observation that the cre^{lck} transgene is not expressed in mature T cells (9).

There may be two main reasons for the incompleteness of *polβ* gene deletion in our experimental system. First, the *lck* proximal promoter is active only at early stages of T cell development (*15*). Earlier data also indicate that the cre^{lck} transgene is expressed only transiently in the thymus (9). It is therefore conceivable that in the transgenic T cells the $polβ^{flox}$ alleles have only a brief period of time to accomplish Cre-*loxP*–mediated recombination. Second, the *cre* gene that we have used corresponds to the wild-type *cre* gene of P1 phage (6). It is known that the expression of this gene in eukaryotic cells is suboptimal, but it can be improved by appropriate genetic manipulation (*7*, *16*). Thus, there are straightforward ways in which our experimental system can be improved to obtain a more efficient deletion of the target gene.

Our data provide no direct evidence at this stage about a possible involvement of *polβ* in the control of TCR gene rearrangements. However, we might interpret the lesser extent of *polβ* deletion in $polβ^{Δ}/polβ^{flox}$; cre^{lck} mice as compared to that in $polβ^{flox}/+$; cre^{lck} transgenic mice (Table 3) to mean that in the former case Cre-*loxP*–mediated *polβ* inactivation results in cell death if it happens to occur before the completion of TCR gene rearrangement.

In principle, Cre-*loxP*–mediated gene targeting should allow the inactivation of any gene in any tissue at any stage of development. It can also be adapted to conditional reconstitution of gene function. Furthermore, through lineage-specific inactivation of genes critical for cell survival, this approach can potentially be used for the ablation of cell lineages in vivo.

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10. A 7.1-kb mouse genomic DNA (Eco RI–Eco RI) fragment containing the promoter and the first and second exon of the DNA *polβ* gene (*17*) was used to produce the targeting construct pMGβ9. A gene cassette containing the neo^r and *HSV-tk* genes flanked by two *loxP* sites was inserted into the Xho I site between the first and second exon. A third *loxP* site was introduced into a Sac I site approximately 2 kb upstream from the first exon. The final targeting construct contains 1 kb of flanking genomic sequences further upstream from the third *loxP* site and a 3-kb fragment including the second exon of the *polβ* gene downstream from the Xho I site (Fig. 2C). To generate homologous recombinants, we transfected E14-1 ES cells [R. Kühn, K. Rajewsky, W. Müller, *Science* **254**, 707 (1991)] with 25 μg of DNA (of the linearized targeting construct) by electroporation. The transfected ES cells were grown on a single layer of mitomycin C–treated embryonic fibroblasts. After 1 week of selection in G418-containing medium, homologous recombinants were identified by Southern blot hybridization based on the strategy depicted in Fig. 2C. A targeted clone should yield a 5.5-kb band in addition to an equally intense 10-kb wild-type band upon hybridization to probe A (Fig. 2). To generate type I and type II deletions, 1 to 3 μg of supercoiled Cre-encoding plasmid, pIC-Cre (*6*), was introduced into the targeted ES cells by electroporation. After selection in ganciclovir-containing medium (1×10^{-6} M) for 5 days, surviving clones were picked and expanded. Genomic DNA was then prepared from the expanded cells for Southern blot analysis. Probe A is a 1-kb genomic DNA fragment (Hind III–Sac I) of the *polβ* gene, and probe B is a 900-bp fragment (Bam HI–Hind III) (*17*).
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Regulation of MHC Class II Expression by Interferon-γ Mediated by the Transactivator Gene CIITA

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Major histocompatibility complex (MHC) class II genes are expressed constitutively in only a few cell types, but they can be induced in the majority of them, in particular by interferon-γ (IFN-γ). The MHC class II transactivator gene CIITA is defective in a form of primary MHC class II deficiency. Here it is shown that CIITA expression is controlled and induced by IFN-γ. A functional CIITA gene is necessary for class II induction, and transfection of CIITA is sufficient to activate expression of MHC class II genes in class II–negative cells in the absence of IFN-γ. CIITA is therefore a general regulator of both inducible and constitutive MHC class II expression.

