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MINIREVIEW

Class II Transactivator: Mastering the Art of Major Histocompatibility Complex Expression

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INTRODUCTION

Major histocompatibility complex (MHC) class II molecules are the predominant presenters of exogenous antigens to T helper cells (reviewed in references 21, 77, and 99). These key molecules are critical for numerous aspects of immune function, including T-cell selection, tolerance induction, antibody production, T-cell-mediated immunity, and the inflammatory response. As principal mediators of transplant rejection, these molecules are often common targets for immune therapies to prevent the rejection of grafted tissues. Class II MHC is implicated as a contributing factor in a host of diseases ranging from rheumatoid arthritis and diabetes to Alzheimer's disease and multiple sclerosis.

Constitutive expression of class II MHC is restricted to "professional" antigen-presenting cells but can be induced on various tissues by gamma interferon (IFN-γ). In humans, a congenital lack of both constitutive and inducible class II results in a profound and generally fatal immunodeficiency (type II bare lymphocyte syndrome [BLS]) (7, 29, 45, 61, 67, 75, 112) marked by a significant reduction of $CD4^+$ T cells. Early molecular forays addressing BLS revealed that the genes encoding class II MHC were not defective. Instead, the defect lay in transcription factors controlling class II MHC gene expression. BLS thus became the first disease known to be caused by defective or absent transcription factors. The availability of patient-derived cell lines with class II MHC transcription defects provided a unique tool of nature to identify the requisite transcription factors.

Transcriptional regulation of class II MHC expression is complex. Class II MHC and related promoters are characterized by the presence of conserved W (or S), X, and Y boxes (Fig. 1) (reviewed in references 10, 67, and 75). The X element is bipartite. The upstream X1 region is recognized by RFX, a trimeric complex of RFX family members including RFX5, RFXANK (RFX-B), and RFXAP (32, 74, 87, 117). The downstream X2 box is bound by X2BP (NF-X2), a complex comprising CREB, and an unidentified 120-kDa protein (83, 84). Another trimeric complex, NF-Y (CBF), which is highly conserved in eukaryotes, binds the Y box (69, 71, 146; reviewed in reference 70). A number of factors interacting with the W box have been described, including the RFX complex (26, 48, 120). The factors involved in X and Y box binding are ubiquitous and expressed constitutively yet fail to account for either constitutive or IFN-γ-inducible class II MHC expression. Somatic cell fusions using BLS patient-derived cells allowed the definition of complementation groups, with each group containing a defect in a single genetic locus. This type of analysis revealed a crucial locus, aIr-1, which in all likelihood encodes the class II transactivator (CIITA), which explained the lack of class II transcription in BLS complementation group A (1, 118). Group A cells express the requisite X and Y binding proteins but fail to transcribe class II. CIITA expression appears to be a nearly absolute requisite for expression of class II MHC, whether constitutive or inducible (17, 19, 23, 47, 85, 103, 114, 118, 119). A number of class II MHC-related genes including genes encoding HLA-DM (H-2M in mice) and invariant chain (Ii), with promoters similar to those for classical class II genes, are also regulated by CIITA (17, 18, 22, 23, 50, 137). CIITA can also upregulate expression of class I MHC genes and beta-2-microglobulin (β_2m) through effects at site α in addition to X- and Y-like sequences in the promoters for these genes (40, 72, 104). These initial observations have led to the view that CIITA is a master, or global, regulator for expression of class II MHC and related genes.

Since the discovery of CIITA, numerous primary articles and several reviews on its role in regulating the class II MHC have been published. In this review, we will discuss the molecular structure of this novel protein, its mechanism of function, and its biologic and clinical relevance, which is broad.

A MASTER REGULATOR?

The father of all master regulators is MyoD, which when placed into 10T1/2 cells can cause these cells to acquire characteristics of muscle cells and the accompanying changes in gene transcription (reviewed in references 135 and 136). By this definition, CIITA is clearly a master regulator of class II MHC genes. Many reports have found that placing CIITA in an array of cell types can result in not only the induction of class II MHC promoters but also the expression of cell surface class II MHC proteins (8, 11, 18, 19, 23, 110, 118). Further, expression of class II MHC is controlled quantitatively by CIITA (96). In $CIITA^{-/-}$ mice, class II MHC is missing in almost all tissues and cells with one exception (see below). Invariant-chain expression is limited, but not absent, in $CIITA^{-/-}$ mice (17, 57). This is likely due to the presence of additional regulatory elements in the invariant-chain promoter (e.g., SP1 and NF-κB) (12, 13, 142). In addition, the expression of H-2O is not affected by the lack of CIITA (17). Most cytokines which alter class II MHC expression, such as IFN-γ, tumor necrosis factor α (TNF-α), transforming growth factor

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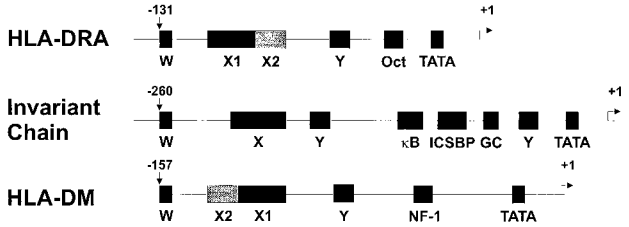


FIG. 1. Organization of W, X, Y, and other motifs in the promoters of class II MHC and related genes. Genes coding for class II MHC and related proteins contain well conserved W, X, and Y boxes, the presence of which correlates with transcriptional regulation by CIITA.

