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Genetic Control of MHC Class II Expression

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The presentation of peptides to T cells by MHC class II molecules is of critical importance in specific recognition by the immune system. Expression of class II molecules is exquisitely controlled at the transcriptional level. A large set of proteins interact with the promoters of class II genes. The most important of these is CIITA, a master controller that orchestrates expression but does not bind directly to the promoter. The transcriptosome complex formed at class II promoters is a model for induction of gene expression.

One of the keys to the development of a specific immune response to a pathogen is held by MHC class II molecules (Cresswell, 1994; Nelson and Fremont, 1999). Unlike class I membrane glycoproteins, which are widely expressed, class II molecules are generally restricted to a subset of antigen presenting cells, such as macrophages, dendritic cells, and B cells. Their expression can be induced on other cells types after stimulation with cytokines such as interferon γ. MHC class II molecules are responsible for presenting peptides derived from extracellular pathogens to T cells bearing the CD4 marker. There are three classical class II molecules in man: HLA-DP, -DQ, and -DR. Mice only express proteins orthologous to the last two, A and E, respectively. In addition to these structures, both species encode so-called nonclassical molecules, namely HLA-DM and -DO in man, and M and O in mouse. These molecules do not normally reside at the cell surface, and they do not present antigens; instead, they modulate binding of peptides to the classical structures. Each class II molecule is a heterodimer of an α chain and a β chain. The transcriptional control of this family of genes has been extensively studied. Numerous DNA binding transcription factors as well as a master coactivator (CIITA, class II transactivator) have been identified. A clear picture of the roles of these factors in the induction of chromatin changes and in the formation of an active transcriptosome has emerged, rendering this a model system to study these issues. In addition, mounting evidence shows that the regulation of class II MHC genes is highly relevant to some important diseases. This review provides a brief background to the genetics, structure, and function of class II, and then focuses on regulation of expression of MHC class II genes.

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Class II Region and Genes

The α and β chains of each class II molecule are encoded by separate genes in the class II region of the MHC (Figure 1A) (Allcock et al., 2000; Beck and Trowsdale, 1999; Gunther and Walter, 2001). In all cases, except for *HLA-DO,* the pairs of genes are encoded adjacently. Some of the genes are duplicated, one copy of each being functional in the case of *DP* and *DQ. DRB* is a special case, as there can be more than one functional copy per haplotype, in addition to nonfunctional pseudogenes. Each mouse haplotype also contains two H-2M β chain genes, *Mb1* and *Mb2*, both of which are functional. Class II sequences obviously arose by repeated duplications. These must have taken place at several different periods throughout evolution of the class II gene family. The DM sequence is only weakly related to other class II sequences and probably resulted from an ancient gene duplication. In contrast, DO sequences are ~60% identical to DR. *DRB* loci are highly similar and must represent recent duplications.

The class II region of the human and rodent MHCs also harbors a small group of genes involved in antigen processing, which encode the TAP transporters as well as interferon-induced proteasome components. Some class I genes are tightly linked to the class II region, at the centromeric end of the MHC, in rodents, but not in humans. This end of the extended class II region also contains the gene for TAPASIN, which is involved in antigen processing for loading class I molecules.

A feature of the MHC is the high degree of linkage disequilibrium across the complex, and the region is divided into extended units, or haplotypes (Dawkins et al., 1999). It is not established whether this is maintained by selection, polarized recombination, or founder effects, but genetic recombination in the MHC class II region is highly focused into hotspots (Cullen et al., 1997; Jeffreys et al., 2001). In terms of class II, explanations could be invoked for maintaining certain combinations of alleles of different genes in *cis* relationship. Both chains of HLA-DQ and H-2A are polymorphic, and particular DQ and H-2A α and β chains do not pair efficiently. In most populations studied to date, one rarely finds α and β alleles encoding these unstable heterodimers on the same haplotype. For example, DQw1-associated β chain is not found together with a DQw-2, -3, or -4 associated α chain and vice versa. The haplotypes containing these unstable heterodimers are generally thought of as "forbidden." Some exceptions to this general rule may be identified, especially in small isolated populations (Grahovac et al., 1998).

Class II Proteins

Each class II heterodimer consists of two integral membrane proteins of around 26 kDa (Figure 1B). The differences in size of the α (32 kDa) and β (29 kDa) chains are mainly attributed to differences in N-linked glycosyl-

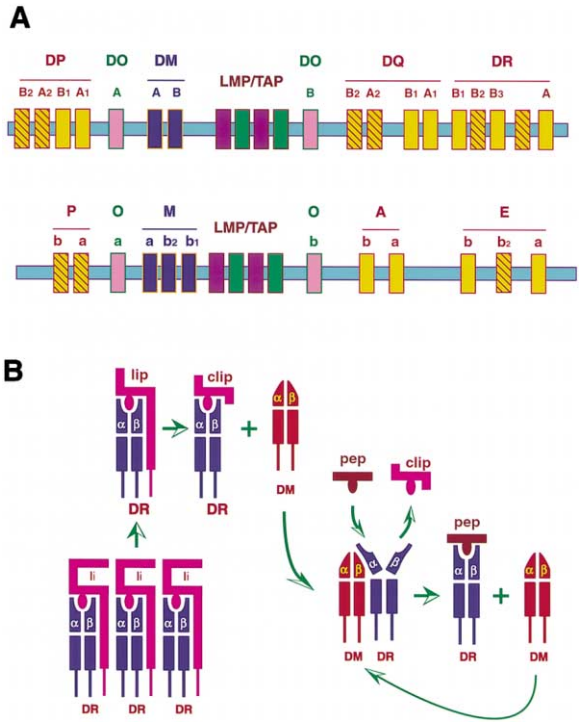

Figure 1. Genetics and Loading of MHC Class II

(A) Schematic maps of the MHC class II regions in man and mouse. The main genes are shown, including classical class II molecules (yellow). Pseudogenes are hatched. Nonclassical class II genes are pink (*DO*) and dark blue (*DM*). Antigen-processing genes for loading peptides onto class I molecules are in purple and green.

(B) Simplified mechanism for DM-mediated peptide exchange on DR molecules. Class II dimers assemble with Ii in the ER to form a nonameric complex of an Ii trimer and three class II dimers. The complexes are transported to specialized lysosome-like compartments for loading of antigenic peptides. In these vesicles, the Ii chain is hydrolyzed to leave class II bound to the Ii derivative, CLIP. DO (not shown) is also associated with DM in the ER and it travels with DM. Peptide exchange is catalyzed by DM, by stabilizing the transition state.

ation. The α and β chains of all classical class II molecules have the same overall conformation, each consisting of two extracellular domains, α1 and α2, and β1 and β2, respectively. The membrane-distal domains combine to form a single peptide binding site composed of two antiparallel α-helical loops supported by a platform of eight antiparallel β strands. These domains feature the high level of polymorphism exhibited by MHC molecules. A conserved disulfide bond connects the α-helical region of the β1 domain to a strand in the platform floor (β10cys to β78cys). The DM molecule contains two additional disulfide bonds (α 24-79 and β 25-35).

The groove of class II, like that of class I molecules, is capable of binding a wide range of peptides. Peptides bind to class II in an extended conformation. In contrast to class I, the N and C termini of class II-bound peptides may extend beyond the ends of the groove. The peptide is held by a series of hydrogen bonds between the peptide backbone and conserved amino acid side chains lining the groove. Since the bonds do not involve peptide side chains, they confer sequence-independent binding. This may explain how class II molecules can bind multiple peptides with high affinity and low specificity. The three-dimensional structure of the nonclassical class II molecule, HLA-DM, reveals its unique function (Alfonso and Karlsson, 2000; Mosyak et al., 1998). The peptide binding site is altered to an almost fully closed groove, and the α helixes of the α1 and β1 domains contact each other over the first and last thirds of their length. A cavity remains at the center of the membrane-distal portion of the molecule, forming a deep, polar pocket, 10 Å wide and 10 Å deep. This pocket is conserved in orthologous molecules from other species, such as H-2M. It could bind the end of a peptide, but is not as large as the lipid binding CD1 pocket. The molecule also has a tryptophan-rich lateral surface that may bind the other nonclassical class II molecule, HLA-DO. MHC class II molecules pack as pairs of heterodimers in some crystals, and the possibility of "dimers of dimers" forming at the cell surface has fuelled speculation about the stoichiometry of interaction with T cell receptors. Evidence for dimer pairs is controversial (Schafer et al., 1995).

Polymorphism

Classical class II sequences exhibit an extraordinary degree of variation that is concentrated on the amino acid residues that shape the peptide binding site. The involvement of selection in the maintenance of the polymorphism is suggested by the finding of a high level of nonsynonymous codon changes. This is in contrast to most other genes, as well as the membrane-proximal domains (α2 and β2), where synonymous variation normally predominates. Except for *DQA* and *Aa,* the sequences encoding the α chains are generally less variable. There are few alleles of *Ea* and *DRA*, with conservative amino acid changes. Generation of the polymorphism could be due to point mutation, but the mutation rate is not especially inflated compared to more conventional genes. It is likely that "allele conversion" (double crossover to replace a short section of an allele) has taken place repeatedly, because the alleles have the semblance of being "patchworks" of each other. Some alleles could have arisen by recombination, using single crossovers. Gene conversion has also been proposed as a possible mechanism for incorporating sequences from other linked class II genes. There is evidence for this mechanism in conversion of class I sequences in mice.

The nonclassical class II molecules are relatively invariant. Some alleles of both *HLA-DM* and *-DO* have been described, but these vary by small numbers of amino acids and, so far, have no known functional significance.

