

## Expression of MHC II Genes

G. Drozina<sup>1</sup> · J. Kohoutek<sup>1</sup> · N. Jabrane-Ferrat<sup>2</sup> · B. M. Peterlin<sup>1</sup> (✉)

<sup>1</sup> Departments of Medicine, Microbiology and Immunology,  
Rosalind Russell Medical Research Center, University of California, San Francisco,  
CA 94143, USA  
*matija@itsa.ucsf.edu*

<sup>2</sup> Structural Immuno-Pharmacology, Institute of Pharmacology and Structural Biology,  
CNRS VMR 5089, 31400 Toulouse, France

<b>1</b>	<b>Innate and Adaptive Immunity</b> . . . . .	148
1.1	Major Histocompatibility Complex Determinants . . . . .	149
1.2	Bare Lymphocyte Syndrome . . . . .	152
1.3	<i>cis</i> -Acting Elements . . . . .	152
1.4	<i>trans</i> -Acting Factors. . . . .	154
1.5	MHC II Enhanceosome and Transcriptosome. . . . .	156
<b>2</b>	<b>Class II Transactivator</b> . . . . .	157
2.1	Regulation of CIITA Gene Expression . . . . .	159
2.2	Post-Translational Modifications of CIITA . . . . .	161
2.3	CIITA Is a Shuttling Protein . . . . .	163
2.4	CIITA Is a Transcriptional Integrator . . . . .	164
<b>3</b>	<b>Conclusions and Future Directions</b> . . . . .	165
	<b>References</b> . . . . .	166

**Abstract** Innate and adaptive immunity are connected via antigen processing and presentation (APP), which results in the presentation of antigenic peptides to T cells in the complex with the major histocompatibility (MHC) determinants. MHC class II (MHC II) determinants present antigens to CD4<sup>+</sup> T cells, which are the main regulators of the immune response. Their genes are transcribed from compact promoters that form first the MHC II enhanceosome, which contains DNA-bound activators and then the MHC II transcriptosome with the addition of the class II transactivator (CIITA). CIITA is the master regulator of MHC II transcription. It is expressed constitutively in dendritic cells (DC) and mature B cells and is inducible in most other cell types. Three isoforms of CIITA exist, depending on cell type and inducing signals. CIITA is regulated at the levels of transcription and post-translational modifications, which are still not very clear. Inappropriate immune responses are found in several diseases, including cancer and autoimmunity. Since CIITA regulates the expression of MHC II genes, it is involved directly in the regulation of the immune response. The knowledge of CIITA will facilitate the manipulation of the immune response and might contribute to the treatment of these diseases.

## 1 Innate and Adaptive Immunity

The immune system is composed of innate and adaptive branches, which are nonspecific and specific, i.e., they target common and unique parts of non-self antigens, respectively (Table 1). They discriminate between self and non-self, and activate appropriate effectors. Although innate and adaptive immunity appear independent, appropriate interactions between them are indispensable for the normal function of the immune response. Indeed, “mistakes” that happen in either branch can affect the organism as a whole.

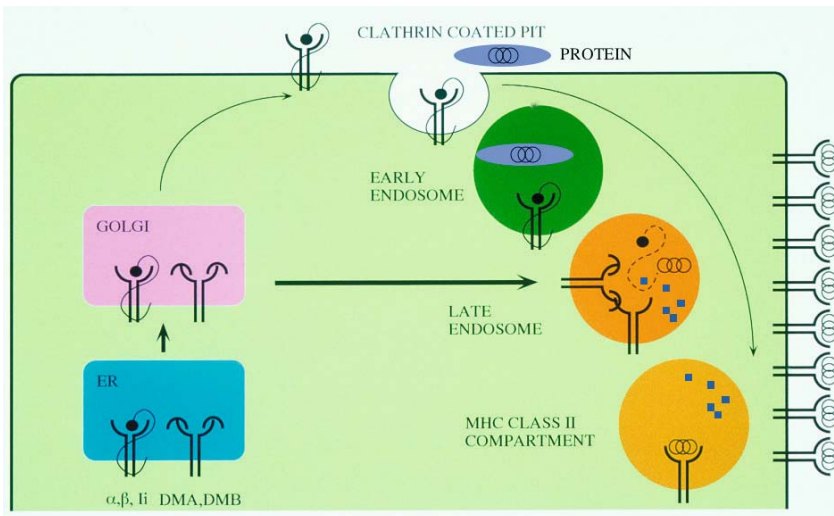
Innate immunity is the first defense against invading pathogens and performs the function of immune surveillance (Calandra et al. 2003; Janeway 2001). Cells that constitute innate immunity are antigen-presenting cells (APCs), including DCs, macrophages and B cells. Innate immunity does not develop any antigen specificity because the discrimination between self and non-self is primarily based on pathogen-associated molecular patterns (PAMP), which are common components of many microorganisms and are not found in humans, i.e., lipopolisaccharides (LPS). Ligation of PAMP with toll-like receptors (TLR) on the surface of APCs (Bachmann and Kopf 2002; Takeda et al. 2003) leads to phagocytosis and antigen processing and presentation (APP). APP results in the presentation of processed antigens to T cells (Cresswell and Lanzavecchia 2001) (Fig. 1) and the establishment of adaptive immunity (Kelly et al. 2002; Smyth et al. 2001).

The function of adaptive immunity is the elimination of non-self antigens and the creation of immune memory. Constituents of adaptive immunity are B and T cells. Adaptive immunity is unique and created only after the encounter with a specific non-self antigen, which is presented to them by APCs. Discrimination between self and non-self at the level of adaptive immunity is complex and involves the elimination or functional inactivation of self-reactive lymphocytes from the repertoire. Es-

**Table 1** Properties of innate and adaptive immunity

	Innate	Adaptive
Tolerance to self	Yes	Yes
Specificity	Low	High
Diversity	Low	High
Memory	None	Yes
Cells	APC	B and T cells

APC, antigen-presenting cells.



**Fig. 1** Pathway of antigen processing and presentation (*APP*). HLA-DP, HLA-DQ, HLA-DR, which are in complex with Ii, and HLA-DM heterodimers are assembled in the endoplasmic reticulum (ER, depicted in *blue*). They travel to the *trans*-Golgi network (depicted in *pink*) and either fuse with the late endosome (depicted in *orange*) or continue to the cell surface. Degradation of phagocytosed antigenic peptides (depicted in *violet*) starts in an early endosome (depicted in *green*). In the late endosome, Ii is removed from the complex with HLA-DP, HLA-DQ, HLA-DR and replaced with antigenic peptides. Loaded with antigens, MHC II molecules travel from MHC class II compartment (MIIC, depicted in *yellow*) to the cell surface, where they activate T cells

establishment of adaptive immunity is slow, but once it is established, it is memorized and able to respond faster to subsequent contacts with the same antigen. However, since innate immunity is faster, it keeps infections under control until the establishment of adaptive immunity (Smyth et al. 2003).

MHC determinants play a very important role in APP, which connects innate and adaptive immunity. Namely, processed antigenic peptides are presented to T cells only in the groove of MHC heterodimers.

