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The Invariant Chain Gene Intrinsic Enhancer Shows Homology to Class II Promoter Elements

Bethany Beilue Moore, Zhu Alexander Cao, Theresa L. McRae, Colleen H. Woo, Sarah Conley, and Patricia P. Jones

Coordinate expression of MHC class II proteins and the class II-associated invariant chain (Ii) is important for proper MHC class II functioning in Ag processing and presentation. The coordinate regulation of these genes results, in part, from the sharing of transcriptional regulatory regions between MHC class II and Ii genes; the Ii has previously been shown to have an upstream enhancer closely related to the essential class II promoter elements. We report here the characterization of a second enhancer in the Ii gene, located within the first intron. This intrinsic enhancer is contained within a 155-bp region, enhances transcription from the Ii minimal promoter, and also contains elements that are homologous to class II promoter elements X1, X2, and Y boxes. The Journal of Immunology, 1998, 161: 1844–1852.

The MHC class II molecules are highly polymorphic cell surface proteins that play key roles in both the selection and activation of CD4+ T cells through their roles in presenting processed Ags (1–3). Multiple MHC class II genes encode the α- and β-chains of the class II heterodimers, and they all share several common sequence motifs in their promoters. These motifs are named S (septamer; also called H, or heptamer) (4–6), X1, X2, and Y boxes (7). Much evidence has been found to support the importance of these motifs for the appropriate expression of MHC class II genes (4–5, 8–12). In addition, these class II promoter motifs can serve as enhancers for some heterologous promoters (10).

Intracellular class II αβ-chain heterodimers are noncovalently associated with a nonpolymorphic glycoprotein called invariant chain (Ii) (13). Ii regulates class II binding of antigenic peptide and targets class II to endosomal compartments where peptide binding occurs. The Ii gene is not in the MHC (14), and analysis of both human (15) and mouse (16) gene sequences indicates that the structure of Ii is not related to that of MHC class II proteins. Consistent with their cooperative roles in the Ag presentation pathway, class II and Ii expression are coregulated. Basal expression of these genes shows the same tissue specificity (B cells, macrophages, dendritic cells, and thymic epithelial cells) (17) and inducibility by cytokines (most notably IFN-γ, TNF-α, and IL-4) (18–20). This coordinate regulation is imperative since both under- and overexpression of Ii has been shown to impair MHC class II transport and presumably function (21). While all cells that express class II also express Ii, some cells are capable of producing Ii in the absence of class II (22). As one might expect, there are some shared elements in the regulatory regions of the MHC class II and Ii genes. For example, it has already been documented that the Ii upstream enhancer shares homology with class II promoters (23, 24). Despite these similarities, differences do exist. The Ii promoter, which contains tissue-specific expression (23), is distinct and appears unrelated to class II (5, 7, 23, 24). In addition, previous evidence from our laboratory suggested a distinct intrinsic enhancer within the Ii gene (23). Thus, regulation of Ii is likely to occur through many cis elements and trans-acting factors.

The Ii gene contains at least three distinguishable transcriptional regulatory regions: 1) an upstream enhancer, 2) a tissue specific promoter, and 3) an intrinsic enhancer (Ref. 23; see Fig. 1A). Characterization of each component in the murine Ii chain gene has revealed the following (23). The Ii promoter fragment from –183 to +88 has been shown to promote transcription of chloramphenicol acetyltransferase (CAT) reporter genes in B cells, L cell fibroblasts, and macrophages, but not in T cells. In the murine Ii promoter, an NF-κB site located between –121 and –110 serves as a cell type-positive regulator in B cells and macrophages. In addition, a CCAAT box is located between –89 and –84, and an Sp1 binding site is present between –76 to –71. These elements are positive cell type regulators, but their presence is not required for basal transcriptional activity. Fragments beginning at –68 are sufficient for low level basal transcriptional activity; this promoter also contains a TATA box at –28 to –24 relative to the transcription start site.

