THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 277, No. 8, Issue of February 22, pp. 5841–5848, 2002

Printed in U.S.A.

NF-Y Recruitment of TFIID, Multiple Interactions with Histone Fold TAF_{II}s*

Received for publication, April 24, 2001, and in revised form, September 26, 2001
Published, JBC Papers in Press, October 31, 2001, DOI 10.1074/jbc.M103651200

Mattia Frontini‡, Carol Imbriano‡, Alberto diSilvio‡, Brendan Bell§, Alessia Bogni‡, Christophe Romier§, Dino Moras§, Laszlo Tora§, Irwin Davidson§, and Roberto Mantovani‡¶

From the ‡Dipartimento di Biologia Animale, Università di Modena e Reggio, Via Campi 213/d, Modena 41100, Italy and the §Institut de Genetique et de Biologie Moleculaire et Cellulaire. 1, Rue L. Fries, BP163, Illkirch C.U. Strasbourg 67404, France

The nuclear factor y (NF-Y) trimer and TFIID contain histone fold subunits, and their binding to the CCAAT and Initiator elements of the major histocompatibility complex class II Ea promoter is required for transcriptional activation. Using agarose-electrophoretic mobility shift assay we found that NF-Y increases the affinity of holo-TFIID for Ea in a CCAAT- and Inr-dependent manner. We began to dissect the interplay between NF-Y- and TBP-associated factors PO1II (TAF_{II}s)-containing histone fold domains in protein-protein interactions and transfections. hTAF_{II}20, hTAF_{II}28, and hTAF_{II}18-hTAF_{II}28 bind to the NF-Y B-NF-YC histone fold dimer; hTAF_{II}80 and hTAF_{II}31-hTAF_{II}80 interact with the trimer but not with the NF-YB-NF-YC dimer. The histone fold α2 helix of hTAF_{II}80 is not required for NF-Y association, as determined by interactions with the naturally occurring splice variant hTAF_{II}80δ. Expression of hTAF_{II}28 and hTAF_{II}18 in mouse cells significantly and specifically reduced NF-Y activation in GAL4-based experiments, whereas hTAF_{II}20 and hTAF_{II}135 increased it. These results indicate that NF-Y (i) recruits purified holo-TFIID *in vitro* and (ii) can associate multiple TAF_{II}s, potentially accommodating different core promoter architectures.

Gene expression is regulated by promoter and enhancer elements recognized by gene-specific DNA-binding proteins and by general transcription factors (1). At a higher level, it is controlled by chromatin structures, whose fundamental unit is the nucleosome, a complex formed by core histones H2A, H2B, H3, H4, which wrap around them 146 base pairs of DNA (2, 3). Histones all share a conserved 65-amino acid histone fold motif (HFM)[1] that has low sequence identity but high structural resemblance (4). Crystallographic analysis showed that this motif is composed of three/four α-helices separated by short loops/strand regions; this structure enables histones to dimerize and form non-sequence-specific interactions with DNA (5). Proteins containing the HFM are also involved in the basic mechanisms of transcription: (i) the two subunits of the TBP-binding NC2, also called Dr1/DRAP1, a global repressor of basal transcription (6, 7); (ii) some of the TBP-associated factors that are part of the TFIID, P/CAF, STAGA, and TFTC complexes (8–17); (iii) one subunit of the P/CAF complex (18); and (iv) two subunits of the CCAAT-binding activator NF-Y (19–21).

TFIID is a general transcription complex composed of TBP, responsible for TATA recognition, and of several associated factors, TAF_{II}s, that constitute a link between gene-specific upstream activators and the general transcription machinery by recognizing TATA and/or initiator elements (reviewed in Refs. 22, 23). Some of the TAF_{II}s appear to be present in specific sub-complexes of TFIID (24–33); 10–12 highly conserved subunits have been identified in yeast, *Drosophila*, and human and biochemically characterized in protein-binding assays and functional *in vitro* transcription experiments. Based on sequence homology, structure-function analysis, and crystallographic studies, hTAF_{II}80/dTAF_{II}60, hTAF_{II}31/dTAF_{II}40, hTAF_{II}28/dTAF_{II}27, and hTAF_{II}18/dTAF_{II}30 have histone-like structures (13, 15); hTAF_{II}20/hTAF_{II}135 and hTAF_{II}30 have also been included in this class (10, 11, 16, 17). Interestingly, gene inactivation of HFM-containing TAF_{II}s in yeast implicates them in a rather broad, if not universal, role in transcriptional activation (34–38). This is unlike other TAF_{II}s, whose inactivation in yeast suggests a more selective role in certain promoters (38–40).

Another protein containing HFMs is NF-Y, also termed CBF, the ubiquitous trimeric protein binding to the widespread CCAAT-box promoter element; it is composed of NF-YA, NF-YB, and NF-YC, all necessary for subunit association and DNA binding (reviewed in refs. 19 and 21). The H2B-H2A-like NF-YB-NF-YC subunits dimerize tightly via their HFMs, forming a complex surface necessary for NF-YA association. The resulting trimer has a high affinity and sequence specificity for the CCAAT sequence. Several types of indications link TFIID to NF-Y. First, their binding sites are either ubiquitous, in the case of TFIID, or quite frequent, because 25% of the promoters have NF-Y sites; both are found at highly conserved positions within a prototypical promoter (20). Second, biochemical evidence of direct interactions has emerged: (i) NF-YB and NF-YC bind to TBP through short subdomains within the larger yeast/human conserved parts and short basic residues in the HS2 of TBP (41); (ii) NF-YB is immunoprecipitated with an anti-TAF_{II}100 antibody from crude nuclear extracts and is present in immunopurified TFIID fractions and in high molecular weight complexes in glycerol gradient experiments, indeed sug-

* This work was in part supported by grants from Ministero dell'Università della Ricerca Scientifica e Tecnologica (PRIN 99) and Associazione Italiana per la Ricerca sul Cancro (to R. M.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom correspondence should be addressed: Tel.: 39-059-205-5542; Fax: 39-059-205-5548; E-mail: mantor@mail.unimo.it.

[1] The abbreviations used are: HFM, histone fold motif; NF-Y, nuclear factor Y; TAF_{II}s, TBP-associated factors PO1II; wt, wild type; EMSA, electrophoretic mobility shift assay; NTA, nitrilotriacetic acid; GST, glutathione *S*-transferase; MHC, major histocompatibility complex; TBP, TATA binding protein; P/CAF, P300 CBP associated factor; SP1, SP protein 1; RFX, DR factor X; Inr, initiator; STAGA, SPT3-TAFII31-GCN5-L acetyltransferase; TFTC, TBP-free TAF complex; CIITA, class II transcriptional activator.

gesting association with additional proteins; (iii) the Q-rich regions of NF-YA and NF-YC interact with $dTAF_{II}110$ *in vitro* (42), a result consistent with the idea that Q-rich activators such as SP1 and cAMP-response element-binding protein function by binding to $dTAF_{II}110$-$hTAF_{II}135$ (43–48). Third, the lack of NF-Y binding has been associated with a closed chromatin configuration of the *Xenopus* HSP70 TATA-box region (49) and, very recently, with the inability to recruit TBP-TFIIB on the γ-globin promoter *in vivo* (50): These results clearly imply that NF-Y binding is essential for TFIID recruitment.