### 1.1 Major Histocompatibility Complex Determinants

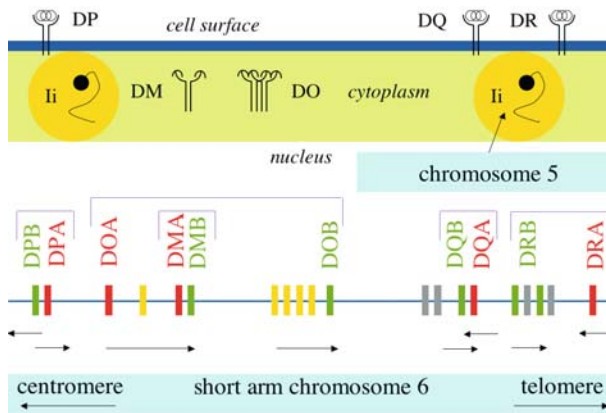
Genes that encode MHC determinants, also known as human leukocyte antigens (HLA), are located on the short arm of chromosome 6 and are extraordinarily polymorphic (Anonymous 1999). They are divided into

two classes: MHC I and MHC II determinants that present intracellular and extracellular antigens, respectively. MHC I determinants are expressed on most nucleated cells, whereas MHC II determinants only on APCs, mature B and activated T cells.

Three different MHC I determinants, namely HLA-A, HLA-B and HLA-C, are assembled and loaded with antigenic peptides, which are generated by protein degradation in the 26S proteasome, in the endoplasmic reticulum (ER) (Fruci et al. 2003; Saveanu et al. 2002). The complex between the MHC I heterodimer and antigenic peptide passes through the *trans*-Golgi network to the cell surface of APCs and activates CD8<sup>+</sup> T cells.

In humans, there are three classic MHC II determinants termed HLA-DP, HLA-DQ and HLA-DR, and two nonclassic determinants called HLA-DM and HLA-DO (Fig. 2). The former are cell-surface heterodimers that present antigenic peptides, whereas the latter are cytoplasmic oligomers that are involved in loading antigenic peptides. HLA-DP, HLA-DQ, HLA-DR and HLA-DM are composed of  $\alpha$  and  $\beta$  chains, whereas HLA-DO form heterotetramers, composed of two  $\alpha$  and two  $\beta$  chains (Fig. 2). Classic MHC II determinants are assembled in the ER in the complex with the invariant chain (Ii), which stabilizes them and prevents premature antigenic peptide loading. This complex is transported to the late endosome that contains degraded antigens. Antigenic peptide loading is a three-step process (Fig. 1). The first two steps, which take place in the late endosome, are the degradation and immediate replacement of Ii by the class II-associated Ii-chain peptide (CLIP). The third step occurs in the MHC class II compartment (MIIC) and is the exchange of CLIP with antigenic peptides. This exchange is mediated by HLA-DM and is regulated by HLA-DO. The complex between MHC II determinants and antigenic peptides then travels to the surface of APCs, where the antigen is presented to CD4<sup>+</sup> T cells, which are the main regulators of the immune response.

Since they present antigens to CD4<sup>+</sup> T cells, MHC II determinants are involved directly in the regulation of the immune response. Therefore, it is not surprising that the precise control of MHC II gene expression, which takes place at the level of transcription, is necessary for the equilibrated function of the immune system. Most of our knowledge about this control has been elucidated from studies of severe combined immunodeficiency called MHC II deficiency or the type II bare lymphocyte syndrome (BLS) (Table 2).



**Fig. 2** The organization and direction of MHC II genes as well as sub-cellular localization of their proteins. Three heterodimers are transcribed from promoters that point in opposite directions (HLA-DPA and HLA-DPB, HLA-DQA and HLA-DQB, HLA-DRA and HLA-DRB), HLA-DO heterotetramer and HLA-DM heterodimer are transcribed from promoters that point towards the telomere. Genes for  $\alpha$  and  $\beta$  chains are shown as red and green bars, respectively. Pseudogenes and MHC II-unrelated genes are shown as grey and yellow bars, respectively. Black arrows point in the direction of transcription. HLA-DP, HLA-DQ and HLA-DR heterodimers are found on the cell surface in complex with antigenic peptides. HLA-DM heterodimers and HLA-DO heterotetramers are cytoplasmic molecules, as well as invariant chain (*Ii*), all of which are also found in MHC class II compartments (MIIC, depicted as yellow circles). Genes coding for HLA-DP, HLA-DQ, HLA-DR, HLA-DM and HLA-DO are located on the short arm of chromosome 6, but gene coding for *Ii* is located on chromosome 5

**Table 2** Properties of bare lymphocyte syndrome

Complementation group	A	B	C	D
MHC II expression	Absent	Absent	Absent	Absent
Enhanceosome	Formed	Not formed	Not formed	Not formed
Mutated gene	<i>MHC2TA</i>	<i>RFXANK/B</i>	<i>RFX5</i>	<i>RFXAP</i>
Mutated protein	CIITA	<i>RFXANK/B</i>	<i>RFX5</i>	<i>RFXAP</i>

*MHC2TA*, major histocompatibility class 2 trans-activator; *RFXANK/B*, regulatory factor X ANK/B; *RFX5*, regulatory factor X 5; *RFXAP*, regulatory factor X AP; CIITA, class II transactivator.

## 1.2 Bare Lymphocyte Syndrome

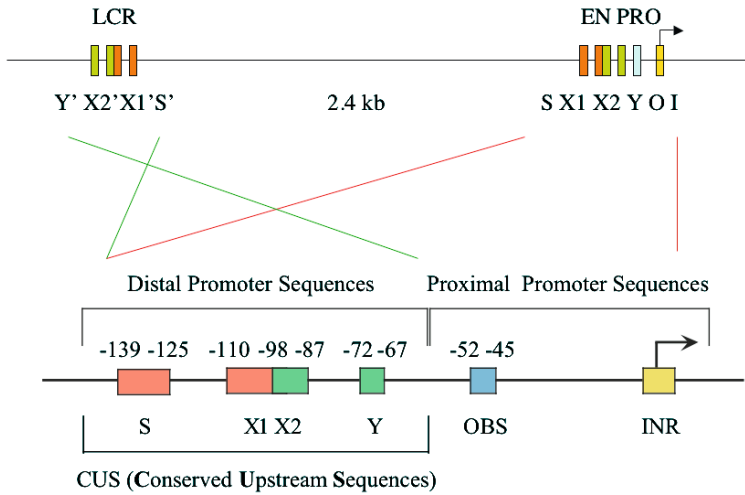
BLS is an autosomal recessive disease characterized by the lack of constitutive and inducible MHC II gene expression (Table 2) (reviewed in Reith and Mach 2001 and Nekrep et al. 2003). It is caused by mutations in factors that direct the transcription from MHC II promoters rather than in MHC II genes themselves.

More than 10 years ago, transcription of MHC II genes was known to require several unidentified proteins. The earliest studies performed to identify these proteins employed *in vitro* assays, which revealed many irrelevant DNA-protein interactions. However, only genetic studies of BLS finally resolved this puzzle. First, CIITA (Steimle et al. 1993) and regulatory factor X5 (RFX5) (Steimle et al. 1995) were identified, which rescued the expression of MHC II determinants in complementation group (CG) A and C, respectively. Subsequently, these findings led to the identification of regulatory factor X, which contains ankyrin repeats (RFXANK/B) (Masternak et al. 1998; Nagarajan et al. 1999) and regulatory factor X associated protein (RFXAP) (Durand et al. 1997), which rescued the expression of MHC II determinants in CGs B and D, respectively. These factors not only account for four CGs of BLS, but also represent all gene-specific *trans*-acting factors that bind *cis*-acting elements for MHC II transcription.

## 1.3 *cis*-Acting Elements

MHC II genes are transcribed from compact promoters (Fig. 3). They contain variable proximal promoter (PPS) and conserved upstream sequences (CUS) (reviewed in Nekrep et al. 2003; Reith and Mach 2001; Ting and Trowsdale 2002).