Peptide Loading, Ii, and the Role of DM

Soon after synthesis, classical class II molecules associate in the endoplasmic reticulum (ER) with a third protein, a type II (i.e., of inverted orientation) membrane glycoprotein called invariant chain (Ii) (Cresswell, 1996) (Figure 1B). The combined proteins form a nonameric structure, consisting of three Ii chains, arranged as a core, surrounded by three classical class II heterodi-

mers. The grooves of the class II molecules are occupied, in the nonamer, by a section of the Ii chain called CLIP. This may help to avoid loading of the groove with ER-resident proteins, as is the case for class I. The main function of invariant chain seems to be as a chaperone to ensure correct folding and egress of class II. It contains a di-leucine targeting signal in its cytoplasmic tail which helps to divert the nonamer from the default secretory pathway to lysosomal-like vesicles, called MIIC (for MHC class II compartment), where peptide is eventually loaded. Before this can take place, however, Ii is degraded by lysosomal proteases such as cathepsin L and S. It is progressively cleaved, leaving just the CLIP fragment itself occupying the groove.

The MIICs are depots where the class II molecules, groomed in this way, meet up with antigenic peptides that come from degradation of exogenous proteins. These may be internalized either by endocytosis or by interaction with surface receptors on antigen presenting cells (APCs). For example, proteins bound to surface antibodies are internalized on B cells. Other cells may take up antibody:antigen complexes using the range of Fc receptors. Lectin-like receptors, such as mannose receptors, may be invoked to deal with glycoproteins. Topologically, peptide loading in MIIC vesicles is "outside" in that it is separated by membrane from the cytoplasm.

Efficient exchange of CLIP for antigenic peptides is mediated by DM (Sanderson and Trowsdale, 1995). The structure of the DM molecule reveals that it is highly unlikely to bind peptides and the groove of DM is effectively sealed (Mosyak et al., 1998). Moreover, at steady state, most of the DM molecules reside in the MIIC vesicles. DM binds transiently to class II:CLIP and stabilizes an intermediate state where CLIP is released, allowing other peptides to bind. A speculative model proposes that DM contacts DR "shoulder to shoulder": a conserved tryptophan residue (α62 Trp) in DM interacts with α51 Phe of DR, at the extended strand where the class II groove differs from that of class I, near pocket 1 (Nelson and Fremont, 1999; Doebele et al., 2000). This interaction could result in destabilization of several peptide:MHC bonds, lowering the free energy barrier to peptide dissociation. DM stabilizes the open transition conformation of DR, favoring faster peptide association, in the MIIC environment that is rich in imported, antigenic peptides. The class II molecule may be quite flexible around the first, hydrophobic pocket in the absence of bound antigenic peptide. A more rigid conformation is probably generated after filling of pocket 1, which would render the molecule less susceptible to the effects of DM (Chou and Sadegh-Nasseri, 2000). The side chains in CLIP could be structured in such a way as to permit binding to all classical class II molecules but release from the groove under appropriate conditions, such as in the presence of DM and in the low pH of the MIIC vesicle. CLIP can be regarded therefore as a disposable stuffer. Once antigenic peptide is stably bound, DM may lose its association for class II. Alternatively, DM may be released at the cell surface, to be retargeted to MIICs. The cytoplasmic tail of DMβ contains a tyrosine-based targeting signal (Copier et al., 1996).

The complex of classical and nonclassical class II molecules in the MIICs associates with tetraspan molecules CD63 and CD82, which may also play a role in the later stages of class II maturation (Hammond et al., 1998).

The Role of DO

The second nonclassical class II molecule HLA-DO arose at a later stage of evolution to HLA-DM (Haas et al., 1987). DO is also monomorphic and, like DM, it resides in MIICs. It appears to require association with DM to access these vesicles. In some hands, DO appears to counteract the effect of DM, in a pH-dependent manner. Its effects may be optimal at pH 6, blocking peptide exchange in early endosomes. DO does not work as well at pH 5, the condition which favors DM-mediated peptide exchange in MIIC vesicles. A simple model to account for the action of DO would be for both DR and DO to compete for the same site on DM.

There is no consensus on the precise function of DO, and in some experiments it seems to enhance peptide exchange (Kropshofer et al., 1999). A clue to the function of DO may be provided by its expression, which is restricted mainly to B cells. Indeed, control of transcription of *DOB* may be less dependent on the CIITA transcription factor and induction by IFN-γ (see below). In B cells, DO may help to refine peptide loading to a restricted subset of class II molecules.

Expression of Class II

Class II molecules are constitutively expressed on cells that serve as APCs for CD4+ T cells, such as macrophages, monocytes, dendritic cells, and B cells; they may be induced on other cells by IFN-γ. Class II expression is also modulated by other agents, such as IL-4, IL-10, IFN-α/β, TNFα, and glucocorticoids. Concomitant expression of all three classical molecules is usually observed, although exceptions exist. Some B cells express solely DQ and others only DR. This raises the important question of whether the different class II isotypes exert distinct T cell functions, or whether they merely enlarge the peptide binding repertoire. Distinct functions have been suggested for HLA-DQ and -DR, the former being more likely to "suppress" some responses (Hirayama et al., 1987). These observations may enjoy renewed interest now that suppression of T cell responses by T cells has finally gained credibility. This issue is of crucial importance in view of the association of class II loci with a vast array of autoimmune conditions—an association which is still not fully explained.

Clearly, precise regulation of class II expression is critical. To address this, much effort has been devoted to the analysis of MHC class II promoters and the transcription factors that are involved in their regulation. The following sections are devoted to these aspects of MHC class II.

Promoter Motifs

One of the outstanding features of MHC class II loci is that not only the structural genes, but also the promoter elements, are remarkably conserved. All the classical and nonclassical class II promoters, including that for Ii, contain three elements—S (also W or Z), X, and Y—which are necessary for optimal constitutive and cytokine-induced gene expression. These sequences have

Table 1. Representative MHC Class II Defective Cell Lines

Complementation group	Patient-derived cell lines	In vitro mutants	MHCII Ag	MHCII promoter activity/mRNA	Genetic defect	RFX binding	Promoter occupancy
A	BLS-2, BCH1,2	RJ2.2.5	—	—	*MHC2TA*	+	+
B	BLS-1, Ra	—	—	—	*RFXANK*	—	—
C	SJO	G1B (IFN-γ)	—	—	*RFX5*	—	—
D	DA, ABI	6.1.6	—	—	*RFXAP*	—	—
Atypical lines							
		G3A (IFN-γ)	—	—	unknown	+	—
	KEN/KER		− for DRB, DQB, DPA	—	unknown	+	− for *DRB, DQB, DPA*

been extensively reviewed elsewhere (Benoist and Mathis, 1990; Glimcher and Kara, 1992), and will not be discussed here in detail. In addition to sequence conservation, the stereospecific alignment (i.e., DNA helical orientation and spacing) of the three elements is also critical (Harton and Ting, 2000). These data strongly implicate a model in which proteins binding to the S/W, X, and Y elements must bind in a spatially-restricted fashion to allow direct interactions among them, and/or interaction with a coactivator to form the active transcriptosome complex (see below).

Bare Lymphocyte Syndrome (BLS)

A discussion of the field of MHC class II regulation would not be complete without considering the heterogeneous group of genetic disorders, collectively called BLS or MHC class II deficiency (MIM209920). Several excellent reviews have appeared elsewhere (DeSandro et al., 1999; Reith and Mach, 2001), and only a brief discussion directly relevant to this review follows. BLS is a rare immunodeficiency inherited as an autosomal recessive disease; it arises due to a high degree of consanguinity in patients' families. Patients typically suffer from frequent, severe bacterial, fungal, or viral infections. Cells from the typical BLS patient lack constitutive and inducible expression of all MHC class II genes, including the α and β chains of DR, DP, and DQ. These patients exhibit severely hampered T cell activation and greatly reduced $CD4^+$ cells, although a recent report has described a family with a L469P mutation in CIITA that presents as an attenuated clinical course accompanied by residual MHC class II expression (Wiszniewski et al., 2001). In all cases that were tested, the MHC class II genes were not structurally defective, since fusions between defective cells and a normal cell invariably lead to surface expression of class II from the genotypes of both cells. EBV-transformed B cell lines obtained from these patients have proven invaluable in deciphering the regulatory pathway of MHC class II genes (Table 1; partly adapted from Reith and Mach, 2001, with permission, from the Annual Review of Immunology, Volume 19. ©2001 by Annual Reviews, www.annualreviews.org). In addition to B cell lines obtained from BLS patients, several mutant cell lines have been generated in vitro, primarily based on the lack either of constitutive MHC class II expression or of IFN-γ-induced MHC class II expression. Somatic cell fusions of BLS-derived cell lines and/or in vitro generated class II-negative cell lines have led to the delineation of four complementation groups. Additionally, two atypical cases represented by twins (KEN/KER) in whose B cells *DRB, DQB,* and *DPA* are not expressed have been reported (Douhan et al., 1996; Hauber et al., 1995). The in vitro generated MHC class $II^{-/-}$ cell line, G3A, also represents an atypical case. In this cell line, although X and Y binding proteins appear to be normal, CIITA induction by IFN-γ is not optimal, and the introduction of exogenous *MHC2TA* restores class II expression (Chin et al., 1994). Except for these atypical cases, where the genetic defect is undefined, it is now clear that each complementation group has a specific defect in a transcription factor that is necessary for MHC class II expression (see below) (Reith and Mach, 2001).