To study the NF-Y-TFIID connections, we employed the mouse MHC class II Ea promoter system (51, 52). Ea is a tissue-specific promoter active in B lymphocytes and other professional antigen-presenting cells (53). Like all other MHC class II promoters, it also requires the ubiquitous trimer RFX and lacks a functional TATA-box. We showed that TFIID binding to the Ea Inr is necessary for function in an *in vitro* transcription system. In this study, we began to dissect NF-Y-TFIID interplay with purified holo-TFIID, recombinant NF-Y, and isolated TAF_{II}s.

MATERIALS AND METHODS

Production and Purification of Recombinant NF-Y, $hTAF_{II}31$, $hTAF_{II}80$, $hTAF_{II}28$, $hTAF_{II}18$, $hTAF_{II}20$, $hTAF_{II}135\Delta1–372$, and holo-$TF_{II}D$—Production and purification of recombinant NF-Y trimer were as described before, using wt NF-YA, wt NF-YB, and a TRX-His-NF-YC fusion protein (54). Recombinant His-tagged $hTAF_{II}28$ and $hTAF_{II}18$, GST-$hTAF_{II}28$ (9), GST-$hTAF_{II}135\Delta1–372$ (12), and GST-$hTAF_{II}20$ (9) were produced in *Escherichia coli* as soluble proteins and purified according to standard procedures. $hTAF_{II}31$, $hTAF_{II}80$, and $hTAF_{II}80\delta$ were produced by the baculovirus expression system, either as single subunits or as a dimer, using standard protocols and purified to homogeneity. $hTAF_{II}80$ contains a FLAG tag. Holo-TFIID was immunopurified from HeLa cells with an anti-TBP antibody as previously detailed (9, 26, 27).

EMSA Analysis—EMSAs of TFIID in agarose gels were as described in Ref. 52: holo-TFIID fractions were incubated in NF-Y buffer (20 mM HEPES, pH 7.9, 50 mM NaCl, 5% glycerol, 5 mM $MgCl_2$, 1 mM dithiothreitol) together with 10,000 cpm of ^{32}P-labeled Ea fragments; the total volume was 10 μl. After incubation for 45 min at 30 °C, we added 2 μl of 1× buffer containing bromphenol blue, and samples were loaded on a 1.5% agarose gel (Bio-Rad Ultrapure) in 0.5× TBE. Gels were run at 140 V for 90 min at 4 °C, transferred onto DE81 paper, vacuum-dried, and exposed. Three independent preparations of purified TFIID were used in EMSAs. The Ea fragments used in EMSA analysis were obtained by PCR and contained sequences from −115 to +60 of the Ea promoter, either wt, mutated in the Y box (Ls17 (51)), or in the Inr (Ls21 (52)).

Antibodies and Supershift EMSA—For supershift experiments, anti-NF-YA and -NF-YB antibodies were purified on antigen columns (55). Monoclonal antibodies against TAF_{II}s were as follows: 24TA and 26TA, anti-$hTAF_{II}80$; 22TA, anti-$hTAF_{II}20$; 15TA, anti-$hTAF_{II}28$; and 16TA, anti-$hTAF_{II}18$ (9, 12, 26). Monoclonal antibodies were purified by caprylic acid precipitation of ascites fluid followed by precipitation with 50% ammonium sulfate, resuspension in phosphate-buffered saline, and dialysis against NDB100 (100 mM KCl, 20 mM HEPES, pH 7.9, 20% glycerol, 0.5 mM EDTA). The $hTAF_{II}31$ rabbit polyclonal was a kind gift of Dr. A. Levine. Supershift experiments were performed by preincubating TFIID, with or without NF-Y, with the indicated antibodies (200 ng of purified monoclonal antibodies, 0.3 μl of the anti-$hTAF_{II}31$ polyclonal, 200 ng of purified anti-NF-YA and anti-YB) for 2 h on ice, before addition of the labeled DNA and further incubation at 30 °C for 45 min.

Protein-Protein Interactions—Interactions with NTA-agarose columns were performed by incubating either crude bacterial extracts or purified His-tagged proteins (1 or 2 μg) in BC100 (100 mM KCl, 20 mM HEPES, pH 7.9, 10% glycerol, 5 mM imidazole, 5 mM β-mercaptoethanol) with NTA-agarose (100–200 μl); the column was washed with BC300 and BC1000, containing 300 mM and 1 M KCl, respectively; proteins were eluted in BC100 buffer containing 300 mM imidazole, dialyzed against BC100, and assayed in Western blots. Immunoprecipitations were performed as follows: The NF-Y trimer (500 ng) and an equivalent amount of the indicated TAF_{II}s were added to 25 μl of Protein G-Sepharose to which 7.5 μg of the purified anti-NF-YA7 monoclonal antibody had been previously bound. Incubation was pursued for 2 h on ice, unbound material was recovered after centrifugation, and the beads were washed with NDB100 with the addition of 0.1% Nonidet P-40. SDS buffer was added, and the samples were boiled at 90 °C for 5 min and loaded onto SDS gels. Western blots were performed according to standard procedures with the indicated primary antibody and a Pierce peroxidase secondary antibody. For multiple interactions, the filter was stripped, blocked with nonfat dry milk, and re-hybridized.

Transfections—The eukaryotic expression vectors for NF-Y, $hTAF_{II}28$, $hTAF_{II}18$, $hTAF_{II}20$, $hTAF_{II}135$, and $hTAF_{II}80$ were described before (9, 12, 56). From PCR-$hTAF_{II}31$ (kindly donated by Dr. Levine) an *Eco*RI insert was cloned into pGAL4poly in-frame with the Gal4 DNA-binding domain, and the latter was excised by cutting with *Xho*I and a partial *Eco*RI digest. Mouse NIH-3T3 fibroblasts were co-transfected with 1–3 μg of activating plasmids, 2 μg of the plasmids containing the Luciferase reporter gene, and 3 μg of pNβGal plasmid for control of transfection efficiency. The total amount of DNA was kept constant (at 15 μg) with Bluescript. All plasmids were purified by centrifugation using cesium chloride gradients. Cells were transfected with the standard calcium-phosphate method, recovered 48 h after transfection, washed in phosphate-buffered saline (150 mM NaCl, 10 mM sodium phosphate, pH 7.4), and resuspended in the Reporter assay reagent (Promega). Luciferase and β-galactosidase activity were measured according standard procedures. A minimum of three independent transfections in duplicate was done; most of the values are based on 8–12 transfections.

RESULTS

Binding of NF-Y and TFIID to the Ea Promoter—We have previously shown that the MHC class II Ea promoter is crucially dependent on a binding of holo-TFIID to a TdT-like initiator (52). We investigated the interactions between NF-Y and holo-TFIID in such a system using recombinant NF-Y and immunopurified holo-TFIID in agarose-EMSA. Fig. 1 (*A* and *B*) shows a dose response of TFIID either alone (*lanes 5–7*), or with a fixed amount of NF-Y (*lanes 2–4*): in Fig. 1*A* the NF-Y dose saturated the labeled fragment (*lane 1*), whereas in Fig. 1*B* lower doses of NF-Y were used. Two major complexes of different mobility, termed IIDa and IIDb, were generated by TFIID; when incubated with NF-Y, both complexes generated dissimilar electrophoretic mobilities, clearly arising as a result of co-incubation of NF-Y and TFIID (Fig. 1*A*, compare *lanes 2–4* with *5–7*; Fig. 1*B*, compare *lanes 1–3* with *5–7*): One complex migrated slightly more slowly than the NF-Y band, and another was further retarded and visible at higher TFIID concentrations. Note that the upper Y/IID complexes were visible at lower TFIID concentrations (Fig. 1*A*, compare *lanes 3* and *6*). As a control for the specificity of the interactions, we used an identical Ea fragment carrying a 10-bp mutation in the Y-box, known to abolish NF-Y binding, as well as *in vivo* and *in vitro* transcriptional activity of the promoter (51): As expected, binding of NF-Y, but not TFIID, was abolished, and the upper bands resulting from simultaneous interactions were not detected (Fig. 1*C*).