β, interleukin 1 (IL-1), IL-4, and IL-10, either up- or down-regulate CIITA and class II MHC accordingly (18, 23, 42, 57, 88, 89, 92, 107, 119). In addition to cytokine-regulated class II MHC expression, the in situ expression of CIITA is also tightly linked to class II MHC gene expression (115, 116).

Developmentally, class II MHC is tightly associated with CIITA. Cell-type-specific and species-specific differences in class II MHC expression can also be explained by differences in CIITA. For example, CIITA is expressed in B cells but not in plasmacytomas where class II is extinguished (109, 114). CIITA is expressed in activated human T cells, which express class II MHC, but not in resting or activated mouse T cells, which lack class II MHC (20). However, one study has found some class II and CIITA message by reverse transcription-PCR (RT-PCR) in activated mouse T cells treated with IL-12 (41). Finally, CIITA regulates not only class II MHC but also Ii and DM molecules. Promoters of genes coding for all the aforementioned proteins have the W, X, and Y motifs, and CIITA works through these motifs (reviewed in references 67 and 126). Nonclassical class II genes are not obligatorily regulated by CIITA (17). Differential expression analyses show that the DNα gene is regulated by CIITA but that DOβ is not (125).

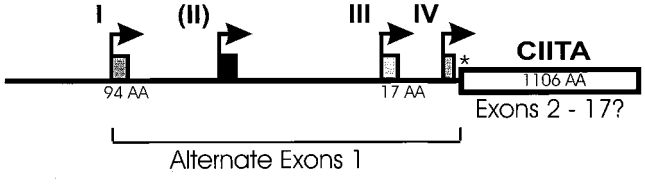
On the other hand, there are a few exceptions where CIITA is not required or associated with classical class II MHC gene expression. For example, it has been shown that in CIITA$^{-/-}$ mice, class II MHC^{+} dendritic cells are detected in lymph nodes and thymus, although the level is significantly lower than that for control mice (17, 139). These studies relied heavily on immunohistochemical staining and RT-PCR. The small amount of class II MHC found in these tissues is apparently not sufficient to permit development of a normal CD4^{+} T-cell population. We and others have found that TNF-α alteration of class II MHC may not be associated with changes in CIITA (30, 91). Indeed, it has been shown that negative modulation of class II MHC can occur via posttranscriptional mechanisms as has been observed with IFN-β (62), and posttranslational mechanisms affecting CIITA also occur with TNF-α (42). Additionally, NK cells have been reported to upregulate HLA-DR in fibrosarcoma cell lines in an IFN-γ- and CIITA-independent fashion dependent on cell contact (28). Finally, some invariant-chain and class II MHC isotype-specific expression in in vitro-generated mutant cell lines has been shown to occur in the absence of functional CIITA, which suggests the existence of isotype-specific transacting factors (31, 122, 149). However, in general, CIITA-independent class II expression appears to be the exception rather than the rule. This may be similar to gene regulation in muscle development, where the addition of MyoD cannot convert all cells to muscle cells and the lack of MyoD does not ablate all muscle development (4, 113). Hence, in a parallel comparison, CIITA compares favorably with MyoD to qualify as a master regulator.

GENETICS

The coactivator function required for class II MHC expression was initially shown to reside on mouse chromosome 16 (the AIR-1 locus) (1). Human CIITA is encoded on chromosome 16 (119). The initial CIITA cDNA, cloned from the RJ2.2.5 B-cell lymphoma, encodes an 1,130-amino-acid protein. Genomic DNA from mice shows that CIITA is encoded by 19 exons (75), although the precise exon organization for human CIITA has not been reported. Expression of human CIITA is controlled by four distinct promoters, each with a distinct product, with three of the forms predominating. In mice only three promoters were identified (86) (Fig. 2). Promoter I is constitutively active in dendritic cells and has a promoter-specific first exon coding for 94 amino acids. In cDNA preparations, promoter II gave rise to a separate product, but this product is not present in significant amounts in any of the individual cell lines tested to date. Promoter III is constitutively active in B cells, responds to IFN-γ via a distal upstream sequence, and has a first exon encoding 17 amino acids (59, 86, 100, 101). The first 300 bp of promoter III is sufficient for B-cell function and contains two important sequences including a transcription elongation factor 2-like element (38). Promoter IV is IFN-γ responsive, active in the monocyte/macrophage lineage, endothelial cells, and fibroblasts, and drives expression of the shortest CIITA transcript (86, 101). Thus constitutive expression of CIITA generally results from promoters I and III. Promoter IV is likely responsible for the majority of IFN-γ-inducible expression, but sequences upstream of promoter III allow IFN-γ-mediated modulation of constitutive CIITA expression. The significance of the various isoforms of CIITA that differ only in the N terminus is presently unknown.