The upstream enhancer, located between –269 to –183, contains sequences homologous to the H-X1-X2-Y boxes that constitute all class II promoters (4–11, 23). The S and X motifs are highly conserved; however, the Y motif is inverted to produce a CAT box (in an opposite orientation) and is termed Y'. Distances between these elements are conserved between Ii and class II. This enhancer is not tissue specific and will enhance the heterogeneous SV40 promoter. The upstream enhancer has been shown to require the Sp1 site in the promoter to fulfill its enhancer function (23).

Previous studies from this laboratory indicated that an intrinsic enhancer is present within a 2.4-kb region in the first intron of the Ii gene (23). This enhancer fragment has been demonstrated to

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Abbreviations used in this paper: S, septamer; Ii, class II-associated invariant chain; CAT, chloramphenicol acetyltransferase; pCAT, chloramphenicol acetyltransferase plasmid; CIITA, class II-associated transactivator; nt, nucleotide; BLS, bare lymphocyte syndrome.
enhance transcription of the Ii promoter (−68 to +88), but does not enhance heterologous SV40 large T Ag promoter-driven transcription.

In this report, we identify the sequence elements that make up the Ii intronic enhancer. Interestingly, the intronic enhancer also contains elements that are homologous to class II promoter region X1, X2, and Y boxes and a sequence showing homology to the S (H) box. Mutational analyses have indicated that these elements are responsible for the enhancing activity of this region. This intronic enhancer is active in constitutive expression in B cells, macrophage lines, and L cells. Furthermore, other studies have indicated that this intronic enhancer contributes to IFN-γ-induced expression of Ii.5

Materials and Methods

Cell lines

The mouse cell lines used in this study were M12, a B cell lymphoma line (25); L cells, fibroblasts (26); and WEHI-3, a myelomonocytic cell line (27). Cell lines were grown in RPMI 1640 medium supplemented with 10% FBS, penicillin/streptomycin, L-glutamine, and 5 × 10⁻³ M 2-ME and were maintained at 37°C in a 5% CO₂ incubator.

DNA constructs

All constructs were made in the pCAT basic vector available from Promega (Madison, WI). The plasmid pCMV was pCAT control containing an SV40 promoter and enhancer driving CAT expression and available from the same vendor. Ii promoter and enhancer fragments were cloned, respectively, into the promoter region polylinker in pCAT basic and into the unique BamHI site3 of the CAT gene. The promoter and enhancer regions for most constructs were generated by PCR amplification or appropriate sequences from the pIiSst2.4 plasmid (14), which contains the Ii promoter region from −843 to +88, and the 2.4-kb Sst intronic enhancer (nucleotides nt 2506–4905) fragment. The sequences and locations of the primers are given in Table I.

The following vectors were made by cloning appropriate fragments amplified using the primer combinations given. Promoter constructs: p284 (primers 12–2), p147 (primers 4–2), p95 (primers 3–2) and p59 (primers 1–2). The 2.4-kb enhancer deletion fragments, named according to their size in basepairs, were cloned into the p59 promoter vector at the BamHI site: 2.4 kb (primers 6–5, intact enhancer fragment), 1.25 kb (primers 6–11), 845 (primers 6–8), 405 (primers 6–7), and 2000 (primers 37–5).

The smaller enhancer-containing constructs were made using the following primer combinations to amplify all or part of the 405-bp enhancer region and clone it into the p59 promoter construct at the BamHI site: A (primers 6–19), B (primers 6–18), C (primers 6–17), D (primers 13–17), E (primers 14–7), F (primers 15–7), G (primers 16–7), H (primers 13–19), J (primers 13–17), K (primers 14–19), L (primers 14–18), and Q (primers 16–19). The X box mutations were made by PCR splicing by overlap extension (28); the first mutated fragment made was from primers 16–40, and the second mutated fragment was from primers 39–19. These two fragments were then mixed at equimolar concentrations and amplified by PCR using primers 16 and 19. The R construct was made using primers 16–18. The T construct was made by first cloning the 405-bp fragment into pUC18 at the Smal site. This construct was then linearized at the 3′ end of the insert and subjected to Bal31 deletion. A deletion mutant that ended at 2767 was used to amplify the 3′ half of the Q fragment. Primer 39 then served as the 5′ primer with primer 19 as the 3′ anchor.

