

CIITA-induced occupation of MHC class II promoters is independent of the cooperative stabilization of the promoter-bound multi-protein complexes

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Abstract

Precise regulation of MHC class II expression plays a crucial role in the control of the immune response. The transactivator CIITA behaves as a master controller of constitutive and inducible MHC class II gene activation, but its exact mechanism of action is not known. Activation of MHC class II promoters requires binding of at least three distinct multi-protein complexes (RFX, X2BP and NF-Y). It is known that the stability of this binding results from cooperative interactions between these proteins. We show here that expression of CIITA in MHC class II⁻ cells triggers occupation of the promoters by these complexes. This observation raised the possibility that the effect of CIITA on promoter occupation is mediated by an effect on the cooperative stabilization of the DNA-bound multi-protein complexes. We show, however, that the presence of CIITA does not affect the stability of the higher-order protein complex formed on DNA by RFX, X2BP and NF-Y. This suggests other mechanisms for CIITA-induced promoter occupancy, such as an effect on chromatin structure leading to increased accessibility of MHC class II promoters. This ability of CIITA to facilitate promoter occupation is undissociable from its transactivation potential. Finally, we conclude that this effect of CIITA is cell-type specific, since expression of CIITA is not required for normal occupation of MHC class II promoters in B lymphocytes.

Introduction

MHC class II molecules are heterodimeric cell surface proteins implicated in at least three crucial processes of the immune response (1). First, they initiate and sustain antigen-specific immune responses by presenting antigenic peptides to the receptor of CD4⁺ T_H lymphocytes. MHC class II expression is also an absolute requirement for superantigen-induced T cell activation. Second, they shape the T cell repertoire by directing positive and negative selection events in the thymus. Finally, they also participate in activation of the antigen-presenting cells (APC) on which they are expressed. In contrast to the relatively ubiquitous cell type distribution of MHC class I molecules, constitutive MHC class II expression is largely restricted to highly specialized APC such as dendritic cells, B lymphocytes and macrophages. In addition, MHC

class II genes can be induced in many other cell types, in particular by IFN- γ .

Both the constitutive and inducible modes of MHC class II expression are regulated at the level of transcription by a short promoter proximal enhancer containing four highly conserved *cis*-acting DNA sequences referred to as the S, X, X2 and Y boxes (2). Most of what is currently known about the molecular mechanisms controlling MHC class II gene transcription via these *cis*-acting DNA sequences has come from studies of genetic defects in MHC class II regulation, in particular from patients having a rare genetic disease called MHC class II deficiency (2–5). Elucidation of the genetic defects underlying this disease has allowed us to clone four transcription factors (CIITA, RFX5, RFXANK and RFXAP)

directly implicated in MHC class II gene regulation. RFX5, RFXANK and RFXAP are three subunits of RFX, a ubiquitously expressed multimeric DNA-binding complex that binds specifically to the X box of MHC class II promoters (3,6–8). In contrast to RFX, CIITA is a non-DNA binding transactivator characterized by a highly regulated pattern of cell type-specific and inducible expression. It is the differential expression of CIITA that controls the constitutive and inducible pattern of MHC class II gene transcription (9,10). Interestingly, the complex control of CIITA expression is achieved by the differential activation of multiple alternative promoters (11).

The RFX complex interacts cooperatively with two other transcription factors, X2BP (12,13) and NF-Y (14), such that binding of all three complexes to MHC class II promoters is strongly stabilized (13,15–18). The formation of these stable higher-order protein–DNA complexes is essential for *in vivo* occupation and activation of MHC class II promoters. In contrast to RFX, CIITA is believed to be recruited to the MHC class II promoter by protein–protein interactions with DNA bound factors such as RFX5 and is thought to activate transcription by contacting the basal transcription machinery via an N-terminal activation domain (19–23). On the other hand, CIITA was until now not thought to be involved in promoter occupation, because MHC class II promoters are occupied normally (24,25) in B cell lines in which the CIITA gene is severely mutated (9,26).

Here, we have analyzed the dependence of MHC class II promoter occupancy on CIITA, and examined the effect of CIITA on cooperative binding between RFX, X2BP and NF-Y in two different situations; constitutive MHC class II expression in B cells and induced expression in MHC class II⁻ cells transfected with CIITA or stimulated with IFN- γ . In contrast to what was previously believed, the results demonstrate that CIITA indeed plays a key role in promoter occupancy, but that this is only evident in certain cell types. While CIITA is dispensable for constitutive promoter occupancy in B cells, it is clearly required for promoter occupancy induced in fibroblasts and other MHC class II⁻ cells by IFN- γ (27,28) or by transfection with CIITA (29 and this study). Since MHC class II promoter occupation is known to be dependent on the cooperative assembly of a strongly stabilized multi-protein complex containing RFX, X2BP and NF-Y (13,15–18), it seemed likely that this CIITA-induced promoter occupation could be due to a direct effect on the stability of this complex. Surprisingly, our results reveal that CIITA-induced promoter occupancy is not the result of an effect of CIITA on the stabilization resulting from cooperative binding between RFX, X2BP and NF-Y. Indeed, both the concentrations of these DNA binding proteins in nuclear extracts and the stability of the higher-order complexes they can form on the MHC class II promoter are independent of the presence of CIITA. These results suggest that CIITA facilitates *in vivo* occupation of MHC class II promoters indirectly by affecting the accessibility of the promoter DNA rather than directly by affecting the concentration or binding affinity of the multi-protein RFX–X2BP–NF-Y complex.