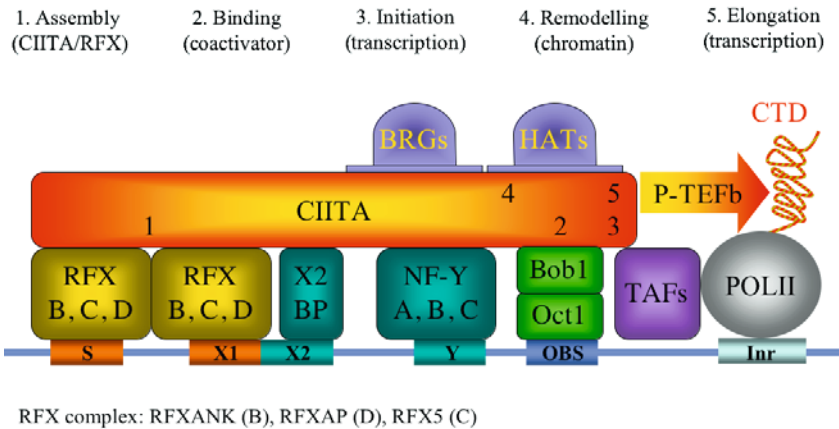
In HLA-DRA PPS are located from position  $-52$  to the transcription start site. From the 5' direction, they contain octamer-binding site (OBS) and initiator (Inr) sequences, but lack a functional TATA box. OBS binds the octamer-binding protein-1 (Oct-1), which recruits the B cell octamer-binding protein 1/Octamer-binding factor 1/Oct co-activator from B cells (Bob1/OBF-1/OCAB) (Matthias 1998), whereas the initiator, which represents the transcription start site, binds RNA polymerase II-associated transcription factor I (TF<sub>II</sub>I) and TATA binding protein (TBP) -associated factor of 250 kDa (TAF<sub>II</sub>250).



**Fig. 3** *cis*-Acting elements in the DRA promoter. Locus control region (LCR), conserved upstream sequences (CUS), and proximal promoter sequences (PPS) are needed for the transcription from the DRA promoter. LCR is located 2.4 kb upstream from CUS. LCR and CUS contain Y', X2', X1', S' and S, X1, X2 and Y boxes, respectively. PPS contain the octamer-binding site (OBS) and the initiator (INR). The spacing between CUS and PPS is presented in the *lower panel*

CUSs are located from positions  $-139$  to  $-67$ . From the 5' direction, they contain S, X and Y boxes. The X box can be further divided into X1 and X2 boxes. Tight spatial constraints are preserved between these boxes (Jabrane-Ferrat et al. 1996), which bind different *trans*-acting factors. S and X1 boxes bind RFX (Fig. 4) (Jabrane-Ferrat et al. 1996; Ting and Trowsdale 2002); the X2 box binds X2 binding protein (X2BP), which can be c-AMP responsive element-binding protein (CREBP) or activator protein 1 (AP-1) (Moreno et al. 1997; Setterblad et al. 1997); and the Y box binds nuclear factor Y (NF-Y) (Fig. 4) (Maity and de Crombrughe 1998; Mantovani 1999).

However, PPS and CUS are not sufficient for HLA-DRA gene expression. A distal locus control region (LCR), which is located approximately 2.4 kb upstream of the CUS, is needed for efficient transcription from the HLA-DRA promoter in the organism (Masternak et al. 2003) (Fig. 3). A LCR is a mirror image of CUS. It contains S', X1', X2' and Y' boxes, but in the opposite orientation from the promoter. Indeed, the LCR binds the same *trans*-acting factors as the CUS.



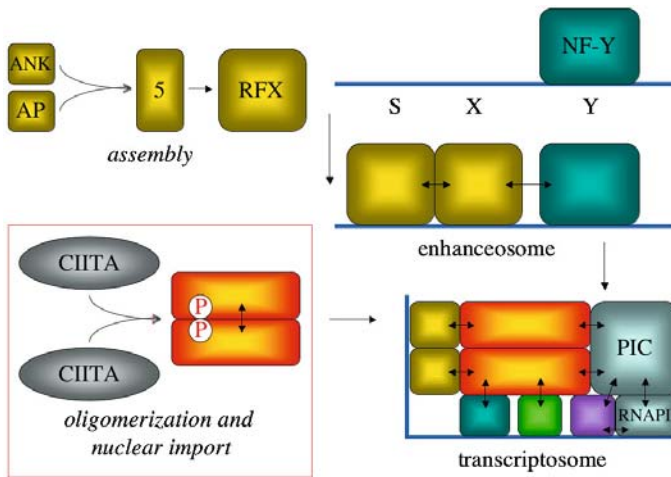
**Fig. 4** *cis*-Acting elements and *trans*-acting factors on MHC II promoters. Conserved upstream sequences (*CUS*) contain S, X and Y boxes. X box is divided into X1 and X2 boxes. In the HLA-DRA gene, the octamer-binding site (*OBS*) and the initiator (*INR*) form the proximal promoter sequences (*PPS*). RFX, which contains RFXANK, RFXAP and RFX5, binds S and X1 boxes. X2 binding protein (*X2BP*) binds X2 box. NF-Y, which contains NF-YA, NF-YB and NF-YC, binds Y box. *OBS* binds octamer binding protein-1 (*Oct-1*), which recruits the B cell octamer-binding protein 1/Octamer binding factor 1/Oct co-activator from B cells (*Bob1/OBF-1/OCAB*). The initiator binds general transcription factors and positions RNA polymerase II (*RNAPII*) on the promoter. CIITA is recruited to the MHC II enhanceosome, which consists of RFX, X2BP and NF-Y on *CUS*. It binds histone acetyl-transferases (*HATs*, *CBP/p300*, *pCAF*) and brahma-related gene (*BRG*) proteins, which remodel the chromatin, *Bob1/OBF-1/OCAB*, GTFs and TAFs, which recruit *RNAPII* and initiate transcription, and positive transcription elongation factor b (*P-TEFb*), which phosphorylates the C terminal domain (*CTD*) of *RNAPII* and facilitates the elongation of transcription. These steps in the transcriptional process are given *above* the drawing

## 1.4 *trans*-Acting Factors

Several *trans*-acting factors are required for MHC II transcription (Fig. 4). Among them, RFX and NF-Y create the platform for other co-activators to access MHC II promoters.

NF-Y is composed of three subunits (reviewed in Matuoka and Yu Chen 1999). NF-YA contains 374 residues. At the N-terminus of NF-YA, there is glutamine-rich region, which is followed by a stretch of prolines, serines and threonines. Its subunit interaction and DNA-binding domains (DBD) are at the C-terminus.





**Fig. 5** Assembly of the MHC II enhanceosome and transcriptosome. RFXANK/B forms complex with RFXAP, which recruits the RFX5 oligomer. RFX binds S and X1 boxes, whereas NF-Y binds Y box. They form the MHC II enhanceosome. The phosphorylation of CIITA leads to its oligomerization and nuclear import. It binds to the MHC II enhanceosome and forms the MHC II transcriptosome, which forms the preinitiation complex (PIC) and recruits as well as modifies RNA polymerase II (RNAPII)

NF-YB contains 207 residues. They form a histone fold and TATA-binding protein (TBP) binding domains. The histone fold is similar to that of histones 2A and 2B (Mantovani 1999), which is responsible for their dimerization. This domain has the same function in NF-Y assembly.

NF-YC contains 335 residues. As with NF-YB, it contains a histone fold and TBP binding domains, and in addition a glutamine-rich region.

RFX is also composed of three subunits (Fig. 5) (reviewed in Reith and Mach 2001). RFXANK/B contains 260 residues that form an acidic domain and four ankyrin repeats, the source of its name.