Transcription Factors and Coactivator

The initial characterizations of proteins that bind directly to MHC class II promoters identified both constitutively and ubiquitously expressed factors (Figure 2A). The Y element, a canonical CCAAT box, is bound by NF-Y/CBF, a molecule that is conserved from yeast to human (Maity and de Crombrugghe, 1998; Mantovani, 1999). NF-Y binds to DNA as a heterotrimer consisting of A, B, and C subunits. The B and C subunits contain histone-fold motifs that are similar to eukaryotic histones H2A and H2B and an archaebacterial histone-like protein. The RFX factor, also a trimer, binds to the X1 element (Durand et al., 1997; Masternak et al., 1998; Nagarajan et al., 1999; Steimle et al., 1995). It consists of RFXANK/RFXB, RFX5, and RFXAP, and defects in each define the BLS complementation groups B, C, and D, respectively (Table 1). RFX5 belongs to the RFX family of DNA binding proteins, and it was identified by complementation cloning using the MHC class II defective cell line, SJO (Steimle et al., 1995). RFX5 has a DNA binding domain and a C-terminal domain that interacts with NF-Y (Reith and Mach, 2001). The other two components of the complex that bind X1 were identified by biochemical purification. RFXANK/RFXB has ankyrin repeats typically thought of as mediating protein-protein interactions. These repeats provide an interaction platform to assemble the RFX complex by interacting with RFXAP, RFX5, and CIITA. A single nucleotide mutation in the ankyrin repeats results in abrogation of the RFXANK-RFXAP interaction in a BLS cell line, affirming the importance of these repeats (Nekrep et al., 2001). RFXAP, or RFX-associated protein, contains acidic, basic, and glutamine-rich sequences (Masternak et al., 1998). A recent report has shown that only the C terminus of RFXAP is essential for function, and different segments within this region are required for allele-specific class II expression

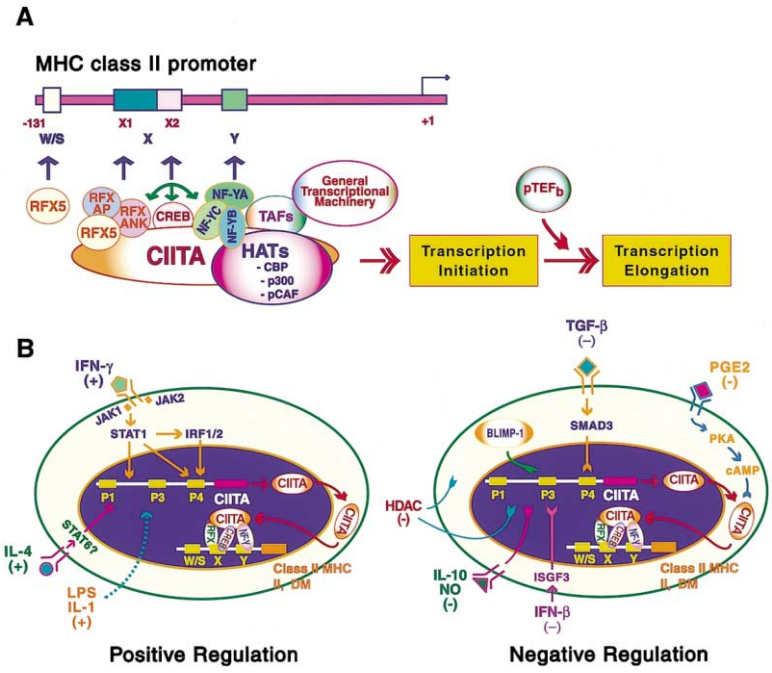

Figure 2. Molecular Regulation of MHC Class II

(A) A prototype MHC class II promoter and transcriptosome. The MHC class II promoter, its cognate DNA binding factors (NF-Y, CREB, and RFX), and the coactivator, CIITA, are shown. Positioning of the RFX subunits is drawn according to Westerheide and Boss (1999). Interactions among the DNA binding factors, CIITA, general transcription factors (TAFs), and HATs are indicated. The transcriptional elongation factor, pTEFb, is also shown.

(B) Negative and positive regulation of MHC class II and CIITA. Positive or negative regulatory processes typically target CIITA transcription or protein, which then targets MHC class II expression. (Left) Positive regulators include IFN-γ, IL-4, LPS, and IL-1. The pathway for IFN-γ is best delineated, involving the induction of P3 through STAT1, and the induction of P4 through IRF-1, IRF-2, and STAT1. (Right) Negative regulators include TGF-β, IL-10, IFN-β, and nitric oxide. Suppression of CIITA P4 expression by TGF-β requires SMAD3, while suppression of CIITA function by IFN-β requires ISGF3. It is unclear which promoters are affected by IL-10, NO, and IFN-β. PGE inhibits CIITA protein function by PKA-mediated phosphorylation. In addition, epigenetic events such as histone deacetylation and DNA methylation, and developmentally expressed molecules such as BLIMP1, negatively regulate CIITA production. HDACs also controls MHC class II expression through a CIITA-independent pathway. Dark blue ovals represent cell nuclei.

(Peretti et al., 2001). In addition to its specificity for the X1 element, the RFX complex also binds the S/W element (Jabrane-Ferrat et al., 1996). Finally, a protein which binds to the X2 box was purified and then identified as CREB (Moreno et al., 1999). CREB is bound to the MHC class II promoter as shown by the chromatin immunoprecipitation (ChIP) assay. It also interacts with CIITA and RFX, forming a final anchor in the large transcriptosome complex.

The DNA binding proteins mentioned above are all constitutively expressed, which cannot explain the cell-specific, cytokine-induced, and developmentally regulated expression of MHC class II genes. The isolation of the MHC class II transactivator gene (*MHC2TA*) solved much of this problem, and remains one of the seminal discoveries in the field (Steimle et al., 1993). *MHC2TA* was identified by complementation cloning of the RJ2.2.5 cell line (see Table 1) using an EBV-based episomal cDNA library. Complementation of RJ2.2.5 with a vector containing *MHC2TA,* encoding CIITA, resulted in the expression of surface class II antigens. Because CIITA does not bind DNA, it is an authentic transcriptional coactivator, defined as a transcription factor that mediates its function through interaction with other proteins. Long thought to be unique, CIITA is now recognized as a founding member of the NACHT protein family, which share several domains, including NTPase and Walker A and B motifs, and have roles in inflammatory responses and apoptosis (Koonin and Aravind, 2000).

Expression and Regulation of CIITA

Unlike RFX and NF-Y, CIITA exhibits cell-specific, cytokine-inducible, and differentiation-specific expression that precisely parallels that of MHC class II synthesis. Likewise, class II^+ cells, such as B cells, monocytes, dendritic cells, and human activated T cells, express CIITA (Harton and Ting, 2000; Reith and Mach, 2001). Additionally, an in vivo study has shown that the expression of CIITA under inflammatory transplantation conditions parallels the expression of MHC class II (Sims and Halloran, 1999). CIITA transcription is upregulated by IFN-γ, LPS, and IL-4, and is downregulated by IFN-β, IL-10, nitric oxide, and TGFβ (Figure 2B) (Harton and Ting, 2000; Reith and Mach, 2001). The induction by IFN-γ and downregulation by TGFβ are best worked out; the former is mediated by IRF-1, IRF-2, USF-1, and STAT1, and the latter by Smad3 (Dong et al., 2001; Xi et al., 1999). The regulation of CIITA expression occurs primarily at the transcriptional level; an exception is the suppression of CIITA activity by prostaglandins in myeloid-monocytic cells when PGE-induced, cAMP-dependent PKA causes the phosphorylation of CIITA (Li et al., 2001). Developmentally, CIITA suppression also occurs when B cells differentiate into plasma cells; this is attributed partly to the BLIMP-1/PRD transcription factor expressed in plasma cells (Ghosh et al., 2001; Piskurich et al., 2000). Another level of regulation is at the epigenetic level, where DNA methylation of the CIITA promoter suppresses its expression in trophoblasts (Morris et al., 2000), while inhibitors of histone deacetylases enhance MHC class II expression through both CIITA-dependent and -independent pathways (Magner et al., 2000).

Cell-Specific Promoters and Isoforms of CIITA

An area of research that has received much attention is the finding that *MHC2TA* contains multiple promoters

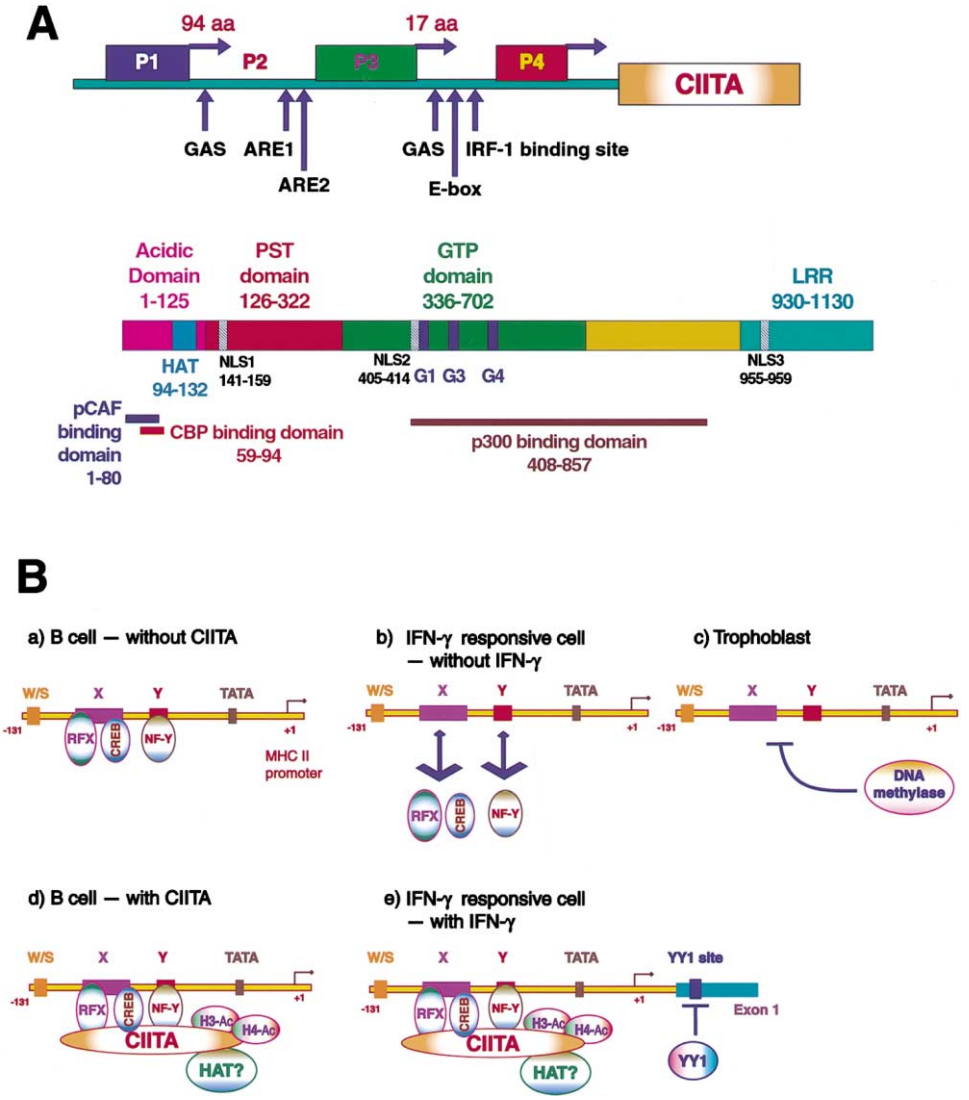

Figure 3. Regulation, Structure, and Function of CIITA

(A) Promoters of *MHC2TA* and structure of CIITA. The promoter of the *MHC2TA* (top) shows the different regulatory elements (arrows) found in the various promoters. A distal GAS site which responds to STAT1 is shown, although the precise location is unclear. The structure of CIITA protein (bottom) shows the different domains described in the text.