Methods

Cell culture and transfections

The B lymphoblastoid cell line Raji, its CIITA-deficient variant RJ2.2.5, the human melanoma cell line GL 19, GL 19 trans-

fectected with CIITA, the monocytic cell line C119/9 [subclone derived from U937 (30)] and C119/9 transfected with CIITA were grown in RPMI 1640. HeLa cells and HeLa transfected with CIITA were grown in DMEM. Both media were supplemented with glutamine, 10% heat-inactivated FCS and antibiotics. Cells were incubated at 37°C in 5% CO₂. For induction of MHC class II expression, cells were incubated for 48–72 h with 500 U/ml of recombinant human IFN- γ . GL 19 melanoma cells were transfected as described using calcium phosphate precipitation followed 4 h later by a glycerol shock (31). C119/9 cells (30) (1×10^7 cells) were washed in cold PBS, resuspended in 0.5 ml PBS containing 10 μ g of linearized CIITA plasmid and transfected by electroporation with a BioRad electropulser using a 300 V/960 μ F pulse. After selection with hygromycin HLA class II⁺ cells were selected with Dynabeads. HeLa cells were transfected as above by electroporation with a 250 V/960 μ F pulse. Stably transfected cells were isolated after selection with hygromycin for 15 days and analyzed for MHC class II expression by FACS (polymorphic HLA-DR mAb 2.06) as previously described (9). Plasmids used for transfections were the empty EBO-Sfi expression vector, EBO-Sfi containing a full-length CIITA cDNA (9), EBO-sfi containing the mutated CIITA-allele 2 of patient BCH (32), EBO-sfi containing the mutated CIITA-gene of patient BLS-2 (9) and EBO-sfi containing the dominant negative NLS-D5 form of CIITA (33).

In vivo footprint analysis

In vivo footprint experiments were performed as described previously (34) using Vent DNA polymerase (New England Biolabs, Beverly, MA) for all steps of the ligation-mediated PCR reaction (LM-PCR). The sequences of the oligonucleotides and the incubation temperatures used for the LM-PCR reactions were as described (34).

Electrophoretic mobility shift assays (EMSA)

Nuclear extracts were prepared from $2\text{--}8 \times 10^8$ cells as described (15,16). The DRA-XY (–150 to –59 bp), DRA-XX2 (–144 to –70 bp) and DRA-Y (–89 to –49 bp) oligonucleotide probes (Fig. 1) were prepared as described (35). With the exception of the following modifications, EMSA experiments were performed essentially as described (15,16). Experiments were performed at room temperature for binding of NF-Y, RFX + NF-Y, RFX + X2BP and RFX + NF-Y + X2BP or on ice for binding of RFX. Binding mixtures were pre-incubated for 30 min prior to addition of 40,000 c.p.m. of the suitable ³²P-labeled oligonucleotide probes. Probes used were DRA-XX2 for binding of RFX and RFX + X2BP, DRA-Y for binding of NF-Y, and DRA-XY for binding of RFX + NF-Y and RFX + X2BP + NF-Y. Reactions were then incubated for a further 45 min to allow binding to proceed to completion, after which they were supplemented with a 500-fold molar excess of unlabeled probe and incubated for various times (Figs 4 and 5) prior to gel electrophoresis. Binding was done in 20 μ l reactions containing 8 μ g of nuclear extract, 5 mM MgCl₂, 50 μ g of BSA, 0.01% NP-40, 10 ng of a methylated pBR322 oligonucleotide (see 15,16) and the following competitor DNAs. Amounts of added poly(dI–dC)·poly(dI–dC) and sonicated denatured *Escherichia coli* DNA were respectively 1 and 0.5 μ g for NF-Y, 0.8 and 0.4 μ g for RFX, 0.6 and 0.3