RFX5 contains 616 residues, which form DBD and PEST domains. It is the fifth member of the RFX family that binds S and X boxes of MHC II promoters, hence its name.

RFXAP contains 272 residues. This protein has acidic, basic and glutamine-rich domains. Because it interacts directly with RFX5, it was named the RFX-associated protein.

Transcription from MHC II promoters starts after productive interactions between *cis*-acting elements and *trans*-acting factors, which lead to the formation of the MHC II enhanceosome and transcriptosome.

## 1.5 MHC II Enhanceosome and Transcriptosome

The MHC II enhanceosome is formed on CUS and LCR. Its assembly requires specific protein–protein and DNA–protein interactions. The formation of the enhanceosome starts off DNA (Fig. 5), where RFX and NF-Y bind loosely. Both RFX and NF-Y are formed in two steps. RFX-ANK/B forms a complex with RFXAP, which binds the RFX5 oligomer (Jabrane-Ferrat et al. 2002). NF-YB and NF-YC first form heterodimer via their histone folds and then bind NF-YA. RFX and NF-Y are linked via interactions between RFXANK/B, NF-YA and NF-YC (Jabrane-Ferrat et al. 2002; Nekrep et al. 2003; Ting and Trowsdale 2002). The binding of the enhanceosome to promoters occurs via RFX5, NF-YB and NF-YC DBDs. Each individual contact between the enhanceosome and DNA takes place on the same side of the double helix. As mentioned earlier, preserved spatial constraints are present in CUS and LCR. Completely invariant spacing between S and X boxes can be explained by the RFX5 oligomers, which bind each other next to their DBDs. These higher-order RFX complexes can occupy S and X boxes only if the exact spacing between them is preserved. Full helical turns are tolerated between X and Y boxes. This finding can be explained because their binding occurs far from their DBDs.

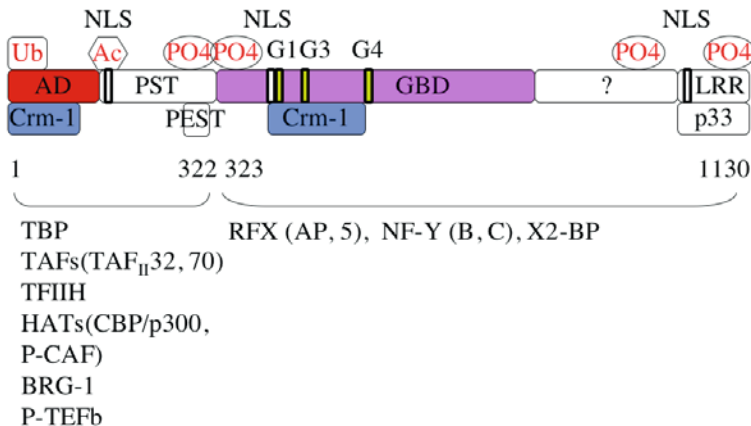
NF-Y not only interacts with RFX, but also with different histone acetyltransferases (HAT) that make chromatin accessible for other co-activators (Fontes et al. 1999; Harton et al. 2001). Moreover, NF-YB and NF-YC bind TBP *in vitro*. This interaction helps to attract general transcription factors (GTF) to promoters, which lack a TATA box. The X2 box is bound by CREBP or AP-1 (Moreno et al. 1995; Setterblad et al. 1997).

The very last step is the recruitment of CIITA to the MHC II enhanceosome, which converts it into the MHC II transcriptosome (Fig. 5). Once the transcriptosome is formed, MHC II genes can be transcribed.

## 2 Class II Transactivator

CIITA is the master regulator of MHC II gene expression. Except for CIITA, all *trans*-acting factors that are needed for the transcription of MHC II genes are expressed ubiquitously. Thus, the synthesis of MHC II determinants correlates directly with the presence of CIITA, which is expressed constitutively only in DCs and mature B cells and is inducible in most other cell types. After CIITA is recruited to the MHC II enhanceosome, it recruits the general transcriptional machinery.

CIITA contains 1,130 residues (Fig. 6). It can be divided into several domains, which are the N-terminal activation (AD), proline/serine/threonine-rich (PST) and GTP-binding domains (GBD), as well as the C-ter-



**Fig. 6** The scheme of CIITA, its post-translational modifications and partner proteins. CIITA is divided into several domains, which are activation (AD), proline/serine/threonine-rich (PST), GTP binding (GBD) domains and leucine-rich repeats (LRR). Additionally, two nuclear localization signals (NLS) are located in the N-terminus and one in the C-terminus of the protein. Within PST domain there is a consensus proline/glutamine/serine/threonine-rich (PEST) sequence. GBD contains three GTP interacting sequences, designated by G1, G3 and G4. Positions of post-translational modifications of CIITA, which are phosphorylation, acetylation and ubiquitylation, are given above the drawing in red letters as PO4, Ac and Ub, respectively. Chromosomal region maintenance-1 protein (Crm-1, depicted as a purple box) interacts with AD and GBD, and p33 (depicted as a white box) binds LRR. Transcriptional co-activators (TBP, TAFs, HATs, BRG-1, P-TEFb) as well as constituents of the MHC II enhanceosome (RFXAP, RFX5, NF-YB, NF-YC and X2BP), which interact with CIITA, are listed under the drawing

minal leucine-rich repeat (LRR). Additionally, CIITA contains three nuclear localization signals (NLS) and two putative nuclear export sequences (NES) (Fig. 6).

The optimal AD of CIITA spans the first 322 residues. Its amino terminal part is rich in aspartic and glutamic acids and resembles the classic acidic AD, similar to that of VP16. Indeed, it can be replaced by AD from VP16 (herpes simplex virus) and E1a (adenovirus) (Riley and Boss 1993). Between residues 145 and 322 is the PST domain. As its name implies, it contains several prolines, serines and threonines, which are targets for post-translational modifications. A consensus PEST sequence is located from positions 283 to 308, but it does not represent a degradation motif or degron (Schnappauf et al. 2003). Rather, degrons are located within the first 99 residues and from positions 230 to 260 of CIITA, respectively (Schnappauf et al. 2003).

A putative GBD resides downstream of AD, from positions 420 to 561. In contrast with the classic GBDs, which contain four GTP-interacting sequences, CIITA contains only three (Harton et al. 1999). These are Walker type A motif (G1), magnesium coordination site (G3) and a site that confers specificity for guanosine (G4). Indeed, although CIITA binds GTP, it does not hydrolyse it in vitro (Harton et al. 1999). This finding suggests that CIITA might be a constitutively active GTP-binding protein. As mutations of either motif reduce the activity of CIITA, the sole function of GTP binding to CIITA may be to alter its conformation.

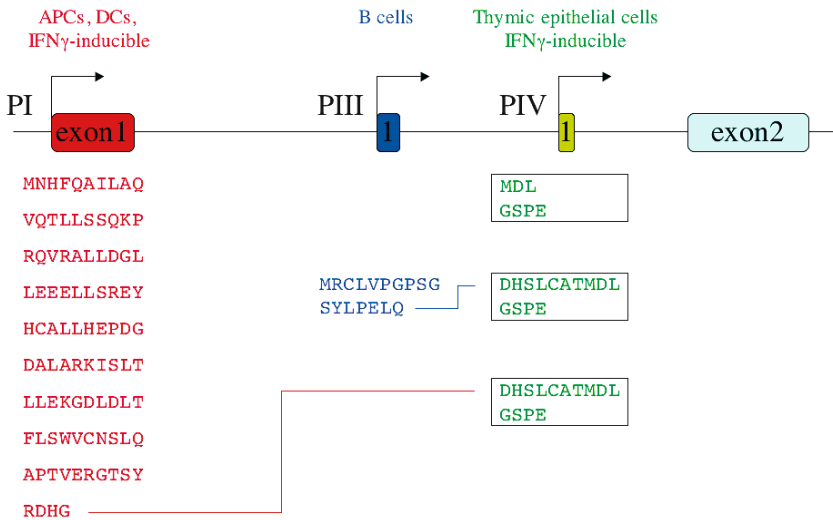
Five or six consensus LRR are located from positions 988 to 1,097 (Harton et al. 2002). They bind a 33-kDa protein of unknown function (Hake et al. 2000). Certain mutations positioned in  $\alpha$  helices of the LRR, but not in  $\beta$  sheets decrease the activity of CIITA. An additional LRR flanking the GBD might be involved in the aggregation of CIITA (Linhoff et al. 2001).

