polymerase (New England Biolabs) was used in the PCR with the supplied Thermopol buffer so that it was necessary to calculate the melting temperature for each primer using the formula given by Ausubel et al. (1994). To optimize the LM-PCR reactions, the DNA was purified by a combination of phenol-chloroform extraction and ethanol precipitation, after both the first-strand synthesis and the ligation reaction.

Electrophoresis Mobility Shift Assay

The nuclear extracts for EMSA were prepared basically according to the methods described (Dignam et al., 1983; Shapiro et al., 1988). The modifications included washing of the nuclei through a 30% sucrose cushion; addition of PMSF (0.5 mM), aprotinin (125 μg/ml), leupeptin (125 μg/ml), and pepstatin A (20 μg/ml) to inhibit proteases; and addition of 1 mM sodium vanadate to inhibit phosphatase activity. To measure the effect of IL-2 stimulation on factor binding to the enhancer, nuclear extracts were prepared from BCL_1 cells exposed to recombinant IL-2 (1.25–14 Takeda U/ml) for 0.5–20 hr at 37°C.

EMSA was performed as described previously (Rinkenberger et al., 1996) by electrophoresis through glycerol-containing 5% polyacrylamide gels (29:1) containing 0.25× Tris-borate-EDTA buffer. For the antibody blocking and/or supershift assays, nuclear extracts were preincubated for 15 min with 1 μl (0.1 μg) of anti-STAT5b (sc-835) or anti-STAT3 (sc-482) rabbit polyclonal IgG (Santa Cruz); or 1 μl of an undiluted anti-CBFβ2 rabbit polyclonal antibody (a generous gift from Y. Ito); or 2 μl of undiluted mouse monoclonal anti-E12 or anti-E47 antibody (a generous gift from C. Murre). Oligonucleotide sequences from the J chain gene enhancer used as probes were as follows: 5′ E2 box, ctagATGAAAAGCCACTGCAGGTGCAAGTTT ATT; 3′ CBF site, gatcAAACATTTTAACCACAAGCCTGCCCTAAG; STAT3 site, CCATGATTCCAGTAATTCAG; and STAT5 site, gatcCAA TAGCAATTCTATGAAAAGCCACTG.

Western Blot

Nuclear extracts (2.5 μg) prepared as described above were resolved by 8% SDS-PAGE and transferred to the nitrocellulose membrane. The blot was then analyzed using anti-STAT5b (C-17), which is specific for both STAT5a and STAT5b (Santa-Cruz, sc-835), and alkaline phosphatase–conjugated anti-rabbit IgG (Promega, S3731).

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References

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J. G., Smith, J.A., and Struhl, K. (1994). Current Protocols in Molecular Biology, K. Janssen, ed. (Boston: Greene Publishing Associates, John Wiley and Sons), pp. 15.5.1–15.5.26.

Berton, M.T., and Vitetta, E.S. (1990). Interleukin 4 induces changes in the chromatin structure of the γ1 switch region in resting B cells before switch recombination. J. Exp. Med. *172*, 375–378.

Bhattacharya, S., Eckner, R., Grossman, S., Oldread, E., Arany, Z., D'Andrea, A., and Livingston, D.M. (1996). Cooperation of Stat2 and p300/CBP in signalling induced by interferon-α. Nature *383*, 344–347.

Blackman, M.A., Tigges, M.A., Minie, M.E., and Koshland, M.E. (1986). A model system for peptide hormone action in differentiation: interleukin 2 induces a B lymphoma to transcribe the J chain gene. Cell *47*, 609–617.

Brooks, K., Yuan, D., Uhr, J.W., Krammer, P.H., and Vitetta, E.S. (1983). Lymphokine-induced IgM secretion by clones of neoplastic B cells. Nature *302*, 825–826.

Brownell, J.E., and Allis, C.D. (1996). Special HAT's for special occasions: linking histone acetylation to chromatin assembly and gene activation. Curr. Opin. Genet. Dev. *6*, 176–184.

Cai, H., and Levine, M. (1995). Modulation of enhancer-promoter interactions by insulators in the *Drosophila* embryo. Nature *376*, 533–536.

Corces, V.G. (1995). Chromatin insulators: keeping enhancers under control. Nature *376*, 462–463.