STRUCTURE AND FUNCTION

CIITA has predicted and apparent molecular masses of 123.5 and 135 to 140 kDa, respectively, suggesting some post-translational modification (118). In those tissues and cell lines tested to date detection of the endogenous CIITA protein has been difficult at best, although some polyclonal-antibody preparations seem able to detect endogenous CIITA in whole-cell lysates of some cell types (14, 24). Intracellular concentrations are insufficient for immunohistochemical staining, and Western blotting requires larger numbers of cells for detection. Structure/function studies to date have been mostly driven by sequence comparison and have focused on the ability of transfected CIITA to activate transcription and subsequent expres-



* = Splice site and portion of exon 2 upstream of promoter IV Kozak consensus AUG that adds 7 AA to promoter I and III proteins

FIG. 2. Promoter organization for CIITA. The CIITA upstream region in humans contains four promoters (I to IV) with independent start sites and alternate first exons. Significant mRNA populations have only been observed for I, III, and IV in vivo. The 3′ splice site for the first exons of promoters I and III is upstream of the translation start site for promoter IV mRNA and thus adds seven amino acid residues (*). The core 1,106 amino acids of CIITA may be encoded by as many as 17 exons.

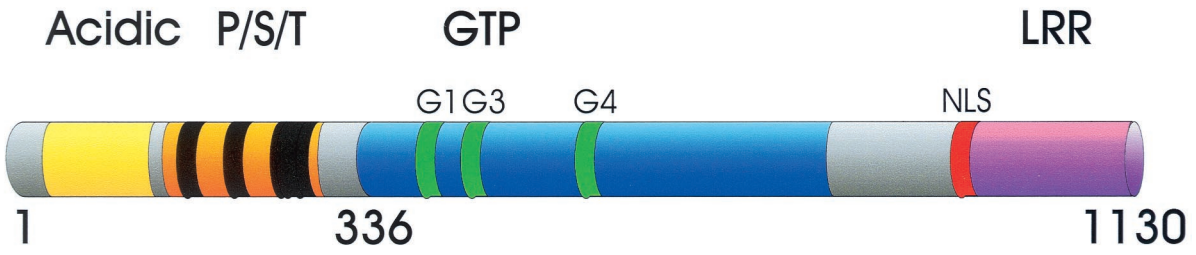


FIG. 3. Schematic representation of protein domain organization for CIITA. Numbering corresponds to the B-cell form of CIITA. The first 336 amino acids of CIITA contain both an acidic domain and the PST domain. Residues 336 to 1130 contain the GTP-binding, NLS, and LRR motifs. G1, G3, and G4 mark the positions of specific motifs required for GTP binding (see text).

sion from endogenous or engineered class II promoters, its compartmentalization, and its capacity to interact with other proteins.

Sequence analyses of CIITA have revealed a complex domain structure (Fig. 3) composed of an amino-terminal acidic domain, proline-, serine-, and threonine-rich (PST) regions, a GTP-binding site, at least one nuclear localization sequence (NLS), and a series of leucine-rich repeats (LRR).

Acidic domain. The N-terminal end of CIITA contains an acidic domain (residues 1 to 125) which through fusion to GAL4 DNA-binding sequences has been demonstrated to act as a transcription activation domain (54, 106, 148). The acidic activation domains of herpes simplex virus type 1α transinducing factor and VP16 (54, 148) can only partially substitute for that of CIITA (~20 to 25% of wild type) using chimeric CIITA. Deletion of the acidic domain of CIITA results in a dominant-negative form of the protein (9, 14, 145) and has been touted as a possible vehicle for suppressing class II gene expression in transgenic animals. Much interest in proteins associating with CIITA has focused on this domain. A large number of proteins shown to interact with CIITA interact with residues in or near this domain. TFIIB, TAFIIs (30, 32, and 70), and CREB-binding protein (CBP) have all been demonstrated to interact with the acidic domain of CIITA in vitro, in vivo, and in some cases both (35, 36, 54, 68). In cotransfection experiments CBP cooperated with CIITA to increase DRA transcription, suggesting that CBP may provide a requisite histone acetyltransferase activity (36, 54).

PST domain. CIITA contains a functionally necessary region with abundant proline, serine, and threonine (Fig. 3; residues 133 to 322) (24, 118). Domains rich in proline are common in a number of transcription factors, with either DNA-binding (16, 80) ability or activation domain (78, 123) properties independent of acidic sequences. In some instances, these proline-rich activation domains also have increased frequencies of serine, threonine, and glutamine as in the case of CTD-1, a prototypical transcription factor not utilizing an acidic activation domain (78). Limited functional data regarding the role of CIITA's PST domain exist. Deletion of the N-terminal or C-terminal half of this domain has no obvious impact on transactivation by CIITA, whereas complete deletion is highly detrimental to function and results in a dominant-negative protein (24). The nonessential carboxy-terminal half of this domain is absent in a CIITA mutant (106) and the published cDNA clone for mouse CIITA (115). Examination of the mouse genomic sequence reveals that the coding sequence for this region is present in a discrete exon of the mouse CIITA gene (J. F. Piskurich and J. P. Y. Ting, unpublished data), suggesting the possibility of splice variants. Deletion of 151 to 160 N-terminal residues from CIITA results in a potent dominant-negative form of the protein (9, 14, 145), indicating that the remaining, seemingly functional portion of the PST domain of CIITA likely fails to mediate activation events independent of the upstream acidic sequences. However, a GAL4-CIITA fusion protein containing residues 104 to 402 can bind CBP and enhance transcription from a GAL4 binding site containing a promoter (54). As some proline-rich sequences bind DNA (16), it is possible that this domain in CIITA may contact and/or bind DNA. The fact that CIITA has failed to bind DNA thus far makes this possibility seem unlikely.