In CIITA, three NLS and two NES have been described. A bipartite NLS is located from positions 141 to 159, and two additional NLS were mapped from positions 405 to 414, next to G1 and 940 to 963 in the LRR (Cressman et al. 1999; Cressman et al. 2001; Nekrep et al. 2002; Spilianakis et al. 2000). NES have been poorly characterized. The first is located in the first 114 residues, the second from positions 408 to 550 (Kretsovali et al. 2001). However, none of these NLS and NES have been examined directly.

## 2.1 Regulation of CIITA Gene Expression

Since the transcription of MHC II genes is dependent on the presence of CIITA, it is not surprising that its expression is highly regulated. In general, there are two types of regulation of gene expression: genetic and epigenetic. CIITA employs both. Whereas specific activators dictate active transcription, epigenetic silencing occurs via chromatin condensation.

Transcription of CIITA can be initiated from up to three different promoters called PI, PIII and PIV (Fig. 7) (Muhlethaler-Mottet et al. 1997). Whereas in myeloid DCs, constitutive expression of CIITA is initiated from PI, in plasmacytoid DCs and B cells, it is initiated from PIII (Muhlethaler-Mottet et al. 1997). IFN- $\gamma$  inducible expression of CIITA is mediated by PI in bone marrow-derived APCs and myeloid DCs and by PIV in somatic cells (Muhlethaler-Mottet et al. 1997; Waldburger et al.



**Fig. 7** CIITA promoters, their composition and specificity. CIITA is transcribed from three different promoters: PI, PIII and PIV with unique exons 1 (depicted as red, purple, and green boxes for PI, PIII, and PIV, respectively), which are spliced into common exon 2 (depicted as a blue box). The additional sequences in CIITA, transcribed from PI is given in red letters and from PIII in purple letters, while a common sequence from all promoters is given in green letters. Cell types and stimuli are given above the scheme. IFN- $\gamma$ , interferon gamma, APCs antigen-presenting cells, DCs dendritic cells

2001). All promoters have a unique exon 1, which is spliced into the common exon 2 (Muhlethaler-Mottet et al. 1997). Translation of CIITA transcripts from PI and PIII starts from the first methionine in exon 1, whereas from PIV it begins from the first methionine in exon 2. This brings unique sequences to the N-terminus of CIITA (Muhlethaler-Mottet et al. 1997) and results in three isoforms (IF) of CIITA: IFI, IFIII and IFIV. IFI and IFIII contain additional 94 and 17 residues, respectively. The additional sequence in IFI bears homology with the caspase recruitment domain (CARD) (Nickerson et al. 2001) and most likely represents a new protein-protein interaction domain. It also has the highest transcriptional activity (Nickerson et al. 2001). Importantly, a single mutation of a conserved leucine in CARD abrogates the activity of CIITA (Nickerson et al. 2001).

Epigenetic regulation of CIITA gene expression is utilized for embryonic survival. Notably, immune responses against paternal antigens in the placenta cause fetal death. In these tissues, promoter hypermethylation and histone deacetylation are proposed mechanisms for silencing the transcription of CIITA (Holtz et al. 2003; Morris et al. 2000; van den Elsen et al. 2000). Derepression of CIITA transcription in trophoblasts after treatment with methylation inhibitors and trichostatin A with IFN- $\gamma$  supports this mechanism.

In immature DCs, the expression of CIITA is observed from PI, but it does not lead to APP. Phagocytosis of an antigen leads to DC maturation, which is accompanied by the cessation of further phagocytosis and repression of de novo CIITA and MHC II transcription and up-regulation of APP (Cella et al. 1997; Landmann et al. 2001; Pierre et al. 1997; Turley et al. 2000). These events might be mediated by TLRs. Because only the specific population of encountered antigens are processed and presented to T cells, such regulation could represent the best way of establishing adaptive immunity. Since the expression of CIITA is shut down after the clearance of a foreign antigen, it also lowers the chance of DCs randomly presenting self-antigens.

In B cells, the constitutive expression of CIITA from PIII is accompanied by the presence of high levels of MHC II determinants on the cell surface. However, after B cells differentiate into highly specialized antibody-producing plasma cells, the expression of CIITA and MHC II determinants is lost (Chen et al. 2002). B lymphocyte inhibitory maturation protein 1 (BLIMP1) begins the process of this silencing (Piskurich et al. 2000).

CIITA gene expression can be induced with IFN- $\gamma$  from PI and PIV. PIV contains gamma-activated sites (GAS), which bind the signal trans-

ducer and activator of transcription 1 (STAT1) and an interferon regulatory factor-1 binding site, which binds interferon regulatory factor-1 (IRF-1) (Muhlethaler-Mottet et al. 1998). Since PI is also responsive to IFN- $\gamma$ , it could contain the same elements. After stimulation with IFN- $\gamma$ , activated, phosphorylated STAT 1 translocates into the nucleus and binds GAS. Activated STAT 1 also induces the expression and accumulation of IRF-1. Binding of STAT 1 to GAS itself causes a weak acetylation of histones 3 and 4 in PIV (Morris et al. 2002). However, the hyperacetylation of histones, which makes transcription more efficient, occurs only after the accumulation and binding of IRF-1 to its site.

Thus, the regulation of transcription of the CIITA gene represents the first level of control of CIITA function. The second level consists of post-translational modifications of CIITA.

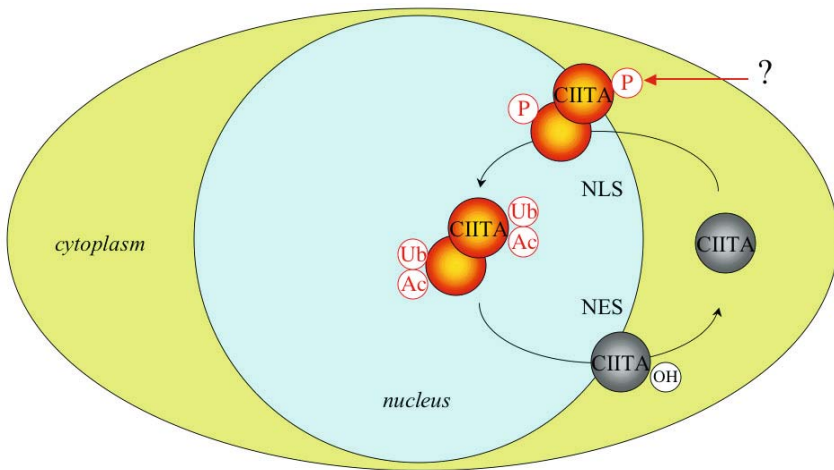
## 2.2

### Post-Translational Modifications of CIITA

CIITA is post-translationally modified by acetylation, phosphorylation and ubiquitylation (Fig. 8). The effects of these modifications are complex and far from being clearly understood, but a general picture can be drawn from several studies. The fate of CIITA in the cell is not random, but each step, starting after its translation and ending with its possible degradation, is regulated precisely by the following modifications.