DNA transfections

Cells (5 × 10⁶) were electroporated with 20 μg of cesium chloride-banded plasmid. The conditions used were 250 V and 960 μF in 300 μl of complete medium using 0.4-cm gap Invitrogen cuvettes (San Diego, CA) and a Bio-Rad GenePulser electroporator (Bio-Rad, Hercules, CA). Cells were then recultured in complete medium and grown for 48 h before harvesting for CAT assays.

CAT assays
CAT assays were performed essentially as described previously by Gorman et al. (29). Briefly, extracts from 5 × 10⁶ cells were quick frozen and thawed three times in 150 μl of 0.25 M Tris, pH 7.5, before heat inactiva-

The Ii intronic enhancer is located in the 5’ region of the first intron

To screen the first intron of the Ii gene for enhancer activity, PCR primers were designed to amplify the 2.4-kb region, originally described as a SacI fragment (23). These primers, designed to introduce convenient BamHI cloning sites, are described in Table I. These enhancer fragments were then cloned 3’ of the CAT gene in the pCAT basic vector containing the −59 to +88 promoter fragment. Figure 2A shows a schematic of the Ii genomic organization and the approximate location of the amplified regions. Transfection of the 2.4-kb enhancer fragment in conjunction with the −59 promoter showed significant enhancement of CAT activity over transfections of the promoter alone (see Fig. 3A). To define further the boundaries of this intronic enhancer, a series of 3’ nested deletions were generated by PCR. These deletions left fragments of 1.25, 845, and 405 bp for analysis. The approximate locations of these fragments are also shown in Figure 2A. Transfections of all of these enhancer fragments in conjunction with the −59 promoter fragment resulted in significant enhancer activity over the transfections of promoter-alone constructs in all cell lines tested (Fig. 3A). The fact that the 845-bp fragment results in less expression than the 405-bp fragment in L cells suggests that there may be negative regulatory elements located between nt 2930 and 3354, which are active in this cell type. These results indicated that the position of the intronic enhancer could be narrowed to the first 405 bp of the Ii first intron and that this enhancer was constitutively active in cell lines representing B cells, monocytes, and fibroblasts. To confirm this result, the region corresponding to the 3’ 2.0 kb of the 2.4-kb intronic enhancer, excluding the 405-bp region, was amplified, cloned, and tested for enhancer activity. This fragment showed minimal enhancement of the −59 promoter when tested in M12 cells (Fig. 3A); not tested in WEHI-3 or L cells, confirming that the activity was localized to the 5’ 405-bp region.

The Ii minimal promoter contains all elements needed to cooperate with the Ii intronic enhancer

The next question investigated was whether the −59 promoter contained all of the promoter elements necessary for maximal enhancement by the 405 bp Ii intronic enhancer. This concern was prompted by previous data showing that the Ii upstream enhancer contained all of the promoter elements necessary for maximal enhancement by the 405 bp Ii intronic enhancer. These constructs were then transiently transfected into M12 cells and assayed for CAT activity. Data for the transfections into M12 cells are presented in Figure 3B. The 405-bp intronic enhancer is at least as active with the −59 promoter as it is with the −284 promoter, giving 17-fold enhancement, indicating that the region of the Ii promoter that is responsive to this enhancer is located in the −59-bp region. As reported earlier for the 2.4-kb intronic enhancer fragment (23), the 405-bp intronic enhancer fragment does not enhance the heterologous SV40 promoter. Transfections into WEHI-3 and L cells showed similar results (data not shown).