Residues C-terminal to the acidic and PST domains (residues 317 to 1130) are necessary to promote transcription from a promoter comprising the W, X, and Y boxes (148). Relatively small perturbations of spacing and sequence in the DRA promoter render it unresponsive to IFN-γ (131), suggesting that CIITA is constrained by the promoter arrangement. An intact X box seems crucial to CIITA's ability to transactivate, suggesting cooperation between CIITA and X box binding proteins (83, 106). These observations suggest that a number of interactions, presumably with X box binding factors and NF-Y, occur with domains other than the acidic domain (see MODE OF ACTION). Amino acids 317 to 1130 of CIITA can be broken down further into a GTP-binding site, nuclear localization signal sequence, and a series of leucine-rich regions discussed below.

GTP-binding site. The presence of a Walker A motif (also known as a P loop or G1 motif) known to be involved in ATP and GTP binding was noted with the initial description of CIITA (118). Further examination revealed GTP-binding motifs similar to those in other GTP-binding proteins, including a magnesium binding site (G3) and a guanine coordination site (G4). CIITA can bind GTP both in vitro and in vivo (43). Deletion or substantial mutation of individual sites (G1, G3, or G4) has a significant impact on both GTP binding and transactivation (14, 24, 43). In contrast, substitution with related sequences from Ras rescued CIITA function (43). Deletions in this region also have an impact on the ability of CIITA to "open" a closed promoter (157). GTP binding regulates a variety of cellular functions by acting as a molecular switch, where GTP binding results in one conformation ("on") and GDP binding results in another ("off"). GTPase activity can be intrinsic or extrinsically provided by GTPase-activating proteins (GAPs) and can promote the off conformation. Exchange activity can be modified by guanine exchange factors (GEFs), which exchange GDP for GTP thus turning the protein on. In vitro, CIITA exhibits an apparent lack of intrinsic GTPase activity, and CIITA mutants that have intrinsic GTPase activity are functionally impaired (43). It is of great interest to determine if GAPs or GEFs regulate CIITA.

Recent experiments demonstrate that residues close to, if not within, the GTP-binding region (residues 336 to 702) can interact with themselves and with residues between 939 and

1130 (see M. W. Linhoff, J. A. Harton, B. J. Conti, D. E. Cressman, and J. P.-Y. Ting, submitted for publication; T. J. Sisk, S. Roys, and C.-H. Chang, submitted for publication). This finding suggests that cooperation within the GTP-binding domain or between the GTP-binding domain and C-terminal LRR, or both, may be important for nuclear localization or transcriptional activation.

NLSs. CIITA is present in both the cytoplasm and the nuclei of transfected cells (28a), and this localization is critical for normal CIITA function. The simian virus 40 (SV40) NLS (KKRKK) recognized by the importins (karyopherins) has become the classic model for nuclear import of protein, with a plethora of nuclear proteins possessing some functionally essential iteration of the motif. A naturally occurring 24-amino-acid deletion in the C terminus of CIITA, identified in a BLS patient, lacks a 5-amino-acid motif very similar to the SV40 NLS. We have shown that this five-amino-acid deletion as well as the original patient-derived CIITA forms are present in the cytoplasm and not the nucleus. Functional studies of this region indicate that this motif in CIITA is essential for nuclear localization of CIITA and can function as an NLS independent of other CIITA-derived sequences (28a). GTP binding by CIITA is also required for nuclear import (43). We have postulated a relationship between the availability of GTP and the ability of CIITA to translocate to the nucleus as a means of regulating CIITA's activity. Elucidating such regulation will require further study. The relationship between NLS-dependent and GTP-dependent nuclear translocation of CIITA is unknown.

Cycling of CIITA between cytoplasm and the nucleus is suggested by cytoplasmic and nuclear expression (28a). CIITA exit from the nucleus, if occurring, should be mediated by nuclear export sequences (NES). Conforming roughly to the consensus LXXXLXXLXL, putative NES abound in CIITA, but functional studies have yet to elucidate which, if any, of these are relevant.