Phosphorylation is the first post-translational modification of CIITA. Two studies have shown that this modification increases the activity of CIITA. The first study mapped phosphorylation sites into the PST region from positions 253 to 321 (Tosi et al. 2002). The second study showed that protein kinase A (PKA) phosphorylates CIITA on one or more serines in the region between PST and GBD (Sisk et al. 2003). The phosphorylation of CIITA leads to its accumulation, oligomerization and nuclear translocation. Most likely, these latter events happen because the phosphorylation changes the conformation of CIITA and exposes its NLS.

After it translocates into the nucleus, CIITA is acetylated on lysines 141 and 144, by CBP/p300 and also by pCAF, which are located in the nucleus (Spilianakis et al. 2000). Acetylation of these residues keeps CIITA in the nucleus and increases the stability of the MHC II transcrip-tosome. Acetylation might also facilitate the subsequent ubiquitylation of CIITA. Indeed, recent data suggest that histone deacetylases (HDACs) are involved in the ubiquitylation of CIITA (Greer et al. 2003).



**Fig. 8** Post-translational modifications of CIITA. After an unknown signal (depicted as the *red arrow*), CIITA is phosphorylated, which causes its oligomerization and nuclear translocation (*black arrow* directed from the cytoplasm into the nucleus). Acetylation and ubiquitylation increase CIITA transcriptional activity. Termination of transcription is accompanied by additional CIITA modifications, possibly phosphorylation that might result in the translocation of CIITA into the cytoplasm (*black arrow* directed from the nucleus into the cytoplasm) or in its degradation. CIITA monomers and oligomers are depicted as *gray* and *orange circles*. CIITA phosphorylation is represented as a *red P* attached to CIITA. CIITA acetylation and ubiquitylation are depicted as *red Ac* and *Ub*, respectively. *NLS* and *NES* stand for nuclear localization signal and nuclear export sequence, respectively

Ubiquitylation, which is a covalent modification of lysines, plays an important role in transcription. Monoubiquitylated transcription factors tend to be more potent activators, whereas polyubiquitylated proteins are destined for degradation by the 26S proteasome. The role of ubiquitylation of CIITA has been addressed in two studies, but clear conclusions on the function of ubiquitin cannot be reached. In the first study, monoubiquitin fused to CIITA prevented the degradation of the modified CIITA protein, but did not affect its transcriptional activity (Schnappauf et al. 2003). In the second study, CIITA co-expressed with ubiquitin had a higher activity than CIITA alone, which was even more pronounced if ubiquitin could not form oligomers (Greer et al. 2003). This effect of ubiquitin was on the stabilization of the MHC II transposome rather than on transcription itself. Interestingly, the same study showed that HDAC prevented the ubiquitylation of CIITA.



Phosphorylation also completes the post-translational modifications of CIITA. Interestingly, PKA inactivates CIITA with phosphorylation of serines 834 and 1050. (Li et al. 2001). Does this modification influence the stability of the enhanceosome? Does it lead to the degradation of CIITA, or does it expose the NES and enable the export of CIITA from the nucleus?

## 2.3

### CIITA Is a Shuttling Protein

Transcription factors and co-factors need to be transported into the nucleus to perform their function. Nucleocytoplasmic shuttling usually requires two types of functionally unique signals, NLS and NES. CIITA contains both, but none of them have been examined adequately (Fig. 6).

At steady state, CIITA is distributed equally between the nucleus and the cytoplasm. Mutant CIITA proteins, which do not follow this pattern, led to the identification of NLS. As mentioned earlier, two NLS are located at the N-terminus and one NLS in the C-terminus of CIITA (Fig. 6) (Cressman et al. 1999, 2001; Spilianakis et al. 2000). However, none of them has been examined directly, i.e., they have not been demonstrated to shuttle a heterologous protein. Moreover, binding to importins has not been investigated.

Two regions in the N-terminus of CIITA have been proposed to function as NES (Fig. 6). The only support comes from interactions between these regions with the chromosomal region maintenance-1 (Crm-1) protein (Kretsovali et al. 2001). Indeed, treatment with leptomycin B (LMB), which blocks Crm-1-dependent nuclear export (Kudo et al. 1998), leads to the nuclear accumulation of CIITA (Cressman et al. 2001; Kretsovali et al. 2001). However, no consensus NES can be found within these sequences. In addition, none of the five putative NES, three of which correspond perfectly to the consensus sequence [Lx(2,3)Lx(2,3)-LxL], functioned in a direct export or bound Crm-1 (G. Drozina, J. Kohoutek, B.M. Peterlin, unpublished observation).

All these results imply the presence of other transport mechanisms. Indeed, several additional regions of CIITA are involved in its nuclear localization, including GBD (Harton et al. 1999; Raval et al. 2003) and LRR (Hake et al. 2000; Harton et al. 2002; Towey and Kelly 2002). Moreover, some mutations in the LRR disrupt the interaction between p33 and CIITA, which interferes with its nuclear localization (Hake et al. 2000). Furthermore, it was suggested that NES from CIITA resemble the snur-

portin-1 NES, which is discontinuous (Paraskeva et al. 1999). In this case, only after a conformational change can Crm-1 bind and transport snurportin-1 from the nucleus to the cytoplasm (Paraskeva et al. 1999).

## 2.4 CIITA Is a Transcriptional Integrator

Once CIITA is modified appropriately and present in the nucleus, it can function. It binds to the MHC II enhanceosome, attracts several transcription factors and co-factors, and integrates initiation and elongation of transcription as well as chromatin remodeling into a process that finally results in the expression of MHC II genes. Moreover, CIITA might also be involved in the dissociation of the MHC II enhanceosome after the termination of transcription.

CIITA recruits RNA polymerase II (RNAPII) to MHC II promoters and increases transcription initiation and elongation rates. For the former function, interactions with TAFs (Fontes et al. 1997), TFIIB (Mahanta et al. 1997) and Bob1/OBF-1/OCAB (Fontes et al. 1996) might be necessary. For the latter, the interaction between CIITA and the positive transcription elongation factor b (P-TEFb), which phosphorylates the CTD of RNAPII, is needed (Kanazawa et al. 2000).

Not surprisingly, CIITA also recruits HATs and chromatin remodeling machinery to the HLA-DRA promoter. The AD of CIITA binds CBP/p300 (Fontes et al. 1999), pCAF (Spilianakis et al. 2000) and Brahma-related gene 1 (BRG-1) (Mudhasani and Fontes 2002). In addition, it has been suggested that CIITA possesses intrinsic HAT activity (Harton et al. 2001; Raval et al. 2001). This CIITA activity has been mapped into AD and bears homology with other HAT domains, e.g., in CBP/p300 (Harton et al. 2001). Interestingly, CIITA that lacks its AD is still able to mediate the acetylation of histone 4, but not of histone 3 (Beresford and Boss 2001). Thus, CIITA could contain another region with direct or indirect HAT activity.

After the termination of transcription, histone deacetylation, which is mediated by HDACs, is involved in chromatin condensation. Binding of CIITA to HDAC1 and HDAC2 decreases the activity of CIITA, most probably because histone deacetylation disrupts the MHC II enhanceosome (Zika et al. 2003). Thus, CIITA might play an active role in the regulation of its own function on MHC II promoters.

### 3 Conclusions and Future Directions

In the last few years, much research has resulted in greater understanding of the function and regulation of CIITA. Since CIITA plays a major role in the regulation of the immune response via MHC II determinants, this is not surprising. Moreover, if the rules of this regulation were understood this knowledge could be used to manipulate the immune system. However, many questions remain.