MHC class II molecules present antigens to T helper lymphocytes, and the tight regulation of their expression is of critical importance for the control of the immune response both in physiological and pathological situations. However, the mechanism of IFN-γ–induced MHC class II expression remains obscure (*1–4*). Induction of MHC class II genes by IFN-γ is characterized by an unusually long lag period and total dependence on de novo protein synthesis (*5–7*). These genes thus exhibit a "secondary" response to IFN-γ (*8*, *9*), which has led to the postulation of an intermediary activator, itself induced by IFN-γ (*5–7*). Despite considerable effort it has not been possible to define "interferon response elements" convincingly in MHC class II genes (*2*). The same DNA sequence elements seem to be required for IFN-γ–induced transcription and constitutive expression in B cells (*2*, *3*). The factors implicated thus far in the regulation of MHC class II genes are expressed constitutively, and their binding to MHC class II

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promoters does not depend on IFN-γ induction (*4*). None of the now well documented IFN-γ–inducible factors has been found to bind to class II promoter sequences. Furthermore, it has been suggested that the constitutive and inducible modes of class II expression are controlled by distinct regulatory mechanisms (*10*).

The MHC class II transactivator CIITA was recently cloned by genetic complementation of an MHC class II mutant B lymphocyte line. CIITA was shown to be defective in a form of primary MHC class II deficiency (or bare lymphocyte syndrome, BLS) and is therefore essential for constitutive expression of MHC class II genes (*11*). Interestingly, CIITA is expressed in a cell type–specific manner, and the pattern of its expression correlates with that of MHC class II molecules in numerous cell lines and tissues (*11, 12*). CIITA expression was studied after IFN-γ stimulation of MHC class II–negative cells. In three different types of IFN-γ–inducible cell lines—a monocytic, a fibroblastic, and a melanoma cell line—CIITA mRNA was indeed detected only after induction by IFN-γ (Fig. 1). Induction of CIITA expression was rapid and preceded by several hours that of MHC class II genes (Fig. 2, A and C): CIITA mRNA appeared in monocytic THP-1 cells as early as 1 to 1.5 hours after the addition of IFN-γ and reached steady-state levels after 6 to 12 hours (Fig. 2A). CIITA is the first MHC class II regulatory factor induced by IFN-γ.

The availability of fibroblast lines from patients belonging to the CIITA-deficient BLS complementation group A (*11, 13*) allowed us to test directly for an obligatory role of CIITA in IFN-γ–induced MHC class II expression. As shown in Fig. 3, the fibroblast line MHV (*13, 14*) could not be induced to express MHC class II molecules by the addition of IFN-γ, whereas MHC class I molecules were induced normally. This indicates that the integrity of CIITA is required for induction by IFN-γ.

Having established that CIITA is required both for constitutive and inducible class II expression and is itself induced by IFN-γ, we addressed whether CIITA expression alone is sufficient to activate MHC class II gene transcription in the absence of IFN-γ. CIITA complementary DNA (cDNA) under the control of a constitutive promoter was therefore introduced into a variety of MHC class II–negative, IFN-γ–inducible cell lines. As shown in Fig. 4A, stable transfection of CIITA cDNA was sufficient to confer constitutive HLA class II expression in monocytic, fibroblastic, as well as melanoma lines. All of these cells acquired, on transfection with CIITA, a cell surface expression of MHC class II molecules similar to that induced by IFN-γ; HLA-DR, -DP, and -DQ were all activated by CIITA (Fig. 4A, panels 1 to 3). Furthermore, transient transfection of CIITA into the CIITA-deficient fibroblast line MHV also resulted in an MHC class II–positive phenotype (Fig. 4B, left). Transfection of CIITA into a fibroblast line (ABL) (*13*) from a different BLS complementation group (group B) had no effect, confirming the specificity of CIITA action (Fig. 4B, right). As expected from the distinct characteristics of MHC class I induction (*15*), CIITA expression did not affect the level of expression of MHC class I molecules (Fig. 4A, panel 6). These results demonstrate that CIITA expression can substitute for IFN-γ to induce MHC class II expression and that CIITA expression rescues MHC class II expression in CIITA-deficient fibroblasts in a specific manner. The results also show that induction of CIITA by IFN-γ is by itself sufficient for activation of MHC class II transcription. Cotransfection of CIITA cDNA and a chloramphenicol acetyltransferase (CAT) reporter gene under the control of the proximal HLA-DRA promoter resulted in a strong transactivation (Fig. 4C), indicating that CIITA transactivation is mediated by the proximal class II promoter.

