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Identification of Class II Transcriptional Activator-Induced Genes by Representational Difference Analysis: Discordinate Regulation of the DNα/DOβ Heterodimer

Debra J. Taxman, Drew E. Cressman, and Jenny P.-Y. Ting

Class II transcriptional activator (CIITA) is a master regulator of MHC class II genes, including DR, DP, and DQ, and MHC class II-associated genes DM and invariant chain. To determine the repertoire of genes that is regulated by CIITA and to identify uncharacterized CIITA-inducible genes, we used representational difference analysis. Representational difference analysis screens for differentially expressed transcripts. All CIITA-induced genes were MHC class II related. We have identified the α subunit, DNα, of the class II processing factor DO as an additional CIITA-inducible gene. Northern analysis confirmed that DNα is induced by IFN-γ in 2TGH fibrosarcoma cells, and CIITA is necessary for high-level expression in B cells. The β subunit, DOβ, is not inducible in fibrosarcoma cells by IFN-γ or exogenous CIITA expression. Moreover, in contrast to other class II genes, DOβ expression remains high in the absence of CIITA in B cells. The promoters for DNα and DOβ contain the highly conserved WXY motifs, and, like other class II genes, expression of both DNα and DOβ requires RFX. These findings demonstrate that both DNα and DOβ are regulated by RFX. However, DNα is defined for the first time as a CIITA-inducible gene, and DOβ as a MHC class II gene whose expression is independent of CIITA. The Journal of Immunology, 2000, 165: 1410–1416.

The MHC is an extensive multigene family that encodes surface glycoproteins involved in the binding and presentation of processed antigenic peptides to T cells. MHC class II is required for the recognition of foreign Ags by Th cells. Three serologically defined isotypes of class II molecules, DP, DQ, and DR, are expressed as αβ heterodimers on the surfaces of B cells, activated T cells, dendritic cells, and many somatic cells following induction by IFN-γ. Intracellularly, class II molecules associate with the invariant chain (II)1 (1). Ii inhibits binding of peptides to class II molecules in the endoplasmic reticulum and directs class II molecules to the endosomal MHC class II compartment (2), where II is degraded and binding of antigenic peptides occurs (3–5). Efficient peptide loading also requires the function of DM, a class II-like heterodimer that resides in the MHC class II compartment (6–8). DM acts as a chaperone for MHC class II molecules, catalyzing the release of an Ii-derived peptide class II-associated peptide (CLIP) from class II molecules, stabilizing the empty peptide-binding grooves and permitting loading of appropriate high stability peptide ligands (for review, see Refs. 9–11).

Two additional genes within the MHC locus, DNα (formerly DZα) and DOβ, also have homology to MHC proteins. The DNα protein originally was presumed to be independent from the DO heterodimer because in fibroblasts DNα, but not DOβ, is induced by IFN-γ (12). However, the association between the two subunits was suggested in studies examining DO localization in human B lymphoma cells. DO was shown to accumulate in lysosomes in a DM-dependent fashion, and accumulation depended upon both DNα and DOβ (13). Association between DNα and DOβ also has been demonstrated directly by copurification of baculovirus-expressed proteins (14) and by immunoprecipitation of endogenous proteins in P815 B cell lysates (15). Virtually all DO molecules are physically associated with DM (14, 16), and DO originally was thought to function primarily as an inhibitor of DM due to its ability to cause accumulation of DR-CLIP complexes when overexpressed (16, 17). The biological role of this inhibition was clarified when gene knockout mice were made for the murine DNα and DOβ. Cells from H2-O-deficient mice had normal levels of class II expression, normal levels of CLIP, and a mixture of peptides like wild-type mice. However, the absence of H2-O resulted in a loss of discrimination between different forms of Ags, and inhibition by DO was shown to occur selectively at high pH. These results suggest that DO functions by decreasing presentation of Ags internalized by fluid-phase endocytosis in favor of Ags internalized via membrane Ig receptors. Consistent with this model, DO has been shown to promote presentation of stable DR-peptide complexes when expressed at higher physiological levels (14). This is especially true for DR alleles that are DM dependent and bind weakly to DM, such as DR4 (14). Thus, whether DO acts as an enhancer or inhibitor of DM may be dependent upon levels of DO expression, the quality of Ag, and the class II allele (for review, see Refs. 19–21).