To verify the effect of TFIID on NF-Y binding, a reciprocal experiment was also performed, namely a dose-response analysis of NF-Y alone, or with two TFIID concentrations (Fig. 1, *D* and *E*, *lanes 2–7*). The TFIID pattern at high concentrations was clearly modified in a dose-dependent manner by NF-Y (Fig. 1*D*, compare *lanes 1* with *2–4*). Interestingly, the lowest amount of NF-Y employed (0.1 ng), which was barely sufficient to generate a visible band, modified the pattern of the TFIIDa and -b bands. When incubated with low amounts of TFIID, insufficient to shift Ea DNA, the NF-Y band was evident at lower concentrations (Fig. 1*E*, compare *lanes 2* and *3* with *6* and *7*). In experiments conceptually similar to those of Fig. 1*C*, we employed a fragment containing a 10-bp mutation in the Ea Inr region, known to cripple TFIID binding and Ea promoter function *in vitro* (52): The NF-Y complex, but not the TFIID or the Y/IID complexes, was generated by co-incubation of the two proteins (Fig. 1*F*), indicating that an intact Inr is required for

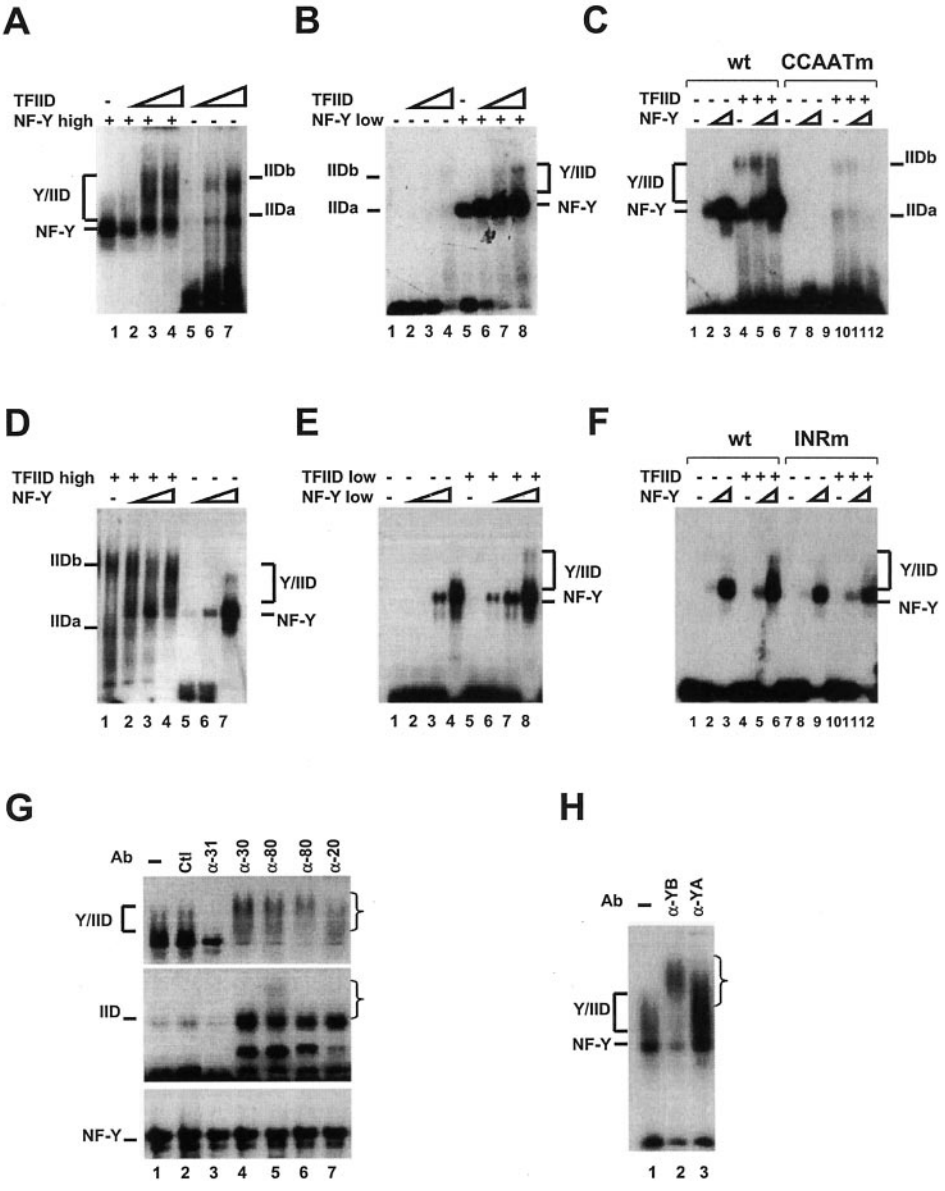

FIG. 1. **EMSA analysis of NF-Y and holo-TFIID binding to Ea core sequences.** *A*, dose-response analysis of holo-TFIID in the presence (*lanes 2–4*) or absence of 1 ng of NF-Y (*lanes 2–4*, 0.2, 1, 3 μl, respectively); in *lane 1*, 1 ng of NF-Y was used. *B*, same as in *A*, except that a low amount of NF-Y (0.1 ng) was used in each of *lanes 5–8*. No protein was added in *lane 1*. *C*, dose response of NF-Y (0.1 ng in *lanes 2*, *5*, *8*, *11*; 0.3 ng in *lanes 4*, *6*, *9*, *12*) with (*lanes 4–6*, *10–12*) or without (*lanes 1–3*, *7–9*) 1 μl of TFIID. The wt Ea −90/+60 promoter fragment was used in *lanes 1–6*, whereas in *lanes 7–12* we used a fragment of identical length containing a 10-bp mutation in the CCAAT box (LS17 (see Ref. 51)). *D*, dose response of NF-Y (0.01, 0.1, 1 ng, *lanes 2–4* and *5–7*, respectively) were incubated alone (*lanes 2–4*) or with 3 μl of TFIID (*lanes 6–8*). *E*, same as in *D*, except that 0.3 μl of TFIID was used. *F*, dose response of NF-Y (0.1 ng in *lanes 2*, *5*, *8*, *11*; 0.3 ng in *lanes 4*, *6*, *9*, *12*) with (*lanes 4–6*, *10–12*) or without (*lanes 1–3*, *7–9*) 0.3 μl of TFIID. In *lanes 1–6* we used the wt Ea −90/+60 promoter fragment; in *lanes 7–12* a fragment of identical length containing a 10-bp mutation in the Initiator (LS21 (see Ref. 52)) was used. *G*, antibody supershift of NF-Y/TFIID, TFIID, and NF-Y complexes: the indicated antibodies (anti-hTAF$_{II}$80 monoclonals were 24TA in *lanes 6* and 26TA in *lanes 7*) were incubated with TFIID on ice before addition of NF-Y (*upper panel*), with TFIID alone (*middle panel*), and with NF-Y (*lower panel*). For details of the antibodies, see "Materials and Methods." The control antibody was an anti-GATA1 monoclonal. *H*, supershift experiments as in *C*, with anti-NF-YB (*lane 2*)- and anti-NF-YA (*lane 3*)-purified polyclonal antibodies.

the formation of the NF-Y-TFIID complexes.