Fusion experiments with human-mouse hybrids have identified several loci that are critical for the induction of MHC class II by IFN-γ. An essential factor for IFN-γ induction of MHC class II molecules was shown to be located on human chromosome 16 (*16*). We have localized the CIITA gene by in situ fluorescence hybridization to chromosome 16 (*12*), which suggests that CIITA is the factor postulated by these earlier cell-fusion experiments (*16*). A second locus, necessary for species specificity of human IFN-γ induction (*16*), has now been identified as the catalytic subunit of the human IFN-γ receptor and is located on chromosome 21 (*17*).

The demonstration that CIITA is an essential mediator of IFN-γ induction of MHC class II genes led us to study the pattern of CIITA induction. In contrast to MHC class II induction (Fig. 2C) (*5–7*), inhibition of protein synthesis by cycloheximide does not prevent CIITA induction by IFN-γ, although a partial reduction

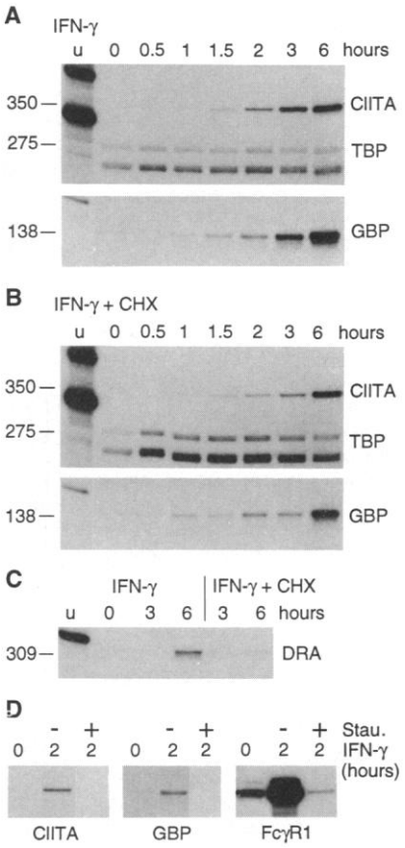

Fig. 2. Time course of CIITA mRNA induction in THP-1 cells with IFN-γ alone or in the presence of inhibitors. The induction of CIITA was compared to that of the IFN-γ–inducible genes GBP and FcγR1 (high-affinity IgG receptor 1). (**A**) Time course of IFN-γ induction. (**B**) IFN-γ induction in the presence of the inhibitor of protein synthesis cycloheximide (CHX, Sigma; 5 μg/ml). Cycloheximide was added to the THP-1 cells 15 min before IFN-γ (time point zero). (**C**) HLA-DRA expression in cells stimulated by IFN-γ with or without cycloheximide (*24*). (**D**) IFN-γ induction in the presence of the inhibitor staurosporine (Stau., Calbiochem; 0.5 μM). THP-1 cells were incubated for 2 hours with IFN-γ in the absence (−) or presence (+) of staurosporine and analyzed for the expression of CIITA, GBP, and FcγR1 (*24*). The GBP probe (*25*) protects a 138-bp fragment. The FcγR1 probe (*26*), generated by polymerase chain reaction amplification of genomic DNA, protects a 201-bp fragment. The size of protected fragments is indicated on the left (in base pairs).