(B) Different states of the MHC class II transcriptosome. (a) In B cells, CIITA is not required for occupancy of the promoter by RFX and NF-Y. (b) In IFN-γ responsive cells, the promoter is bare or weakly bound in the absence of CIITA. (c) In cells which are not known to express class II antigens, such as trophoblasts, the promoter is silenced by DNA methylation. (d) The presence of CIITA in B cells causes H3 and H4 acetylation. Whether this is through tethered HATs, or through CIITA's intrinsic HAT activity, or both, is unclear. (e) Introduction of CIITA or induction by IFN-γ causes promoter occupancy and H3/H4 acetylation in IFN-γ responsive cells. YY1, a known HDAC, blocks IFN-γ-induced class II mRNA expression through a YY1 binding site found in the first exon.

directing the synthesis of at least three different 5′ coding sequences (Muhlethaler-Mottet et al., 1997). This indicates that CIITA function is regulated in a complex fashion, controlled by both cell-specific promoters as well as cell-specific isoforms. Although the precise expression of these isoforms is still undergoing revision, the current understanding is summarized as follows (Figure 3A, top). Promoter 1 (referred to as P1) is used in dendritic cells and leads predominantly to the expression of the longest isoform of 132 kDa (Landmann et al., 2001). It is extinguished when immature dendritic cells differentiate into a mature phenotype, and the extinction can be reversed by an inhibitor of histone deacetylases (HDACs). The extra 94 aa found in this isoform, as compared to the P3 isoform, encodes a caspase activation and recruiting domain (CARD) (Nickerson et al., 2001). This isoform is quantitatively more efficient than the P3 isoform in activating a DR promoter, perhaps explaining the higher concentration of class II molecules on dendritic cells. P2 is not clearly defined. P3 causes the generation of a 124 kDa isoform which is constitutively expressed in B cells. A short promoter region of 200+ bp for P3 is required for expression in B cells; two in vivo footprints in this region correspond

to two functional promoter elements, ARE-1 (a TEF-like element) and ARE-2 (Ghosh et al., 1999). On the other hand, a long promoter for P3 that extends 6 kb upstream is required for activation in IFN-γ-treated macrophage/monocytic lines in a STAT1-dependent fashion, and it is also abundantly expressed in IFN-γ activated melanomas and glioblastomas (Piskurich et al. 1999, Deffrennes et al., 2001; Goodwin et al., 2001). It is also expressed by immature dendritic cells, and is similarly silenced upon maturation (Landmann et al., 2001). P4, originally suggested to represent the primary IFN-γ-inducible promoter, is responsive to a combination of IRF-1, STAT1, and USF transcription factors, and produces the shortest isoform of 121 kDa. However, a targeted deletion of this promoter region in mice showed that it is crucial for the expression of MHC class II in nonhematopoietic cells, including cortical thymic epithelial cells, but not for hematopoietic cells (Waldburger et al., 2001). The most straightforward explanation is that this form is normally not required for CIITA expression in hematopoietic cells, although there is a possibility that other promoter-isoform pairs may have compensated for the loss of this form in hematopoietic tissues.

In summary, this complex array of *MHC2TA* promoters suggests that the fine-tuning of MHC class II expression must be crucial to assure a balance of selective immunity to foreign pathogens/antigens and tolerance to self-antigen. The timing and regulation of CIITA isoforms in distinct tissues have to be tightly regulated to assure proper immune function. The complexity of environmental signals, developmental programs, and cell-specific information must be interpreted accurately at the level of *MHC2TA* regulation to achieve appropriate MHC class II expression.

Structure and Function of CIITA

Structure-function analyses of CIITA have revealed the presence of both conventional domains expected of transcriptional activators, as well as unorthodox motifs (see Harton and Ting, 2000) (Figure 3A, bottom). The acidic domain at the N terminus (residues 1–125) is required for transactivation function, which may be achieved by providing an interaction surface for the histone acetylase CBP and RFXANK (Fontes et al., 1999; Kretsovali, 1998; Zhu et al., 2000). This segment also contains an intrinsic histone acetyltransferase (HAT) domain (Raval et al., 2001) (see below). A proline-serine-threonine-rich domain (residues 126–336) that contains multiple potential phosphorylation sites then follows. The midsection of the protein contains an unusual sequence for a transcription coactivator, the GTP binding domain (residues 337–702), that is involved in protein self-association and is important in nuclear import (Kretsovali et al., 2001; Linhoff et al., 2001; Sisk et al., 2001). Finally, a leucine-rich region (LRR) that also affects nuclear translocation and the self-association process resides at the C terminus. It associates with a 33 kDa protein; however, the identity and significance of this protein are presently unclear (Hake et al., 2000). Scattered amidst the protein are three nuclear translocation sequences, including two conventional nuclear localization sequences (NLS) and a bipartite NLS (Cressman et al., 1999, 2001; Kretsovali et al., 2001). An emerging theme is the shuttling of CIITA in and out of the nucleus, as evidenced by its sensitivity to the nuclear export inhibitor, leptomycin B, and by the identification of two sequences that are similar to nuclear export motifs which interact with the nuclear export protein CRM1.

Protein Complex Formation and Transcriptosome Assembly

Extensive protein-protein interactions involving all of the players described above occur across the MHC class II promoter to form an active and more stabilized transcriptosome (Figure 2A). Several reports have demonstrated interactions among peptides that bind to the X elements and NF-Y, and binding at these sites in an in vitro gel shift assay has a mutually enhancing effect on protein-DNA interactions (Harton and Ting, 2000; Reith and Mach, 2001). Analysis of the X2 binding protein, CREB, shows that it also interacts with RFX to form a stable complex. These interactions are in agreement with the analysis of in vivo or genomic footprint analysis which allows the visualization of protein-DNA interactions in intact cells. These latter studies show that the in vivo binding of transcription factors to X1 and Y is interdependent, while occupancy of X2 is dependent on binding of both X1 and Y. A generally accepted working model is that NF-Y, RFX, and CREB all interact, and this interaction likely promotes the formation of a stable transcriptosome complex.

From its discovery, it was presumed that CIITA must interact with the DNA binding transcription factors, since it is not a DNA binding protein. Indeed, several groups have used different approaches to reveal such interactions. CIITA interacts with NF-YB and NF-YC, but only weakly with NF-YA; it also interacts with RFXANK/RFXB and RFX5. In addition, an insightful in vivo approach, namely the ChIP assay, used to examine protein components of a transcriptosome, has begun to reveal important information. This procedure utilizes antibody directed at a component of the transcriptosome to pull down interacting proteins, and hence their respective cognate DNA sequences. A ChIP analysis has shown that CIITA coprecipitates DNA sequences that correspond to the X and Y elements, and hence CIITA directly or indirectly interacts with X and Y binding proteins (Masternak et al., 2000).

In addition to the specific players described above, the MHC class II transcriptosome has other interacting partners. By yeast two-hybrid analysis, CIITA interacts with the basal transcription factor $TAF_{II}32$, a component of TFIID, and indirect evidence also indicates the involvement of $TAF_{II}250$ in CIITA-mediated transactivation. CIITA has also been observed to promote transcriptional elongation, presumably through its interaction with cyclin T1, which together with CDK9 forms the positive transcription elongation factor b (P-TEFb) (Kanazawa et al., 2000). Aside from the interaction of CIITA with basal transcription factors, NF-Y has been shown to recruit the TFIID complex and to enhance the affinity of holo-TFIID for a MHC class II promoter through interactions with a number of TAFs (Mantovani, 1999). Additionally, NF-Y also interacts with histones H3 and H4 as well as the HAT p300 (Caretti et al., 1999). Notably, CBP, a homolog of p300, is a CREB binding protein, and CREB

binds X2. Whether all these associations occur at the chromatin level at the MHC class II promoter is an important area of investigation.

Chromatin Modification

One of the hallmarks of gene expression is the alteration of chromatin accessibility and DNA methylation status. Earlier studies of the chromatin structure of MHC class II promoters utilized the genomic footprinting approach to show that different subgroups of BLS-derived B cell lines differ in the in vivo occupancy of their promoters by DNA binding factors (Figure 3B). In cells lacking RFX, all MHC class II promoters are bare (i.e., in vivo footprints are lacking), while in cells lacking CIITA, the promoters are occupied normally (Kara and Glimcher, 1991). This would suggest that RFX is crucial for promoter accessibility, while CIITA is not. Indeed, in an RFX-defective, IFN-γ responsive cell line (G1B), the promoter is also bare despite IFN-γ treatment, which verifies the important role of the RFX protein in promoter assembly in both constitutive and inducible model systems (Brickey et al., 1999). In contrast, the role of CIITA in promoter occupancy in B cells and in an IFN-γ inducible system is in disagreement. In an IFN-γ inducible system, where CIITA is not expressed, or expressed at a minimal level prior to cytokine treatment, MHC class II promoters are minimally occupied; the addition of IFN-γ causes accessibility of the promoter in a time-dependent fashion (Harton and Ting, 2000). This would suggest that CIITA is the crucial factor for promoter accessibility. Indeed, when CIITA was transfected into MHC class II-negative cells, the nonclassical *DM* as well as *Ii* promoters became bound by factors. The precise reason for the different dependency on CIITA between B cells and IFN-γ responsive cells remains unresolved. One reasonable model is that the ubiquitous factors such as RFX and/or NF-Y may be expressed at higher concentrations in B cells, which obviates the absolute need for CIITA to stabilize the transcriptsome. This possibility was suggested by a report which noted higher levels of RFX in B cell lines than in IFN-γ responsive lines (Moreno et al., 1997). Despite these differences, CIITA remains pivotal for the transcription of MHC class II genes in both B cells and IFN-γ responsive cells.