Leucine-rich regions. Through sequence analysis of portions of CIITA it has become apparent that CIITA contains a number of leucine-rich sequences. These include leucine-charged domains (LCDs) (14), a series of leucine-rich repeats (LRR) (reviewed in reference 15) in the C terminus with similarity to those of Nod1, a nucleotide-dependent activator of caspases (46). Presumably these motifs mediate protein-protein interactions. Alanine substitution mutations in the LCD motifs of CIITA diminish class II MHC transcription (14). Some C-terminal deletions in CIITA (which happen to remove one or more LRR sequences) both abrogate transactivation function and confer a dominant-negative effect (9, 14, 22). While CIITA has fourLRR which conform precisely to the published consensus (15), CIITA also contains a number of LRR-like sequences (some of which only vary from the consensus by a single residue). Surprisingly, a point mutation changing F at 961 to S (within an LRR-like sequence adjacent to the identified NLS mentioned above) is responsible for an unusual BLS case. This particular patient was not diagnosed until his late twenties and died early in his thirties (102). In recent experiments examining CIITA self-association, specific point mutations within the LRR of CIITA diminished both transactivation and the ability of this region to self-associate with residues 336 to 702 (Linhoff et al., submitted); it remains unclear if these associations are direct or indirect. Mutations at various points in the LRR of CIITA affect the ability of CIITA to activate transcription, largely due to a defect in nuclear translocation (S. B. Hake, K. Masternak, C. Kammerbauer, C. Janzen, W. Reith, and V. Steimle, submitted for publication; J. Harton and J. P.-Y. Ting, unpublished data). Together these observations support an important role for CIITA's LRR.

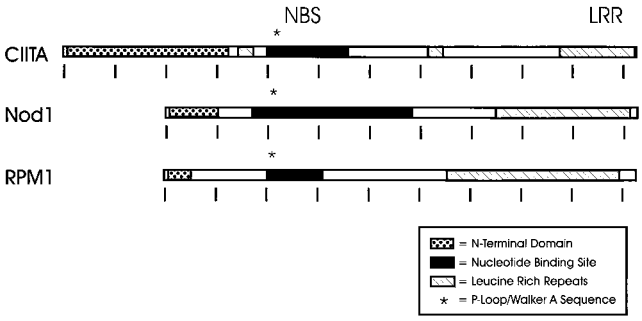


FIG. 4. The domain organization of CIITA is similar to those of other NBS- and LRR-containing proteins. CIITA is compared to two NBS- and LRR-containing proteins, mammalian Nod1 and the plant disease resistance protein RPM1 (see text). The N-terminal domains of these proteins are dissimilar. Tick marks, intervals of approximately 100 amino acids.

The LRR of CIITA have homology to a number of LRR-containing proteins. An example is the RNase inhibitor family, which includes human and pig RNase inhibitor and *Schizosaccharomyces pombe* rna1p (44, 52, 98). Surprisingly, Nod1 (see above) is similar to CIITA and contains a nucleotide binding site (NBS) motif upstream of its LRR (46). More intriguing still is the apparent conservation of NBS-LRR proteins, which function as disease resistance products in numerous species of plants (reviewed in reference 33). Remarkably, these proteins have NBS motifs and LRR with spacing and sizes similar to those of CIITA (Fig. 4), suggesting a divergent family of genes with a similar domain structure. It is tempting to speculate that these motifs are crucial for proteins which protect against infectious agents in both mammals and plants.

MODE OF ACTION

The mode of action of CIITA has been an enigma since its discovery, due to the lack of consensus DNA-binding motifs and its inability to bind W, X, and Y elements. However, CIITA requires intact W, X, and Y elements and requires their stereospecific alignment. Thus changes in X and Y by half a helical turn destroy the ability of CIITA to upregulate these genes, while insertion of a whole helical turn does not (150). This parallels early data demonstrating that IFN-γ induction of class II MHC promoters also requires an aligned promoter (130, 131). These data suggest that proteins binding to W, X, and Y may interact with CIITA in a highly specific three-dimensional structure allowing proper binding and intermolecular interactions. Indeed two lines of evidence now indicate that this is occurring. The first is indirect, showing that exogenously expressed CIITA can result in the in vivo protein binding of W, X, and Y of class II MHC, Ii, and DM promoters, as shown by in vivo footprinting (105, 132, 141). Second and more directly, in vitro and in vivo analyses have shown that CIITA interacts with RFX5, RFXANK, CREB, NF-YB, and NF-YC (75, 111, 150; Hake et al., submitted). Furthermore, recruitment of CIITA into the transcription complex requires the multiple, synergistic interactions provided by these transcription factors (76). Interactions between these factors and CIITA have been observed within the following residues of CIITA: RFX5, 335 to 612; RFXANK, 1 to 335; CREB, 1 to 612; NF-YB, 518 to 612; NF-YC, 218 to 335 (150). The recent observation that deleterious mutations in the LRR fail to disrupt RFX5, RFXANK, NF-YB, and NF-YC (Hake et al., sub-

