Immunity, Vol. 8, 157–166, February, 1998, Copyright ©1998 by Cell Press

Activation of the MHC Class II Transactivator CIITA by Interferon-γ Requires Cooperative Interaction between Stat1 and USF-1

Annick Muhlethaler-Mottet, Wilma Di Berardino, Luc A. Otten, and Bernard Mach*
Louis Jeantet Laboratory of Molecular Genetics
Department of Genetics and Microbiology
University of Geneva Medical School
1, rue Michel-Servet
CH-1211 Geneva 4
Switzerland

Summary

CIITA is the mediator of MHC class II gene induction by interferon-γ (IFNγ). The CIITA gene is itself selectively activated via one of its four promoters (PIV). We show here that three *cis*-acting elements, the GAS, the E box, and the IRF-1–binding site, as well as the *trans*-acting factors Stat1 and IRF-1, are essential for activation of CIITA promoter IV by IFNγ. Stat1 binds to the GAS site only in the presence of the ubiquitous factor USF-1, which binds to the adjacent E box. Indeed, Stat1 and USF-1 bind to the GAS/E box motif in a cooperative manner. The specificity for CIITA activation by IFNγ is thus dictated by the GAS/E box motif and by the selective interaction of IFNγ-activated Stat1 and USF-1. This clarifies the missing link in the overall pathway of IFNγ activation of MHC-II expression.

Introduction

Major histocompatibility complex class II (MHC-II) molecules present exogenous antigenic peptides to $CD4^+$ helper T lymphocytes. A very tight regulation of MHC-II expression is crucial for the control of the immune response. Constitutive expression is restricted to specialized antigen-presenting cells, such as dendritic cells and B lymphocytes. In addition, MHC-II expression can be induced by a number of different stimuli, particularly by interferon-γ (IFNγ), in a variety of MHC-II–negative cells (Glimcher and Kara, 1992; Mach et al., 1996).

Progress in understanding the regulation of MHC-II genes has come from studies of various regulatory mutant cell lines affected in MHC-II regulatory factors and of cell lines isolated from patients suffering from hereditary MHC-II deficiency (Griscelli et al., 1989; Mach et al., 1994). This disease, also referred to as bare lymphocyte syndrome, is characterized by a lack of MHC-II expression due to mutations in *trans*-acting regulatory factors and thus it represents a disease of gene regulation (Mach et al., 1996). Genetic and molecular analyses of these cell lines, corresponding to different complementation groups, have allowed us to identify and characterize three transcription factors essential for the control of MHC-II regulation: CIITA, RFX-5, and RFX-AP. RFX-5 (Steimle et al., 1995) and RFX-AP (Durand et al., 1997) are components of the RFX complex, which binds to the X box of MHC-II promoters (Reith et al., 1988), and are present in an ubiquitous manner.

CIITA is the general regulator of MHC-II gene expression, both constitutive and inducible (Steimle et al., 1993, 1994), and it is likely to function as a transcriptional coactivator interacting with the transcription factors bound to MHC-II promoters (Zhou and Glimcher, 1995). CIITA expression pattern correlates with that of MHC-II genes with a constitutive expression in MHC-II–positive cells and tissues (Steimle et al., 1993, 1994). CIITA is the obligatory mediator of IFNγ-inducible MHC-II expression (Steimle et al., 1994). Moreover, in plasmocytes the extinction of MHC-II expression corresponds to CIITA repression (Silacci et al., 1994). CIITA and MHC-II gene expression are not only qualitatively but also quantitatively correlated (Otten et al., 1998). Therefore, the regulation of MHC-II gene expression, constitutive as well as inducible, and its tissue specificity are entirely dependent on the control of CIITA gene expression itself.

The CIITA gene is controlled by multiple promoters, leading to multiple CIITA transcripts with different first exons (Muhlethaler-Mottet et al., 1997). More importantly, these promoters are activated in a selective manner. Two promoters direct specific constitutive expression in dendritic cell (promoter I [PI]) and in B lymphocytes (PIII), whereas another promoter (PIV) mediates IFNγ-inducible expression in most MHC-II–negative IFNγ-inducible cells (Muhlethaler-Mottet et al., 1997).

Recently, progress was made in understanding the signaling cascade of gene activation in response to IFNγ. Binding of IFNγ to its cell-surface receptor activates the protein tyrosine kinases Jak1 and Jak2. The latent cytoplasmic transcription factor Stat1 is then phosphorylated on tyrosines by the Jak kinases and translocates to the nucleus, where it binds to the GAS element of IFNγ-responsive promoters, leading to gene activation (Darnell et al., 1994; Schindler and Darnell, 1995; Darnell, 1997). Different studies involved the Jak/STAT pathway in CIITA induction by IFNγ. Indeed, CIITA, like other IFNγ-inducible genes, cannot be induced in Jak1-deficient cell lines (Chang et al., 1994). Moreover, CIITA mRNA was not induced by IFNγ in bone marrow macrophages derived from $Stat1^{-/-}$ mice (Meraz et al., 1996), indicating a role of Stat1 in CIITA induction by IFNγ.

Analyses of the IFNγ-responsive CIITA promoter (PIV) and sequence comparison between human and mouse lead to the identification of several conserved *cis*-regulatory elements such as a NF-GMa–binding site, a GAS element, an E box, and an IRF-1–binding site (Muhlethaler-Mottet et al., 1997). The presence of the GAS element suggests the direct binding of Stat1 to CIITA PIV, leading to CIITA expression. The IRF-1–binding site can be bound by the members of the interferon regulatory factor (IRF) family of transcription factors, which includes, among others, IRF-1, IRF-2, ISGF3γ, and ICSBP (Boehm et al., 1997).

The presence of the canonical E box CACGTG within the CIITA promoter IV suggests that a member of basic–helix-loop-helix/leucine zipper (bHLH-zip) class of transcription factors might be involved in IFNγ-induced

*To whom correspondence should be addressed (e-mail:mach@medecine.unige.ch).

CIITA expression. This family includes the constitutively expressed transcription factors TFE3 (Beckmann et al., 1990), TFEB (Carr and Sharp, 1990), USF-1 (Gregor et al., 1990), and USF-2 (Sirito et al., 1994), as well as proteins involved in the Myc network, including Myc-Max and Mad-Max (Blackwood and Eisenman, 1991; Ayer et al., 1993).

Here, we report on the mechanisms controlling CIITA promoter IV activation by IFNγ. By mutagenesis and functional analyses we show that the GAS element, the E box, and the IRF-1–binding site are all essential for CIITA PIV activation by IFNγ. We demonstrate that Stat1 binds directly to CIITA PIV-GAS element, which completes the entire cascade of MHC-II induction by IFNγ. We also show that the ubiquitous bHLH-zip transcription factor USF-1 binds to CIITA PIV-E box. More importantly, we demonstrate that Stat1 binding to the GAS site is strongly stabilized by USF-1 and that both factors bind cooperatively to the GAS/E box motif of CIITA PIV. We conclude that it is the cooperative interaction between a ubiquitous factor, USF-1, and an IFNγ-activated protein, Stat1, that controls the specific activation of CIITA promoter IV by IFNγ.