How chromatin accessibility occurs over MHC class II promoters is an increasing focus of study. Both CIITA and NF-Y can interact with HATs, the former with p300, CBP, and pCAF, the latter with p300 (Fontes et al., 1999; Kretsovali, 1998). Cotransfection with CIITA and CBP, pCAF, or p300 can lead to increased activation of a MHC class II promoter-reporter construct. Despite this enhancement, the HAT domains of CBP and pCAF are dispensable for this activation (Harton et al., 2001). This would agree with the findings of two reports. The first shows that CIITA has intrinsic HAT activity, and thus may render other HATs dispensable (Raval et al., 2001). CIITA's HAT domain shares sequence homology with CBP and can substitute for the HAT function of $TAF_{II}250$. A second study found that two acetylated lysine residues within CIITA are important for nuclear import and that CBP and CAF may serve an alternate function in facilitating CIITA import into the nucleus (Spilianakis et al., 2000). More detailed analysis of the relevance of CBP/p300/CAF in promoter assembly will require analysis of the endogenous promoter under most physiologic conditions. A recent study has begun to address this issue by employing the ChIP assay in conjunction with real time PCR to assess the relationship between histone acetylation and CIITA occupancy (Beresford and Boss, 2001). They found that the presence of CIITA correlated with the acetylation of H3 and H4 at the endogenous MHC class II promoter in both B cells and an IFN-γ-inducible cell line. This result should be interpreted in light of the in vivo footprint analysis described above where binding of factors to endogenous MHC class II promoters in B cells does not require CIITA, while CIITA is required in IFN-γ responsive cells. This new study shows that regardless of cell type, CIITA is essential for proper histone acetylation in both cell groups. These results distinguish between factor binding to promoters and histone acetylation, and conclude that the former can occur without the latter, although transactivation does not occur in the absence of CIITA or histone acetylation (Figure 3B).

Finally, the role of histone deacetylase complexes (HDACs) in MHC class II gene control has begun to emerge. The general HDAC inhibitor, TSA, can rescue class II expression in tumor cells and mature dendritic cells where CIITA promoters are silenced (Landmann et al., 2001; Magner et al., 2000). Similarly, in a system where class II expression is silenced in the absence of the retinoblastoma protein (Rb), the addition of TSA restored expression and YY1 was identified as one of the HDACs (Osborne et al., 2001). Prior to the inhibition of HDAC, the promoter was found to exist in a nucleosome-free, DNase hypersensitive configuration, indicating that HDAC can exert its effect despite the establishment of a chromatin environment favorable for transcription initiation, and presumably with proteins bound to the promoter. This again suggests that modification of the acetylation state of histones and binding of proteins to the promoter occur independently.

Specificity of CIITA and RFX for MHC Class II Gene Expression

Two questions have been raised regarding the specificity of CIITA and RFX: (1) Do these factors control other genes? (2) Does MHC class II expression persist when either of these factors is missing? The first question has to be considered against the backdrop that the specificity of transcription factors is frequently invoked when they are first described, but this specificity fails to withstand more extensive investigation. CIITA has largely escaped this fate, with rare exceptions; many of its effects are specific for MHC class II molecules or its associated proteins. This is supported by representation difference analysis (RDA), which shows that most, if not all, of the genes induced by CIITA are within the class II pathway (Taxman et al., 2000). The control of the *DOA* and *DOB* genes by CIITA is less straightforward. Two reports showed common findings in the regulation of these two genes: the first used the aforementioned RDA method (Taxman et al., 2000), while the second used a chip array analysis (Nagarajan et al., 2002). Both found that in B cells, *DOA* is dependent on CIITA for expression, while *DOB* is expressed even in cells lacking CIITA.

Additionally, *DOA* and *DOB* are dependent on RFX for gene expression. However, the array analysis accompanied by real-time PCR showed a 2-fold enhancement of DOB in the presence of CIITA, which was not detected by RDA. This is reasonable, as the latter is best for detecting all-or-none differences, while array analysis detects more quantitative differences. The array paper also showed that the introduction of CIITA into an IFN-γ-inducible system does not greatly enhance *DOB* expression, in contrast to the great enhancement of *DOA* and *DRA* by CIITA. This agrees with an earlier finding that IFN-γ likewise significantly induces *DOA* but not *DOB* (Tonnelle et al., 1985). It is reasonable to conclude that *DOB* is less affected by CIITA than other class II MHC genes, although an effect can be detected, and that a CIITA-independent pathway exists for its expression. To further complicate the picture, CIITA$^{-/-}$ mice retain expression of both *H-2OA* and *B* as observed by RT-PCR. Whether this represents differences between humans versus mice, in vitro cell lines versus in vivo primary tissues, or simply RT-PCR versus real-time PCR is unclear.

CIITA is known to control a few genes other than MHC class II, although none are as strongly induced as class II genes. Class I MHC promoter and antigen expression is enhanced by CIITA in both human and murine lines (reviewed in van den Elsen and Gobin, 1999). CIITA regulation of class I is mapped to a region that has S/W, X, and Y homologs; ChIP analysis also shows the presence of CIITA at the endogenous β*2M* promoter, which likewise contains X and Y elements (Masternak et al., 2000; Riegert et al., 1996). A reduction of class I MHC has been observed in human Group A BLS patients but not in CIITA$^{-/-}$ mice. The reason for this discrepancy is unclear. In addition to class I MHC, a handful of genes have been found to be downregulated by the presence of CIITA, including *IL-4, fas*, and collagen (Gourley and Chang, 2001; Sisk et al., 2000; Zhu and Ting, 2001). In all these cases, CIITA mediates suppression by squelching general HATs. One of these studies compared the defective G3A cell line, in which IFN-γ induction of CIITA is suboptimal, to its normal parent, thus allowing the investigation of endogenous CIITA in gene suppression (Zhu and Ting, 2001). The observation was made that CIITA induction by IFN-γ can lead to the suppression of genes that are known suppressive targets of this cytokine. Hence, CIITA represents a dual-function factor, both as a strong inducer of immune response genes, and a repressor of general histone acetyltransferases for certain genes that may not be of immediate use during an IFN-γ response.

The second question regarding CIITA and RFX is whether they are indispensable for MHC class II expression. Mice lacking functional CIITA or RFX5 have been used to address this issue. CIITA$^{-/-}$ mice are largely devoid of class II, although some residual expression remains. Analyses of two CIITA$^{-/-}$ mice revealed substantial (20%) residual class II expression on dendritic cells limited to the s.c. lymph nodes detected by immunohistochemistry (Williams, 1998). The analysis of a third CIITA$^{-/-}$ strain revealed very low levels of class II mRNA only detected by RT-PCR, but not Northern hybridization, in the lymph nodes and spleen (Itoh-Lindstrom et al., 1999). A more recent study utilized one of the CIITA$^{-/-}$ mice used in the first study and found that class II expression on dendritic cells is reduced 99% as assessed by real-time PCR, and agrees with the notion of CIITA serving as a master regulator (Landmann et al., 2001).

The analysis of RFX5$^{-/-}$ mice showed class II expression on thymic medulla, mature dendritic cells, and activated B cells, but not B cells or IFN-γ activated macrophages (Clausen et al., 1998). Despite this residual expression, both CIITA$^{-/-}$ and RFX5$^{-/-}$ mice show severe immunodeficiency and CD4^{+} T cell defects, replicating the findings in humans. However, the CIITA$^{-/-}$ mice do respond differently from mice lacking Aβ, which are generally considered to be class II defective. In the nonobese diabetes (NOD) model, CIITA$^{-/-}$ NOD mice have pancreatic cellular infiltrates, while Aβ$^{-/-}$ mice do not (Mora et al., 1999), yet neither display symptoms of diabetes. Whether this difference between the two strains can be attributed to residual class II expression, to other class II-associated genes (*Ii, M, O*), or to nonassociated genes that are selectively controlled by CIITA remains to be determined.

Disease and Physiologic Relevance

The MHC class II loci are associated with more diseases than any other region of the genome of equivalent size. Class II-related autoimmune conditions are suspected to be due to a failure of tolerance. Expression of class II genes must be tightly and subtly controlled to ensure appropriate vigorous responses to pathogens while minimizing collateral damage to host tissues.

Considering that CIITA is a master regulator of MHC class II gene transcription, it represents an ideal target for pathogens to evade the immune system. Indeed, a variety of pathogens (*cytomegalovirus* (CMV), *Mycobacterium bovis, Chlamydia, varicella-zoster* virus, parainfluenza virus, and Epstein Barr virus (EBV)) have evolved several pathways to alter the expression of CIITA (reviewed in Harton and Ting, 2000; Reith and Mach, 2001; Accolla et al., 2001; see also Gao et al., 2001; Morrison et al., 2001). Furthermore, HIV-1 infection also suppresses class II expression by interfering with both CIITA and NF-YA functions (Rakoff-Nahoum et al., 2001). One common feature is that most of the pathways utilized by pathogens affect a more general target upstream of CIITA expression, such as the Jak/Stat pathway, the USF-1 transcription factor, IFN-γ receptor expression, and other cytokines which can affect class II expression; thus, the effects are far-reaching beyond MHC class II regulation. Additionally, it was found that statin, a drug used in the treatment of heart disease to control lipid levels, also decreases CIITA expression by interfering with P4 function (Kwak et al., 2000). This raises the possibility that these drugs might be useful to regulate immune activation involving the hyperexpression of class II antigens, as in autoimmune or autoinflammatory disorders.