The Ii intronic enhancer is contained within a 150-bp region

Having established that the −59 promoter was a suitable minimal promoter to use in these analyses, the 405-bp fragment was subdivided to identify the essential enhancer elements. A series of new PCR primers were designed (Table I) to PCR amplify a set of nested deletions of the 405-bp fragment, as shown in Figure 2B. Constructs containing fragments of the 405-bp intronic enhancer-containing region corresponding to A, B, C, D, E, F, G, H, J, K, L,
and Q were tested in M12 cells. Figure 4 shows the results of these transfections normalized to the pCAT control plasmid. Transfection of the p59 (−59 to +88) promoter alone gave minimal activity. Introduction of the 405-bp intronic enhancer showed a 25-fold enhancement. Transfection of all of the alphabet subfragment constructs, except for C and J, showed a high level of enhancement. Transfection of the C enhancer fragment resulted in a 2-fold induction over the −59 promoter alone in four different experiments. This result suggested that the enhancer activity probably resided downstream of the binding sites for primers 16 and 17 (nt 2720–2875; see Fig. 2B). In addition, the J fragment showed almost no activity. The Q fragment, which was the smallest region tested, initially gave high enhancer activity. This result suggested that the intronic enhancer could be narrowed down to this 150-bp region delineated by primers 16 and 19 (between nt 2720 and 2875). Although the B and L fragments, which share a 3′ boundary at oligo 18, showed enhancer activity, individual experiments that directly compared fragment B, L, and Q consistently showed the activity of the Q fragment to be higher. This led us to believe that enhancer motifs were entirely encompassed by the Q fragment (position 2720–2875 in the genomic sequence (33)).

The Ii intronic enhancer contains elements homologous to both class II promoter regions and the Ii upstream enhancer

Analysis of the sequence of the Q fragment (nt 2721–2875) revealed several interesting homologies (Table II; Fig. 5A) (33). The most striking of these was the presence of a CRE.1 site between positions 2805 and 2810. This CGTCAT sequence turned out to be highly homologous to the X2 box, previously described for class II promoter regions, and the Ii upstream enhancer (23, 24, 34). In addition, the sequence GCAACA appears between positions 2795 and 2800. This sequence aligns well with previously identified X1 boxes (Table II). A classical Y box is also located 15 bp downstream of X2. These striking homologies suggested that the classical class II promoter elements had been duplicated as transcriptional control regions in both the Ii upstream and intronic enhancer.

**FIGURE 1.** Regulatory regions in the Ii gene. A, Schematic diagram of the Ii 5′ region showing the relative positions of cis-acting regulatory elements (not to scale). The position of the intron enhancer is identified in this study. p284, p147, p95, and p59 are promoter fragments used in this study. Nucleotides are numbered relative to the start site of transcription (23). B, Analysis of effects of 5′ deletions on Ii promoter activity in M12, WEHI-3, and L cells. CAT activity is expressed as a percentage of pCAT control (SV40-driven CAT) transfection. Data are expressed as mean and SD of at least four experiments performed in triplicate.
In an effort to identify whether the X boxes were critical for the intronic enhancer to function, a site-directed mutation was made in this region. The original sequence of GCAACATGTGCGTCAT was changed to GCAACATGTCGACCAG within the context of the entire 155-bp Q fragment. This mutation sequence was selected to introduce a SalI site (GTCGAC) for ease in manipulation as well as disruption of the conserved X box sequences. This X box mutant enhancer (fragment X) was then tested for activity relative to the 59 promoter alone and the wild-type Q fragment. As seen in Figure 5, this mutation (X fragment) ablated the enhancing activity of the Q fragment, suggesting that the X box region is important for this enhancer’s function in a manner analogous to that seen in both class II promoter regions and the Ii upstream enhancer (23, 24).

Alignment of the sequences upstream of the X boxes in the Q fragment revealed modest homology to some of the previously identified S (also called H or W) motifs (24, 34). To test the involvement of this region, two new fragments were made and tested. These fragments were named R and T. The R fragment was made by PCR amplification using primers 6 bp 3′ of the GGGCATTT (S box) sequence. The T fragment extends from nt 2767 to 2876. The T fragment preserves the X and Y boxes but lacks the putative S motif. These fragments were subcloned as enhancer fragments downstream of the 59 promoter CAT vector and tested in M12 cells. Figure 5 shows that both the R and T fragments have some enhancer activity; however, neither one is as effective as the Q fragment. These results are consistent with previously published data for class II genes showing that both the X1/X2 boxes, as well as the S region, are needed for full promoter activity (24, 34). These elements in the Ii intronic enhancer are not cell type specific, and they contribute to the activity of the Q fragment enhancer in monocytes and L cells as well (data not shown).