The majority of MHC class II-related genes are coregulated. W, X, and Y boxes in their promoters are conserved in sequence and spacing, and this triad motif is known to govern B cell-specific and IFN-γ-inducible gene expression of DR, DP, DQ, DM, and Ii. Four complementation groups from bare lymphocyte syndrome (BLS)
patients have defined two master regulators that are essential for cell-specific class II expression, RFX and class II transcriptional activator (CIITA). RFX is a trimeric protein composed of RFXS (complementation group C) (22), RFXAP (group D) (23), and RFXANK/RFX-B (group B) (24, 25). RFX is thought to direct B cell-specific and IFN-γ-inducible expression of MHC class II genes through binding to the X box (26) and possibly the W box (27). CIITA (group A) (28) is the only MHC class II transcription factor known to be induced in response to IFN-γ, and its expression alone is sufficient to activate expression of the MHC class II genes (29–31). CIITA is a nonclassical transactivator that requires intact W, X, and Y boxes, yet functions without apparent DNA binding. CIITA has several functional domains typically associated with transcriptional activation, including an amino terminal acidic domain (32–34), proline-serine/threonine-rich regions (28, 35), a nuclear localization sequence (36), and several leucine-rich repeats (37). However, its primary mode of action is thought to involve the bridging of multiple transcription factors together at the class II promoter. CIITA has been shown to interact with several other transcriptional activators, including TAFI132, Bob-1, RFXS, RFXANK, CREB, CREB binding protein, PTEFb, and NF-YB and C (34, 38–42, 58, 59). CIITA also is unique in that it is the only transcriptional activator known to be regulated by GTP binding (43). Though the DNa and DOβ gene promoters also contain W, X, and Y boxes, expression of these genes is often incongruous with other MHC class II-associated genes (12, 15, 44), and whether DNa and DOβ are induced by RFX or CIITA remains an open question.

In the present study, we have performed representational difference analysis (RDA) to identify uncharacterized CIITA-inducible genes. RDA is a powerful methodology that combines subtractive hybridization and PCR amplification to identify differentially expressed genes (45–48). Enrichment of differentially expressed genes is 2-fold: subtractive enrichment is obtained through hybridization with an excess of driver cDNA, and kinetic enrichment is obtained through higher rates of annealing and removal of the more abundant DNA species (47). Subsequent PCR amplification allows recovery of genes that are expressed even at very low level. Successive rounds of hybridization/subtraction and PCR also can be done, resulting in the exponential enrichment of differentially expressed genes. We have performed RDA using cDNA from G3A fibrosarcoma cells stably transfected with CIITA vs cells transfected with a vector control. All CIITA-induced genes were found to be MHC class II related. DNa was identified for the first time as a CIITA-inducible gene. We show that DNa is induced by IFN-γ in 2TGH cells and that its high-level expression in B cells requires CIITA. In contrast, DOβ is shown to be refractive to induction by CIITA. Implications for the coordinate regulation of these two genes are discussed.

Materials and Methods
Cell culture
2TGH and G3A fibrosarcoma cells (49) were grown in Dulbecco’s Modified Eagles Medium supplemented with 10% FCS (Life Technologies). G3A is a mutant cell line derived from 2TGH that is deficient in IFN-γ induction of MHC class II. CIITA transcript is absent in uninduced G3A cells, and is detectable in IFN-γ-induced cells at a very low level, which does not lead to MHC expression (30). The preparation of G3A-p4 and G3A-CIITA cell lines has been described (50). Briefly, pREP4 (Invitrogen, San Diego, CA) and pREP4-CIITA (50) episomal expression plasmids were transfected into G3A cells, and cells were selected for 2 wk in media containing 250 μg/ml hygromycin B (Boehringer Mannheim, Mannheim, Germany). Media was changed every 3 days. Cells were cultured subse-
quent to 250 μg/ml hygromycin B. Expression of CIITA in G3A-CIITA cells was confirmed by Northern blotting for CIITA, and induction of MHC class II expression was confirmed by Northern analysis and FACS staining for DR. Where indicated, 2TGH, G3A, or G3A-p4 cells were induced for 24 h with 500 U/ml IFN-γ (Genzyme, Cambridge, MA). Jurkat E6–1 T lymphocytes (TIB 152; American Type Culture Collection, Manassas, VA), Raji B lymphocytes (CCLS6; American Type Culture Collection), R2.J2.5 and BCH CIITA-mutant B cells (28), BLS-1 RFXANK-deficient B cells (24), and SJ0 RFX5-deficient B cells (22) were cultured in RPMI 1640, 10% FCS. Cells were incubated at 37°C in 5% CO2.