Next, we wished to determine whether the Y/IID complexes observed in our EMSAs truly contain TAF$_{II}$s. To this aim, we used antibodies specific for different TAF$_{II}$s in supershift experiments. Fig. 1*G* indicates that anti-hTAF$_{II}$31, anti-hTAF$_{II}$30, anti-hTAF$_{II}$20, and two different anti-hTAF$_{II}$80 antibodies all modify the Y/IID complexes, whereas an irrelevant anti-GATA1 antibody had no effect (Fig. 1*G*, *upper panel*, compare *lanes 1* and *2* with *3–7*). In parallel, when challenged with the IIDa complex, these antibodies all showed interactions (Fig. 1*G*, *middle panel*), whereas none recognized the NF-Y complex (Fig. 1*G*, *lower panel*). Note that the anti-hTAF$_{II}$31 antibody, rather than supershifting the IIDa or Y/IID complexes, apparently inhibited binding of TFIID to DNA. Finally, we verified whether the Y/IID complexes also contain NF-Y, by challenging them with anti-NF-YA and anti-NF-YB antibodies. Indeed, both antibodies modified the mobilities of the complexes (Fig. 1*H*). The experiments shown in Fig. 1 (*A–E*) also suggest that the presence of NF-Y improves the binding capacity of holo-TFIID. We verified whether NF-Y can facilitate binding of TFIID by performing on-rate experiments: Binding of TFIID to DNA, in fact, is known to be a slow process, and the on-rates even on high affinity TATA-Inr elements are on the order of 20–40 min (Ref. 57, and references therein). On the contrary, NF-Y

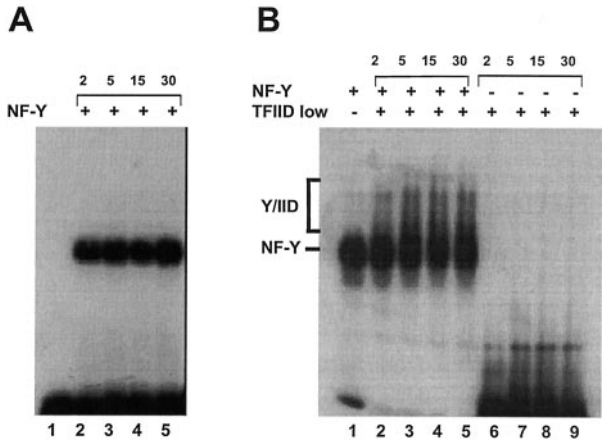

FIG. 2. **On-rates of TFIID and NF-Y on the Ea promoter.** *A*, EMSA on-rate experiments of NF-Y (1 ng). *B*, same as in *A*, except that NF-Y and TFIID (0.1 μl) were used. Experiments were performed by preincubating NF-Y alone (*lane 1*) for 15 min at RT and then adding holo-TFIID for the indicated time before loading a running agarose gel (*lanes 2–5*). Incubations of TFIID alone for the corresponding times are shown in *lanes 6–9*.

binding under our experimental conditions is extremely rapid, being completed after 1–2 min (Fig. 2*A*; see also Ref. 58). We incubated suboptimal amounts of TFIID (see Fig. 1*E*) with and without saturating amounts of NF-Y: A weak IID band was seen only after 30 min of incubation in the absence of NF-Y (Fig. 2*B*, *lanes 6–9*), whereas the upper Y/IID complexes were evident already after 2 min of incubation at 30 °C, and maximal after 5 min (Fig. 2*B*, *lanes 2* and *3*), strongly suggesting that NF-Y-CCAAT complexes recruit TFIID onto the Ea initiator.

From this set of *in vitro* experiments with purified holo-TFIID and recombinant NF-Y, we conclude that: (i) complexes of different mobilities are formed upon simultaneous binding of TFIID and NF-Y to the Ea promoter; (ii) these complexes contain both TAF$_{II}$s and NF-Y; (iii) binding of NF-Y to the CCAAT box and of TFIID to the initiator is required; and (iv) TFIID binding is remarkably facilitated when NF-Y is bound to DNA.

Binding of NF-YB-NF-YC to Histone Fold TAF$_{II}$s—A large number of subunits are present in holo-TFIID, and full reconstitution of the holo-TFIID complex with recombinant proteins has not been achieved yet. We therefore decided to dissect NF-Y-TFIID interactions by taking a reductionist approach, investigating the interactions between isolated HFM subunits of the two complexes. Recombinant proteins were produced in *E. coli* or *Baculovirus* and purified (Fig. 3). Note that the GST-hTAF$_{II}$135Δ1–372 protein used hereafter is a mutant containing the C-terminal region with the HFM (16) but lacking the N-terminal 372 Q-rich region that mediates binding to Q-rich activators, such as SP1 and cAMP-response element-binding protein (46), and possibly contact NF-Y (42). The recombinant NF-YB-NF-YC dimer, containing the histone folds, was incubated with bacterial extracts containing GST-hTAF$_{II}$28, GST-hTAF$_{II}$135Δ1–372, GST-hTAF$_{II}$20, or GST-hTAF$_{II}$20-GST-hTAF$_{II}$135Δ1–372, and Sf9 extracts containing hTAF$_{II}$80-hTAF$_{II}$31. The complexes were purified over a nickel NTA-agarose column, exploiting the presence of His tags on NF-YC. Columns were washed with buffers containing 0.3 M and 1 M KCl, and eluted with 0.3 M imidazole. Flow-through, wash, and bound material were checked in Western blots with the respective antibodies. Results of the experiments are shown in Fig. 4. As expected, NF-YB was efficiently bound to the column, despite the lack of His tags (Fig. 4, *upper panels*). GST-hTAF$_{II}$20 and GST-hTAF$_{II}$28 were efficiently retained on the columns but were in the FT fraction in the absence of NF-YB-NF-YC, thus ruling out that any of the HFM proteins tested had any intrinsic affinity for NTA-agarose (Fig. 4 and data not shown). On the other hand, GST-hTAF$_{II}$135Δ1–372, GST-hTAF$_{II}$20-GST-hTAF$_{II}$135Δ1–372, and hTAF$_{II}$80-hTAF$_{II}$31 were not retained by the affinity column. The reverse approach was also tested, namely His-tagged hTAF$_{II}$28, hTAF$_{II}$18, and hTAF$_{II}$18-hTAF$_{II}$28 were incubated with an NF-YB-NF-YC5 dimer lacking His tags. In this experiment, we used an NF-YC mutant, YC5, that contains only the evolutionarily conserved part of NF-YC, fully capable to associate NF-YB. The NF-Y HFM subunits were found in the bound fractions with hTAF$_{II}$28 and hTAF$_{II}$18-hTAF$_{II}$28 but not with hTAF$_{II}$18.

From this set of experiments we conclude that only hTAF$_{II}$28, hTAF$_{II}$18-hTAF$_{II}$28, and hTAF$_{II}$20, but not the HFM containing hTAF$_{II}$20-hTAF$_{II}$135Δ1–372, nor hTAF$_{II}$80-hTAF$_{II}$31, are capable to associate the NF-Y HFM dimer in solution.