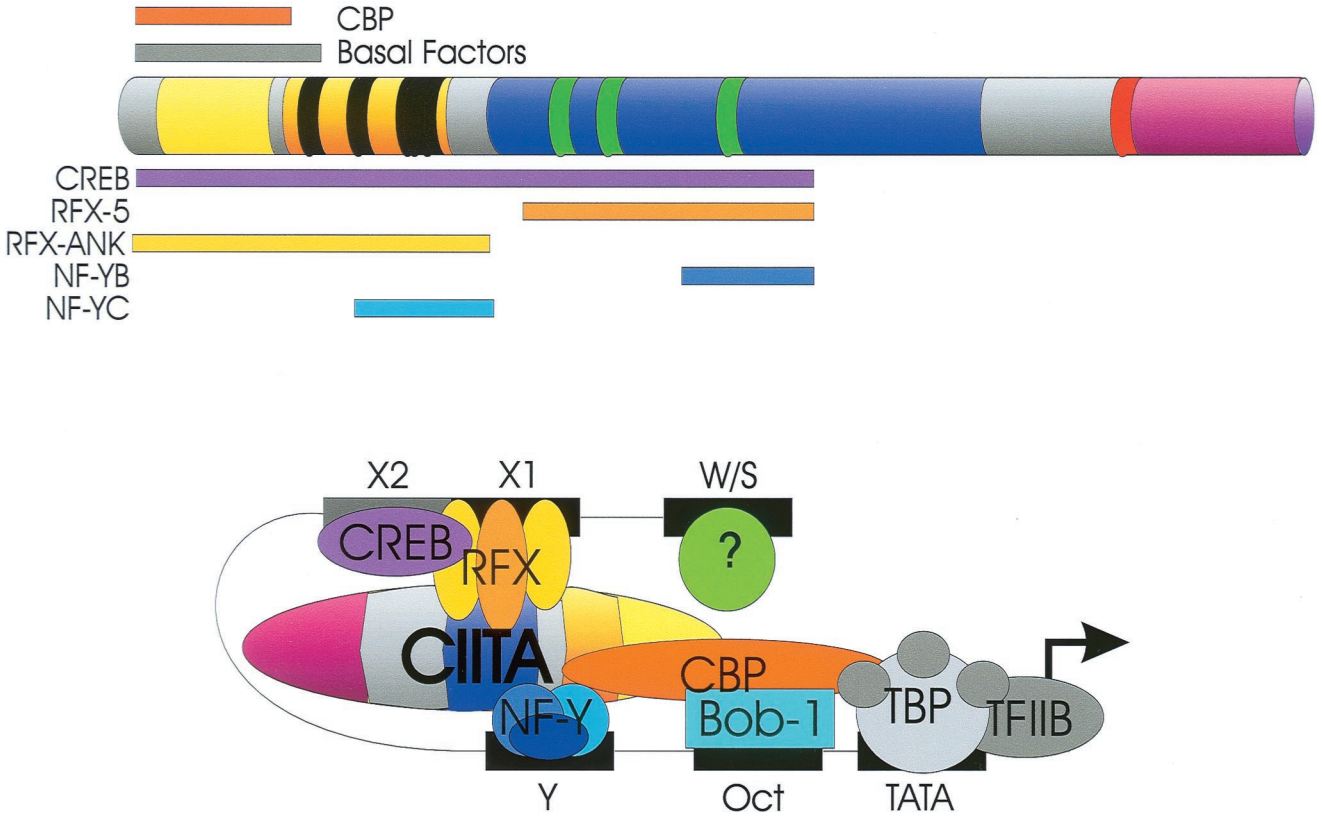


FIG. 5. Multiple transcription factor contacts allow CIITA to function as a transcriptional scaffold and integrator. CIITA contacts components of the basal transcription machinery via its acidic domain. Interactions with RFX, RFXANK, CREB (X2BP), NF-YB, and NF-YC have been mapped. The region of CIITA required for each interaction is shown (see text). Domain markings for CIITA are the same as in Fig. 3. TBP, TATA-binding protein.

mitted) is consistent with the mapping result above. Interactions between CIITA, RFX, CREB, and NF-Y are likely to be important as suggested by the observation that a C-terminal domain of RFX is required for cooperative binding between RFX and NF-Y (138). CIITA interactions with the B-cell-specific protein Bob-1 (OcaB) (34) have been described, but the domain involved is unknown. Thus many of these proteins could be contacting independent domains of CIITA (acidic, PST, or GTP binding), the exact sites of which will hopefully be informative. Considering the interactions between CIITA and components of the basal transcription machinery (see Structure and Function, "Acidic domain"), these observations have led to a firm view of CIITA as a scaffold which acts as an integrator or enhanceosome for class II transcription (37, 76, 127, 150; S. J. P. Gobin, M. van Zutphen, S. D. Westerheide, J. M. Boss, and P. van den Elsen, submitted for publication). Thus a composite picture would show all these proteins binding to CIITA and localized to the promoter (Fig. 5). A question of whether this binding is direct or indirect still has not been completely resolved and awaits the isolation of these proteins in pure form.

BIOLOGICAL SIGNIFICANCE

The implications of a master regulator controlling expression of class II MHC genes are immense. Aside from the obvious importance of CIITA to BLS, the regulation of CIITA largely determines the presence or absence of class II MHC and its degree of expression and thus the nature of the immune response. Consequently, CIITA is exceedingly important whenever class II MHC is required and will impact infectious diseases, neurologic disorders, autoimmune diseases, tumor rejection, graft acceptance, and perhaps even normal development. Furthermore, as CIITA is a single protein that affects a large family of genes, it represents an ideal target for pharmacological intervention in the class II antigen presentation pathway, whether for immune suppression in transplantation and autoimmunity or for enhancement for BLS and tumor rejection.