Representational difference analysis
Cytosolic mRNA was purified from G3A-p4 and G3A-CIITA cells by two passes over oligotex particles (Qiagen, Chatsworth, CA), and mRNA integrity was confirmed by Northern blotting with 32P labeled GAPDH and DRα. CDNA was prepared using the Superscript Choice System for cDNA Synthesis (Life Technologies). Complete cDNA synthesis was confirmed by low-level incorporation of [32P]dCTP, electrophoresis, and autoradiography. RDA was performed essentially as described using the Bgl-24 and Bgl-12 oligo series (47, 48). The steps taken to isolate CIITA-induced genes are outlined in Fig. 1. Representations of G3A-p4 and G3A-CIITA CDNA were prepared by digesting 2 μg of cDNA with DpnII, ligating paired adaptor/primer oligonucleotides, and PCR amplification. Three rounds of hybridization/subtraction and PCR amplification were completed using G3A-CIITA representation as tester and G3A-p4 representation as driver. Adaptors were changed for each round. Tested:driver ratios of 1:100, 1:800, and 1:400,000 were used in successive rounds, yielding difference products (DP) 1–3. For DP4 and 5, the MHC driver was prepared by combining DNa, CIITA, and known CIITA-induced genes, including DRα, DBβ, DPr, DPβ, DQα, DQβ, DMα, DMβ, and I (American Type Culture Collection). Tester and driver were combined at ratios of 1:1,000 and 1:200,000 for DP4 and DP5, respectively. Gene fragments from DP3 and DP5 were cloned by digestion with DpnII and ligation into BamHI-digested Bluescript II vector (Stratagene, La Jolla, CA). Following transformation, known CIITA-induced genes were further eliminated by colony purification (51) using radiolabeled MHC driver. Southern hybridization of plasmids from unlabelled colonies with 32P MHC driver was performed as a final step to eliminate as many known genes as possible. Cloned gene fragments were sequenced and identified through blast searches. All manipulations of mRNA and CDNA were done with aerosol tips to avoid cross contamination of samples.

Northern and Southern analysis
Northern analysis was performed using 100 ng mRNA. The mRNA was isolated using Oligotex direct purification (Qiagen), electrophoresed on formaldehyde gels, and blotted onto nylon membranes. Probes were prepared by random priming of isolated gene fragments (Prime-it II; Stratagene) for DRα, CIITA, and known CIITA-induced genes, including DRα, DBβ, DPr, DPβ, DQα, DQβ, DMα, DMβ, and I (American Type Culture Collection). Tester and driver were combined at ratios of 1:100 and 1:200,000 for DP4 and DP5, respectively. Gene fragments from DP3 and DP5 were cloned by digestion with DpnII and ligation into BamHI-digested Bluescript II vector (Stratagene, La Jolla, CA). Following transformation, known CIITA-induced genes were further eliminated by colony purification (51) using radiolabeled MHC driver. Southern hybridization of plasmids from unlabelled colonies with 32P MHC driver was performed as a final step to eliminate as many known genes as possible. Cloned gene fragments were sequenced and identified through blast searches. All manipulations of mRNA and CDNA were done with aerosol tips to avoid cross contamination of samples.

Results
Representational difference analysis to identify CIITA-induced genes
To identify uncharacterized CIITA-induced genes, stable cell lines were prepared containing a CIITA expression plasmid (pREP4-CIITA) or a vector control plasmid (pREP4) (50). G3A fibrosarcoma cells were chosen as a background for expression because deficient MHC class II induction in these cells correlates with suboptimal induction of CIITA (30). To avoid any potential back- 
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of G3A-p4 driver to G3A-CIITA tester. The G3A-p4 driver, G3A-CIITA tester, and difference products (DP) from successive rounds of subtraction/PCR amplification were analyzed by electrophoresis and ethidium bromide staining (Fig. 2A). As expected, the G3A-p4 driver and G3A-CIITA tester each appeared as continuous smears of bands (lanes p4 and CII). DP1 to 3 were progressively subtracted to a ladder of discrete bands (lanes DP1–3). This is consistent with the effective selection of a subset of gene fragments from the G3A-CIITA tester.