Binding of NF-Y to Histone Fold TAF$_{II}$s—Because NF-YA is necessary for CCAAT-box binding and is known to recognize determinants in the HFMs of both NF-YB and NF-YC (19), it was important to test whether the interactions with TAF$_{II}$s would also be scored in the context of the trimeric complex. NF-YA has an intrinsic affinity for NTA-agarose and is unsuitable for the protein-protein interaction approach taken above, most likely because of the high number of His residues in the conserved domain (Not shown). We thus switched to immunoprecipitations with the purified recombinant NF-Y trimer and the different TAF$_{II}$s, either as single subunits or dimers. The complexes were incubated with Mab7, a monoclonal antibody that recognizes the NF-YA Q-rich activation domain (55), previously bound to a Protein G-Sepharose matrix; in parallel, equivalent amounts of recombinant proteins were incubated with a control Protein G-Sepharose resin associated with an irrelevant anti-MHC class II antibody. After washing, bound material was recovered by boiling samples in SDS buffer and analyzed in Western blots with the anti-NF-Y and anti-TAF$_{II}$ antibodies. As expected, NF-YA and NF-YB are immunoprecipitated with Mab7 but not with the control antibody (Fig. 5*A*, *upper panel*); the same was true for NF-YC (not shown, see below). Incubation of NF-Y with hTAF$_{II}$28, but not with TAF$_{II}$18, retained the TAF$_{II}$ in the bound material. Surprisingly, unlike the previous experiments with the NF-YB-NF-YC dimer, when incubated together with the NF-Y trimer, interactions of the hTAF$_{II}$28-hTAF$_{II}$18 dimer were not observed, implying that the presence of NF-YA prevents hTAF$_{II}$28-hTAF$_{II}$18 binding to the NF-Y HFM dimer. On the other hand, hTAF$_{II}$20 and the hTAF$_{II}$80-hTAF$_{II}$31 dimer, but not hTAF$_{II}$20-hTAF$_{II}$135Δ1–372, were bound to the NF-Y trimer. hTAF$_{II}$80, but not hTAF$_{II}$31, was immunoprecipitated when incubated alone with NF-Y. In this experimental setting, we also used a differentially spliced form of hTAF$_{II}$80, termed hTAF$_{II}$80δ, in which 10 amino acids of the HFM α2 are missing. This isoform is incapable of interacting with hTAF$_{II}$31.[2] We tested this protein in the immunoprecipitation assays and found that, as for hTAF$_{II}$80, it is able to interact with NF-Y. To confirm that hTAF$_{II}$20-hTAF$_{II}$135Δ1–372 and hTAF$_{II}$28-hTAF$_{II}$18 are present in dimeric form in our assays, we immunoprecipitated these dimers, in the absence of NF-Y subunits, with anti-hTAF$_{II}$135 and anti-hTAF$_{II}$18 monoclonal antibodies, respectively. Fig. 5*B* shows that both hTAF$_{II}$20 and hTAF$_{II}$28 are indeed associated with their respective partners, as assessed in Western blots.

From the immunoprecipitation analysis of the NF-Y trimer, we conclude that hTAF$_{II}$18 and hTAF$_{II}$31 do not bind NF-Y, whereas

[2] B. Bell and L. Tora, submitted.

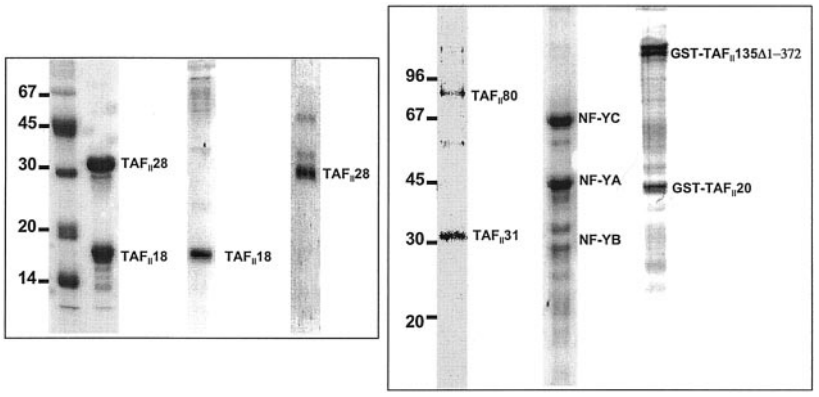

FIG. 3. **Purification of recombinant proteins.** SDS gels stained with Coomassie Blue showing the indicated purified recombinant proteins used for protein-protein assays. The GST-hTAF$_{II}$135Δ1–372 is a mutant lacking the 372 amino acids at the N-terminal of the protein and containing the HFM.

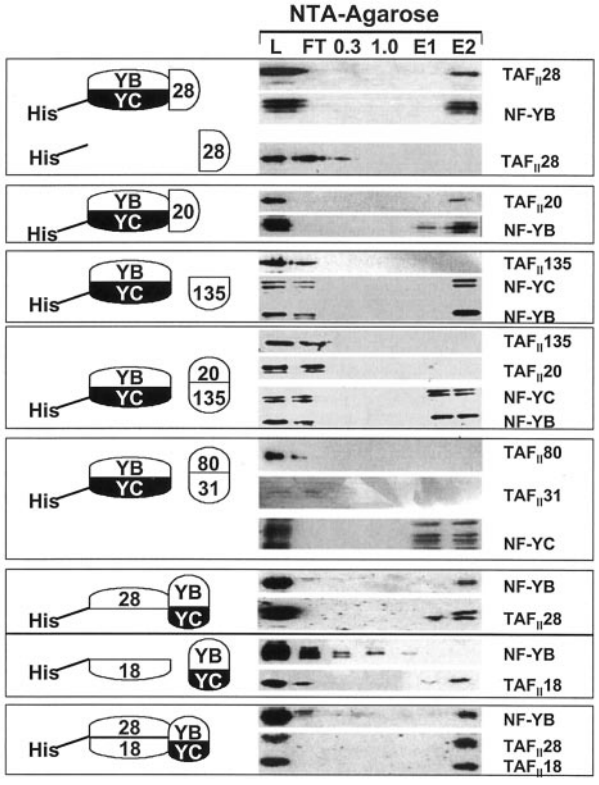

FIG. 4. **Protein-protein interactions of TAF$_{II}$s and NF-Y HFM subunits.** *A*, in the different *panels*, wt NF-YB-NF-YC His-tagged were incubated with GST-hTAF$_{II}$18 and GST-hTAF$_{II}$28, before loading onto NTA-agarose. *Load*, flow-through, 300 mM, and 1.0 M KCl washes and the two imidazole-eluted fractions are indicated. The fractions were run in SDS gels, and the proteins are revealed by Western blotting with the indicated antibody. Similar experiments are shown for GST-hTAF$_{II}$20, GST-hTAF$_{II}$135Δ1–372, GST-hTAF$_{II}$20-GST-hTAFI$_{I}$135Δ1–372, and hTAF$_{II}$80-hTAF$_{II}$31. In the *lower panels*, an NF-YB-NF-YC dimer devoid of the His tag, consisting of wt NF-YB and of the HFM-containing YC5 mutant (54), was incubated with the indicated hTAF$_{II}$s. From *top* to *bottom*: His-tagged hTAF$_{II}$28, His-tagged hTAF$_{II}$18, and hTAF$_{II}$28-hTAF$_{II}$18 (both His-tagged).