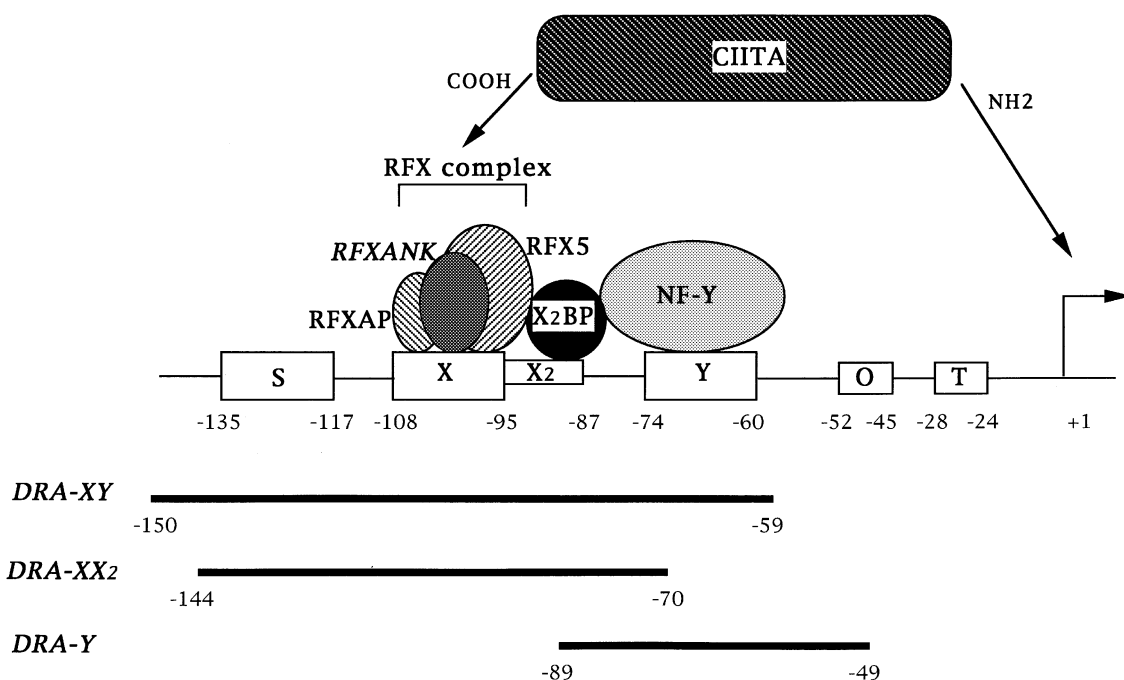


Fig. 1. Schematic representation of the HLA-DRA promoter. The S, X, X2 and Y boxes are present in all MHC class II promoters, and are crucial *cis*-acting regulatory sequences controlling transcription of MHC class II genes. T represents the TATA box. The octamer sequence (O) is present in the HLA-DRA promoter but not in other MHC class II genes. The DRA-XY, DRA-XX2 and DRA-Y oligonucleotides used in EMSA experiments are shown below. The RFX complex binding to the X box is composed of three subunits (RFX5, RFXANK and RFXAP) (4,6–8). NF-Y and X2BP are nuclear proteins that bind to the Y and X2 boxes respectively. CIITA is believed to be recruited to MHC class II promoters by protein–protein interactions with one or more of the DNA bound factors.

μg for RFX + X2BP, 1 and 0.5 μg for RFX + NF-Y, and 0.5 and 0.25 μg for RFX + NF-Y + X2BP. An aliquot of 50 ng of an X2 box competitor oligonucleotide was also included for binding of RFX and RFX + NF-Y.

Results

Induction of CIITA expression in MHC class II⁻ cells leads to the occupation of MHC class II promoters

In vivo footprint experiments were performed to analyze promoter occupation. The promoter is occupied constitutively in the MHC class II⁺ B cell line Raji (Fig. 2, lanes 15 and 16). Protections are particularly clear at the guanosine residues –104 in the X box, –98 and –93 in the X2 box, –71, –68, and –67 in the Y box, and –53 and –48 in the octamer sequence. This pattern is identical to what has been described previously (24). In RJ2.2.5, a CIITA-deficient and MHC class II⁻ variant derived from Raji, the promoter is also occupied normally (Fig. 2, lanes 13 and 14). The same has been observed in other CIITA-deficient B cell lines (24,25). Clearly, therefore, promoter occupation in B cells is not dependent on CIITA. It is also not dependent on transcriptional activity because the MHC class II genes are not expressed in these CIITA-deficient B cells.

The situation is very different for cell types in which expression of MHC class II genes is induced by stimuli such as IFN- γ . The promoter is bare in the uninduced human melanoma cell line GL 19 (Fig. 2, lanes 1 and 2) and the monocytic cell

line C119/9 (Fig. 2, lanes 9 and 10). Two approaches for studying the effect of CIITA on promoter occupancy in these cells were explored; induction with IFN- γ and transfection with a CIITA expression vector. Induction of MHC class II gene expression in GL 19 by treatment with IFN- γ is accompanied by the appearance of an occupied promoter (Fig. 2, lanes 3 and 4). As judged by the pattern of protected G residues, occupation of the X, X2 and Y boxes in the induced GL 19 cells is identical to that observed in Raji B cells. As described previously (27) occupation of the octamer sequence is less evident in the induced cells than in B cells. Similar results have been observed for other IFN- γ inducible cell lines, including HeLa (27,28).