Both class II MHC and CIITA promoters exhibit polymorphisms that may be relevant to disease. This is not surprising, since the expression level or control dynamics of a class II molecule could help to modulate the immune response (Baumgart et al., 1998). It may influence cytokine profiles or the ratio of Th1 to Th2 re-

sponses, for example. Conversely, promoter variation could influence susceptibility to disease independent of coding region variation. A number of variant nucleotides have been described in promoter regions upstream of class II genes. Some of these are in canonical sequences identified as binding elements for transcription factors, and in a few cases the changes have been shown to affect transcription in reporter assays (Andersen et al., 1991). In addition to promoter variation, intergenic regions several kb upstream of class II loci may exhibit marked variation. The *DQB1* gene is marked by variation in the presence or absence of retroviral LTRs that could in principle influence expression levels (Kambhu et al., 1990). Finally, more recent reports have shown polymorphisms in the CIITA promoter and 3′ untranslated region, although the significance to disease is not clear (Rasmussen et al., 2001).

The utilization of CIITA as a means to enhance cancer immunotherapy has also been investigated. There is some disagreement concerning the ability of CIITA-transfected cells to present exogenous antigens (Sartoris et al., 1998; Siegrist et al., 1995), and this may simply vary with the cell line examined. However, two studies which directly examined antitumor activity of different CIITA-transfected tumors have failed to show an efficacy of CIITA in enhancing tumor control, despite the presence of costimulatory molecules such as CD80 and CD86 (Armstrong et al., 1997; Martin et al., 1999). These studies showed a correlation with the poor ability of CIITA-induced class II complexes to present antigens targeted through the endogenous pathway, which may represent the route traveled by many tumor antigens. Overcoming such issues will be necessary to exploit the utilization of CIITA as a potential therapeutic in tumor control. As an alternate strategy, an adenoviral construct containing *MHC2TA* has been introduced into dendritic cells with enhanced immunostimulatory effects (Marten et al., 2001), which warrants the testing of this strategy in animal models.

Summary

The MHC class II system has evolved into a complex array of surface molecules which are required to bind peptides of a wide ranging number of pathogens. The classical and nonclassical MHC class II genes, in addition to Ii, have one predominant function: presenting antigenic peptides and activating CD4 T cells in hematopoietic as well as extrahematopoietic tissues. The molecular control of these molecules is highly complex and intricate. Strict coordination of MHC class II gene control has been implemented to deal efficiently and appropriately with foreign antigens, while minimizing autoreactive responses against self-antigens. A master transcriptional regulator, CIITA, which is highly specific in its function, controls the expression of this entire class of genes. This is performed by coordinating the functions of DNA binding proteins and histone acetylases to modify local chromatin and promoter accessibility. DNA methylases and HDAC are also involved, and these are likely to represent areas of intense research focus. Future endeavors to harness this knowledge to modulate disease outcome, whether infectious diseases, autoimmunity, or cancer therapy, should be of tremendous interest and potential.

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References

Accolla, R.S., De Lerma Barbaro, A., Mazza, S., Casoli, C., De Maria, A., and Tosi, G. (2001). The MHC class II transactivator: prey and hunter in infectious diseases. Trends Immunol. *22*, 560–563.

Alfonso, C., and Karlsson, L. (2000). Nonclassical MHC class II molecules. Annu. Rev. Immunol. *18*, 113–142.

Allcock, R.J., Martin, A.M., and Price, P. (2000). The mouse as a model for the effects of MHC genes on human disease. Immunol. Today *21*, 328–332.

Andersen, L.C., Beaty, J.S., Nettles, J.W., Seyfried, C.E., Nepom, G.T., and Nepoom, B.S. (1991). Allelic polymorphism in transcriptional regulatory regions of *HLA-DQB* genes. J. Exp. Med. *173*, 181–192.

Armstrong, T.D., Clements, V.K., Martin, B.K., Ting, J.P.Y., and Ostrand-Rosenberg, S. (1997). Major histocompatibility complex class II-transfected tumor cells present endogenous antigen and are potent inducers of tumor-specific immunity. Proc. Natl. Acad. Sci. USA *94*, 6886–6891.

Baumgart, M., Moos, V., Schuhbauer, D., and Muller, B. (1998). Differential expression of major histocompatibility complex class II genes on murine macrophages associated with T cell cytokine profile and protective/suppressive effects. Proc. Natl. Acad. Sci. USA *95*, 6936–6940.

Beck, S., and Trowsdale, J. (1999). Sequence organisation of the class II region of the human MHC. Immunol. Rev. *167*, 201–210.

Benoist, C., and Mathis, D. (1990). Regulation of major histocompatibility complex class-II genes: X, Y, and other letters of the alphabet. Annu. Rev. Immunol. *8*, 681–715.

Beresford, G.W., and Boss, J.M. (2001). CIITA coordinates multiple histone acetylation modifications at the HLA-DRA promoter. Nat. Immunol. *2*, 652–657.

Brickey, W.J., Wright, K.L., Zhu, X.S., and Ting, J.P. (1999). Analysis of the defect in IFN-γ induction of MHC class II genes in G1B cells: identification of a novel and functionally critical leucine-rich motif (62-LYLYLQL-68) in the regulatory factor X 5 transcription factor. J. Immunol. *163*, 6622–6630.

Caretti, G., Motta, M.C., and Mantovani, R. (1999). NF-Y associates of H3–H4 tetramers and octamers by multiple mechanisms. Mol. Cell. Biol. *19*, 8591–8603.

Chin, K.-C., Mao, C., Skinner, C., Riley, J.L., Wright, K.L., Moreno, C.S., Stark, G.R., Boss, J.M., and Ting, J.P.-Y. (1994). Molecular analysis of G1B and G3A IFN-γ mutants reveals that defects in CIITA or RFX result in defective class II MHC and Ii gene induction. Immunity *1*, 687–697.

Chou, C.L., and Sadegh-Nasseri, S. (2000). HLA-DM recognizes the flexible conformation of major histocompatibility complex class II. J. Exp. Med. *192*, 1697–1706.

Clausen, B.E., Waldburger, J.M., Schwenk, F., Barras, E., Mach, B., Rajewsky, K., Forster, I., and Reith, W. (1998). Residual MHC class II expression on mature dendritic cells and activated B cells in RFX5-deficient mice. Immunity *8*, 143–155.

Copier, J., Kleijmeer, M.J., Ponnambalam, S., Oorschot, V., Potter, P., Trowsdale, J., and Kelly, A. (1996). Targeting signal and subcellular compartments involved in the intracellular trafficking of HLA-DMB. J. Immunol. *157*, 1017–1027.

Cressman, D.E., Chin, K.C., Taxman, D.J., and Ting, J.P. (1999). A defect in the nuclear translocation of CIITA causes a form of type II bare lymphocyte syndrome. Immunity *10*, 163–171.

Cressman, D.E., O'Connor, W.J., Greer, S.F., Zhu, X.S., and Ting, J.P. (2001). Mechanisms of nuclear import and export that control

the subcellular localization of class ii transactivator. J. Immunol. *167*, 3626–3634.

Cresswell, P. (1994). Assembly, transport and function of MHC class II molecules. Annu. Rev. Immunol. *12*, 259–293.

Cresswell, P. (1996). Invariant chain structure and MHC class II function. Cell *84*, 505–507.

Cullen, M., Noble, J., Erlich, H., Thorpe, K., Beck, S., Klitz, W., Trowsdale, J., and Carrington, M. (1997). Characterization of recombination in the HLA class II region. Am. J. Hum. Genet. *60*, 397–407.

Dawkins, R., Leelayuwat, C., Gaudieri, S., Tay, G., Hui, J., Cattley, S., Martinez, P., and Kulski, J. (1999). Genomics of the major histocompatibility complex: haplotypes, duplication, retroviruses and disease. Immunol. Rev. *167*, 275–304.

Deffrennes, V., Vedrenne, J., Stolzenberg, M.C., Piskurich, J., Barbieri, G., Ting, J.P., Charron, D., and Alcaide-Loridan, C. (2001). Constitutive expression of MHC class II genes in melanoma cell lines results from the transcription of class II transactivator abnormally initiated from its B cell-specific promoter. J. Immunol. *167*, 98–106.

DeSandro, A., Nagarajan, U.M., and Boss, J.M. (1999). The bare lymphocyte syndrome: molecular clues to the transcriptional regulation of major histocompatibility complex class II genes. Am. J. Hum. Genet. *65*, 279–286.

Doebele, C.R., Busch, R., Scott, M.H., Pashine, A., and Mellins, D.E. (2000). Determination of the HLA-DM interaction site on HLA-DR molecules. Immunity *13*, 517–527.

Dong, Y., Tang, L., Letterio, J.J., and Benveniste, E.N. (2001). The Smad3 protein is involved in TGF-beta inhibition of class II transactivator and class II MHC expression. J. Immunol. *167*, 311–319.

Douhan, J., 3rd, Hauber, I., Eibl, M.M., and Glimcher, L.H. (1996). Genetic evidence for a new type of major histocompatibility complex class II combined immunodeficiency characterized by a dyscoordinate regulation of HLA-D alpha and beta chains. J. Exp. Med. *183*, 1063–1069.

Durand, B., Sperisen, P., Emery, P., Barras, E., Zufferey, M., Mach, B., and Reith, W. (1997). RFXAP, a novel subunit of the RFX DNA binding complex is mutated in MHC class II deficiency. EMBO J. *16*, 1045–1055.

Fontes, J.D., Kanazawa, S., Jean, D., and Peterlin, B.M. (1999). Interactions between the class II transactivator and CREB binding protein increase transcription of major histocompatibility complex class II genes. Mol. Cell. Biol. *19*, 941–947.