To determine whether enrichment was selective for CIITA-induced genes, the contents of gene fragments in the above fractions were assessed by Northern analysis using the DRα gene as a model CIITA-inducible gene (Fig. 2B). Results indicated that DRα gene fragments were enriched significantly in the first round of subtraction (lane DP1). Levels of DRα remained high in the subsequent two rounds (Fig. 2B, lanes DP2 and DP3), while the total DNA content was reduced (Fig. 2A, lanes DP2 and DP3). This is consistent with the progressive enrichment of CIITA-induced genes. Levels of the housekeeping gene GAPDH as assessed by dot blot hybridization appeared to decrease during subtraction, indicating that enrichment was specific for MHC class II genes (data not shown).

Table I. Gene fragments recovered by RDA

<table>
<thead>
<tr>
<th>Gene Fragment</th>
<th>No. of Clones</th>
<th>Differential Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRα</td>
<td>108–300</td>
<td>1</td>
</tr>
<tr>
<td>DRβ</td>
<td>231–587</td>
<td>Yes</td>
</tr>
<tr>
<td>DQα</td>
<td>48–543</td>
<td>Yes</td>
</tr>
<tr>
<td>DQβ</td>
<td>534–857</td>
<td>Yes</td>
</tr>
<tr>
<td>Ii</td>
<td>43–264</td>
<td>Yes</td>
</tr>
<tr>
<td>DNα</td>
<td>453–781</td>
<td>Yes</td>
</tr>
<tr>
<td>Misc.</td>
<td>(3′ utl)</td>
<td>11</td>
</tr>
</tbody>
</table>

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<td>453–781</td>
<td>Yes</td>
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a Confirmed by Northern analysis.

b NT, Not tested.

Our original proposal was to identify novel genes that may be MHC class II related either in sequence or in function. Thus, to
eliminate known CIITA-induced genes, two further rounds of subtraction/PCR amplification were performed using pooled MHC genes, including DRα, DRβ, DPα, DPβ, DQα, DQβ, DMα, DMβ, Ii, CIITA, and DNα as driver (Fig. 1). As seen by ethidium bromide staining, the ladder of fragments appeared to reduce in complexity even further during these two additional rounds (Fig. 2A, lanes DP4 and DP5). Moreover, Southern analysis for DRα indicated significant reduction of known MHC genes from the pool during this process (Fig. 2B, lanes DP4 and DP5). Gene fragments from DP5 were cloned, and known MHC genes were further filtered out by colony hybridization with radiolabeled MHC genes. Eighty colonies that hybridized poorly with radiolabeled MHC were cultured, plasmids were isolated, and an additional round of negative selection was performed to screen out known MHC genes by Southern analysis with pooled MHC. Gene fragments that survived these four additional screening steps then were sequenced. Despite extensive negative screening, several known CIITA-induced genes remained in the pool, including DRα, DQβ, and Ii, as well as the DNα 3′ untranslated fragment (Table I). This likely is due to the selectively high abundance of these genes in CIITA-induced cells. Several ribosomal genes and a mitochondrial gene were also identified during the analysis. We have observed these genes commonly in other RDA searches and thus they are likely artifacts of the procedure. We also recovered six nonhousekeeping genes that could not be confirmed to be differentially expressed by Northern hybridization. Thus, the only genes induced by CIITA that could be detected by this protocol are MHC class II genes, and the recovery of fragments from DNα strongly suggest that it is an additional CIITA-inducible gene.

DNα and DOβ are discoordinately regulated by CIITA

Northern analysis was performed to confirm DNα mRNA induction by CIITA. Consistent with the RDA results, DNα transcript was found in G3A-CIITA cells, but not in G3A-p4 cells (Fig. 3). Expression was seen for both the full-length 1.1-kb transcript and the alternatively spliced 3.5-kb transcript. To determine whether DNα transcription is induced by endogenous CIITA expression, mRNA was collected from wild-type 2TGH cells following IFN-γ induction. DNα transcripts were detected in IFN-γ-treated 2TGH cells, but not in untreated cells. DNα levels also were low in IFN-γ-treated G3A-p4 cells, indicating that IFN-γ induction of DNα requires CIITA. To test whether DNα expression correlates with CIITA expression in B cells, mRNA was tested from wild-type Raji B lymphoblasts vs its CIITA-defective counterpart, RJ2.2.5 (28). An additional CIITA-deficient cell line, BCH, also was tested. As expected, DNα 3.5-kb and 1.1-kb transcripts were expressed in Raji cells. Levels were reduced in RJ2.2.5 cells and absent in BLS-2 cells. Reduction in RJ2.2.5 of the 1.1-kb transcript was 3-fold, while the 3.5-kb transcript was 1.6-fold. However, this disparity is of unknown significance because both transcripts yield the same protein (52). These findings indicate that DNα expression is inducible by CIITA in fibrosarcoma cells and is partially to fully dependent upon CIITA in B cells.