Results

Mutagenesis and Functional Assays Define Three Essential *Cis*-Acting Elements for CIITA Induction by IFNγ

Sequence analysis of CIITA promoter IV indicated the presence of four potential *cis*-acting elements conserved between human and mouse: a GAS element, an E box, an IRF-1–binding site, as well as a NF-GMa–binding site (Figure 1A) (Muhlethaler-Mottet et al., 1997). To analyze the functional relevance of these *cis*-acting elements, site-directed mutagenesis was performed, followed by functional analyses by promoter-reporter gene assays, measured by quantitative competitive reverse transcriptase-polymerase chain reaction (RT-PCR) (Sperisen et al., 1992). A 308 bp fragment of CIITA promoter PIV (PIV-308 wt), which contains all the elements necessary for responsiveness to IFNγ, as well as various mutated PIV-308 constructs (Figure 1A) were analyzed for transcriptional activation in response to IFNγ under experimental conditions described earlier (Muhlethaler-Mottet et al., 1997) (Figure 1B). Transient transfections of PIV-308 wt in the IFNγ-inducible melanoma cell line Me67.8 showed a very low basal transcriptional activity of the β-globin reporter construct. Treatment of cells with IFNγ-induced PIV-308 wt promoter construct activity, which was referred as 100% of stimulation index (Figure 1B). In contrast, mutations in either the GAS element (Gm), the E box (Em), or the IRF-1–binding site (Im) of CIITA promoter IV resulted in an almost complete abolition of promoter inducibility by IFNγ, with stimulation index equivalent to 19%, 16%, and 23% of wild-type (wt) stimulation index, respectively (Figure 1B). The same reduction of promoter responsiveness to IFNγ was observed with the double mutant GmEm (17% of wt stimulation index) (Figure 1B). Conversely, a mutation in the NF-GMa box showed an increased response to IFNγ (3-fold compared to wt), suggesting a role as a negative control element. However, we cannot exclude

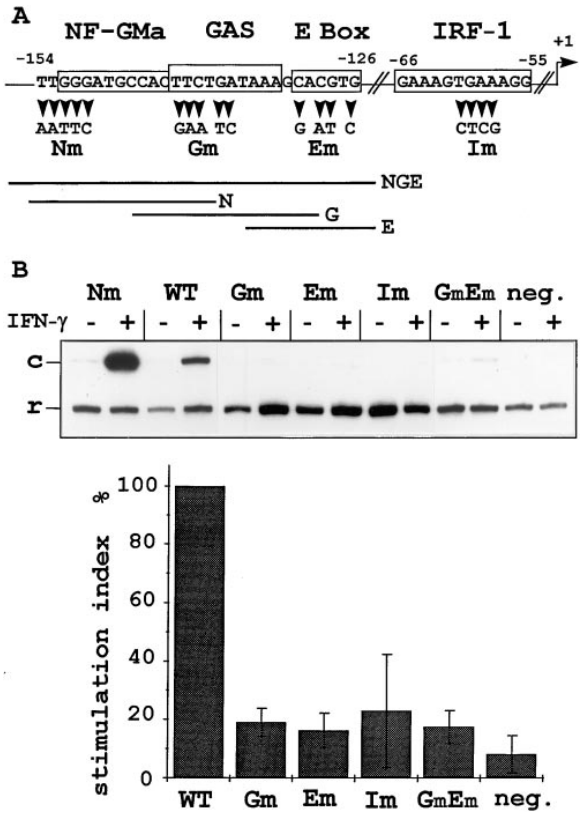

Figure 1. Functional Analysis of CIITA Promoter IV

(A) The organization of the human wt and mutants CIITA promoter IV. Sequences and positions of conserved *cis*-acting elements are indicated. Nucleotides modified for reporter gene assays are shown below the wt sequence with names of mutant constructs. Oligonucleotides used as probes for EMSA and in competition experiments are indicated.

(B) Functional analysis of wt and mutagenised CIITA promoter IV. Transient transfections of Me67.8 cells with plasmids PIV-308 wt, Nm, Gm, Em, Im, GmEm, or the promoterless plasmid pGβG(+) and the reference plasmid pGβAcβGID. c, RT-PCR signals from CIITA PIV-308/β-globin reporter gene construct; r, signals derived from reference plasmid. Stimulation index is determined by the ratio between promoter activity after culture with IFNγ and after culture in medium alone, as described earlier (Muhlethaler-Mottet et al., 1997). Plotted results are means of three independent experiments with standard deviations.

the alternative and unlikely explanation that the mutations introduced in this site have created a cryptic binding site for a positive transcription factor. As a control, the promoterless construct pGβG(+) showed no transcriptional activity upon IFNγ induction (8% of wt stimulation index) (neg. in Figure 1B). These results show that each of these *cis*-acting elements are functionally relevant. Moreover, the strong decrease of promoter inducibility by IFNγ clearly demonstrates that the GAS element, the E box, and the IRF-1–binding site each play a functionally important role in the induction of CIITA gene expression by IFNγ.

IRF-1 Is Functionally Essential for CIITA Promoter IV Inducibility by IFNγ

Because we have demonstrated the functional importance of the IRF-1–binding site of CIITA PIV and because

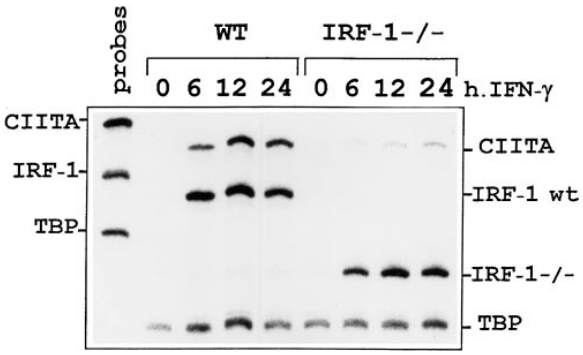

Figure 2. IRF1 Is Essential for CIITA Induction by IFNγ

CIITA and IRF-1 mRNA were analyzed by RNase protection assays in RNAs from embryonic fibroblasts from wt and IRF-$1^{-/-}$ mice, either unstimulated (O) or stimulated for 6 hr, 12 hr, or 24 hr with IFNγ. A TBP probe was used as internal control. The positions of the protected fragments and of the undigested probes are indicated. The protected fragment corresponding to IRF-1 mRNA is shorter in RNA from IRF-$1^{-/-}$ mice, because the probe covers a part of the region deleted in the IRF-$1^{-/-}$ mice.

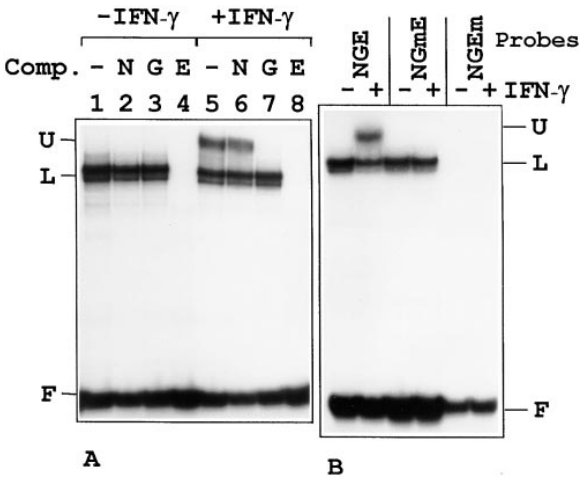

Figure 3. Two Distinct Complexes Can Bind to the NGE Probe

EMSA experiments were performed with the NGE probe and nuclear extracts from Me67.8 cell line unstimulated (−) or stimulated with IFNγ (+). U, upper complex; L, lower complex; F, free probe. (A) Competition experiments. Competitor DNA (200-fold molar excess, comp.) added during the binding reactions are indicated. (B) EMSA performed on mutated NGE probes.

IRF-1 was shown to be involved in the induction of several IFNγ-inducible genes, such as GBP (Briken et al., 1995), we investigated the role of IRF-1 in the induction of CIITA by IFNγ. RNAs from embryonic fibroblasts (EF) derived from wt and from IRF-$1^{-/-}$ mice were compared for CIITA mRNA expression after stimulation by IFNγ. RNase protection assays revealed that, in contrast to wt EF, IFNγ-induced CIITA mRNA expression was strongly reduced in IRF-$1^{-/-}$ EF (Figure 2). The same inhibition of IFNγ stimulation was observed, as expected, for GBP mRNA (data not shown). The results indicate that IRF-1 is an essential factor for CIITA induction by IFNγ in EF.

Two Distinct Protein Complexes Can Be Formed on the GAS/E Box Region

To analyze the protein–DNA complexes that can be formed within the region containing the three *cis*-acting elements, NF-GMa/GAS/E box, electrophoretic mobility shift assay (EMSA) experiments were performed with a DNA probe covering this region (NGE) and nuclear extracts from Me67.8 cells, either uninduced or induced by IFNγ (Figures 1A and 3A). With nuclear extracts from unstimulated cells, a DNA/protein complex (lower [L]) was observed (Figure 3A, lane 1). Following stimulation by IFNγ, an additional complex (upper [U]) with a lower electrophoretic mobility was observed (Figure 3A, lane 5).