An emerging pathway by which pathogens escape the immune system involves modulating CIITA expression. Cytomegalovirus, for example, downregulates expression of CIITA by altering the IFN-γ signal pathway, thus diminishing the immune stimulatory function of class II MHC (60, 79). During *Mycobacterium bovis* BCG infection of murine macrophages, a mycobacterial N gene product diminishes IFN-γ-mediated phosphorylation of STAT-1α, thus reducing CIITA and class II MHC expression (140). Chlamydia similarly interferes with CIITA transcription through the degradation of upstream stimulatory factor 1 (147).

In contrast to examples where CIITA is downregulated during infection, the human immunodeficiency virus (HIV) utilizes CIITA to upregulate HIV long terminal repeat (LTR) function (108). There is a correlation between expression of class II MHC and higher HIV expression and replication. The introduction of CIITA into T cells leads to increased viral replication and transcription from the HIV LTR. CIITA is expressed by activated human T cells and macrophages, and both of these cell types are the primary targets of HIV, both having developed different receptors for HIV. This correlation

is intriguing and suggests that CIITA, not unlike other known positive regulators of HIV LTR (i.e., Tat and NF-κB), regulates the transcription and replication of HIV. In contrast, mutations in the cysteine-rich portion of HIV Tat (C22S37G and C37G) decrease class II MHC transcription, leading to diminished class II MHC expression in the THP-1 (monocytic) and H-9 (T-lymphocyte) cell lines, an effect not seen with wild-type Tat (128). Interestingly these mutations can decrease class II MHC transcription without affecting CIITA expression (128), likely through disruptive interaction with transcription elongation factor b (49). This suggests a clever mechanism to evade immune detection (via loss of class II MHC) while potentially maintaining CIITA-dependent HIV LTR transcription. A seeming dilemma exists in the literature. Tosi et al. show that mutant HIV Tat, but not the wild type, interferes with class II MHC transcription and expression, whereas Kanazawa et al. present the opposite with a different mutation in the cysteine-rich region of HIV Tat (49, 128). This difference may be due simply to differential effects of HIV Tat and its mutants on expression of class II MHC in THP-1 and H-9 cells versus COS cells.

As discussed above, a lack or decrease in class II MHC can prove advantageous to invading pathogens. Conversely, induced class II MHC expression has an implicated role in inflammatory processes (reviewed in references 2, 27, 66, and 138) and the role of class II MHC in autoimmune disease has been extensively studied (reviewed in references 77, 90, and 124). The role of CIITA in these diseases is currently being studied. In nonobese diabetic mice it has been shown that the lack of CIITA prevented diabetes despite noticeable pancreatic infiltration (82), whereas class II MHC deficiency alone was insufficient to prevent cytotoxic T-cell-mediated disease pathology in a model of lymphocytic choriomeningitis virus-induced diabetes (55). In autoimmune thyroiditis, potential interactions between CIITA and single-strand binding protein 1, a regulator of thyroid-stimulating hormone gene expression, may contribute to increased class II MHC expression (5, 81), thus allowing thyroid cell antigen presentation to T cells (143).

Correction of the BLS defect is an obvious use of CIITA in gene therapy. Retroviral transfer of CIITA was recently employed to correct class II deficiency in cells from a BLS patient (11). Another significant application is the potential use of dominant-negative forms of CIITA in the production of class II MHC-deficient organs for transplantation (9, 22, 145). We have found that the lack of CIITA in heart donor grafts also greatly enhances graft survival in totally allogeneic hosts (W. J. Brickey, N. J. Felix, and J. P.-Y. Ting, unpublished data). This level of enhancement is beyond that produced by $A_{\beta}^{-/-}$ grafts, perhaps owing to repression of all class II MHC expression including Ii and DM in CIITA knockout mice.

Tumors often lack class II MHC and thus have diminished immunogenicity. The expression of class II MHC and/or costimulatory molecules has shown promise in increasing tumor immunogenicity and tumor rejection (6, 94, 95). Loss of the retinoblastoma protein (Rb), a tumor suppressor, has been linked with loss of class II inducibility in retinoblastoma, non-small-cell lung carcinoma and bladder carcinoma (63, 65, 93). Rb is thought to in some way allow or enhance the accessibility of class II MHC promoters (63). Whether CIITA requires Rb to function is unclear and may depend on the relative expression of the individual proteins or other cell-type-specific factors (64, 129). In tumors with intact Rb, a failure to upregulate CIITA in response to IFN-γ is the most common explanation for absent class II MHC (64). CIITA is absent in small-cell lung cancer, and transfection of CIITA can restore class II expression (144). Restoring class II expression to tumor lines by introduction of either the IFN-γ gene (97) or the gene for CIITA (73) can improve the immunogenicity of the tumor. However, overexpression of CIITA leading to high levels of class II MHC correlated with increased tumorigenicity (73), suggesting that overexpression of CIITA may be detrimental in tumor therapy approaches. Other experiments suggest that class II-transfected tumors are more immunogenic than CIITA transfectants due to an ability of class II-transfected tumors to present endogenous antigen whereas CIITA expression also induces Ii, which favors presentation of exogenous antigens (3, 25).