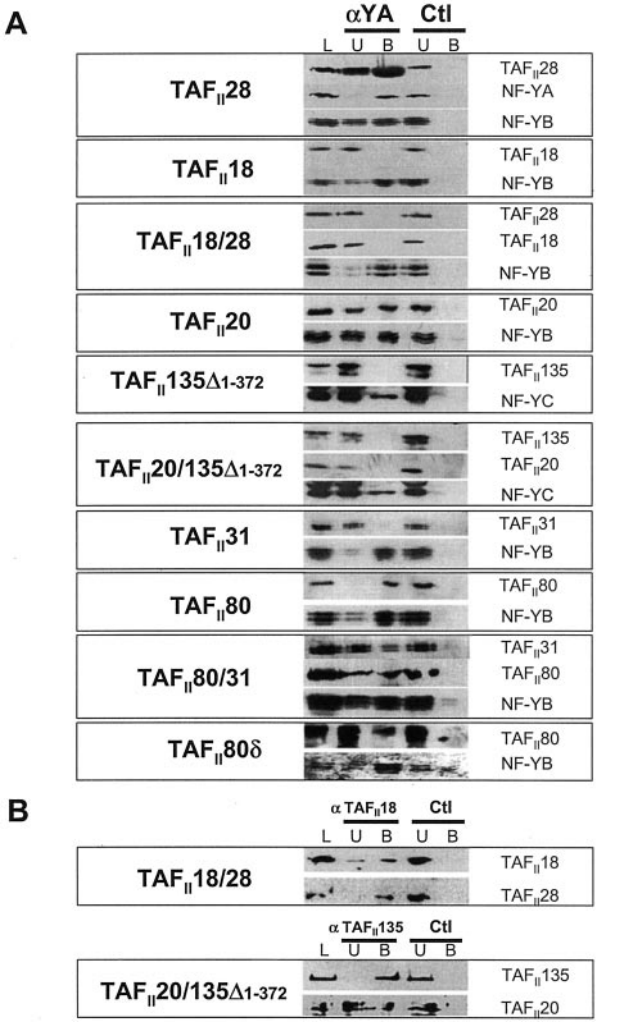

FIG. 5. **Immunoprecipitations of NF-Y and HFM TAF$_{II}$s.** *A*, equivalent amounts of recombinant NF-Y and the indicated hTAF$_{II}$s were incubated and immunoprecipitated with the anti-NF-YA monoclonal antibody 7 (55), or with an anti-MHC class II control antibody. Load (*L*), unbound (*U*), and bound (*B*) materials were loaded onto SDS gels and checked in Western blots with the indicated antibody. *B*, Western blots of immunoprecipitations of the His-hTAF$_{II}$18-His-hTAF$_{II}$28 dimer with the anti-hTAF$_{II}$18 antibody 16TA. Similarly, Western blot analysis of immunoprecipitations of preparations containing GST-hTAF$_{II}$20 and GST-hTAF$_{II}$135Δ1–372 with the 20TA monoclonal antibody against hTAF$_{II}$135.

hTAF$_{II}$80, hTAF$_{II}$80δ, hTAF$_{II}$28, and hTAF$_{II}$20 are capable of associating with the trimer. Upon dimerization, hTAF$_{II}$31-hTAF$_{II}$80, but not hTAF$_{II}$28-hTAF$_{II}$18, can bind to NF-Y.

Effect of TAF$_{II}$s Overexpression on NF-Y Activation in Mammalian Cells—Having established that NF-Y is capable of increasing the binding of holo-TFIID and associated multiple TAF$_{II}$s *in vitro*, we sought to investigate the *in vivo* effects with "co-activator" assays used for other activators (12, 59–62). The system is based on the co-expression in NIH-3T3 fibroblasts of GAL4-NF-YA together with NF-YB and NF-YC, activating a promoter containing five GAL4 sites driving the Luciferase reporter gene: Transcription is strictly dependent upon co-transfection of all NF-Y subunits, requiring at least one of the NF-Y Q-rich domains (56; see Fig. 6*A*). Co-transfections of different amounts of vectors expressing the TAF$_{II}$s used in our *in vitro* analysis gave the results outlined in Fig. 6*B*: hTAF$_{II}$20

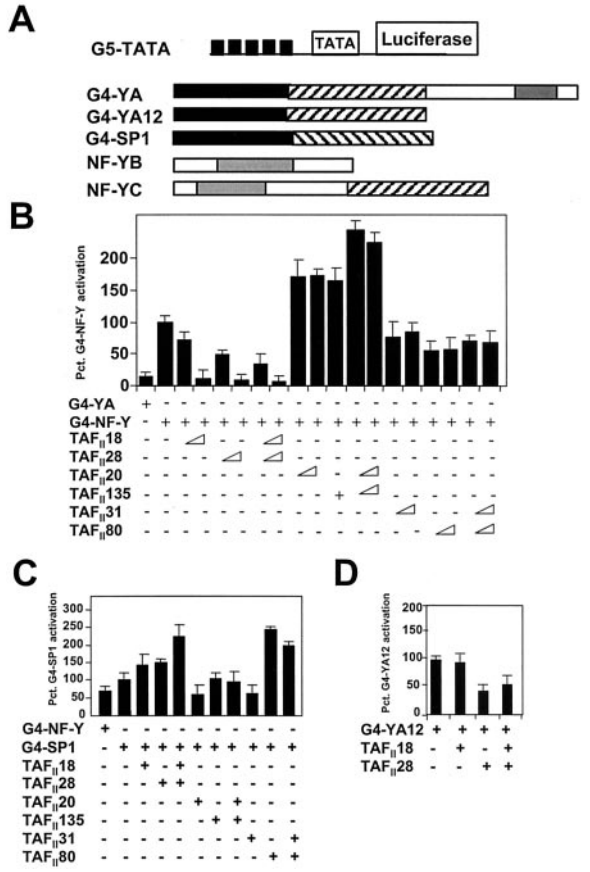

FIG. 6. **TAF$_{II}$s-mediated transcriptional modulation of NF-Y activity.** *A*, scheme of the GAL4 vectors used in the transfection experiments (56). *Black boxes* indicate the GAL4 DNA-binding domain; *hatched boxes* represent the Q-rich regions; *gray boxes* the homology domains. *B*, NIH-3T3 fibroblasts were transfected with a GAL4-driven Luciferase reporter and 1 or 3 μg of TAF$_{II}$s plasmids as indicated. *C* and *D*, same as *B*, except that GAL-SP1 and GAL4-YA12 (56) were used with the indicated co-transfected TAF$_{II}$s.

and hTAF$_{II}$135 had small, 2-fold-positive effects on GAL4-NF-Y activation; hTAF$_{II}$31 and hTAF$_{II}$80, either alone or in combination showed no effect; whereas expression of hTAF$_{II}$18 and hTAF$_{II}$28, alone or together, had a clear dose-dependent negative effect. To verify whether this inhibition was specific for the NF-Y trimer, or exerted indirectly through the basal promoter, we tested in similar assays an NF-YA mutant, G4-YA12, containing the isolated activation domain of NF-YA fused to the DNA-binding domain of GAL4, and GAL4-SP1, also a Q-rich activator. Co-expression of hTAF$_{II}$18 and/or hTAF$_{II}$28, or of the other TAF$_{II}$s, had minor effects on GAL4-YA12 or GAL4-SP1 (Fig. 6, *C* and *D*): if anything, hTAF$_{II}$18 and/or hTAF$_{II}$28 slightly increased GAL4-SP1 activation. Because the reporter construct was identical in all these experiments, these latter experiments rule out that the strong inhibitory effect of hTAF$_{II}$18-hTAF$_{II}$28 observed on the GAL4-NF-Y trimer is due to an unspecific repression of core promoter activity; moreover, inhibition is specific for the NF-Y trimer and not for the Q-rich activation domains, such as those of NF-YA, or of SP1.