Gao, J., De, B.P., Han, Y., Choudhary, S., Ransohoff, R., and Banerjee, A.K. (2001). Human parainfluenza virus type 3 inhibits gamma interferon-induced major histocompatibility complex class II expression directly and by inducing alpha/beta interferon. J. Virol. *75*, 1124–1131.

Ghosh, N., Piskurich, J.F., Wright, G., Hassani, K., Ting, J.P., and Wright, K.L. (1999). A novel element and a TEF-2-like element activate the major histocompatibility complex class II transactivator in B-lymphocytes. J. Biol. Chem. *274*, 32342–32350.

Ghosh, N., Gyory, I., Wright, G., Wood, J., and Wright, K.L. (2001). Positive regulatory domain I binding factor 1 silences class II transactivator expression in multiple myeloma cells. J. Biol. Chem. *276*, 15264–15268.

Glimcher, L.H., and Kara, C.J. (1992). Sequences and factors: a guide to MHC class-II transcription. Annu. Rev. Immunol. *10*, 13–49.

Goodwin, B.L., Xi, H., Tijiram, R., Eason, D.D., Ghosh, N., Wright, K.L., Nagarajan, U., Boss, J.M., and Blanck, G. (2001). Varying functions of specific major histocompatibility class II transactivator promoter III and IV elements in melanoma cell lines. Cell Growth Differ. *12*, 327–335.

Gourley, T.S., and Chang, C.H. (2001). Cutting edge: the class II transactivator prevents activation-induced cell death by inhibiting Fas ligand gene expression. J. Immunol. *166*, 2917–2921.

Grahovac, B., Sukernik, R.I., O'hUigin, C., Zaleska-Rutczynska, Z., Blagitko, N., Raldugina, O., Kosutic, T., Satta, Y., Figueroa, F., Takahata, N., and Klein, J. (1998). Polymorphism of the HLA class II loci in Siberian populations. Hum. Genet. *102*, 27–43.

Gunther, E., and Walter, L. (2001). The major histocompatibility complex of the rat (Rattus norvegicus). Immunogenetics *53*, 520–542.

Haas, D.A., Boss, J.M., Strominger, J.L., and Spies, T. (1987). A highly diverged beta 1 exon in the DR region of the human MHC: sequence and evolutionary implications. Immunogenetics *25*, 15–20.

Hake, S.B., Masternak, K., Kammerbauer, C., Janzen, C., Reith, W., and Steimle, V. (2000). CIITA leucine-rich repeats control nuclear localization, In vivo recruitment to the major histocompatibility complex (MHC) class II enhanceosome, and MHC class II gene transactivation. Mol. Cell. Biol. *20*, 7716–7725.

Hammond, C., Denzin, L.K., Pan, M., Griffith, J.M., Geuze, H.J., and Cresswell, P. (1998). The tetraspan protein CD82 is a resident of MHC class II compartments where it associates with HLA-DR, -DM, and -DO molecules. J. Immunol. *161*, 3282–3291.

Harton, J.A., and Ting, J.P. (2000). Class II transactivator: mastering the art of major histocompatibility complex expression. Mol. Cell. Biol. *20*, 6185–6194.

Harton, J.A., Zika, E., and Ting, J.P. (2001). The histone acetyltransferase domains of CREB-binding protein (CBP) and p300/CBP-associated factor are not necessary for cooperativity with the class II transactivator. J. Biol. Chem. *276*, 38715–38720.

Hauber, I., Gulle, H., Wolf, H.M., Maris, M., Eggenbauer, H., and Eibl, M.M. (1995). Molecular characterization of major histocompatibility complex class II gene expression and demonstration of antigen-specific T cell response indicate a new phenotype in class II-deficient patients. J. Exp. Med. *181*, 1411–1423.

Hirayama, K., Matsushita, S., Kikuchi, I., Iuchi, M., Ohta, N., and Sasazuki, T. (1987). HLA-DQ is epistatic to HLA-DR in controlling the immune response to schistosomal antigen in humans. Nature *327*, 426–430.

Itoh-Lindstrom, Y., Piskurich, J.F., Felix, N.J., Wang, Y., Brickey, W.J., Platt, J.L., Koller, B.H., and Ting, J.P. (1999). Reduced IL-4-, lipopolysaccharide-, and IFN-γ-induced MHC class II expression in mice lacking class II transactivator due to targeted deletion of the GTP-binding domain. J. Immunol. *163*, 2425–2431.

Jabrane-Ferrat, N., Fontes, J.D., Boss, J.M., and Peterlin, B.M. (1996). Complex architecture of major histocompatibility complex class II promoters: reiterated motifs and conserved protein-protein interactions. Mol. Cell. Biol. *16*, 4683–4690.

Jeffreys, A.J., Kauppi, L., and Neumann, R. (2001). Intensely punctate meiotic recombination in the class II region of the major histocompatibility complex. Nat. Genet. *29*, 217–222.

Kambhu, S., Falldorf, P., and Lee, J.S. (1990). Endogenous retroviral long terminal repeats within the HLA-DQ locus. Proc. Natl. Acad. Sci. USA *87*, 4927–4931.

Kanazawa, S., Okamoto, T., and Peterlin, B.M. (2000). Tat competes with CIITA for the binding to P-TEFb and blocks the expression of MHC class II genes in HIV infection. Immunity *12*, 61–70.

Kara, C.J., and Glimcher, L.H. (1991). *In vivo* footprinting of MHC class II genes: Bare promoter in the Bare Lymphocyte Syndrome. Science *252*, 709–712.

Koonin, E.V., and Aravind, L. (2000). The NACHT family—a new group of predicted NTPases implicated in apoptosis and MHC transcription activation. Trends Biochem. Sci. *25*, 223–224.

Kretsovali, A. (1998). Involvement of CREB binding protein in expression of major histocompatibility complex class II genes via interaction with the class II transactivator. Mol. Cell. Biol. *18*, 6777–6783.

Kretsovali, A., Spilianakis, C., Dimakopoulos, A., Makatounakis, T., and Papamatheakis, J. (2001). Self-association of class II transactivator correlates with its intracellular localization and transactivation. J. Biol. Chem. *276*, 32191–32197.

Kropshofer, H., Hammerling, G.J., and Vogt, A.B. (1999). The impact of the non-classical MHC proteins HLA-DM and HLA-DO on loading of MHC class II molecules. Immunol. Rev. *172*, 267–278.

Kwak, B., Mulhaupt, F., Myit, S., and Mach, F. (2000). Statins as a newly recognized type of immunomodulator. Nat. Med. *6*, 1399–1402.

Landmann, S., Muhlethaler-Mottet, A., Bernasconi, L., Suter, T., Waldburger, J.M., Masternak, K., Arrighi, J.F., Hauser, C., Fontana,

A., and Reith, W. (2001). Maturation of dendritic cells is accompanied by rapid transcriptional silencing of class II transactivator (CIITA) expression. J. Exp. Med. *194*, 379–391.

Li, G., Harton, J.A., Zhu, X., and Ting, J.P. (2001). Downregulation of CIITA function by protein kinase a (PKA)-mediated phosphorylation: mechanism of prostaglandin E, cyclic AMP, and PKA inhibition of class II major histocompatibility complex expression in monocytic lines. Mol. Cell. Biol. *21*, 4626–4635.

Linhoff, M.W., Harton, J.A., Cressman, D.E., Martin, B.K., and Ting, J.P. (2001). Two distinct domains within CIITA mediate self-association: involvement of the GTP-binding and leucine-rich repeat domains. Mol. Cell. Biol. *21*, 3001–3011.

Magner, W.J., Kazim, A.L., Stewart, C., Romano, M.A., Catalano, G., Grande, C., Keiser, N., Santaniello, F., and Tomasi, T.B. (2000). Activation of MHC class I, II, and CD40 gene expression by histone deacetylase inhibitors. J. Immunol. *165*, 7017–7024.

Maity, S.N., and de Crombrugghe, B. (1998). Role of the CCAAT-binding protein CBF/NF-Y in transcription. Trends Biochem. Sci. *23*, 174–178.

Mantovani, R. (1999). The molecular biology of the CCAAT-binding factor NF-Y. Gene *239*, 15–27.

Marten, A., Ziske, C., Schottker, B., Weineck, S., Renoth, S., Buttgereit, P., Schakowski, F., Konig, S., von Rucker, A., Allera, A., et al. (2001). Transfection of dendritic cells (DCs) with the CIITA gene: increase in immunostimulatory activity of DCs. Cancer Gene Ther. *8*, 211–219.

Martin, B.K., Frelinger, J.G., and Ting, J.P. (1999). Combination gene therapy with CD86 and the MHC class II transactivator in the control of lung tumor growth. J. Immunol. *162*, 6663–6670.

Masternak, K., Barras, E., Zufferey, M., Conrad, B., Corthals, G., Aebersold, R., Sanchez, J.C., Hochstrasser, D.F., Mach, B., and Reith, W. (1998). A gene encoding a novel RFX-associated transactivator is mutated in the majority of MHC class II deficiency patients. Nat. Genet. *20*, 273–277.

Masternak, K., Muhlethaler-Mottet, A., Villard, J., Zufferey, M., Steimle, V., and Reith, W. (2000). CIITA is a transcriptional coactivator that is recruited to MHC class II promoters by multiple synergistic interactions with an enhanceosome complex. Genes Dev. *14*, 1156–1166.

Mora, C., Wong, F.S., Chang, C.H., and Flavell, R.A. (1999). Pancreatic infiltration but not diabetes occurs in the relative absence of MHC class II-restricted CD4 T cells: studies using NOD/CIITA-deficient mice. J. Immunol. *162*, 4576–4588.

Moreno, C.S., Rogers, E.M., Brown, J.A., and Boss, J.M. (1997). Regulatory factor X, a bare lymphocyte syndrome transcription factor, is a multimeric phosphoprotein complex. J. Immunol. *158*, 5841–5848.

Moreno, C.S., Beresford, G.W., Louis-Plence, P., Morris, A.C., and Boss, J.M. (1999). CREB regulates MHC class II expression in a CIITA-dependent manner. Immunity *10*, 143–151.