De novo expression of CIITA is an obligatory intermediate step in the signal transduction pathway leading to the induction of MHC class II genes by IFN- γ . To determine whether the induction of promoter occupation by IFN- γ is a direct consequence of CIITA expression, uninduced GL 19 cells were transfected with a CIITA expression vector. Cells transfected with empty expression vector were used as controls. The results demonstrate that transfection with CIITA is sufficient to obtain normal promoter occupancy (Fig. 2, lanes 7 and 8) and activate expression of MHC class II genes. The pattern of protected G residues observed in the CIITA-transfected cells is identical to that observed in the IFN- γ -induced cells (Fig. 2, lanes 3 and 4). The same results are observed in the monocytic cell line C119/9, where transfection with CIITA also induced an occupied promoter (Fig. 2, lanes 11 and 12). In contrast to what is observed in B cells, these

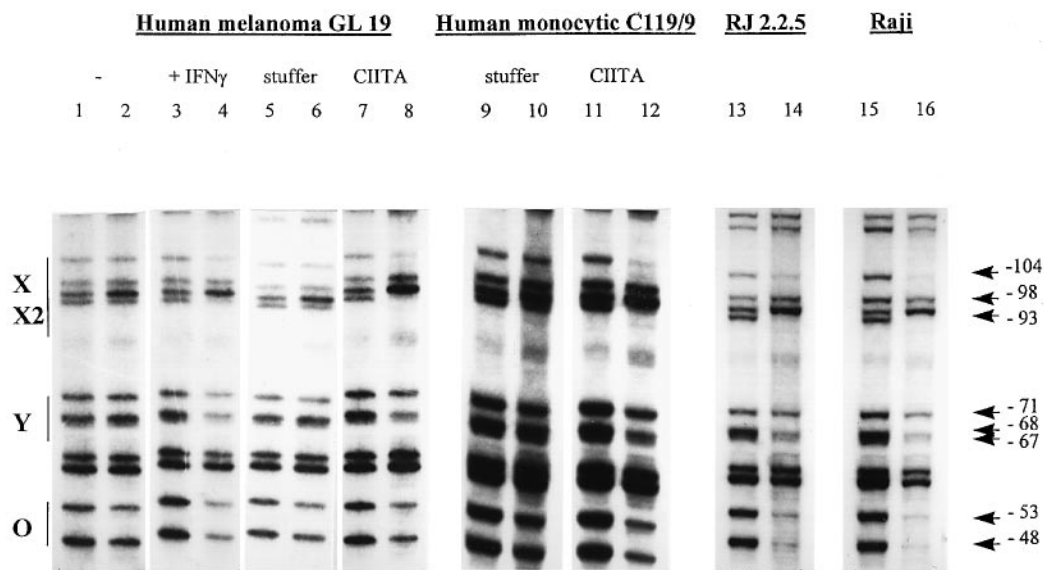


Fig. 2. *In vivo* footprint analysis of the HLA-DRA promoter in cells lacking or expressing CIITA. *In vivo* footprint analysis was performed with uninduced GL 19 cells (lanes 1 and 2), GL 19 cells induced with IFN- γ (lanes 3 and 4), GL 19 cells transfected with an empty expression vector (stuffer, lanes 5 and 6), GL 19 cells transfected with a CIITA expression vector (lanes 7 and 8), C119/9 monocytic cells transfected with an empty vector (lanes 9 and 10), C119/9 transfected with CIITA (lanes 11 and 12), RJ2.2.5 cells (lanes 13 and 14) and Raji cells (lanes 15 and 16). Lanes 2, 4, 6, 8, 10, 12, 14 and 16: the cells were treated with DMS, the DNA was purified and cleaved at methylated nucleotides with piperidine, and cleaved fragments spanning the DRA promoter were amplified by LM-PCR and analyzed by sequencing gel electrophoresis. Lanes 1, 3, 5, 7, 9, 11, 13 and 15: control samples representing unoccupied DNA were obtained by performing the procedure with naked DNA purified from the same cells. Positions of the X, X2, Y and O *cis*-acting sequences, and the protected positions observed in IFN- γ -treated GL 19 cells (lane 4), GL 19 cells transfected with CIITA (lane 8), C119/9 cells transfected with CIITA (lane 12), RJ2.2.5 (lane 14) and Raji (lane 16) are indicated.

results demonstrate that in other cells types, occupation of MHC class II promoters requires CIITA. MHC class II promoter occupancy thus exhibits a cell type-dependent requirement for CIITA.