To analyze the specificity and the DNA-binding sites of each of these two complexes, we carried out competition experiments with oligonucleotides specific for either the NF-GMa–binding site (N), the GAS site (G), or the E box (E) (Figure 1A). The results show that the L complex was disrupted only by competitor oligo E, whether the nuclear extracts came from unstimulated cells or from IFNγ-stimulated cells (Figure 3A, lanes 4 and 8). In contrast, the U complex was disrupted by competitor oligos covering either the GAS site (G) or the E box (E) (Figure 3A, lanes 7 and 8). The fact that the oligo covering the NF-GMa–binding site (N) was not able to compete for any of these two complexes indicated that no protein binds to the NF-GMa–binding site under these experimental conditions (Figure 3A, lanes 2 and 6).

We then analyzed protein-binding activity on mutated DNA target sequences, using probes containing the same mutations as the reporter gene constructs Gm or Em in either the GAS site or the E box, respectively. When the GAS element was mutated (NGmE probe), only the L complex was formed, even with nuclear extract from IFNγ-stimulated cells (Figure 3B). By contrast, when the E box was mutated (NGEm probe), neither the L nor the U complex could be formed (Figure 3B). Taken together, these EMSA data show that complex L consists of an E box–binding protein, which is present in unstimulated cells, and that, following stimulation by IFNγ, a novel U protein complex appears, whose DNA binding activity depends on both an intact GAS and E box. We conclude that this U complex is composed of at least an E box–binding protein and a protein activated by IFNγ, which binds to the GAS site. An obvious candidate is Stat1, because it has been shown to bind GAS element following activation by IFNγ (Decker et al., 1991).

Stat1 Controls CIITA Expression by Direct Binding to the GAS Site of CIITA Promoter IV

Because of its known activation following exposure of cells to IFNγ, STAT1 has been thought to play a role in the induction of CIITA (Steimle et al., 1994; Lee and Benveniste, 1996; Muhlethaler-Mottet et al., 1997). To evaluate directly the role of Stat1 in the regulation of the CIITA gene by IFNγ, the Stat1-deficient cell line U3A was compared to the parental fibrosarcoma cell line 2FTGH for it ability to induce CIITA expression upon IFNγ stimulation. In contrast to 2FTGH, the U3A cell line showed neither induction of CIITA mRNA expression nor CIITA promoter IV activation after IFNγ stimulation, as determined by RNase protection assays and promoter-reporter gene assays, respectively (data not shown). These results show that Stat1 controls IFNγ-induced activation of CIITA promoter IV. They are in agreement

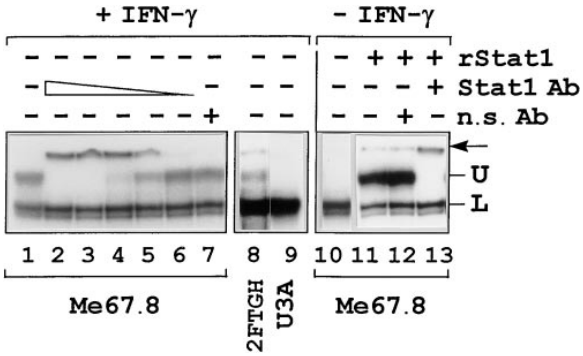

Figure 4. Stat1 Is Present in the U Complex

Anti-Stat1 antibodies can supershift the U complex in EMSAs. EMSAs with the NGE probe and nuclear extracts from IFNγ-stimulated Me67.8 (lanes 1–7), from IFNγ-stimulated 2FTGH (lane 8), from IFNγ-stimulated U3A (lane 9), or from unstimulated Me67.8 (lanes 10–13), activated rStat1 (10 ng, lanes 11–13), and either no added antibody (−, lanes 1, 8–11), different amounts of anti-human Stat1 antibodies (+, lanes 2–6, 13), or nonspecific antibodies (+, lanes 7 and 12). Monoclonal antibodies dilutions were 1:20 for lanes 2, 7, 12, and 13; 1:200 for lane 3; 1:1000 for lane 4; 1:2000 for lane 5; and 1:10000 for lane 6. Only the region of the gel containing the upper (U) and lower (L) complexes is shown. Arrow indicates supershifted complexes. In lanes 8, 11, and 12, a small part of the probe is blocked in the well where the supershifted complex is also blocked.

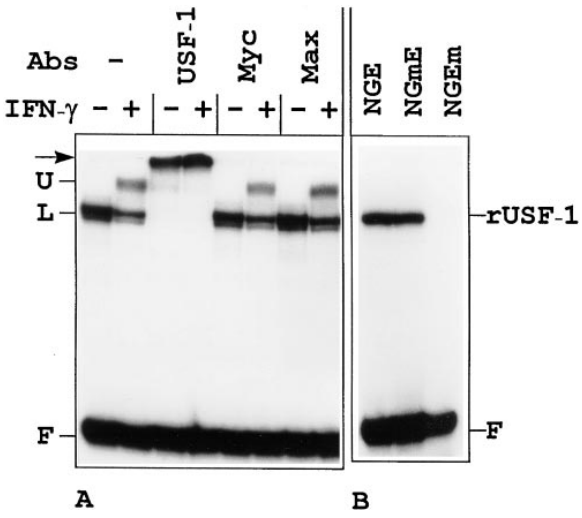

Figure 5. USF-1 Binds to the E Box of CIITA PIV

(A) Anti-USF-1 antibodies can supershift the U and L complexes in EMSA. EMSA were performed with the NGE probe, nuclear extracts from unstimulated (−) or IFNγ-stimulated (+) Me67.8, and either no antibody (−) or anti-USF-1, anti-Myc, or anti-Max antibodies. Arrow indicates supershifted complexes; U, upper complex; L, lower complex; F, free probe.

(B) Analysis of USF-1–binding site. EMSA with rUSF-1 (5 ng) and either wt NGE, NGmE, or NGEm probes are shown.

with reports showing that CIITA mRNA cannot be induced by IFNγ in bone marrow macrophages from Stat1$^{-/-}$ mice (Meraz et al., 1996).

To investigate the role of Stat1 at the level of CIITA promoter IV and therefore its presence in the IFNγ-inducible U complex, we analyzed the protein complexes binding to the NGE probe by supershift experiments using monoclonal antibodies specific for Stat1. While nonspecific monoclonal antibodies (mAbs) could not retard migration of any complex (Figure 4, lanes 7 and 12), Stat1-specific antibodies supershifted the U complex, even when diluted to 1/2000 (Figure 4, lanes 2–6). By contrast, the L complex was not supershifted by anti-Stat1 mAbs (Figure 4, lanes 2–6 and 13). These experiments indicate that Stat1 is present in the IFNγ-dependent GAS/E box U complex. Finally, EMSA performed on the NGE probe with nuclear extract from the U3A Stat1–deficient cell line did not lead to the formation of the U complex following IFNγ induction, whereas this U complex was observed with nuclear extract from the 2FTGH-induced cell line (Figure 4, lanes 8 and 9).

Addition of recombinant activated Stat1 protein (rStat1) to nuclear extracts from unstimulated Me67.8 cells allowed us to reconstitute the U complex (normally seen only after IFNγ induction). This reconstituted U complex was specifically retarded by anti-Stat1 mAbs (Figure 4, lanes 11–13). We conclude that Stat1 is the IFNγ-activated component that mediates the formation of the U complex. Together these results clearly demonstrate that Stat1 controls IFNγ induction of CIITA gene by direct binding to the GAS element of CIITA promoter IV.