Why is CIITA transcribed from three distinct promoters? Are different CIITA IFs modified differently, thus influencing their activity and regulation?

Are post-translational modifications independent from each other or are they carefully orchestrated or sequential? CIITA phosphorylation increases or decreases CIITA activity, depending on phosphorylated residues. What signals regulate this phosphorylation that results in activation and inactivation of CIITA, and how? Why does the acetylated CIITA protein accumulate in the nucleus? What is the role of its ubiquitylation? Where is CIITA ubiquitylated? These and probably many more questions regarding the post-translational modifications of CIITA still need to be answered.

The shuttling of CIITA is a big puzzle. Since CIITA has not been shown to bind importins and since no NES can be found, how does CIITA shuttle? And why does it shuttle at all?

A lot is known about the *cis*-acting elements in the MHC II promoters, but the role of LCR needs more attention. What is the mode of action of these LCR? Do they also bring P-TEFb to RNAPII? How many LCR are there in the MHC cluster?

Once these questions have been answered, the manipulation of the immune system could become possible and eventually applicable in clinical medicine. Cancer and inadequate vaccination are examples of weak immune responses. Introduction of the constitutively active CIITA proteins could turn cancer cells into APCs. They would then present their own antigenic peptides to CD4<sup>+</sup> T cells to activate and direct immune response against transformed cells. Vaccination could also be more effective with the addition of CIITA. Autoimmunity is caused by the inappropriate responses to self-antigens. Dominant negative CIITA proteins could attenuate immune responses as well as the need for other immunosuppressive therapies. Thus, CIITA could become a new tool for immunotherapy in humans.

## References

- Anonymous (1999) Complete sequence and gene map of a human major histocompatibility complex. The MHC sequencing consortium [comment]. *Nature* 401:921–923
- Bachmann ME, Kopf M (2002) Balancing protective immunity and immunopathology. *Curr Opin Immunol* 14:413–419
- Beresford GW, Boss JM (2001) CIITA coordinates multiple histone acetylation modifications at the HLA-DRA promoter. *Nat Immunol* 2:652–657
- Calandra T, Froidevaux C, Martin C, Roger T (2003) Macrophage migration inhibitory factor and host innate immune defenses against bacterial sepsis. *J Infect Dis* 187 [Suppl 2]:S385–S390
- Cella M, Engering A, Pinet V, Pieters J, Lanzavecchia A (1997) Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells. *Nature* 388:782–787
- Chen X, Laur O, Kambayashi T, Li S, Bray RA, Weber DA, Karlsson L, Jensen PE (2002) Regulated expression of human histocompatibility leukocyte antigen (HLA)-DO during antigen-dependent and antigen-independent phases of B cell development. *J Exp Med* 195:1053–1062
- Cressman DE, Chin KC, Taxman DJ, Ting JP (1999) A defect in the nuclear translocation of CIITA causes a form of type II bare lymphocyte syndrome. *Immunity* 10:163–171
- Cresswell P, Lanzavecchia A (2001) Antigen processing and recognition. *Curr Opin Immunol* 13:11–12
- Cressman DE, O'Connor WJ, Greer SF, Zhu XS, Ting JP (2001) Mechanisms of nuclear import and export that control the subcellular localization of class II transactivator. *J Immunol* 167:3626–3634
- Durand B, Sperisen P, Emery P, Barras E, Zufferey M, Mach B, Reith W (1997) RFXAP, a novel subunit of the RFXDNA binding complex is mutated in MHC class II deficiency. *EMBO J* 16:1045–1055
- Fontes JD, Jabrane-Ferrat N, Toth CR, Peterlin BM (1996) Binding and cooperative interactions between two B cell-specific transcriptional coactivators. *J Exp Med* 183:2517–2521
- Fontes JD, Jiang B, Peterlin BM (1997) The class II trans-activator CIITA interacts with the TBP-associated factor TAFII32. *Nucleic Acids Res* 25:2522–2528
- Fontes JD, Kanazawa S, Jean D, Peterlin BM (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
- Fruci D, Lauvau G, Saveanu L, Amicosante M, Butler RH, Polack A, Ginhoux F, Lemonnier F, Firat H, van Endert PM (2003) Quantifying recruitment of cytosolic peptides for HLA class I presentation: impact of TAP transport. *J Immunol* 170:2977–2984
- Greer SF, Zika E, Conti B, Zhu XS, Ting JP (2003) Enhancement of CIITA transcriptional function by ubiquitin. *Nat Immunol* 4:1074–1082
- Hake SB, Masternak K, Kammerbauer C, Janzen C, Reith W, Steimle V (2000) CIITA leucine-rich repeats control nuclear localization, in vivo recruitment to the major

- histocompatibility complex (MHC) class II enhanceosome MHC class II gene transactivation. *Mol Cell Biol* 20:7716–7725
- Harton JA, Cressman DE, Chin KC, Der CJ, Ting JP (1999) GTP binding by class II transactivator: role in nuclear import [comment]. *Science* 285:1402–1405
- Harton JA, Zika E, Ting JP (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
- Harton JA, O'Connor W Jr, Conti BJ, Linhoff MW, Ting JP (2002) Leucine-rich repeats of the class II transactivator control its rate of nuclear accumulation. *Hum Immunol* 63:588–601
- Holtz R, Choi JC, Petroff MG, Piskurich JF, Murphy SP (2003) Class II transactivator (CIITA) promoter methylation does not correlate with silencing of CIITA transcription in trophoblasts. *Biol Reprod* 69:915–924
- Jabrane-Ferrat N, Fontes JD, Boss JM, Peterlin BM (1996) Complex architecture of major histocompatibility complex class II promoters: reiterated motifs and conserved protein-protein interactions. *Mol Cell Biol* 16:4683–4690
- Jabrane-Ferrat N, Nekrep N, Tosi G, Esserman LJ, Peterlin BM (2002) Major histocompatibility complex class II transcriptional platform: assembly of nuclear factor Y and regulatory factor X (RFX) on DNA requires RFX5 dimers. *Mol Cell Biol* 22:5616–5625
- Janeway CA Jr (2001) How the immune system works to protect the host from infection: a personal view. *Proc Natl Acad Sci USA* 98:7461–7468
- Kanazawa S, Okamoto T, Peterlin BM (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
- Kelly JM, Takeda K, Darcy PK, Yagita H, Smyth MJ (2002) A role for IFN-gamma in primary and secondary immunity generated by NK cell-sensitive tumor-expressing CD80 in vivo. *J Immunol* 168:4472–4479
- Kretsovali A, Spilianakis C, Dimakopoulos A, Makatounakis T, Papamatheakis J (2001) Self-association of class II transactivator correlates with its intracellular localization and transactivation. *J Biol Chem* 276:32191–32197
- Kudo N, Wolff B, Sekimoto T, Schreiner EP, Yoneda Y, Yanagida M, Horinouchi S, Yoshida M (1998) Leptomycin B inhibition of signal-mediated nuclear export by direct binding to CRM1. *Exp Cell Res* 242:540–547
- Landmann S, Muhlethaler-Mottet A, Bernasconi L, Suter T, Waldburger JM, Masternak K, Arrighi JE, Hauser C, Fontana A, 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 JA, Zhu X, Ting JP (2001) Downregulation of CIITA function by protein kinase A (PKA)-mediated phosphorylation: mechanism of prostaglandin E, cyclic AMP, PKA inhibition of class II major histocompatibility complex expression in monocytic lines. *Mol Cell Biol* 21:4626–4635
- Linhoff MW, Harton JA, Cressman DE, Martin BK, Ting JP (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
- Mahanta SK, Scholl T, Yang FC, Strominger JL (1997) Transactivation by CIITA, the type II bare lymphocyte syndrome-associated factor, requires participation of