Morris, A.C., Spangler, W.E., and Boss, J.M. (2000). Methylation of class II trans-activator promoter IV: a novel mechanism of MHC class II gene control. J. Immunol. *164*, 4143–4149.

Morrison, T.E., Mauser, A., Wong, A., Ting, J.P., and Kenney, S.C. (2001). Inhibition of IFN-γ signaling by an Epstein-Barr virus immediate-early protein. Immunity *15*, 787–799.

Mosyak, L., Zaller, D.M., and Wiley, D.C. (1998). The structure of HLA-DM, the peptide exchange catalyst that loads antigen onto class II MHC molecules during antigen presentation. Immunity *9*, 377–383.

Muhlethaler-Mottet, A., Otten, L.A., Steimle, V., and Mach, B. (1997). Expression of MHC class II molecules in different cellular and functional compartments is controlled by differential usage of multiple promoters of the transactivator CIITA. EMBO J. *16*, 2851–2860.

Nagarajan, U.M., Louis-Plence, P., DeSandro, A., Nilsen, R., Bushey, A., and Boss, J.M. (1999). RFX-B is the gene responsible for the most common cause of the bare lymphocyte syndrome, an MHC class II immunodeficiency. Immunity *10*, 153–162.

Nagarajan, U.M., Lochamy, J., Chen, X., Beresford, G.W., Nilsen, R., Jensen, P.E., and Boss, J.M. (2002). Class II transactivator is required for maximal expression of HLA-DOB in B cells. J. Immunol. *168*, 1780–1786.

Nekrep, N., Geyer, M., Jabrane-Ferrat, N., and Peterlin, B.M. (2001). Analysis of ankyrin repeats reveals how a single point mutation in RFXANK results in bare lymphocyte syndrome. Mol. Cell. Biol. *21*, 5566–5576.

Nelson, C.A., and Fremont, D.H. (1999). Structure principles of MHC class II antigen presentation. Rev. Immunogenet. *1*, 47–59.

Nickerson, K., Sisk, T.J., Inohara, N., Yee, C.S., Kennell, J., Cho, M.C., Yannie, P.J., Nunez, G., and Chang, C.H. (2001). Dendritic cell specific CIITA contains a caspase-recruitment domain that confers potent transactivation activity. J. Biol. Chem. *276*, 19089–19093.

Osborne, A., Zhang, H., Yang, W.M., Seto, E., and Blanck, G. (2001). Histone deacetylase activity represses gamma interferon-inducible HLA-DR gene expression following the establishment of a DNase I-hypersensitive chromatin conformation. Mol. Cell. Biol. *21*, 6495–6506.

Peretti, M., Villard, J., Barras, E., Zufferey, M., and Reith, W. (2001). Expression of the three human major histocompatibility complex class II isotypes exhibits a differential dependence on the transcription factor RFXAP. Mol. Cell. Biol. *21*, 5699–5709.

Piskurich, J.F., Linhoff, M.W., Wang, Y., and Ting, J.P.-Y. (1999). Two distinct gamma interferon-inducible promoters of the major histocompatibility complex class II transactivator gene are differentially regulated by STAT1, interferon regulatory factor *1*, and transforming growth factor beta. Mol. Cell. Biol. *19*, 431–440.

Piskurich, J.F., Lin, K.I., Lin, Y., Wang, Y., Ting, J.P., and Calame, K. (2000). BLIMP-I mediates extinction of major histocompatibility class II transactivator expression in plasma cells. Nat. Immunol. *1*, 526–532.

Rakoff-Nahoum, S., Chen, H., Kraus, T., George, I., Oei, E., Tyorkin, M., Salik, E., Beuria, P., and Sperber, K. (2001). Regulation of class II expression in monocytic cells after hiv-1 infection. J. Immunol. *167*, 2331–2342.

Rasmussen, H.B., Kelly, M.A., and Clausen, J. (2001). Genetic susceptibility to multiple sclerosis: detection of polymorphic nucleotides and an intron in the 3′ untranslated region of the major histocompatibility complex class II transactivator gene. Hum. Immunol. *62*, 371–377.

Raval, A., Howcroft, T.K., Weissman, J.D., Kirshner, S., Zhu, X.S., Yokoyama, K., Ting, J., and Singer, D.S. (2001). Transcriptional coactivator, CIITA, is an acetyltransferase that bypasses a promoter requirement for $TAF_{II}250$. Mol. Cell *7*, 105–115.

Reith, W., and Mach, B. (2001). The bare lymphocyte syndrome and the regulation of MHC expression. Annu. Rev. Immunol. *19*, 331–373.

Riegert, P., Andersen, R., Bumstead, N., Dohring, C., Dominguez-Steglich, M., Engberg, J., Salomonsen, J., Schmid, M., Schwager, J., Skjodt, K., and Kaufman, J. (1996). The chicken beta 2-microglobulin gene is located on a non-major histocompatibility complex microchromosome: a small, G+C-rich gene with X and Y boxes in the promoter. Proc. Natl. Acad. Sci. USA *93*, 1243–1248.

Sanderson, F., and Trowsdale, J. (1995). Antigen presentation: kissing cousins exchange CLIP. Curr. Biol. *5*, 1372–1376.

Sartoris, S., Valle, M.T., Barbaro, A.L., Tosi, G., Cestari, T., D'Agostino, A., Megiovanni, A.M., Manca, F., and Accolla, R.S. (1998). HLA class II expression in uninducible hepatocarcinoma cells after transfection of AIR-1 gene product CIITA: acquisition of antigen processing and presentation capacity. J. Immunol. *161*, 814–820.

Schafer, P.H., Pierce, S.K., and Jardetzky, T.S. (1995). The structure of MHC class II: a role for dimer of dimers. Sem. Immunol. *7*, 389–398.

Siegrist, C.A., Martinez-Soria, E., Kern, I., and Mach, B. (1995). A novel antigen-processing-defective phenotype in major histocompatibility complex class II-positive CIITA transfectants is corrected by interferon-gamma. J. Exp. Med. *182*, 1793–1799.

Sims, T.N., and Halloran, P.F. (1999). MHC class II regulation *in vivo* in the mouse kidney. Microbes Infect. *1*, 903–912.

Sisk, T.J., Gourley, T., Roys, S., and Chang, C.H. (2000). MHC class II transactivator inhibits IL-4 gene transcription by competing with

NF-AT to bind the coactivator CREB binding protein (CBP)/p300. J. Immunol. *165*, 2511–2517.

Sisk, T.J., Roys, S., and Chang, C.H. (2001). Self-association of CIITA and its transactivation potential. Mol. Cell. Biol. *21*, 4919–4928.

Spilianakis, C., Papamatheakis, J., and Kretsovali, A. (2000). Acetylation by PCAF enhances CIITA nuclear accumulation and transactivation of major histocompatibility complex class II genes. Mol. Cell. Biol. *20*, 8489–8498.

Steimle, V., Otten, L.A., Zufferey, M., and Mach, B. (1993). Complementation cloning of an MHC class II transactivator mutated in hereditary MHC class II deficiency (or bare lymphocyte syndrome). Cell *75*, 135–146.

Steimle, V., Durand, B., Barras, E., Zufferey, M., Hadam, M.R., Mach, B., and Reith, W. (1995). A novel DNA-binding regulatory factor is mutated in primary MHC class II deficiency (bare lymphocyte syndrome). Genes Dev. *9*, 1021–1032.

Taxman, D.J., Cressman, D.E., and Ting, J.P. (2000). Identification of class II transcriptional activator-induced genes by representational difference analysis: discoordinate regulation of the DNα/DOβ heterodimer. J. Immunol. *165*, 1410–1416.

van den Elsen, P.J., and Gobin, S.J. (1999). The common regulatory pathway of MHC class I and class II transactivation. Microbes Infect. *1*, 887–892.

Tonnelle, C., DeMars, R., and Long, E.O. (1985). DOβ: a new β chain gene in HLA-D with a distinct regulation of expression. EMBO J. *4*, 2839–2847.

Waldburger, J.M., Suter, T., Fontana, A., Acha-Orbea, H., and Reith, W. (2001). Selective abrogation of major histocompatibility complex class II expression on extrahematopoietic cells in mice lacking promoter IV of the class II transactivator gene. J. Exp. Med. *194*, 393–406.

Westerheide, S.D., and Boss, J.M. (1999). Orientation and positional mapping of the subunits of the multicomponent transcription factors RFX and X2BP to the major histocompatibility complex class II transcriptional enhancer. Nucleic Acids Res. *27*, 1635–1641.

Williams, G.S. (1998). Mice lacking the transcription factor CIITA—a second look. Int. Immunol. *10*, 1957–1967.

Wiszniewski, W., Fondaneche, M.C., Le Deist, F., Kanariou, M., Selz, F., Brousse, N., Steimle, V., Barbieri, G., Alcaide-Loridan, C., Charron, D., et al. (2001). Mutation in the class II trans-activator leading to a mild immunodeficiency. J. Immunol. *167*, 1787–1794.

Xi, H., Eason, D.D., Ghosh, D., Dovhey, S., Wright, K.L., and Blanck, G. (1999). Co-occupancy of the interferon regulatory element of the class II transactivator (CIITA) type IV promoter by interferon regulatory factors 1 and 2. Oncogene *18*, 5889–5903.

Zhu, X.S., and Ting, J.P. (2001). A 36-amino-acid region of CIITA is an effective inhibitor of CBP: novel mechanism of gamma interferon-mediated suppression of collagen α(2)(I) and other promoters. Mol. Cell. Biol. *21*, 7078–7088.

Zhu, X.S., Linhoff, M.W., Li, G., Chin, K.C., Maity, S.N., and Ting, J.P. (2000). Transcriptional scaffold: CIITA interacts with NF-Y, RFX, and CREB to cause stereospecific regulation of the class II major histocompatibility complex promoter. Mol. Cell. Biol. *20*, 6051–6061.