Nuclear extracts contain factors that bind to the Ii intronic enhancer region

To determine whether nuclear proteins bind to these regulatory elements, a series of electrophoretic mobility shift assays were performed on the intronic enhancer fragments. The Q fragment was end labeled and incubated with nuclear extracts from M12 B lymphoma cells. As shown in Figure 6, the band pattern shows three major protein complexes binding to the Q fragment. By analyzing the banding pattern of the cold competitor-containing reactions, it is possible to assign these complexes to particular motifs. Cold competition with a fragment that spans both the S and X1

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**FIGURE 2.** Schematic of the Ii first intron enhancer region showing approximate locations of PCR primer binding sites used to generate amplified fragments for analysis. A. Nested 3′ deletions of the 2.4-kb SalI intronic enhancer-containing region. B. Nested deletions of the 405-bp intronic enhancer-containing region showing the relationship between primer binding sites (primer numbers correspond to those in Table I) and the combinations of primers used to generate the enhancer subfragment regions. The shaded area indicates the boundaries of the intronic enhancer identified in this study.
elements eliminates the highest mobility band, No. 1 (2805 fragment, lane 3). A fragment that spans S, X, and Y elements eliminates both band 1 and the middle band, No. 2 (2834 fragment, lane 8). A cold probe that is initiated within the X region and spans the Y box competes the second band (fragment 4, lane 4). This probe would not be expected to compete with X box-binding factors, because the X motif is so close to the 5’ end of fragment 4, and the X box-binding proteins only bind efficiently to longer fragments that contain the X motif within the center (W. Reith, unpublished observations; and our unpublished observations). Furthermore, this No. 4 fragment does not contain the pyrimidine region just 5’ of the X box corresponding to half of the EFC/MDBP site, which may be necessary for X box binding activity (reviewed in Ref. 35). The fastest migrating species, No. 3, is competed only by the intact Q fragment and fragment 4 (lanes 4 and 7). Based on these results, it is possible to assign the fastest migrating species to a region downstream of the Y box, most likely to a polypyrimidine tract in this location in probe Q. This band is seen only in B cell lines (data not shown). The middle band, No. 2, is most likely due to factors that bind to the Y box, and the slowest migrating band, No. 1, is probably due to X box-binding factors. We have been unable to shift any factors binding to the S region, either on the intact Q fragment or on isolated smaller fragments from this region.

We next analyzed whether the sequence elements from the Ii upstream enhancer or the Aα promoter could compete for factors binding to the Ii intronic enhancer. The slowest migrating species, No. 1, seen in lane 2, is greatly diminished by cold competition using a DNA fragment that contains the upstream enhancer region (lane 5), or it is somewhat diminished using a fragment from the class II Aα promoter region containing S, X1, X2, and Y (lane 6). Cold competition with the Ii upstream enhancer (lane 5) decreased binding of all three protein species. Competition with the Aα promoter fragment decreased binding of only the X- and Y-binding proteins.

Although the Ii intronic enhancer shares regulatory elements and transacting factors with MHC class II, one important distinction is that the Ii gene does not require CIITA for expression. This is demonstrated by the fact that this enhancer is active in L cells that are CIITA deficient. Figure 7 demonstrates RT-PCR analysis of our L cell fibroblast line as compared with spleen cells. As seen in this figure, spleen cells have a readily apparent 700-bp amplification product corresponding to CIITA, which titrates from 5 μg to 40 ng of input RNA, most likely representing mRNA from splenic B cells. However, no such band is detected in the L cell RT-PCR reactions, even with the highest concentration of RNA tested (5 μg) and 40 cycles of PCR. These results demonstrate that the L cells used in these studies are CIITA deficient.

Discussion

The results presented in this paper have confirmed that the murine Ii gene contains at least three distinct regulatory elements. An upstream enhancer, a tissue-specific promoter region, and as characterized here, an intronic enhancer region. The existence of an intronic enhancer was originally detected with a 2.4-kb fragment from the Ii first intron (23). We have narrowed that region to 155 bp and have identified putative core regulatory motifs. Interestingly, this intronic enhancer is similar to the previously described Ii upstream enhancer, in that it contains elements that are homologous to class II promoter S, X1, X2, and Y. However, it is highly
likely that these duplications arose from separate transposition events, since the S, X, and Y boxes in each location are distinct from each other (see Table II). First, the upstream enhancer contains an inverted Y box (which itself contains an inverted CCAAT box), while the intronic enhancer contains a more traditional Y box; in fact, the intronic enhancer Y box is 90% homologous to the Aα and Eα Y box sequences (see Table II). Second, the X1/X2 boxes of the intronic enhancer are similar to the same region in the