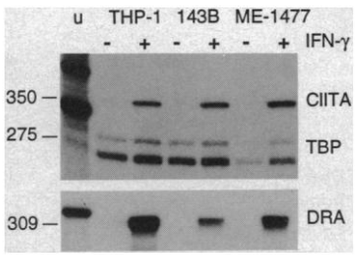

Fig. 1. CIITA mRNA is expressed only in IFN-γ–induced, MHC class II–positive cells. The cell lines THP-1 (monocytic, ATCC TIB202), 143B (*6*) (fibroblastic osteosarcoma), and ME1477 (melanoma) were grown in RPMI 1640 (THP-1) or Dulbecco's minimum essential medium (143B and ME1477) supplemented with 10% fetal calf serum either without or with IFN-γ (250 U/ml) for 48 hours. CIITA and HLA-DRA expression was analyzed in total RNA from unstimulated (−) or IFN-γ–induced (+) cells by ribonuclease protection analysis with a TBP-probe as internal control for the CIITA hybridization (u, undigested probes) (*23*). The size of protected fragments is indicated on the left (in base pairs).

of CIITA mRNA is found at intermediary time points (1.5 to 3 hours, Fig. 2B). In THP-1 cells, the pattern of CIITA induction by IFN-γ is very similar to that of the well-studied guanylate binding protein (GBP) gene, whose promoter contains the first described gamma activated sequence (GAS) element (*8*, *18*). In these cells, GBP mRNA also appears after 1.5 hours of IFN-γ stimulation and exhibits a partial sensitivity to cycloheximide (Fig. 2, A and B). Induction of the CIITA, GBP, and FcγR1 [high-affinity immunoglobulin G (IgG) receptor] genes by IFN-γ is completely inhibited by staurosporine (Fig. 2D), which has been shown to block the functionally critical phosphorylation of p91 (*19*, *20*). The p91 protein has recently been shown to mediate the induction of all IFN-γ–inducible genes, including MHC class II genes (*9*, *19*, *21*). In contrast to immediate early genes, which require only latent preexisting factors for induction (*22*), both CIITA and GBP exhibit intermediary kinetics and partial sensitivity to cycloheximide in THP-1 cells. This probably reflects the fact that in certain cell types the synthesis of one or more additional factors is necessary for full stimulation (*22*). Such factors may themselves be induced by IFN-γ, as for example the DNA-binding component of IFN-stimulated gene factor–3, p48 (*9*).

Taken together, the differential expression of CIITA, the induction of CIITA mRNA by IFN-γ (Figs. 1 and 2), the demonstration of the noninducibility of CIITA-deficient fibroblasts (Fig. 3), and the demonstration that CIITA expression is sufficient to induce MHC class II expression both in normal inducible cells and in CIITA-defective cells (Fig. 4) provide a complete chain of evidence for the essential role of CIITA in IFN-γ–inducible MHC class II expression. We accordingly propose a model for the regulation of MHC class II gene transcription in which the IFN-γ–induced intracellular signals activate expression of the CIITA gene as an obligatory intermediate, rather than having a direct effect on MHC class II promoters (Fig. 5). In cells constitively positive for class II, CIITA is also essential and is constitutively expressed (*11*). These results indicate that control of both constitutive and inducible MHC class II expression is mediated by the transactivator CIITA. Because there is no evidence for direct binding of CIITA to the MHC class II promoter (*11*, *12*), CIITA might act as a gene- and tissue-specific coactivator. Identification of CIITA as a modulator of MHC class II gene expression in multiple cell types opens new perspectives for the manipulation of the immune system. This can be envisaged either by up- or down-regulation of CIITA expression or activity, and hence of MHC class II expression, or by introduction of the recombinant CIITA gene to increase immunogenicity.

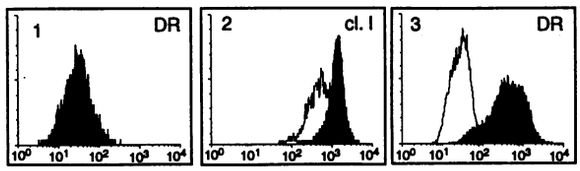

Fig. 3. MHC class II gene expression cannot be induced by IFN-γ in a CIITA-deficient BLS fibroblast cell line. Fibroblasts from patient M.H.V. were cultivated for 48 hours in the absence (open profiles) or presence (shaded profiles) of IFN-γ (500 U/ml) and analyzed by flow cytofluorimetry for the expression of HLA-DR (panel 1) and HLA class I (panel 2). Panel 3 shows HLA-DR induction in a normal control.