Dignam, J.D., Levovitz, R., and Roeder, R.G. (1983). Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. *11*, 1475–1489.

Forrester, W.C., van Genderen, C., Jenuwein, T., and Grosschedl, R. (1994). Dependence of enhancer-mediated transcription of the immunoglobulin μ gene on nuclear matrix attachment regions. Science *265*, 1221–1225.

Giannini, S.L., Singh, M., Calvo, C.-F., Ding, G., and Birshtein, B.K. (1993). DNA regions flanking the mouse Ig 3′ α enhancer are differentially methylated and DNaseI hypersensitive during B cell differentiation. J. Immunol. *150*, 1772–1780.

Gilman, M.Z., Wilson, R.N., and Weinberg, R.A. (1986). Multiple protein-binding sites in the 5′-flanking region regulate c-*fos* expression. Mol. Cell. Biol. *6*, 4305–4316.

Grosschedl, R., and Baltimore, D. (1985). Cell-type specificity of immunoglobulin gene expression is regulated by at least three DNA sequence elements. Cell *41*, 885–897.

Grunstein, M. (1997). Histone acetylation in chromatin structure and transcription. Nature *389*, 349–352.

Hagman, J., Rudin, C.M., Haasch, D., Chaplin, D., and Storb, U. (1990). A novel enhancer in the immunoglobulin λ locus is duplicated and functionally independent of NFκB. Genes Dev. *4*, 978–992.

Henderson, A.J., and Calame, K.L. (1995). Lessons in transcriptional regulation learned from studies on immunoglobulin genes. Crit. Rev. Eukaryot. Gene Express. *5*, 255–280.

Horvai, A.E., Xu, L., Korzus, E., Brard, G., Kalafus, D., Mullen, T.-M., Rose, D.W., Rosenfeld, M.G., and Glass, C.K. (1997). Nuclear integration of JAK/STAT and Ras/AP1 signaling by CBP and p300. Proc. Natl. Acad. USA *94*, 1074–1079.

Hou, J., Schindler, U., Henzel, W.J., Wong, S.C., and McKnight, S.L. (1995). Identification and purification of human Stat proteins activated in response to interleukin-2. Immunity *2*, 321–329.

Jenuwein, T., Forrester, W.C., Fernandez-Herrero, L.A., Laible, G., Dull, M., and Grosschedl, R. (1997). Extension of chromatin accessibility by nuclear matrix attachment regions. Nature *385*, 269–272.

Lamson, G., and Koshland, M.E. (1984). Changes in J chain and μ chain RNA expression as a function of B cell differentiation. J. Exp. Med. *160*, 877–892.

Lansford, R.D., McFadden, H.J., Siu, S.T., Cox, J.S., Cann, G.M., and Koshland, M.E. (1992). A promoter element that exerts positive and negative control of the interleukin 2-responsive J-chain gene. Proc. Natl. Acad. Sci. USA *89*, 5966–5970.

Lecine, P., Algarte, M., Rameil, P., Beadling, C., Bucher, P., Nabholz, M., and Imbert, J. (1996). Elf-1 and Stat5 bind to a critical element in a new enhancer of the human interleukin-2 receptor α gene. Mol. Cell. Biol. *16*, 6829–6840.

Lu, J., Maruyama, M., Satake, M., Bae, S.-C., Ogawa, E., Kagoshima, H., Shigesada, K., and Ito, Y. (1995). Subcellular localization of the α and β subunits of the acute myeloid leukemia-linked transcription factor PEBP2/CBF. Mol. Cell. Biol. *15*, 1651–1661.

Madisen, L., and Groudine, M. (1994). Identification of a locus control region in the immunoglobulin heavy-chain locus that deregulates c-*myc* expression in plasmacytoma and Burkitt's lymphoma cells. Genes Dev. *8*, 2212–2226.

Matsui, K., Nakanishi, K., Cohen, D.I., Hada, T., Furuyama, J.-I., Hamaoka, T., and Higashino, K. (1989). B cell response pathways regulated by IL-5 and IL-2: secretory μH chain-mRNA and J chain mRNA expression are separately controlled events. J. Immunol. *142*, 2918–2923.

McFadden, H.J., and Koshland, M.E. (1991). Interleukin 2- and interleukin 5-induced changes in the binding of regulatory factors to the J-chain gene promoter. Proc. Natl. Acad. Sci. USA *88*, 11027–11031.