DISCUSSION

TFIID Recruitment—The TATA-box is the most frequent promoter element. When absent, polymerase II positioning is in general assured by an initiator; *i.e.* both these core elements are recognized by TFIID (1). NF-Y binding sites are found in 25% of eukaryotic promoters, invariably playing an important and sometimes essential role. Both boxes are normally found at a fixed position, TATA at −25/−30 and CCAAT at −60/−100. The mean CCAAT-box position and orientation in TATA and TATA-less promoters pointed to a small, but significant difference (20),[3] suggesting that the proteins binding to these sites could directly or indirectly interplay. In keeping with this, we previously presented biochemical evidence showing that the NF-Y HFM subunits are associated with holo-TFIID and can bind TBP directly (41). The aim of our study was to examine their relationships, using a model system, the MHC class II Ea promoter, in which the functional importance of the two complexes is well established. We have previously shown, in fact, that holo-TFIID binds to a TdT-like initiator in the Ea promoter in a sequence-specific way. Our EMSA analysis is the first indication that NF-Y helps recruit holo-TFIID, thereby adding experimental proof to the hypothesis that NF-Y and TFIID have intimate relationships. It should be noted that other upstream factors are thought to recruit TBP and associated factors, as an important step in the formation of a transcriptional competent complex (1). Concerning the mechanisms of such DNA-binding facilitation, an obvious hypothesis is that there are direct protein-protein contacts between TAF$_{II}$s and NF-Y. We already detailed the binding of NF-YB-NF-YC to TBP (41), and the *in vitro* analysis presented here is strongly supportive of this, because a number of TAF$_{II}$s show affinity for NF-Y. Indeed, the multiple interactions of HFM TAF$_{II}$s with NF-Y observed in solution invite the speculation that TFIID binding might be influenced, if not dictated, by one or more transcription factor combinations binding nearby. Interestingly, we evidenced holo-TFIID complexes showing differential mobilities in our EMSAs, depending on the presence or absence of NF-Y: The upper TFIIDb, for example, migrates faster with NF-Y, possibly suggesting that a different composition of subunits might be present and that a DNA-bound NF-Y might select specific sub-complexes. The apparent multiplicity of TFIID combinations could then reflect into a plasticity of DNA binding, because the presence of adjacent activators might select subtypes of holo-TFIIDs with subunit compositions particularly suited to fit within the context of a given promoter. Antibody supershifting experiments, although showing that "core" TAF$_{II}$s are present in the IID/Y complexes, cannot yet provide us with a complete description of the composition of these complexes.

TFIID is known to be capable of sequence-specific interactions, and multiple subunits within TFIID can contact DNA (63, 64 and references therein). In the course of our analysis we found no evidence of sequence-specific binding of the isolated HFM TAF$_{II}$s considered here to the Ea promoter (data not shown). Non-HFM TAF$_{II}$s with well documented core promoter DNA-binding specificity, such as hTAF$_{II}$150 and/or hTAF$_{II}$250 (64), could then be considered for the Inr-binding activity.

NF-Y Interactions with HFM TAF$_{II}$s—Complete reconstitution of the holo-TFIID complex with recombinant subunits has not been achieved yet. We therefore dissected NF-Y interactions with individual TAF$_{II}$s. Given the common structural features of many TAF$_{II}$s with NF-Y subunits, we decided to start with HFM TAF$_{II}$s. In general, our analysis showed several interactions between NF-Y and TAF$_{II}$s: Minimally, hTAF$_{II}$28, hTAF$_{II}$80, and hTAF$_{II}$20 have affinity for either the HFM NF-YB-NF-YC dimer, the trimer, or both (Table I). hTAF$_{II}$135 should be added to this list (42), because the negative results obtained here probably reflect the absence of the N-terminal Q-rich region. Therefore, the interactions of the trimer with hTAF$_{II}$20 and hTAF$_{II}$80 are possibly relevant for the function of the CCAAT-binding trimer. On the other hand,

[3] R. Mantovani, unpublished.

TABLE I
Summary of NF-Y/TAF$_{II}$ interactions

	NF-Y	NF-YB-NF-YC
TAF$_{II}$28	+	+
TAF$_{II}$18	−	−
TAF$_{II}$18-TAF$_{II}$28	−	+
TAF$_{II}$31	−	−
TAF$_{II}$80	+	ND[a]
TAF$_{II}$80-TAF$_{II}$31	+	−
TAF$_{II}$20	+	+
TAT$_{II}$135Δ1 –372	−	−
TAF$_{II}$20-TAF$_{II}$135Δ1–372	−	−

[a] ND, not determined.

the interactions of hTAF$_{II}$28-hTAF$_{II}$18 with the HFM NF-YB-NF-YC dimer, but not with the trimer, should be considered in the light of the presence of the HFM dimer, without NF-YA, in several cell types, including monocytes and differentiated myotubes (21). This finding might point to a role in the basic mechanisms of activation on promoters that lack the CCAAT target site.

The HFM TAF$_{II}$s are present not only in TFIID but also in other complexes: (i) hTAF$_{II}$135, hTAF$_{II}$80, and hTAF$_{II}$20 are found in TFTC, a TAF$_{II}$s-containing complex lacking TBP (27, 30); (ii) hTAF$_{II}$20 and hTAF$_{II}$31 (and the hTAF$_{II}$80-like PAF65α) are in the P/CAF complex (18); and (iii) hTAF$_{II}$31 is found in STAGA (28). A histone octamer-like structure within TFIID has been hypothesized (10); indeed, core histones and hTAF$_{II}$80, hTAF$_{II}$31, and hTAF$_{II}$20 interact through their HFMs in ways that are consistent with histones rules: The H4-like hTAF$_{II}$80 with H3 and H2B, the H3-like hTAF$_{II}$31 with H4, and the H2B-like hTAF$_{II}$20 with H2A and H4. These interactions are fully in agreement with previous findings on the binding of H3-H4 and H2A-H2B subfamilies of histone folds. Crystallographic analysis of the nucleosome found details for residues that are required for H4-H2B interactions: H4 His-75 and H4 Lys-91, and H2B Glu-90 and H2B Glu-73 (5). These residues are conserved in hTAF$_{II}$80 and in the related PAF65α. In NF-YB, the Asp-115 and Glu-98 residues corresponding to H2B Glu-90 and Glu-73 are among the relatively few amino acids present in all 26 NF-YB sequences from different species (65). We found that NF-Y is able to interact with an isoform of hTAF$_{II}$80-hTAF$_{II}$80δ, which lacks 10 amino acids in the α2 helix of the HFM (66), a fact that is mirrored by the interaction of hTAF$_{II}$80δ with the H2B-like hTAF$_{II}$20.[4] Under these circumstances, it is unclear what the structure of the HFM might be, but the α2 subunit is crucial for the formation of heterodimers and, indeed, hTAF$_{II}$80δ is unable to bind to hTAF$_{II}$31: Other parts of the HFM might be involved in the contacts with the H2B-likes. Alternatively, a domain of hTAF$_{II}$80 distinct from the HFM might be implicated in contacting NF-Y. Concerning the hTAF$_{II}$28/hTAF$_{II}$18 dimer, structural analysis detailed somewhat different sorts of HMFs not easily assigned to any of the core histone sub-classes (15). Thus it is difficult to rationalize our interactions data with available structural information.