The ability of CIITA to induce promoter occupation is undissociable from its transactivation potential

The ability of CIITA to activate transcription of MHC class II genes relies on two different functions that map to two different regions of the protein. The N-terminal region of CIITA contains a transcription activation domain that is believed to contact the basal transcription machinery (19–23). The C-terminus of CIITA, on the other hand, is believed to mediate protein–protein interactions required for recruitment to the MHC class II promoter (20–22). To determine whether promoter occupation induced by CIITA could be attributed to one or both of these functional domains we analyzed three different mutants of CIITA. In B cell lines from patients having mutations in the CIITA gene (BLS-2 and BCH) MHC class II promoters are normally occupied even though the genes are transcriptionally silent. It therefore seemed possible that the mutated forms of CIITA of these patients could retain the capacity to drive occupation of the promoter. In both patients, CIITA is disrupted by a small deletion in the C-terminal part of the gene (Fig. 3A). The NLS-D5 mutant of CIITA has been demonstrated to function as a dominant negative molecule (Fig. 3B) (33). This mutant has a deletion of the N-terminal part of the gene and has lost the activation domain of CIITA (3,20,21,33). On the other hand, NLS-D5 retains intact the C-terminal region of

CIITA that is believed to be required for its recruitment to MHC class II promoters (20,21). We therefore thought that the capacity to induce promoter occupation might be maintained by this mutant. To determine whether the NLS-D5, BCH and BLS-2 versions retained the ability to induce promoter occupation, they were transfected into HeLa cells. These three mutated forms of CIITA are unable to activate expression of MHC class II genes (Fig. 3B). In addition NLS-D5 has a strong dominant negative effect that severely hinders induction of MHC class II expression by IFN- γ (Fig. 3B). Promoter occupation could not be observed in any of the three transfectants, while promoter occupation could be induced normally by transfection with wild-type CIITA (Fig. 3C). These results suggest that the transactivation activity of CIITA is undissociable from its ability to induce the occupation of MHC class II promoters and requires the same functional domains. The same conclusion has recently been arrived at by another study using a different series of CIITA mutants (29).

Stability of the complexes bound to MHC class II promoters is independent of CIITA

Previous *in vitro* binding studies have demonstrated that RFX binds cooperatively with two other MHC class II promoter binding proteins, X2BP and NF-Y (13,15–18). These cooperative binding interactions strongly enhance the stability with which RFX, X2BP and NF-Y are bound to their respective X, X2 and Y box target sites. The strongly stabilized binding observed in the higher order RFX + X2BP + NF-Y complex is essential for promoter occupation *in vivo*. Indeed, in cell

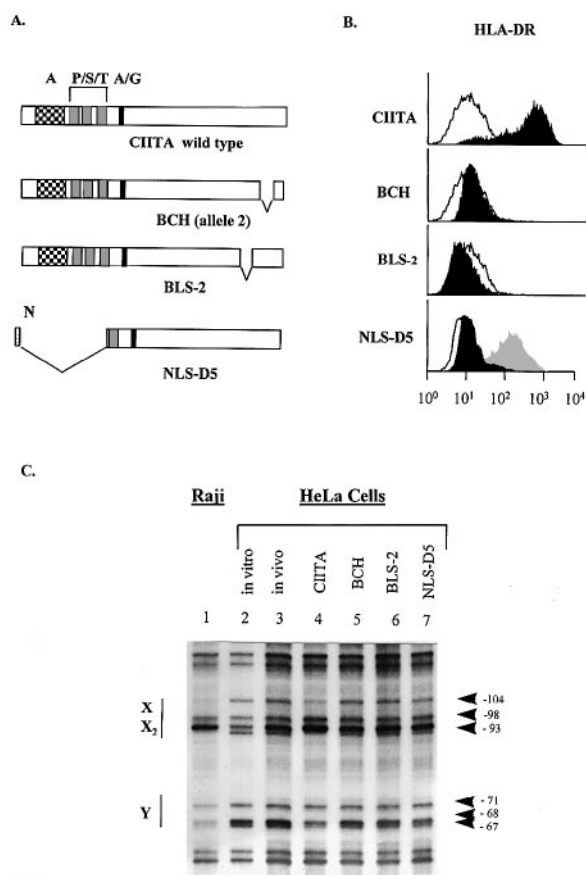


Fig. 3. *In vivo* footprint analysis of the HLA-DRA promoter in HeLa cells expressing mutated CIITA proteins. (A) Schematic view of CIITA and the different mutants analyzed. Mutant BCH (allele 2) has a deletion of 27 amino acids in the C-terminus (Δ 1079–1106). Mutant BLS-2 has a deletion of 23 amino acids in the C-terminus (Δ 940–963). The dominant-negative NLS-D5 mutant has a nuclear localization signal (NLS) at its N-terminus and has a deletion of the first 294 amino acids. A, acidic region; P/S/T, Pro/Ser/Thr-rich regions; A/G, ATP/GTP-binding motif. (B) Cell surface expression of HLA-DR in HeLa cells transfected with the mutated CIITA constructs. Black profiles: cells transfected with the wild-type or mutated forms of CIITA. Open profiles: cells transfected with empty expression vector. To demonstrate the dominant negative effect of NLS-D5, the NLS-D5 (black profile) and empty vector transfected cells (gray profile) were treated with IFN- γ to induce MHC class II expression. (C) *In vivo* footprint analysis was performed as outlined in Fig. 2 with Raji cells (lane 1) and HeLa cells transfected with empty vector (lanes 2 and 3), wild-type CIITA (lane 4), the BCH mutant (lane 5), the BLS-2 mutant (lane 6) and the NLS-D5 mutant (lane 7). In the case of the empty vector transfected samples, DMS treatment was done directly with the cells (lane 3, *in vivo*) or with the purified DNA (lane 2, *in vitro*). Positions that are protected in Raji (lane 1) and wild-type CIITA-transfected cells (lane 4), but not in the other transfectants (lanes 3 and 5–7) are indicated at the right.

lines deficient in RFX the formation of these higher-order complexes is abolished and the entire promoter remains bare (16,17,25).