Minie, M.E., and Koshland, M.E. (1986). Accessibility of the promoter sequence in the J-chain gene is regulated by chromatin changes during B-cell differentiation. Mol. Cell. Biol. *6*, 4031–4038.

Montminy, M. (1997). Something new to hang your HAT on. Nature *387*, 654–655.

Murre, C., McCaw, P.S., and Baltimore, D. (1989). A new DNA binding and dimerization motif in immunoglobulin enhancer binding, *daughterless*, *MyoD*, and *myc* proteins. Cell *56*, 777–783.

Nakanishi, K., Cohen, D.I., Blackman, M., Nielsen, E., Ohara, J., Hamaoka, T., Koshland, M.E., and Paul, W.E. (1984). Ig RNA expression in normal B cells stimulated with anti-IgM antibody and T cell-derived growth and differentiation factors. J. Exp. Med. *160*, 1736–1751.

Neurath, M.F., Max, E.E., and Strober, W. (1995). Pax5 (BSAP) regulates the murine immunoglobulin 3′α enhancer by suppressing binding of NF-αP, a protein that controls heavy chain transcription. Proc. Natl. Acad. Sci. USA *92*, 5336–5340.

Niles, M.J., Matsuuchi, L., and Koshland, M.E. (1995). Polymer IgM assembly and secretion in lymphoid and nonlymphoid cell lines: evidence that J chain is required for pentamer IgM synthesis. Proc. Natl. Acad. Sci. USA *92*, 2884–2888.

Ogryzko, V.V., Schiltz, R.L., Russanova, V., Howard, B.H., and Nakatani, Y. (1996). The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell *87*, 953–959.

Rinkenberger, J.L., Wallin, J.J., Johnson, K.W., and Koshland, M.E. (1996). An interleukin-2 signal relieves BSAP (Pax5)–mediated repression of the immunoglobulin J chain gene. Immunity *5*, 377–386.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed. (Cold Spring Harbor: Cold Spring Harbor Laboratory Press).

Shapiro, D.J., Sharp, P.A., Wahli, W.W., and Keller, M.J. (1988). A high-efficiency HeLa cell nuclear transcription extract. DNA *7*, 47–55.

Soldaini, E., Pla, M., Beermann, F., Espel, E., Corthesy, P., Barange, S., Waanders, G.A., MacDonald, H.R., and Nabholz, M. (1995). Mouse interleukin-2 receptor alpha gene expression: delimitation of cis-acting regulatory elements in transgenic mice and by mapping of DNAse-I hypersensitive sites. J. Biol. Chem. *270*, 10733–10742.

Speck, N.A., and Stacy, T. (1995). A new transcription factor family associated with human leukemias. Crit. Rev. Eukaryot. Gene Express. *5*, 337–364.

Sperisen, P., Wang, S.M., Soldaini, E., Pla, M., Rusterholz, C., Bucher, P., Corthesy, P., Reichenbach, P., and Nabholz, M. (1995). Mouse interleukin-2 receptor α gene expression (interleukin-1 and interleukin-2 control transcription via distinct *cis*-acting elements). J. Biol. Chem. *270*, 10743–10753.

Tanguay, R.L., Pfeifer, G.P., and Riggs, A.D. (1990). PCR-aided DNaseI footprinting of single copy gene sequences in permeabilized cells. Nucleic Acids Res. *18*, 5902.

Turner, C.A., Jr., Mack, D.H., and Davis, M.M. (1994). Blimp-1, a novel zinc finger-containing protein that can drive the maturation of B lymphocytes into immunoglobulin-secreting cells. Cell *77*, 297–306.

Wotton, D., Ghysdael, J., Wang, S., Speck, N.A., and Owen, M.J. (1994). Cooperative binding of Ets-1 and core binding factor to DNA. Mol. Cell. Biol. *14*, 840–850.

Zhang, J.J., Vinkemeier, U., Gu, W., Chakravarti, D., Horvath, C.M., and Darnell, J.E., Jr. (1996). Two contact regions between Stat1 and CBP/p300 in interferon γ signaling. Proc. Natl. Acad. Sci. USA *93*, 15092–15096.