Identification of USF-1 as the Essential E Box–Binding Protein on CIITA Promoter IV

Functional assays have revealed the major importance of the E box for inducibility by IFNγ (Figure 1B), and this site is also involved in the formation of the two DNA/protein complexes U and L (Figure 3). The nucleotide sequence of the E box of CIITA PIV matches the consensus binding site (CACGTG) of several bHLH-zip DNA-binding proteins, such as c-Myc, Max, or USF-1/2. To determine which of these E box–binding factors interacts with CIITA promoter IV, we tested several antibodies specific for different proteins of the bHLH-zip family (Figure 5A). Addition of antibodies directed against c-Myc and Max to the binding reactions did not supershift any of the two complexes. On the other hand, polyclonal antibodies specific for USF-1 lead to a supershift of both the U and L complexes (Figure 5A). This indicates that the E box–binding protein USF-1 is present in both the U and L complexes.

To demonstrate directly the capacity of USF-1 to bind to CIITA promoter IV, we performed EMSA with recombinant USF-1 protein (rUSF-1). As shown in Figure 5B, rUSF-1 alone was capable of binding to the NGE probe. Moreover, mutations in the E box of NGE probe (NGEm) abolished rUSF-1 binding, whereas mutations in the GAS element of NGE probe (NGmE) did not (Figure 5B). This demonstrates that USF-1, which is present in U and L complexes, can specifically bind to the E box of CIITA promoter IV.

Close Proximity between Stat1 and USF-1 Is Necessary for CIITA Promoter IV Activity

To analyze the precise DNA contact points involved in the formation of U and L complexes on the CIITA PIV GAS/E box motif, methylation interference analyses were performed with nuclear extract from IFNγ-stimulated Me67.8 (data not shown). USF-1 contact points span the entire E box and two nucleotides downstream

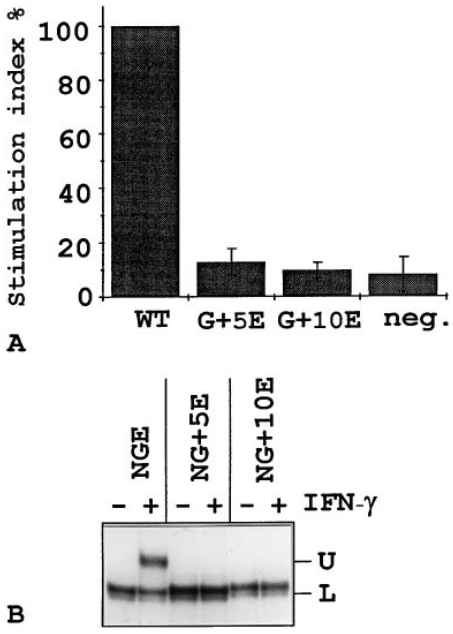

Figure 6. USF-1 and Stat1 Need Close Proximity to Control CIITA PIV Activity

(A) Increasing the distance between the GAS site and E box inhibits CIITA PIV activity. Functional analyses of wt and mutagenised CIITA promoter IV by transient transfections of Me67.8 cells with plasmids PIV-308 wt, G+5E, G+10E, or the promoterless plasmid pGβG(+) and the reference plasmid pGβAcβGID (see Figure 1B). Plotted results are means of three independent experiments with standard deviations.

(B) Distance between GAS site and E box inhibits Stat1/USF-1 binding. EMSA were performed with nuclear extracts from unstimulated (−) or IFNγ-stimulated (+) Me67.8 and either wt NGE, NG+5E, or NG+10E probes. Only the region of the gel containing the upper (U) and the lower (L) complexes is shown.

of it and are similar in positions and intensity for complexes U and L. The binding characteristics of USF-1 are thus identical when bound alone or together with Stat1. Stat1 binding involves contact points on the entire GAS site.

As the GAS site and the E box are juxtaposed on CIITA promoter IV, it was interesting to study if this close proximity is necessary for USF-1 and Stat1 to bind to CIITA promoter IV. We inserted 5 or 10 bp between their respective DNA-binding elements in PIV-308 reporter constructs (G+5E and G+10E). Me67.8 cells were transfected with the wt and mutant constructs for functional analysis. The results showed that insertion of half-helical turn as well as full-helical turn almost abolished CIITA PIV reporter construct inducibility by IFNγ (13% and 9% of wt stimulation index, respectively) (Figure 6A). To analyze the binding capacity of Stat1 and USF-1 on these mutated regions, we carried out EMSA on NGE probes bearing the same nucleotides addition as reporter constructs (NG+5E and NG+10E). In contrast to the wt NGE probe, insertion of 5 or 10 nucleotides between the GAS and E box did not allow the formation of Stat1/USF-1 complex (U, Figure 6B). Nevertheless, USF-1 was still capable of binding alone to these mutated probes. These results indicate that the spatial orientation of bound factors relative to each other as well as the distance between them are limiting features in the formation of the U complex and therefore in promoter response to IFNγ. A very close proximity between Stat1 and USF-1 is thus necessary for their binding to CIITA promoter IV and therefore for its activation.

Cooperative Binding between Stat1 and USF-1 to CIITA Promoter IV

USF-1 and Stat1 bind to juxtaposed *cis*-acting elements, and their binding is strongly influenced by the distance and the spatial orientation relative to each other. Therefore, it was of interest to analyze if the two proteins USF-1 and Stat1 cooperate in the formation of the IFNγ-induced U complex on CIITA promoter IV. EMSA analyses revealed that, in contrast to rUSF-1, which can bind alone to the NGE probe, no complex attributable to rStat1 bound on its own was detected on the NGE probe (Figure 7A, lanes 3 and 7). Moreover, addition of increasing amount of activated rStat1 to rUSF-1, in excess of free probe, generated more rStat1/rUSF-1 complex bound than rUSF-1 bound alone (Figure 7A, lanes 4–6). Quantification of the amount of probe bound indicated that the binding was cooperative, because the amount of probe bound by rStat1/rUSF-1 complex (17%) was 2.2-fold greater than the sum of the probe bound by rUSF-1 alone (7.6%) and rStat1 alone (0%) (Figure 7A, lanes 6, 3, and 7, respectively). The cooperative binding was similarly observed with nuclear extract from unstimulated Me67.8 cells and activated rStat1, with a percentage of probe bound equivalent to 39% with rStat1/USF-1 complex, to 14.7% with USF-1 alone, and 0% with rStat1 alone (data not shown). The complexes formed with recombinant proteins have the same migration profiles as those made with nuclear extracts from Me67.8, L and U (Figure 7A, lanes 1, 2, and 6). Therefore, activated rStat1 either with nuclear extract or with rUSF-1 gave similar migration profiles and cooperative binding. This indicates that USF-1 is not only capable of binding to CIITA-E box but also capable of cooperating with Stat1 to promote its binding to CIITA promoter IV. Also it suggests that no other protein is necessary for the formation of the U complex.

To examine if the cooperative binding of Stat1 and USF-1 might have an effect on the stability of the complexes, we performed off-rate measurements to determine the half-lives of Stat1 alone, USF-1 alone, and the Stat1/USF-1 complex on the DNA probes. The half-life of Stat1 was determined with activated rStat1 on the GAS probe (G), because Stat1 did not bind on its own to the NGE probe under these conditions. rStat1 was very unstable on CIITA-GAS site, with a half-life of less than 1 min (Figure 7B, top and bottom). The half-lives of USF-1 and USF-1/Stat1 were determined with nuclear extract from IFNγ-stimulated cells on the NGE probe. The half-life of USF-1 on the NGE probe was around 7 min. In contrast, USF-1 and Stat1 together formed a much more stable complex with a half-life of 20 min (Figure 7B, middle and bottom).

In conclusion, binding of Stat1 on CIITA-GAS site is strongly stabilized by USF-1. Moreover, binding of USF-1 is also stabilized by Stat1, as the half-life of the Stat1/USF-1 complex is higher than that of USF-1 alone.