Considering this key role of cooperative binding in MHC class II promoter occupancy and the fact that CIITA expression can trigger promoter occupancy, it was logical to explore if

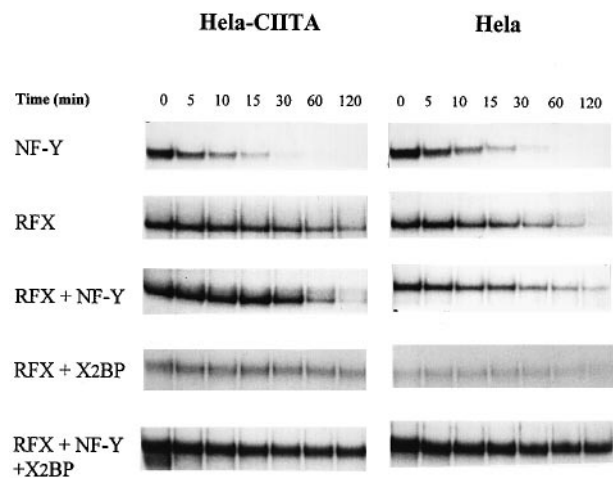


Fig. 4. Dissociation rate experiments performed with extracts from HeLa and HeLa transfected with CIITA. Reactions optimized for binding of RFX or NF-Y on their own, or for the simultaneous binding of RFX + X2BP, RFX + NF-Y and RFX + X2BP + NF-Y (see Methods), were first incubated to allow binding to proceed to completion, and were then supplemented with an excess of unlabeled competitor DNA and continued for 0, 5, 10, 30, 60 or 120 min prior to gel electrophoresis. Nuclear extracts were from HeLa or HeLa transfected with CIITA. The experiment for RFX was done on ice in order to prolong the half-life of the RFX-DNA complex, which is <5 min at room temperature (15). All other experiments were performed at room temperature. Only the regions of the gels containing the protein–DNA complexes are shown.

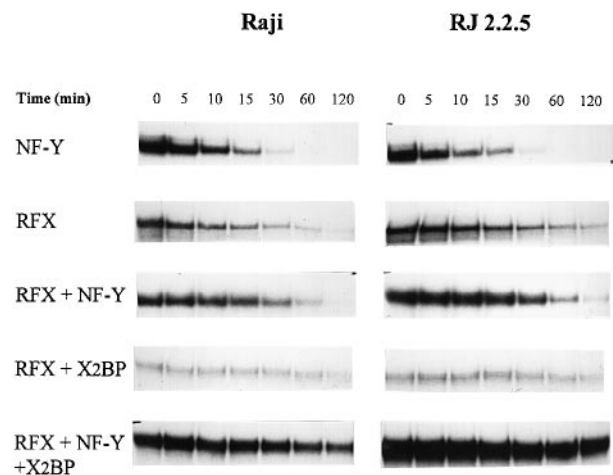


Fig. 5. Dissociation rate experiments performed with extracts from Raji and RJ2.2.5. Dissociation rate experiments with nuclear extracts from Raji and RJ2.2.5 were done as described in Fig. 4.

the effect of CIITA was mediated by either an increase in the concentration of RFX, X2BP or NF-Y, or by an enhanced stability in the interactions between these proteins and their target sites. The abundance and stability of various different protein–DNA complexes were therefore analyzed by EMSA experiments. As an indication of stability, the off-rates of the protein–DNA complexes were examined. Four different cell types were included in the analysis (Figs 4–6). Two of these