- multiple regions of the TATA box binding protein. *Proc Natl Acad Sci USA* 94:6324–6329
- Maity SN, de Crombrughe 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
- Masternak K, Barras E, Zufferey M, Conrad B, Corthals G, Aebersold R, Sanchez JC, Hochstrasser DF, Mach B, 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, Peyraud N, Krawczyk M, Barras E, Reith W (2003) Chromatin remodeling and extragenic transcription at the MHC class II locus control region. *Nat Immunol* 4:132–137
- Matthias P (1998) Lymphoid-specific transcription mediated by the conserved octamer site: who is doing what? *Semin Immunol* 10:155–163
- Matuoka K, Yu Chen K (1999) Nuclear factor Y (NF-Y) and cellular senescence. *Exp Cell Res* 253:365–371
- Moreno CS, Emery P, West JE, Durand B, Reith W, Mach B, Boss JM (1995) Purified X2 binding protein (X2BP) cooperatively binds the class II MHCX box region in the presence of purified RFX, the X box factor deficient in the bare lymphocyte syndrome. *J Immunol* 155:4313–4321
- Moreno CS, Rogers EM, Brown JA, Boss JM (1997) Regulatory factor X, a bare lymphocyte syndrome transcription factor, is a multimeric phosphoprotein complex. *J Immunol* 158:5841–5848
- Morris AC, Spangler WE, Boss JM (2000) Methylation of class II trans-activator promoter IV: a novel mechanism of MHC class II gene control. *J Immunol* 164:4143–4149
- Morris AC, Beresford GW, Mooney MR, Boss JM (2002) Kinetics of a gamma interferon response: expression and assembly of CIITA promoter IV and inhibition by methylation. *Mol Cell Biol* 22:4781–4791
- Mudhasani R, Fontes JD (2002) The class II transactivator requires brahma-related gene 1 to activate transcription of major histocompatibility complex class II genes. *Mol Cell Biol* 22:5019–5026
- Muhlethaler-Mottet A, Otten LA, Steimle V, 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
- Muhlethaler-Mottet A, Di Berardino W, Otten LA, Mach B (1998) Activation of the MHC class II transactivator CIITA by interferon-gamma requires cooperative interaction between Stat1 and USF-1. *Immunity* 8:157–166
- Nagarajan UM, Louis-Pence P, DeSandoro A, Nilsen R, Bushey A, Boss JM (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
- Nekrep N, Jabrane-Ferrat N, Wolf HM, Eibl MM, Geyer M, Peterlin BM (2002) Mutation in a winged-helix DNA-binding motif causes atypical bare lymphocyte syndrome. *Nat Immunol* 3:1075–1081

- Nekrep N, Fontes JD, Geyer M, Peterlin BM (2003) When the lymphocyte loses its clothes. *Immunity* 18:453–457
- Nickerson K, Sisk TJ, Inohara N, Yee CS, Kennell J, Cho MC, Yannie PJ 2nd, Nunez G, Chang CH (2001) Dendritic cell-specific MHC class II transactivator contains a caspase recruitment domain that confers potent transactivation activity. *J Biol Chem* 276:19089–19093
- Paraskeva E, Izaurralde E, Bischoff FR, Huber J, Kutay U, Hartmann E, Luhrmann R, Gorlich D (1999) CRM1-mediated recycling of snurportin 1 to the cytoplasm. *J Cell Biol* 145:255–264
- Pierre P, Turley SJ, Gatti E, Hull M, Meltzer J, Mirza A, Inaba K, Steinman RM, Mellman I (1997) Developmental regulation of MHC class II transport in mouse dendritic cells. *Nature* 388:787–792
- Piskurich JF, Lin KI, Lin Y, Wang Y, Ting JP, Calame K (2000) BLIMP-1 mediates extinction of major histocompatibility class II transactivator expression in plasma cells. *Nat Immunol* 1:526–532
- Raval A, Howcroft TK, Weissman JD, Kirshner S, Zhu XS, Yokoyama K, Ting J, Singer DS (2001) Transcriptional coactivator CIITA, is an acetyltransferase that bypasses a promoter requirement for TAF(II)250. *Mol Cell* 7:105–115
- Raval A, Weissman JD, Howcroft TK, Singer DS (2003) The GTP-binding domain of class II transactivator regulates its nuclear export. *J Immunol* 170:922–930
- Reith W, Mach B (2001) The bare lymphocyte syndrome and the regulation of MHC expression. *Annu Rev Immunol* 19:331–373
- Riley JL, Boss JM (1993) Class II MHC transcriptional mutants are defective in higher order complex formation. *J Immunol* 151:6942–6953
- Saveanu L, Fruci D, van Endert P (2002) Beyond the proteasome: trimming, degradation and generation of MHC class I ligands by auxiliary proteases. *Mol Immunol* 39:203–215
- Schnappauf F, Hake SB, Camacho Carvajal MM, Bontron S, Lisowska-Grospierre B, Steimle V (2003) N-terminal destruction signals lead to rapid degradation of the major histocompatibility complex class II transactivator CIITA. *Eur J Immunol* 33:2337–2347
- Setterblad N, Peterlin BM, Andersson G (1997) Role of the X2 box in activated transcription from the DRA promoter in B cells. *Immunogenetics* 46:318–325
- Sisk TJ, Nickerson K, Kwok RP, Chang CH (2003) Phosphorylation of class II transactivator regulates its interaction ability and transactivation function. *Int Immunol* 15:1195–1205
- Smyth MJ, Godfrey DI, Trapani JA (2001) A fresh look at tumor immunosurveillance and immunotherapy. *Nat Immunol* 2:293–299
- Smyth MJ, Takeda K, Hayakawa Y, Peschon JJ, van den Brink MR, Yagita H (2003) Nature's TRAIL—on a path to cancer immunotherapy. *Immunity* 18:1–6
- Spilianakis C, Papamatheakis J, 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 LA, Zufferey M, 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 MR, Mach B, 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
- Takeda K, Kaisho T, Akira S (2003) Toll-like receptors. *Ann Rev Immunol* 21:335–376
- Ting JP, Trowsdale J (2002) Genetic control of MHC class II expression. *Cell* 109 [Suppl]:S21–S33
- Tosi G, Jabrane-Ferrat N, Peterlin BM (2002) Phosphorylation of CIITA directs its oligomerization, accumulation and increased activity on MHCII promoters. *EMBO J* 21:5467–5476
- Towey M, Kelly AP (2002) Nuclear localisation of CIITA is controlled by a carboxy terminal leucine-rich repeat region. *Mol Immunol* 38:627–634
- Turley SJ, Inaba K, Garrett WS, Ebersold M, Unternaehrer J, Steinman RM, Mellman I (2000) Transport of peptide-MHC class II complexes in developing dendritic cells. *Science* 288:522–527
- Van den Elsen PJ, van der Stoep N, Vietor HE, Wilson L, van Zutphen M, Gobin SJ (2000) Lack of CIITA expression is central to the absence of antigen presentation functions of trophoblast cells and is caused by methylation of the IFN-gamma inducible promoter (PIV) of CIITA. *Hum Immunol* 61:850–862
- Waldburger JM, Suter T, Fontana A, Acha-Orbea H, 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 [comment]. *J Exp Med* 194:393–406
- Zika E, Greer SF, Zhu XS, Ting JP (2003) Histone deacetylase 1/mSin3A disrupts gamma interferon-induced CIITA function and major histocompatibility complex class II enhanceosome formation. *Mol Cell Biol* 23:3091–3102