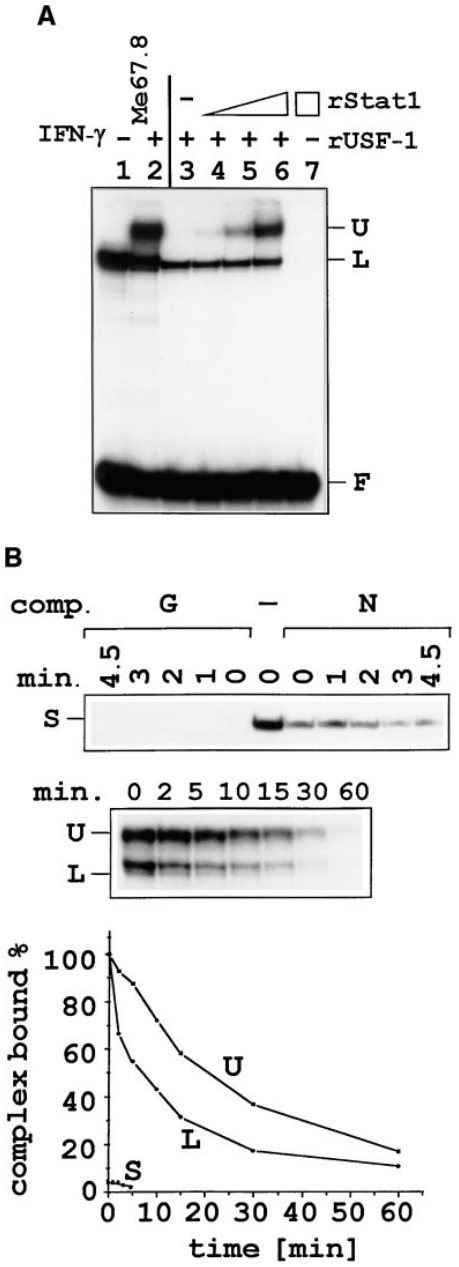

Figure 7. Binding of Stat1 and USF-1 to the NGE Probe Is Strongly Cooperative

(A) EMSA showing the cooperative binding between activated rStat1 and rUSF-1 to NGE probe. EMSA with large excess of NGE probe and rUSF-1 protein (2 ng) (lanes 3–6), either alone (lane 3), or with increasing amount of rStat1 (lane 4, 5 ng; lane 5, 20 ng; and lane 6, 50 ng), or rStat1 alone (lane 7, 50 ng). EMSA with nuclear extract from Me67.8 (lane 1, −IFNγ; lane 2, +IFNγ).

(B) Stat1 is strongly stabilized by USF-1 on the CIITA PIV GAS site. Top: activated rStat1 (10 ng) was incubated with the G probe to allow complexes formation and then supplemented with a 500-fold molar excess of unlabeled G or N competitor oligonucleotides. After 0, 1, 2, 3, or 4.5 min, a sample was directly loaded on a 5% polyacrylamide gel. Middle: the Stat1/USF-1 complex is more stable than USF-1 alone on NGE probe. Nuclear extract from Me67.8 stimulated with IFNγ was first incubated with NGE probe to allow complexes formation, supplemented with a 500-fold excess of unlabeled NGmE competitor oligonucleotide (competing U and L complexes), and then continued for 0, 2, 5, 10, 15, 30, and 60 min before gel electrophoresis. Bottom: off-rates measurements of Stat1 and USF-1 either alone or as a Stat1/USF-1 complex. Gels were quantified by PhosphorImager analysis. The percentage of complexes bound to DNA after adjunction of the competitor is plotted as a function of time. U, upper complex; L, lower complex; S, Stat1 competed with G oligo. For Stat1, 100% of complex bound corresponds to 0 min with the N oligonucleotide as competitor.

Based on the requirement of both the wt E box and GAS site for the formation of the Stat1/USF-1 U complex and promoter activity, as well as the cooperative binding of Stat1 and USF-1 in vitro, we propose that activation of CIITA promoter IV by IFNγ is dependent on the cooperative interaction of Stat1 and USF-1, an interaction that can only take place once the two factors have been recruited to their specific DNA-binding site.

Discussion

Both constitutive and inducible MHC-II gene expression, in different cellular and functional compartments, is quantitatively regulated by the same transactivator CIITA and is therefore dependent on the control of CIITA gene expression itself. In a previous study (Muhlethaler-Mottet et al., 1997), we have shown that this highly complex regulation results from the alternative usage of several distinct promoters of the CIITA gene, which control either constitutive expression, in dendritic cells via promoter I, in B lymphocytes via promoter III, or inducible expression in a variety of other cell types via promoter IV. Functional analysis of CIITA promoter III and IV have shown that in both cases the proximal promoter is sufficient to confer tissue-specific expression of a reporter gene. Sequence conservation between human and mouse and comparison with known *cis*-acting elements indicated the presence of several potential *cis*-regulatory elements (Muhlethaler-Mottet et al., 1997). The purpose of this study was to characterize, functionally and biochemically, the *cis*-acting elements and the *trans*-acting factors that participate in the induction of CIITA promoter IV by IFNγ and thus control the induction of MHC-II expression.

By mutagenesis and analysis with reporter gene assays, we demonstrate the functional importance of the GAS site, the E box, and the IRF-1–binding site of CIITA promoter IV, because mutations of each site separately reduce promoter inducibility by IFNγ dramatically. The double mutant (GmEm) does not exhibit more inhibition compared to the single mutants (Gm or Em), suggesting that the GAS/E box motif functions as a single *cis*-regulatory element. This was observed in different cell types (data not shown). Moreover, functional analyses revealed that the GAS/E box motif and the IRF-1–binding site synergise for CIITA PIV activation, because the sum of the activity resulting from each motif alone (IRF-1 motif with GmEm construct or GAS/E box motif with Im construct) is lower than that observed with both motifs together (wt).

The functionally essential role of the IRF-1–binding site of CIITA PIV, as well as the drastic inhibition of CIITA mRNA induction by IFNγ in IRF-1–deficient cells, indicate a functional role of IRF-1 in CIITA promoter IV

activation, very likely through binding to CIITA PIV. The observed requirement for IRF-1 is in agreement with a previous report showing a reduced induction of CIITA mRNA in IRF-$1^{-/-}$ mice (Hobart et al., 1997). IRF-1 is involved in the regulation of other IFNγ-inducible genes, such as GBP and iNOS (Kamijo et al., 1994; Briken et al., 1995). Because the synthesis of IRF-1 is itself rapidly induced by IFNγ, CIITA dependence on IRF-1 explains the slight delay in the kinetics of IFNγ induction and the partial dependence on de novo protein synthesis observed similarly in the case of GBP and CIITA induction by IFNγ (Steimle et al., 1994).

Different studies implicated Stat1 in the control of CIITA expression without addressing its mode of action. First, CIITA mRNA was not expressed in IFNγ-stimulated bone marrow macrophages from Stat$1^{-/-}$ mice (Meraz et al., 1996). Second, Stat1 antisense experiments showed a reduction of Stat1 protein synthesis leading to a reduction in CIITA induction by IFNγ (Lee and Benveniste, 1996). Here, we have analyzed the role of Stat1 in the induction of CIITA by IFNγ directly at the level of CIITA promoter IV. We demonstrate with reporter gene assays that CIITA PIV is unresponsive to IFNγ in the Stat1-deficient cell line U3A (data not shown). Moreover, we show, by EMSA and with competitor oligonucleotides, antibodies directed against Stat1 and activated rStat1 that Stat1 binds directly to the CIITA PIV-GAS site.

The essential role of the canonical E box within the CIITA promoter IV, as demonstrated by mutagenesis experiments, strongly suggested that a member of bHLH-zip class of transcription factor may be involved in IFNγ-induced CIITA expression. Among the multiple members of this family of transcription factors, the ubiquitous factor USF-1 has been shown here to be a key player in the induction of CIITA expression. Indeed, EMSA with competitor oligonucleotides, USF-1–specific antibody, and recombinant USF-1 protein demonstrate the binding of USF-1 to the E box of CIITA PIV. USF-1/USF-2 can bind to DNA as homo- or heterodimers, and the ratio can vary depending on the cell type. EMSA with α-USF-1 antibody show that all the U and L complexes are supershifted, indicating that no USF-2 homodimers are bound to the NGE probe. In addition, experiments with USF-1 recombinant protein demonstrate that rUSF-1 homodimers are able to bind to the NGE probe on their own, without USF-2, and to cooperate with Stat1 to form the Stat1/USF-1 U complex.