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**Table II. A comparison of regulatory motifs within class II and Ii genes**

<table>
<thead>
<tr>
<th>S Motif</th>
<th>X1&lt;sup&gt;b&lt;/sup&gt; and X2&lt;sup&gt;c&lt;/sup&gt; Motifs</th>
<th>Y Motif</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGGCCTC</td>
<td>17 bp GCTGGGACAAGGTACGGTCA</td>
<td>15 bp CTTAGTGTTT</td>
</tr>
<tr>
<td>AGGCCTC</td>
<td>17 bp GCTAGTGACAAGGTACGGTCA</td>
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</tr>
<tr>
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<tr>
<td>GGGGATT</td>
<td>16 bp CCTAGGCGAAGGTACGGTCA</td>
<td>14 bp TCTAGTGTTT</td>
</tr>
</tbody>
</table>

<sup>a</sup> Compiled from References 4, 14, and 34, and this study.
<sup>b</sup> Italics indicate the region corresponding to the X1 motif.
<sup>c</sup> Underline represents the region corresponding to the X2 motif.
<sup>d</sup> The region of the Q fragment sequence shows begins at the 5' end of the S box (nt 2792) and ends at the 3' end of the Y box (nt 2834).

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**FIGURE 5.** Intropic enhancing activity is maintained on small fragments containing regions homologous to class II promoter X1, X2, Y, and S boxes. A. The sequence of the Q enhancer fragment from nt 2721 through 2875. The putative S box is indicated with uppercase letters. The X1 region is in bold and is underlined. The X2 region is outlined. The Y box is in bold italics. B. The Q fragment and its derivative fragments, showing the positions of the S, X1, X2, and Y boxes. C. Transfections of constructs containing the p59 promoter alone or p59 with enhancer fragments.

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**FIGURE 6.** EMSA analysis demonstrates nuclear proteins capable of binding X and Y elements. EMSA analysis performed with 0.5 ng of labeled Q fragment and 20 μg of M12 extract. Lane 1, End-labeled Q fragment probe; lane 2, Q probe incubated with M12 extract; lane 3, Q probe incubated with M12 extract and cold competed with the a DNA fragment ending at bp 2805 (40-fold excess); lane 4, Q probe incubated with M12 nuclear extract and cold competed with fragment 4, which spans the X2 box, Y box, and further 3' (20-fold excess); lane 5, Q probe incubated with M12 extract and cold competed with a fragment from the Ii upstream enhancer (the p284 promoter fragment, 20-fold excess); lane 6, Q probe incubated with M12 extract and cold competed with unlabeled Q fragment (20-fold excess); lane 7, Q probe incubated with M12 extract and cold competed with a fragment that extends to nt 2834, spanning the X1, X2, and Y boxes (150-fold excess).
Aa gene, but somewhat different from the upstream enhancer. However, the putative S region from the intronic enhancer is quite distinct from other S regions, but may have strong enhancing activity even on its own (see Fig. 5B, R fragment). Furthermore, there is preliminary evidence that the class II DQα gene contains an intronic enhancer, and it may also represent a duplication of the H-X-Y regulatory motifs (12).

Thus, the Ii has duplicated the class II promoter region in at least two regulatory motifs. Although the sequences that comprise these motifs are different from each other, preliminary evidence suggests that the same nuclear factors may be able to recognize both the upstream and the intronic enhancer regions. Evidence for this is provided by the cold competition experiments shown in Figure 6.

Despite these similarities in sequence elements, there are unique aspects to the transcriptional regulation from these sites. For example, the upstream enhancer requires the Sp1 binding site (GC box) in the promoter for activity and can enhance Sp1-containing heterologous promoters such as SV40, whereas the intronic enhancer does not require Sp1 binding sites and cannot enhance SV40 promoter-driven transcription (Ref. 23; and these results). Clearly then, the promoter requirements for these two enhancing activities are different, suggesting distinct interactions between promoter-binding proteins and/or the transcription initiation complex and the proteins binding to the two enhancers. These results indicate that the Ii intronic enhancer may be uniquely designed to cooperate only with its cognate promoter.