As mentioned above, class I MHC and β_2m can also be transcriptionally regulated by CIITA (40, 72, 104). Both class I and β_2m promoters contain W, X, and Y motifs (40, 107), consistent with the requirement of these elements for transactivation by CIITA. In cell lines, CIITA has been observed to increase and in some cases induce class I expression following transfection of CIITA (78). However, class I MHC expression in CIITA-deficient mice is apparently normal. An answer to this conspicuous paradox may lie in the genetics of development. Trophoblasts lack expression of the classical class I and class II MHC (reviewed in references 56 and 134, expressing instead the nonclassical class I molecule HLA-G (53). CIITA fails to transactivate the HLA-G promoter in extravillous cytotrophoblast cells but does transactivate HLA-A and B promoters (39). Expression of CIITA can induce class I promoter activity in trophoblast-derived choriocarcinoma cells (58). Thus CIITA appears to be under tight control early in development. As CIITA-deficient mice develop normally, suggesting that the lack of CIITA does not interfere with development, one is left to propose that the effects of CIITA on class I transcription might have subtle roles during normal development. Alternatively, it could be easily argued that class I MHC expression in vivo is not regulated by CIITA. This interpretation is teleologically unsatisfying and begs the question of the necessity of obvious W, X, and Y elements within the class I MHC. Furthermore, recent chromatin immunoprecipitation experiments reveal that CIITA is bound to class I MHC and β_2 promoters in vivo (76). It may be best to conclude that a physiologically relevant role for CIITA in regulating class I MHC expression will likely be subtle and possibly restricted to limited developmental or cell-specific events.

These early studies suggest that, in principle, modulation of CIITA, whether by suppression to generate better transplants or avoid autoimmune disease or by introduction to improve immune responses to pathogens or tumors, is a promising field of study with enormous potential.

SUMMARY

Great progress in understanding the relative importance of various portions of CIITA for transcriptional activation of class II MHC genes has been made since CIITA's discovery in 1993. Emerging from these studies is a fairly consistent picture where CIITA is expressed, binds GTP, translocates to the nucleus, and interacts with specific DNA-binding transcription factors and basal transcription components, thus opening and activating class II MHC and related promoters. Despite these strides, this model is essentially unchanged from that initially espoused. The observation that class II MHC promoters in some B cells are bound to X and Y box binding proteins and thus open even in the absence of CIITA, whereas these same promoters in non-B cells are closed until CIITA is present, is provocative. One potential explanation is that CIITA possesses two distinct functions, the ability to direct the opening of responsive promoters (presumably through some form of remod-

eling) and the ability to activate transcription through its activation domain and protein-protein interactions (132, 141). The presence of a locus control region responsive to a B-cell-specific factor is another possibility, yet CIITA must, in some fashion, be directing chromatin remodeling in cells which can be induced to express CIITA. While CBP is an obvious candidate for mediating remodeling, no conclusive experiments have shown that CBP is required for the remodeling of class II MHC promoters. The studies above support interactions between CIITA and transcription factors, but does CIITA merely bind these factors to place the activation domain appropriately? Why has it been difficult to demonstrate a role for CIITA in a transcription complex? Is GTP binding only essential for nuclear import? Is nuclear export of CIITA occurring and is it relevant? What aspect of class II MHC transcription requires that retinoblastoma protein Rb be present? Is CIITA a prototype for a family of transcriptional coactivators? Why is limited class II expression observed in the absence of CIITA? The evolutionary conservation of W-, X-, and Y-containing promoters in mammals, birds (104), amphibians (51), and fish (121) suggests that CIITA may be extremely old; what are its origins?

All remaining questions aside, CIITA is truly a remarkable protein. Controlled by up to four separate promoters, CIITA has been imparted a complex pattern of inducible and constitutive expression that can be regulated in developmental pathways. Through exercising specific control over the transcription of every major component of class II MHC antigen presentation pathway, CIITA gains the title of a master regulator. As CIITA appears to be class II MHC specific, it can be thought of as the core transcription factor of which all the remaining components are but cofactors. This is central to the concept of CIITA as a scaffolding protein or integrator and perhaps alters our view of transcriptional control away from promoters and individual factors towards a more unified enhanceosome perspective.

The view of CIITA as a master regulator has implications for practical applications that are staggering. Successful engineering of dominant-negative CIITAs may lead to the production of transplant tissues unable to express class II MHC and the associated self peptides which contribute so significantly to graft rejection. A thorough understanding of CIITA's molecular mechanisms may lead to therapeutics which allow temporary enhancement or suppression of class II MHC, thus favorably altering the immune response during critical events in pathogenesis, autoimmune disease, tumorigenesis, and neuroinflammation.

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