USF is expressed ubiquitously and contributes to the regulation of multiple genes, some of which are expressed in a tissue-specific or inducible manner. The human growth hormone gene (Peritz et al., 1988), the immunoglobulin λ2 chain gene (Chang et al., 1992), the human β-globin control region (Caterina et al., 1994), and the *p53* gene (Reisman and Rotter, 1993) are all regulated to some extent by USF. USF was also implicated in the TGF-β1–responsive element in the human plasminogen activator inhibitor gene (Riccio et al., 1992). Because USF is expressed ubiquitously, the tissue-specific expression of the target gene must therefore be specified by the contribution of other transcription factors. In the case of CIITA gene activation, it is the activation of Stat1 by IFNγ that confers to USF-1 its specific activity, as the result of their cooperative binding to promoter IV. Indeed, USF-1 without Stat1 is not able to activate CIITA PIV, as shown by reporter gene assay with a mutated GAS site and by the use of Stat1 mutant cells.

Modification of the spatial orientation (+5) or distance (+10) between GAS and E box abolishes cooperative binding of Stat1 and USF-1, as well as inducibility of promoter IV to IFNγ. Therefore, cooperative binding of USF-1 and Stat1 and subsequent promoter activation require not only the integrity of the GAS site and of the E box sequences but also their relative position and distance. The simultaneous addition of activated rStat1 and USF-1 generates an increased amount of rStat1/USF-1 complex, compared to the amount of rStat1 and USF-1 bound alone, indicative of highly cooperative interactions. Indeed, cooperativity is defined by an increase in the number of promoter-bound complexes when both factors are present in comparison to the number observed with either factor alone.

The strong cooperativity between Stat1 and USF-1 for binding to CIITA PIV was also demonstrated by an analysis of the stability of the complexes bound to DNA. Off-rate experiments showed that, although Stat1 has a dramatically short half-life on the CIITA-GAS site, the half-life of the Stat1/USF-1 complex is considerably extended. Binding of Stat1 to CIITA PIV is therefore strongly stabilized by the presence of USF-1. The binding of USF-1 is also stabilized by the presence of Stat1, because the half-life of the Stat1/USF-1 complex is three times that of USF-1 alone. This strong cooperativity between Stat1 and USF-1 is in agreement with the results obtained by functional assays and EMSA, showing that in the absence of USF-1 binding, Stat1 alone is neither capable to bind nor to activate CIITA promoter IV. It also explains why the GAS/E box motif functions as a single *cis*-regulatory element.

The principle of cooperative binding of two or more transcription factors on DNA implies that such proteins do not spontaneously interact until they are recruited to their respective DNA target, which allows them to retain their full potential for binding to different promoters and for association with different protein partners, as is known to be the case for Stat1 and USF-1. Initial binding of individual factors to their DNA target, even at low affinity, is thus a prerequisite for subsequent interactions between the two protein partners, which results in the stabilization of the multiprotein complex on DNA and eventually in gene activation. In an alternative but not exclusive model, binding of one factor might produce a structural modification in an adjacent DNA site that facilitates binding of a second factor. Such an effect is considered unlikely on such a short DNA probe and without any chromatin structure.

The case of CIITA promoter IV is, to our knowledge, the first example of cooperative interaction between Stat1 and an ubiquitous transcription factor, such as USF-1, in the selective activation of a given promoter. The same applies to USF-1. Stat1 can interact with CBP/p300, a transcriptional coactivator known to potentiate the activity of different transcription factors (Zhang et al., 1996). Stat1 has also been shown to synergise with other transcription factors like Sp1 (Look et al., 1995)

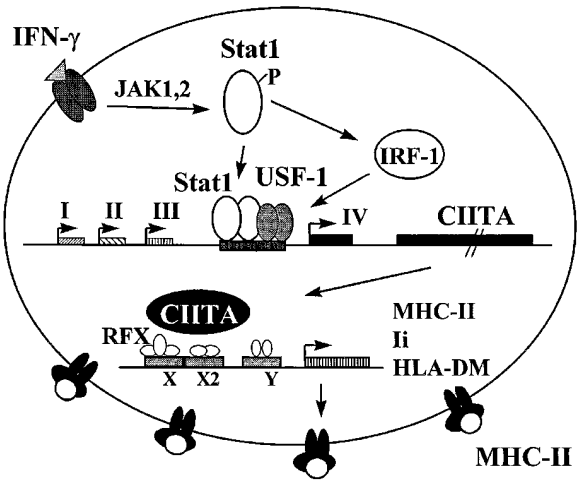

Figure 8. The Entire Cascade of the Induction of MHC-II Genes Expression by IFNγ

or NFkB (Ohmori et al., 1997) to increase promoter activity, but in these cases, there was no evidence of cooperativity in the binding of the two factors.

The mechanism of CIITA induction by IFNγ can be distinguished from that of other IFNγ-inducible genes by the strict requirement for USF-1, as an essential partner for Stat1 binding and activity. This unique and functionally essential cooperative interaction between Stat1 and USF-1 for the activation of CIITA expression and thus for the induction of MHC class II molecules points to a highly selective novel target for potential immunomodulators. Indeed, it should be possible to affect this cooperative interaction selectively, ideally without compromising the role of each of the two factors in the activation of other promoters, or to selectively block the binding of the Stat1/USF-1 complex (Choo et al., 1994). The physiological implications of a selective experimental inhibition of inducible expression of MHC class II genes, via the inducible promoter of CIITA, without compromising MHC class II expression on professional antigen-presenting cells, might be of great biological and pharmacological interest.

The identification of the key players and the mechanisms of their involvement, including the demonstration that Stat1 controls CIITA induction by direct binding to the functionally essential GAS element of CIITA promoter IV cooperatively with USF-1, allows us to fill the last missing gap in the entire cascade of MHC-II genes induction by IFNγ (Figure 8). This cascade begins with the binding of IFNγ to its receptor, leading to dimerization of the receptor, activation of Janus kinase 1 and 2 by tyrosine phosphorylation, and tyrosine phosphorylation of receptors cytoplasmic domains allowing interaction with Stat1. Stat1 is then activated by tyrosine phosphorylation, dimerizes, and translocates to the nucleus. There it binds to the GAS element of CIITA promoter IV, where it is stabilized by its cooperative interaction with USF-1, and activates the transcription of CIITA mRNA. CIITA then operates as the essential mediator of MHC-II gene induction (Steimle et al., 1993; Steimle et al., 1994). It activates MHC-II gene expression as a coactivator interacting with the promoter-bound transcription factors (Figure 8).

Activation of CIITA promoter IV by IFNγ represents an additional example where specificity in gene activation depends on composite DNA elements. Specificity relies on both DNA–protein and protein–protein interactions, in this case between the IFNγ induced Stat1 and the ubiquitous bHLH-zip USF-1. We had observed a similar phenomenon in the case of MHC class II promoters, where cooperative interactions between the RFX complex and other factors (X2bp and NF-Y) were shown to be essential for stable binding of these complexes and activation of transcription (Reith et al., 1994a, 1994b; Mach et al., 1996). Combinatorial interactions between tissue-specific, developmentally restricted, and ubiquitous transcription factors is an efficient way to achieve selective activation of multiple individual genes, as the result of specific combination of a restricted set of *cis*-acting elements, each capable of binding a limited number of transcription factors.

Experimental Procedures

Cells

The cell lines Me67.8 (melanoma cell line, provided by S. Carrell) and THP1 (monocytic cell line) were grown in RPMI-1640 medium. The cell lines 2FTGH (fibrosarcoma cell line) and U3A (Stat1 mutant from 2FTGH), both a gift from I. Kerr, were grown in Dulbecco's modified Eagle's medium. Mediums were supplemented with 10% fetal calf serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 2 mM L-glutamine and incubated at 37°C in 5% CO_2.