The intronic enhancer not only is important for basal transcription but is also involved in cytokine-induced gene regulation. Recent work in our laboratory by Cao et al.5 has shown that this intronic region of the gene is partially responsible for the increased transcription of the Ii gene in response to IFN-γ, consistent with the roles of the homologous regions in the Ii upstream enhancer and class II promoters on IFN-γ induction of transcription. This suggests that the IFN-γ-induced coactivator of MHC and Ii expression, CIITA, is able to interact with X and Y box-binding proteins associated with the intronic enhancer, as well as the upstream enhancer, to activate Ii transcription. Furthermore, the severely diminished activity of the mutated X box fragment (Fig. 5) suggests that, in the context of the entire enhancer, higher order protein interactions are probably necessary for full enhancer activity, in a manner analogous to that seen for class II promoters (reviewed in Ref. 35).

In patients suffering from some forms of the disease bare lymphocyte syndrome (BLS), it has long been noted that they display greatly reduced levels of mRNA for class I and class II proteins (35, 36). However, it is of interest that the level of Ii message was merely reduced, but not absent (reviewed in Ref. 24). One possibility is that the intronic region of the Ii gene may play some role in this residual expression. As BLS-inducing mutations have identified X box-binding proteins (37) and CIITA (35), it made sense that class I and class II promoter regions would be impaired without these components. To address the question of whether or not CIITA expression was necessary for Ii intronic enhancer function, we tested our L cell line for CIITA expression. L cells have previously been reported to lack CIITA expression (38), and our results corroborate this finding, yet the Ii intronic enhancer still functions effectively in these cells. Therefore, it seems that the X box-binding factors may be critical for basal Ii expression, however, CIITA may only be required for IFN-γ-induced Ii expression. Therefore, either the Ii promoter alone or the intronic enhancer may be sufficient to give a reduced level of Ii expression in either BLS cell lines or in the CIITA knock-out mouse (39). This supposition is further supported by the data shown in Figure 1 (p-95 construct). The observations that Ii expression is maintained in class II-deficient cell lines and that some nonprofessional APCs express Ii in the absence of class II (reviewed in Ref. 24) could be used to suggest that the Ii gene is an ancestrally more primitive gene. It is possible that the Ii gene first evolved as a chaperone for some protein(s) other than class II, and that when the class II genes evolved, they acquired the transcriptional regulatory regions from one or both of the Ii enhancers.

While the 155-bp region we have identified in the Ii intronic enhancer contains the majority of the enhancing activity seen within the intronic region, it is possible that there are additional positive regulatory elements (for example in enhancer fragments E, F, and H) as well as negative regulatory elements (for example, fragment A) contained within the 405-bp intronic region. Searches for sequences homologous to known cis-acting elements suggest that there is an AP-1 site centered at nt 2565 in the 5′ end of the 405-bp region. The 3′ end of the 405-bp enhancer contains two PU boxes or Ets-1 sites centered at positions 2874 and 2888. In addition, there are several homologies to γ-IFN response elements (γIRE) centered at positions 2695, 2813, and 2903. Scanning mutations in these regions should be informative for dissecting the

FIGURE 7. CIITA expression is not necessary for basal Ii enhancer activity. Total RNA from splenic B cells or L cells was isolated and subjected to first-strand cDNA synthesis and RT-PCR. Decreasing amounts of total RNA from either spleen or L cells, corresponding to 5, 2.5, 1.25, 0.63, 0.31, 0.16, 0.08, and 0.04 μg, were subjected to RT-PCR analysis for CIITA and β-actin. The 100-bp DNA ladder is shown in the far left lane. The arrow denotes the position of the 600-bp marker. The expected sizes of the amplified products are 700 bp for CIITA and 510 bp for β-actin.
functions and possible interplay among these regions. Characterization of the proteins binding to the regulatory regions of the class II promoters and the two Ii chain enhancers should reveal the mechanisms regulating class II expression and Ag presenting function.

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References