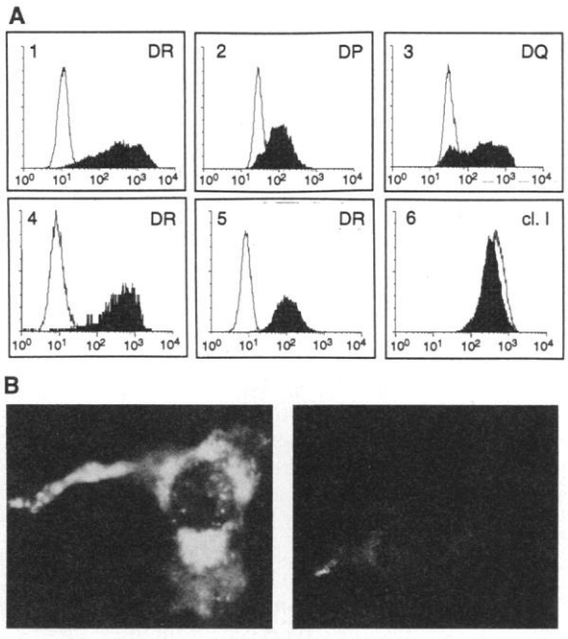

Fig. 4. CIITA expression induces constitutive HLA class II expression. (**A**) Stable transfectants of the cell lines ME1477 (panels 1, 2, 3, and 6), THP-1 (panel 4), and 143B (panel 5) with either the expression vector EBO-Sfi alone (open profiles) or a full-length CIITA cDNA cloned into EBO-Sfi (EBO-CIITA, shaded profiles) (*11*) were analyzed by flow cytofluorimetry for the expression of HLA-DR (mAb 2.06; panels 1, 4, and 5), HLA-DP (mAb B7/21; panel 2), HLA-DQ (mAb Tü22; panel 3) (*27*), and HLA–class I (mAb W6/32, Serotec; panel 6) (*28*). CIITA cDNA in EBO-Sfi is under the control of the SV40 promoter. Profiles of EBO-Sfi–transfected cell lines are superposable with those of untransfected, noninduced cells. (**B**) (**Left**) The CIITA-deficient fibroblast line MHV was transfected transiently with vector EBO-CIITA. (**Right**) Transfection of fibroblast ABL from BLS complementation group B (*13*) with EBO-CIITA. Transfection of EBO-Sfi into these cells has no effect on MHC class II expression. Cells were grown for 48 hours after transfection, fixed, and analyzed for HLA-DR expression (*29*). (**C**) CIITA cDNA expression activates the 150-bp DRA promoter. ME1477 cells were cotransfected with the indicated amounts (in micrograms) of pDRsyn (*30*) (containing the 150-bp DRA promoter fused to the CAT coding sequence), either EBO-As (a full-length CIITA cDNA cloned in the reverse orientation into EBO-Sfi) or EBO-CIITA and 3 μg of pSVAP (*31*) (containing the alkaline phosphatase gene under control of the SV40 promoter) to monitor transfection efficiencies. CAT assays were carried out 48 hours after transfection. Cell extracts were prepared, assayed, and corrected for alkaline phosphatase activity (*31*) and then assayed for CAT activity (*32*).

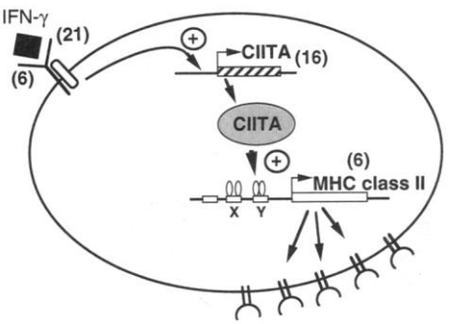

Fig. 5. Model of MHC class II induction by IFN-γ. Numbers in parentheses indicate the chromosomal assignments of the various factors on human chromosomes known to be essential for the induction of MHC class II genes by IFN-γ.