DNα exists intracellularly as a heterodimer with DOβ (termed DO) (15). To test whether DOβ is similarly regulated by CIITA in the same cell. Northern blots were probed with radiolabeled DOβ cDNA. Surprisingly, DOβ was not induced either endogenously by IFN-γ in 2TGH, or by exogenous CIITA expression in G3A-CIITA cells (Fig. 3, panel DOβ). As previously observed (12), DOβ is expressed in Raji B lymphocytes, yet levels of expression remain high even in the absence of CIITA in RJ2.2.5. DOβ expression was also high in CIITA-deficient BLS-2 cells. However, expression was absent in Jurkat T cells, suggesting that, while not dependent upon CIITA, DOβ expression is B cell specific. These findings suggest that the DO heterodimer subunits are regulated discoordinately.

**Table II. Conserved W, X, and Y box sequences of DOβ and CIITA-inducible genes**

<table>
<thead>
<tr>
<th>Consensus</th>
<th>W Box</th>
<th>X Box</th>
<th>Y Box</th>
</tr>
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<tbody>
<tr>
<td>DOβ</td>
<td>-C-</td>
<td>A-----</td>
<td>T------</td>
</tr>
<tr>
<td>DRα</td>
<td>------</td>
<td>C------</td>
<td></td>
</tr>
<tr>
<td>DRβ</td>
<td>------</td>
<td>C------</td>
<td></td>
</tr>
<tr>
<td>DPα</td>
<td>CA-C</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>DPβ</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>DQα</td>
<td>G-C</td>
<td>------</td>
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</tr>
<tr>
<td>DQβ</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>DMα</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>DMβ</td>
<td>G-----</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Ii</td>
<td>G-----</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>DNα</td>
<td>TC------</td>
<td>------</td>
<td>AC-----</td>
</tr>
</tbody>
</table>

* Based on sequences in Genbank and Refs. 54, 56, and 57.
RFX is essential for Dα and DOβ expression in B cells

The DOβ promoter, similar to other known CIITA-inducible promoters, is comprised of conserved W, X, and Y elements (Table II). In part, CIITA derives its activity through interaction with the trimeric transcription factor RFX, which is essential for transactivation from the X box and is thought to bind the W box as well (27, 40). Voliva et al. have shown that the X box of DOβ can substitute functionally in the DRα promoter (54). However, in gel shift assays, the DOβ X box is unable to compete for X-binding complexes on the DRα X box. Based on these findings, the authors have hypothesized that a different protein complex may bind to the X box of DOβ (54). To directly test whether RFX is involved in the expression of Dα and DOβ in B cells, mRNA from the RFX mutant B cell lines BLS-1 and SJO was examined. BLS-1 is deficient in the RFXANK/RFX-B subunit of RFX, and SJO is deficient in the RFX5 subunit.

Discussion

RDA is an extremely powerful methodology for identifying differences in gene expression between cells (45–48). In the present study, we have used RDA to determine the repertoire of genes that is regulated by CIITA. All of the CIITA-induced genes identified were MHC class II related. The specificity of CIITA for MHC class II genes and MHC class II processing genes is remarkable given the size and complexity of the CIITA protein and the large number of transcriptional activators with which it can interact (28, 32–42, 58, 59). The Dα subunit of MHC processing factor DO also was defined for the first time as a CIITA-inducible gene, whereas the DOβ gene is CIITA independent. While we cannot rule out the possibility that there may be additional unknown CIITA-inducible genes, Dα was the only one found in this system. Several rounds of screening were done to filter out known CIITA genes. Initially, two additional rounds of subtraction were done using a pool of all known CIITA-inducible genes. Next, colony hybridization was done using pooled radiolabeled MHC cDNA to eliminate residual CIITA-induced genes. Finally, gene fragments from unlabelled/lightly labeled colonies were assayed by Southern blotting with pooled radiolabeled MHC cDNA. Despite these efforts to eliminate known MHC genes from the final difference product, the genes recovered were those previously known to be CIITA induced, Dα, or artifacts. Note, however, that this procedure may not be exhaustive. It is possible that some CIITA-induced genes were lost during the PCR amplification or subtraction steps. Because CIITA was expressed exogenously in G3A cells in the absence of IFN-γ, genes whose induction depends both on CIITA and additional IFN-γ-inducible components may be missed. Also, it is possible that there are additional CIITA-inducible genes that are expressed in APCs in vivo.