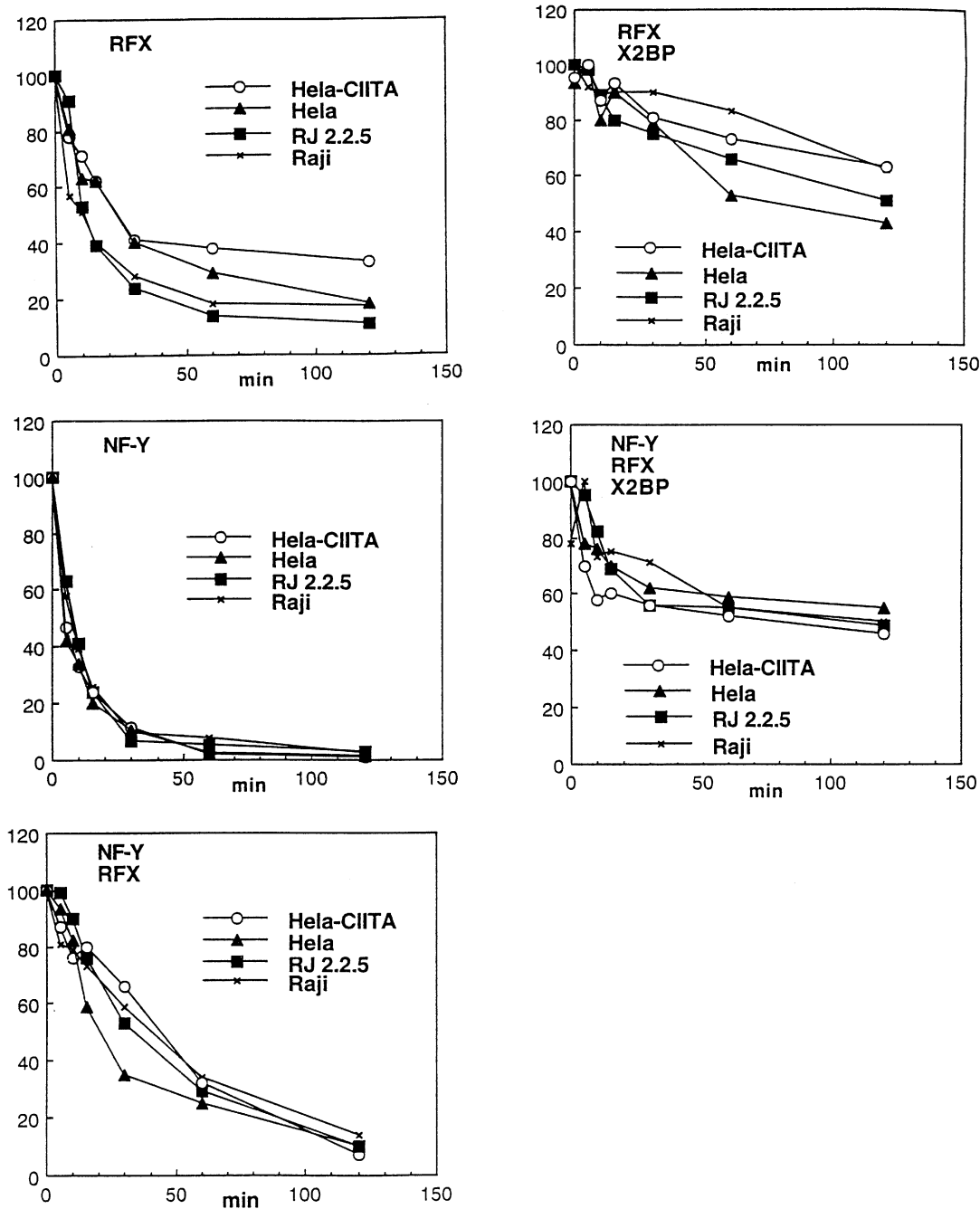


Fig. 6. Quantification of the dissociation rate experiments. The gels shown in Figs 4 and 5 were quantified by PhosphorImager analysis. The percent of the remaining protein-DNA complexes is plotted as a function of time. The amount of complex bound at time zero (addition of unlabeled competitor DNA) was taken as 100%.

lack CIITA (HeLa and RJ2.2.5) while the other two express CIITA (HeLa cells transfected with CIITA and Raji). For each cell type, off-rates were determined for complexes containing only RFX or NF-Y, and for the higher-order complexes containing RFX + NF-Y, RFX + X2BP and RFX + X2BP + NF-Y. The off-rate for X2BP bound on its own could not be determined because its affinity for the X2 box in the absence of the other two proteins is too low to permit detection of a stable complex in EMSA experiments (15). Representative gels for HeLa and

HeLa transfected with CIITA are shown in Fig. 4, those for Raji and RJ2.2.5 are shown in Fig. 5, and quantifications of the results are shown in Fig. 6. Identical results were also obtained in experiments comparing RJ2.2.5 with CIITA-transfected RJ2.2.5 and uninduced HeLa with IFN- γ -treated HeLa (data not shown). Four major conclusions can be drawn from the data shown here. First, expression of CIITA does not change the levels of RFX, X2BP and NF-Y detectable in the nuclear extracts. This holds true both for B cells (Raji) and

HeLa. Second, as observed previously (13,15,16), in all cell types the interaction of RFX, X2BP and NF-Y with the promoter is strongly stabilized in the higher-order RFX + X2BP, RFX + NF-Y and RFX + X2BP + NF-Y complexes. Third, there are no significant differences between B cells (Raji and RJ2.2.5) and HeLa cells (transfected or not with CIITA) with respect to the stability of any of the different complexes. Fourth, no differences in off-rates are observed between cells that lack CIITA (uninduced HeLa and RJ2.2.5) and those that express it (Raji and HeLa transfected with CIITA), indicating that CIITA does not influence the stability of any of the complexes. Taken together these results demonstrate that the effect of CIITA on promoter occupancy is not mediated by an increase in the expression levels of RFX, X2BP and NF-Y, or by an enhancement in the efficiency with which these proteins can form stable higher-order complexes on the promoter.