Reporter Gene Assays

Reporter gene expression was measured by quantitative RT-PCR as previously described (Sperisen et al., 1992). Transfections, RNA preparations, and RT-PCR analyses were performed as previously described (Muhlethaler-Mottet et al., 1997). Plasmid PIV-308 contains the −308 to +75 fragment of the 5′ flanking region of CIITA promoter PIV subcloned upstream of the rabbit β-globin gene of plasmid pGβG(+). Mutagenesis was performed by introduction of restriction endonuclease sites by PCR, generating four to five nucleotide modifications per mutated binding site. The input ratio between CIITA constructs and the reference plasmid was 9:1. RT-PCR products were denatured and separated by electrophoresis on 6% polyacrylamide 8 M urea gel. mRNA-derived signals were quantified by PhosphorImager. Promoter activity was determined by normalizing the mRNA signal derived from CIITA construct to that obtained with the reference plasmid.

RNase Protection Assays

RNAs from EF from wt and IRF-$1^{-/-}$ mice were a gift from M. Matsumoto and T. Taniguchi (Kimura et al., 1994). For the IRF-1 probe, a fragment was constructed that covers nucleotides 797 (NdeI) to 1061 (XhoI), protecting 264 bp of IRF1 mRNA. In IRF-$1^{-/-}$ EF, this probe covers nucleotides 874 to 1061 (XhoI), protecting 187 bp of IRF-$1^{-/-}$ mRNA. CIITA and TBP probes were described elsewhere (Otten et al., 1998). RNase protection assays with 10 μg of RNA per reaction were carried out as previously described (Steimle et al., 1993).

Oligonucleotides

NGE: 5′-ggCCAGGCAGTTGGGATGCCACTTCTGATAAAGCACGTG GTGGCCACAG-3′; N: 5′-ggCAGTTGGGATGCCACTTCT-3′; G: 5′-gg CCACTTCTGATAAAGCAC-3′; E: 5′-gggAAAGCACGTGGTGGCC-3′; NGmE: 5′-ggCCAGGCAGTTGGGATGCCACGAATTCTAAAGCACG TGGTGGCCACAG-3′; NGEm: 5′-ggCCAGGCAGTTGGGATGCCAC TTCTGATAAAGGAATTCGTGGCCACAG-3′; NG+5E: 5′-ggCCAGGC AGTTGGGATGCCACTTCTGATAAACTCGAGCACGTGGTGGCCAC AG- 3′; and NG+10E: 5′-ggCCAGGCAGTTGGGATGCCACTTCTGA TAAACTCGAGCTAGGCACGTGGTGGC CACAG-3′. Mutations are underlined.

EMSA and Methylation Interference Analysis

Cells were stimulated or not by IFNγ (500 U/ml) for 30 min prior to nuclear extract preparation as described by Harroch et al. (1994). The oligonucleotides were annealed with their complementary sequences, end-labeled by [γ-^{32}P]ATP with T4 PNK, and gel-purified.

For binding reaction with the NGE probes, 6 μg of nuclear extract proteins was mixed with 2 × 10^4 cpm DNA probe, 1.25 μg poly (dI)(dC) (Pharmacia), 0.5 μg *Escherichia coli* single-stranded DNA, with or without competitor, in a final volume of 20 μl containing 20 mM Tris-HCl (pH 7.9), 50 mM NaCl, 1 mM EDTA, 5% (v/v) glycerol, 5 mM dithiothreitol, 1 mM spermidine, and 100 μg bovine serum albumin. Binding reactions with the G probe were performed with the following modifications: no poly(dI)(dC), no *E. coli* single-stranded DNA, in a final volume of 20 μl containing 20 mM Tris-HCl (pH 7.9), 40 mM KCl, 2.5 mM $MgCl_2$, 1 mM EDTA, 5% (v/v) glycerol, 5 mM dithiothreitol, 1 mM spermidine, and 100 μg bovine serum albumin. Purified recombinant activated Stat1 (a gift from R. Schreiber) (Greenlund et al., 1995) and purified recombinant USF-1 (a gift from P. Pognonec) (Roy et al., 1991) were used in the same conditions. After addition of the labeled probe, the mixture was incubated 30 min at 20°C. For supershift experiments, antibodies were added (as described in figure legends) and left 20 min at 4°C before gel electrophoresis in 5% acrylamide/bisacrylamide (29:1) gels with 0.25 × TBE for 3 hr at 200 V at 4°C with recirculating buffer. Gels were dried and subjected to autoradiography. Quantifications were carried out by PhosphorImager.

Antibodies against Stat1 (SATO-20.1, a gift from R. Schreiber) and negative control (a gift from S. Izui) are mAbs from hybridoma culture supernatants. Antibodies against USF-1 (provided by R.G. Roeder and P. Pognonec) (Pognonec and Roeder, 1991), Myc and Max (both a gift from B. Amati) (Littlewood et al., 1992) are polyclonal antisera.

Methylation interference analysis were performed as previously described (Kobr et al., 1990), except that binding reactions were set up as described above.

Acknowledgments

We thank P. Staeheli for IRF-1 cDNA and T. Taniguchi for RNAs from wt and IRF-$1^{-/-}$ mice. We are grateful to I. Kerr for providing cell lines, R. Schreiber and P. Pognonec for purified proteins and antibodies, and B. Amati, S. Izui, and R. G. Roeder for antibodies. We are grateful to B. Amati for discussions concerning bHLH proteins. We thank V. Steimle, W. Reith, P. Emery, M. Strubin, and other members of the Mach lab for helpful discussions and advice and W. Reith and V. Steimle for critical reading of the manuscript. This work was supported by grants from the Swiss National Foundation and the Louis Jeantet Foundation.

Received October 10, 1997; revised December 12, 1997.

References

Ayer, D.E., Kretzner, L., and Eisenman, R.N. (1993). Mad: a heterodimeric partner for Max that antagonizes Myc transcriptional activity. Cell *72*, 211–222.

Beckmann, H., Su, L., and Kadesch, T. (1990). TFE3: a helix-loop-helix protein that activates transcription through the immunoglobulin enhancer μE3. Genes Dev. *4*, 167–179.

Blackwood, E.M., and Eisenman, R.N. (1991). Max: a helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc. Science *251*, 1211–1217.

Boehm, U., Klamp, T., Groot, M., and Howard, J.C. (1997). Cellular responses to interferon-gamma. Ann. Rev. Immunol. *15*, 749–795.

Briken, V., Ruffner, H., Schultz, U., Schwarz, A., Reis, L.F., Strehlow, I., Decker, T., and Staeheli, P. (1995). Interferon regulatory factor 1 is required for mouse Gbp gene activation by gamma interferon. Mol. Cell Biol. *15*, 975–982.

Carr, C.S., and Sharp, P. (1990). A helix-loop-helix protein related to the immunoglobulin Ebox-binding proteins. Mol. Cell. Biol. *10*, 4384–4388.

Caterina, J.J., Ciavatta, D.J., Donze, D., Behringer, R.R., and Townes, T.M. (1994). Multiple elements in human beta-globin locus control region 5′ HS 2 are involved in enhancer activity and position-independent, transgene expression. Nucleic Acids Res. *22*, 1006–1011.

Chang, L.A., Smith, T., Pognonec, P., Roeder, R.G., and Murialdo, H. (1992). Identification of USF as the ubiquitous murine factor that binds to and stimulates transcription from the immunoglobulin lambda 2-chain promoter. Nucleic Acids Res. *20*, 287–293.

Chang, C.H., Fontes, J.D., Peterlin, M., and Flavell, R.A. (1994). Class II transactivator (CIITA) is sufficient for the inducible expression of major histocompatibility complex class II genes. J. Exp. Med. *180*, 1367–1374.

Choo, Y., Sanchez Garcia, I., and Klug, A. (1994). In vivo repression by a site-specific DNA-binding protein designed against an oncogenic sequence. Nature *372*, 642–645.

Darnell, J.E., Jr. (1997). STATs and gene regulation. Science *277*, 1630–1635.

Darnell, J.E., Jr., Kerr, I.M., and Stark, G.R. (1994). Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science *264*, 1415–1421.