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29. Transient transfection of BLS fibroblasts was carried out in situ on sterile microscope slides with a JOUAN GHT 128/A electropulser (*13*). The cells were transfected in 100 μl of 10 mM sodium phosphate (pH 7.2), 1 mM $MgCl_2$, 250 mM sucrose with 1 μg of plasmid DNA. Five pulses (100 ms, 600 V, 1 Hz, E = 1.5 kV/cm) were delivered, and 2 min later the slides were placed in complete medium and cultivated for 48 hours. Antibody binding [monoclonal antibody (mAb) 2.06] on ethanol-fixed fibroblasts was revealed with biotynilated sheep antibody to mouse Ig (Amersham) followed by avidin–fluorescein isothiocyanate (Southern Biotechnology Associates). Immunofluorescence analysis was carried out with a Leitz Orthoplan optical microscope.
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Pattern Formation and Eyespot Determination in Butterfly Wings

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Butterfly wings display pattern elements of many types and colors. To identify the molecular processes underlying the generation of these patterns, several butterfly cognates of *Drosophila* appendage patterning genes have been cloned and their expression patterns have been analyzed. Butterfly wing patterns are organized by two spatial coordinate systems. One system specifies positional information with respect to the entire wing field and is conserved between fruit flies and butterflies. A second system, superimposed on the general system and involving several of the same genes, operates within each wing subdivision to elaborate discrete pattern elements. Eyespots, which form from discrete developmental organizers, are marked by *Distal-less* gene expression. These circular pattern elements appear to be generated by a process similar to, and perhaps evolved from, proximodistal pattern formation in insect appendages.

Most of the more than 17,000 species of butterflies can be distinguished by their wing color patterns. The diversity of butterfly wing pattern elements has stimulated comparative, theoretical, and experimental study [reviewed in (*1*)]. One pattern element, the eyespot, occurs in varying sizes and numbers on the upper or lower wing surfaces and is used to confuse or warn off predators. This circular pattern of pigmentation is controlled by a patterning focus at the center (*1–3*). In *Precis coenia* (*Nymphalidae*), the best studied model species, cautery of this organizer ablates the eyespot (*2*), whereas transplantation to an ectopic site induces a new eyespot (*3*). Although the pattern-organizing properties of the eyespot focus may be related to other developmental organizers, nothing is known at the molecular level about butterfly wing pattern elements.

Flies diverged from butterflies approximately 200 million years ago and possess wings that are much smaller, have a simpler suborganization, and develop from imaginal discs with a distinct style of growth and morphogenesis. However, the fruit fly *Drosophila melanogaster* is the closest relative of butterflies for which there is detailed developmental and genetic information. Much progress has been made in the characterization of genes involved in *Drosophila* wing organization and patterning [(*4–9*), reviewed in (*10*)]; therefore we examined the potential roles of the butterfly cognates of such *Drosophila* genes in butterfly wing patterning. We cloned and examined the expression of the *Precis coenia* homologs of the *Drosophila wingless* and *decapentaplegic* signaling molecules and the *apterous*, *invected*, *scalloped*, and *Distal-less* transcription factors.

The largest *P. coenia* complementary DNAs (cDNAs) representing the *apterous* (*ap*, 3.2 kb), *scalloped* (*sd*, 3.8 kb), *wingless* (*wg*, 3.0 kb), *decapentaplegic* (*dpp*, 0.9 kb), and *invected* (*inv*, 1.5 kb) genes were isolated and analyzed. Genomic Southern blot analyses demonstrated that the cDNAs represent single copy sequences and are the closest *P. coenia* relatives, at the DNA level, of the respective *D. melanogaster* genes. Conserved regions of these cDNAs were sequenced, and the deduced amino acid translations were aligned with the *D. melanogaster* proteins (Fig. 1). Sequence conservation occurs within and outside (*11*) the *ap* homeodomains, and these residues are also present in an apparent vertebrate *ap* cognate (*12*). The *P. coenia en/inv* gene homolog contains

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