Discussion

A combination of biochemical and genetic evidence has formally demonstrated that the DNA binding complex RFX and the non-DNA binding transactivator CIITA are both key components of the molecular machinery that controls the activity of the MHC class II promoters (3,4,36,37). Although both are essential for MHC class II expression, their respective modes of action are believed to be quite different. RFX binds cooperatively with two other MHC class II promoter binding proteins, X2BP (12,13) and NF-Y (14), to generate a very stable higher-order protein–DNA complex and this stable binding is required for promoter activation (13,15–18). Formation of this higher-order complex is clearly required for occupation of MHC class II promoters *in vivo*, because these promoters remain bare (24,25) in cells in which formation of the higher-order complex is abrogated by mutations destroying RFX (3,6) or by mutations in the Y box of MHC class II promoters (38). In the case of CIITA, existing evidence suggests that it is recruited to the promoter by protein–protein interactions with one or more DNA bound factors such as the RFX5 subunit of RFX and that it activates transcription by contacting components of the basal transcription machinery via an acidic activation domain (19–22). In contrast to RFX, CIITA has been believed to be dispensable for promoter occupation *in vivo* because occupancy remains essentially normal in mutant B cell lines lacking functional CIITA (24,25). However, we now demonstrate here that CIITA does in fact also play a role in promoting promoter occupation, but that this is not evident in all cell types: while CIITA is not necessary for promoter occupation in B cells, it is required in cell types in which MHC class II expression depends on induction with stimuli such as IFN- γ (Fig. 2). This is consistent with similar results presented in another recent study using cell types distinct from those used here (29). The analysis of different mutants of CIITA suggests that the transactivation potential of CIITA and its ability to influence promoter occupation map to the same functional domains of the protein (Fig. 3). Moreover, we demonstrate that CIITA affects promoter occupation by a mechanism that is distinct from the stabilization that results from the cooperative binding between RFX, X2BP and NF-Y (Figs 4–6).

Two different MHC class II promoter occupancy phenotypes

can be observed in cells lacking CIITA (Fig. 2). Promoter occupancy is normal in B lymphoblastoid cells such as RJ2.2.5 which lack functional CIITA (Fig. 2). On the other hand, the promoter is bare in CIITA⁻ cells such as uninduced GL 19 melanoma cells, C119/9 monocytic cells and HeLa cells (Figs 2 and 3) (24,25,27,28). This cannot be explained by a difference in the expression levels of proteins known to be required for occupation, i.e. RFX, X2BP and NF-Y (Figs 4 and 5). Surprisingly, it is also not the result of differences in the stability of the higher-order protein–DNA complexes formed by cooperative binding between these three proteins (Figs 4–6). It can also be excluded that promoter occupancy simply reflects active transcription, because neither mutant B cells lacking CIITA (RJ2.2.5) nor the uninduced melanoma cells, monocytes and HeLa cells express MHC class II genes. Consequently, it is likely that the difference in promoter occupancy observed between these two MHC class II⁻ cell types reflects a difference in the accessibility of the DNA within the chromatin structure at the promoter.

A difference in promoter accessibility is not observed only when comparing B cells and MHC class II⁻ cells. Changes in promoter accessibility also appear to occur during differentiation within the B cell lineage. Developmental extinction of MHC class II expression in plasmocytes is accompanied by the appearance of a bare promoter phenotype that can be reversed by transfection with CIITA (34 and data not shown). As described here for IFN- γ inducible cells, the bare promoter in plasmocytes is not a consequence of the absence of RFX, X2BP or NF-Y, or of a reduced ability to form stable higher-order protein–DNA complexes (34). Compared to MHC class II⁻ B cells, plasmocytes thus seem to acquire a tighter chromatin structure prohibiting occupation of MHC class II promoters by RFX, X2BP and NF-Y.

Transfection with CIITA is sufficient to overcome the accessibility barrier opposing binding of RFX, X2BP and NF-Y *in vivo* to the MHC class II promoter in MHC class II⁻ cells. How CIITA achieves this remains a matter of speculation. Several possibilities come to mind. One possibility is that interaction of CIITA with RFX, X2BP and/or NF-Y contributes an additional stabilizing effect that is not detectable by *in vitro* binding assays. It is also possible that this interaction induces a conformational change in the RFX–X2BP–NF-Y complex that is required to maintain stable binding of these proteins *in vivo*. Alternatively, perhaps CIITA facilitates access of the DNA binding proteins to the DNA because it has a direct or indirect effect on the chromatin structure at the promoter: it could, for example, recruit chromatin remodeling factors such as histone acetylases (39,40) or the Swi/Snf complex (41,42). Clearly, the precise mode of action of CIITA is far from elucidated and the novel observations made here point to several potential mechanisms that need to be explored.

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Abbreviations

APC	antigen-presenting cell
EMSA	electrophoretic mobility shift assay
LM-PCR	limited-mobility polymerase chain reaction
NLS	nuclear localization signal

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