Decker, T., Lew, D.J., Mirkovitch, J., and Darnell, J.E. (1991). Cytoplasmic activation of GAF, an IFNγamma-regulated DNA-binding factor. EMBO J *10*, 927–932.

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.

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

Greenlund, A.C., Morales, M.O., Viviano, B.L., Yan, H., Krolewski, J., and Schreiber, R.D. (1995). Stat recruitment by tyrosine-phosphorylated cytokine receptors: an ordered reversible affinity-driven process. Immunity *2*, 677–687.

Gregor, P.D., Sawadago, M., and Roeder, R.G. (1990). The adenovirus major late transcription factor USF is a member of the helix-loop-helix group of regulatory proteins and binds to DNA as a dimer. Genes Dev. *4*, 1730–1740.

Griscelli, C., Lisowska-Grospierre, B., and Mach, B. (1989). Combined immunodeficiency with defective expression in MHC class II genes. Immunodefic. Rev. *1*, 135–153.

Harroch, S., Revel, M., and Chebath, J. (1994). Induction by interleukin-6 of interferon regulatory factor 1 (IRF-1) gene expression through the palindromic interferon response element pIRE and cell type-dependent control of IRF-1 binding to DNA. EMBO J. *13*, 1942–1949.

Hobart, M., Ramassar, V., Goes, N., Urmson, J., and Halloran, P.F. (1997). IFN regulatory factor-1 plays a central role in the regulation of the expression of class I and II MHC genes in vivo. J. Immunol. *158*, 4260–4269.

Kamijo, R., Harada, H., Matsuyama, T., Bosland, M., Gerecitano, J., Shapiro, D., Le, J., Koh, S.I., Kimura, T., Green, S.J., et al. (1994). Requirement for transcription factor IRF-1 in NO synthase induction in macrophages. Science *263*, 1612–1615.

Kimura, T., Nakayama, K., Penninger, J., Kitagawa, M., Harada, H., Matsuyama, T., Tanaka, N., Kamijo, R., Vilcek, J., Mak, T.W., and Taniguchi, T. (1994). Involvement of the IRF-1 transcription factor in antiviral response to interferons. Science *264*, 1921–1924.

Kobr, M., Reith, W., Herrero Sanchez, C., and Mach, B. (1990). Two DNA-binding proteins discriminate between the promoters of different members of the major histocompatibility complex class II multigene family. Mol. Cell. Biol. *10*, 965–971.

Lee, Y.J., and Benveniste, E.N. (1996). Stat1 alpha expression is involved in IFN-gamma induction of the class II transactivator and class II MHC genes. J. Immunol. *157*, 1559–1568.

Littlewood, T.D., Amati, B., Land, H., and Evan, G.I. (1992). Max and c-Myc/Max DNA-binding activities in cell extracts. Oncogene *7*, 1783–1792.

Look, D.C., Pelletier, M.R., Tidwell, R.M., Roswit, W.T., and Holtzman, M.J. (1995). Stat1 depends on transcriptional synergy with Sp1. J. Biol. Chem. *270*, 30264–30267.

Mach, B., Steimle, V., and Reith, W. (1994). MHC class II-deficient

combined immunodeficiency: a disease of gene regulation. Immunol. Rev. *138*, 207–221.

Mach, B., Steimle, V., Martinez-Soria, E., and Reith, W. (1996). Regulation of MHC class II genes: lessons from a disease. Annu. Rev. Immunol. *14*, 301–331.

Meraz, M.A., White, J.M., Sheehan, K.C., Bach, E.A., Rodig, S.J., Dighe, A.S., Kaplan, D.H., Riley, J.K., Greenlund, A.C., Campbell, D., R.D., et al. (1996). Targeted disruption of the *Stat1* gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway. Cell *84*, 431–442.

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.

Ohmori, Y., Schreiber, R.D., and Hamilton, T.A. (1997). Synergy between interferon-gamma and tumor necrosis factor-alpha in transcriptional activation is mediated by cooperation between signal transducer and activator of transcription 1 and nuclear factor kappaB. J Biol. Chem. *272*, 14899–14907.

Otten, L.A., Steimle, V., Bontron, S., and Mach, B. (1998). Quantitative control of MHC class II expression by the transactivator CIITA. Eur. J. Immunol. *28*, 473–478.

Peritz, L.N., Fodor, E.J., Silversides, D.W., Cattini, P.A., Baxter, J.D., and Eberhardt, N.L. (1988). The human growth hormone gene contains both positive and negative control elements. J. Biol. Chem. *263*, 5005–5007.

Pognonec, P., and Roeder, R.G. (1991). Recombinant 43-kDa USF binds to DNA and activates transcription in a manner indistinguishable from that of natural 43/44-kDa USF. Mol. Cell Biol. *11*, 5125–5136.

Reisman, D., and Rotter, V. (1993). The helix-loop-helix containing transcription factor USF binds to and transactivates the promoter of the p53 tumor suppressor gene. Nucleic Acids Res. *21*, 345–350.

Reith, W., Satola, S., Herrero Sanchez, C., Amaldi, I., Lisowska-Grospierre, B., Griscelli, C., Hadam, M.R., and Mach, B. (1988). Congenital immunodeficiency with a regulatory defect in MHC class II gene expression lacks a specific HLA-DR promoter binding protein, RF-X. Cell *53*, 897–906.

Reith, W., Kobr, M., Emery, P., Durand, B., Siegrist, C.A., and Mach, B. (1994a). Cooperative binding between factors RFX and X2bp to the X and X2 boxes of MHC class II promoters. J. Biol. Chem. *269*, 20020–20025.

Reith, W., Siegrist, C.A., Durand, B., Barras, E., and Mach, B. (1994b). Function of major histocompatibility complex class II promoters requires cooperative binding between factors RFX and NF-Y. Proc. Natl. Acad. Sci. USA *91*, 554–558.

Riccio, A., Pedone, P.V., Lund, L.R., Olesen, T., Olsen, H.S., and Andreasen, P.A. (1992). Transforming growth factor beta 1-responsive element: closely associated binding sites for USF and CCAAT-binding transcription factor-nuclear factor I in the type 1 plasminogen activator inhibitor gene. Mol. Cell Biol. *12*, 1846–1855.

Roy, A.L., Meisterernst, M., Pognonec, P., and Roeder, R.G. (1991). Cooperative interaction of an initiator-binding transcription initiation factor and the helix-loop-helix activator USF. Nature *354*, 245–248.

Schindler, C., and Darnell, J.E. (1995). Transcriptional responses to polypeptide ligands: the JAK-STAT pathway. Annu. Rev. Biochem. *64*, 621–651.

Silacci, P., Mottet, A., Steimle, V., Reith, W., and Mach, B. (1994). Developmental extinction of major histocompatibility complex class II gene expression in plasmocytes is mediated by silencing of the transactivator gene CIITA. J. Exp. Med. *180*, 1329–1336.

Sirito, M., Lin, Q., Maity, T., and Sawadogo, M. (1994). Ubiquitous expression of the 43- and 44-kDa forms of transcription factor USF in mammalian cells. Nucleic Acids Res. *22*, 427–433.

Sperisen, P., Wang, S.M., Reichenbach, P., and Nabholz, M. (1992). A PCR-based assay for reporter gene expression. PCR Meth. Appl. *1*, 164–170.

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., Siegrist, C., Mottet, A., Lisowska-Grospierre, B., and Mach, B. (1994). Regulation of MHC class II expression by Interferon-gamma mediated by the transactivator gene CIITA. Science *265*, 106–109.

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 and Dev. *9*, 1021–1032.

Zhang, J.J., Vinkemeier, U., Gu, W., Chakravarti, D., Horvath, C.M., and Darnell, J.E. (1996). Two contact regions between Stat1 and CBP/p300 in interferon gamma signaling. Proc. Natl. Acad. Sci. USA *93*, 15092–15096.

Zhou, H., and Glimcher, L.H. (1995). Human MHC class II gene transcription directed by the carboxyl terminus of CIITA, one of the defective genes in type II MHC combined immune deficiency. Immunity *2*, 545–553.