The identification of Dα as a CIITA-induced gene is consistent with the IFN-γ induction of Dα in human fibroblasts (12) and neuroblastoma cells (55). We also have confirmed the requirement for CIITA for Dα expression by Northern analysis: Dα is induced both by exogenous CIITA expression in G3A cells and by endogenous CIITA in 2TG17H cells following IFN-γ treatment. Furthermore, Dα gene expression is reduced in complementation group A cell lines. However, the CIITA-dependent expression of Dα we observe is seemingly in contrast to the expression of its murine homologue, H2-0β, in the spleen of CIITA knockout mice (44). We have shown that Dα is partially expressed in CIITA-defective RJ2.2.5 cells. Thus, it is possible that in B cells there is an alternate mechanism of regulation that can partially activate Dα. Alternatively, regulation may differ in mouse and human B cells.

Whereas expression of Dα is regulated by CIITA, we have shown that its heterodimeric partner, DOβ, is not CIITA inducible in G3A cells. This is consistent with the lack of DOβ observed in IFN-γ-induced fibroblasts (12). We also have shown that DOβ expression is independent of CIITA in B cells, consistent with the expression of H2-0β in CIITA knockout mice (44). The refractoriness of the DOβ gene to CIITA is of interest for several reasons. First, the Dα and DOβ genes comprise a unique MHC-related heterodimer in that they appear to be regulated independently. The biological role of discordant expression is unclear. It is especially intriguing that Dα is expressed in the absence of DOβ in IFN-γ-induced cells (Ref. 12 and Fig. 3). It is possible that Dα has a function that is independent of DOβ. Alternately, it may pair with an unknown β subunit in IFN-γ-induced cells. To address these possibilities, it will be necessary to perform localization and immunoprecipitation experiments using anti-Dα Abs.

A second point of interest is that DOβ is unusual in its ability to be expressed independent of CIITA. This is a surprising finding because the promoter of DOβ, like all MHC class II genes, has conserved W, X, and Y boxes. Additionally, we have shown that expression of DOβ requires the X-binding protein, RFX. CIITA is thought to act as a transcriptional scaffold by recruiting transcription factors that bind W, X, and Y boxes. It is possible that in B cells there is an unknown transcriptional coactivator that can bridge proteins onto the DOβ promoter in the absence of CIITA. It is likely that sequences outside of the proximal promoter region are involved because a 250-bp fragment of the DOβ promoter is not sufficient to confer activation of a reporter gene in Raji cells (54). Nevertheless, the DOβ promoter provides an interesting model for studying mechanisms of MHC gene regulation without the overwhelming effects of CIITA. This promoter might be used to more precisely define functional interactions between W, X, and Y factors on the MHC promoter that occur independent of CIITA. Furthermore, the DOβ promoter may serve as a model for identifying new transcriptional activators, transcriptional repressors, and/or chromatin remodeling factors involved in MHC class II gene activation. The DO heterodimer is also expressed in some, but not all, cultured dendritic cells (15, 20, 21), and it would be of interest to determine the parameters that determine dendritic cell expression. Lastly, it would be of interest to determine whether CIITA is involved in the LAN-5 neuroblastoma model in which DOβ is induced by IFN-γ (55).
In summary, we have used RDA for the first time to identify CIITA-inducible genes. We have shown that all genes induced in this system are MHC class II genes or MHC class II-associated genes. We have characterized DNRs for the first time as an additional CIITA-induced gene, and have demonstrated an interesting disparity in DNRs and DOβ control by CIITA. Finally, we show that despite this disparity, both DNR and DOβ are controlled by RFX. It will be of interest to identify additional factors controlling the expression of DOβ and to elucidate the function of DNR expression independent of DOβ.

Acknowledgments

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References