TAF$_{II}$s are known to contact the activation domains of gene-specific upstream factors, and indeed the capacity of a given factor to activate transcription *in vitro* correlates well with its TAF$_{II}$-binding ability (1). Conditional inactivation of yeast histone fold TAF$_{II}$s, yTAF$_{II}$17/hTAF$_{II}$31, yTAF$_{II}$60/hTAF$_{II}$80, and yTAF$_{II}$68/hTAF$_{II}$20, provided compelling genetic evidence for their general role in promoter activation (34–38). However, only a limited set of studies focused on the effect of TAF$_{II}$s overexpression in mammalian cells. In some cases, positive effects were seen: hTAF$_{II}$28 on the activation factor 2 of retinoic X receptor and hTAF$_{II}$135 on retinoic acid receptor, vitamin D receptor and thyroid receptor (12, 59). In other reports, *in vitro* interactions, SP1-hTAF$_{II}$135, E1A-hTAF$_{II}$135, and p53-dTAF$_{II}$40-dTAF$_{II}$60-dTAF$_{II}$230, were matched by strong repression in co-transfections of the TAF$_{II}$s with GAL4 fusions containing the activation domains (61, 67), although the same TAF$_{II}$s interactions with GAL4-p53 resulted in activation *in vitro* (68). In our GAL4 assays, we observe a similar negative effect by overexpressing hTAF$_{II}$28 and/or hTAF$_{II}$18 on the activation of the NF-Y trimer. It should be noted that this is specific, both for the target, it is not seen with GAL4-SP1 and the Q-rich activation domain of NF-YA, and for these two TAF$_{II}$s, because hTAF$_{II}$31-hTAF$_{II}$80 have negligible effects and hTAF$_{II}$20-hTAF$_{II}$135 have small positive effects; moreover, hTAF$_{II}$28-hTAF$_{II}$18 do not inhibit the natural Ea promoter (not shown). What might be the reason for the inhibition of GAL4-NF-Y fusions? Our *in vitro* results indicate that the hTAF$_{II}$28-hTAF$_{II}$18 dimer interacts with NF-YB-NF-YC, but not with the NF-Y trimer, implying that NF-YA, which recognizes determinants in the HFMs of both subunits (20), prevents the association of hTAF$_{II}$28-hTAF$_{II}$18 to NF-YB-NF-YC. Because formation of the trimer *in vivo* is essential for GAL4-NF-YA activation (56), one can imagine that the co-transfected TAF$_{II}$s could compete for binding to NF-YA by associating NF-YB-NF-YC. However, other interpretations must reconcile our findings that the NF-Y-interacting hTAF$_{II}$28 and the non-interacting hTAF$_{II}$18 both inhibit when transfected alone. It is possible that overexpression of some TAF$_{II}$s alters the stoichiometry of endogenous TFIID complexes, impairing their capacity to mediate activation through the artificial GAL4 constructs. In this respect, it should be noted that certain mouse tissues do have with lower amounts of hTAF$_{II}$28 and hTAF$_{II}$18 (33).

TAF$_{II}$s and MHC Class II Transcription—In addition to NF-Y, binding the trimeric complex RFX is important for MHC class II promoters (53). The NF-Y-RFX trimers make cooperative interactions. In particular, NF-Y binding improves the otherwise rather inefficient binding of RFX. Two non-DNA-binding co-activators are also crucial: the ubiquitous p300/CBP (69, 70) and the tissue-specific CIITA (reviewed in Ref. 71). A network of protein-protein interactions is emerging, in particular, CIITA can interact with hTAF$_{II}$31. Our finding that the hTAF$_{II}$80-hTAF$_{II}$31 dimer can associate NF-Y through hTAF$_{II}$80 suggests that hTAF$_{II}$80-hTAF$_{II}$31 could be contacted at the same time by two of the MHC class II activators. The interactions of NF-Y with HFM TAF$_{II}$s should be considered in the light of recent findings showing that both complexes can associate HATs: p300, P/CAF, and hGCN5 interact with NF-Y (21), whose NF-YB subunit is acetylated by p300 (72). Similarly, CBP/p300 can interact with CIITA. It has been suggested that TAF$_{II}$s could be chaperones of the histone-modifying machines in the proximity of core promoters. NF-Y could recruit acetylase complexes not only through direct interactions with P/CAF, GCN5, or CBP/p300, but also by contacting P/CAF complexes, via the HFM TAF$_{II}$s. From its privileged location at −60, NF-Y is clearly a pivotal factor at the cross-road of multiple connections: It helps upstream factors such as RFX on MHC class II promoters bind DNA, and it recruits TFIID by binding to TBP and to core TAF$_{II}$s. At a higher level, NF-Y reaches its site efficiently in the context of a pre-formed nucleosome, interfacing well with H3-H4 tetramers (54). In summary, NF-Y represents an excellent candidate for penetrating chromatin structures, allowing other upstream activators bind their sites, organizing TFIID complexes and recruiting co-activators that further modify, by acetylation, surrounding nucleosomes. Studies aimed at clarifying the complexity of NF-Y-

[4] B. Bell and L. Tora, unpublished.

TFIID interplay by taking into account the interactions with other TAF_{II}s are currently underway.

Acknowledgments—We are grateful to A. Levine, L. Lania, and G. Caretti for gift of reagents. We thank M. Minuzzo for technical assistance.

REFERENCES

1. Lemon, B., and Tjian, R. (2000) *Genes Dev.* **14,** 2551–2569
2. Wolffe, A. P., and Pruss, D. (1996) *Trends Genet.* **12,** 58–62
3. Workman, J. L., and Kingston, R. E. (1998) *Annu. Rev. Biochem.* **67,** 745–779
4. Baxevanis, A. D., Arents, G., Moudrianakis, E. N., and Landsman, D. (1995) *Nucleic Acids Res.* **23,** 2685–2691
5. Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997) *Nature* **389,** 251–260
6. Inostroza, J. A., Mermelstein, F. H., Ha, I., Lane, W. S., and Reinberg, D. (1992) *Cell* **70,** 477–489
7. Goppeldt, A., Steltzer, G., Lottspeich, F., and Meisterernst, M. (1996) *EMBO J.* **15,** 3105–3116
8. Hisatake, K., Ohta, T., Takada, R., Guermah, M., Horikoshi, M., Nakatani Y., and Roeder, R. G. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 8195–8199
9. Mengus, G., May, M., Jacq, X., Staub, A., Tora, L., Chambon, P., and Davidson, I. (1995) *EMBO J.* **14,** 1520–1531
10. Hoffman, A., Chiang, C.-M., Oelgeschlager, T., Xie, X., Burley, S. K., Nakatani, Y., and Roeder, R. G. (1996) *Nature* **380,** 356–359
11. Hoffman, A., and Roeder, R. G. (1996) *J. Biol. Chem.* **271,** 18194–18202
12. Mengus, G., May, M., Carré, L., Chambon, P., and Davidson, I. (1997) *Genes Dev.* **11,** 1381–1395
13. Xie, X. Kokubo, S. L., Cohen, U., Mirza, A., Hoffmann, A., Chait, B. T., Roeder, R. G., Nakatani, Y., and Burley, S. K. (1996) *Nature* **380,** 316–322
14. Bando, M., Ijuin, S., Hasegawa, S., and Horikoshi, M. (1997) *Biochem. J.* **121,** 591–597
15. Birck, C., Poch, O., Romier, C., Ruff, M., Mengus, G., Lavigne, A.-C., Davidson, I., and Moras, D. (1998) *Cell* **94,** 239–249
16. Gangloff, Y.-G., Werten, S., Romier, C., Carré, L., Poch, O., Moras, D., and Davidson, I. (2000) *Mol. Cell. Biol.* **20,** 340–351
17. Gangloff, Y.-G., Sanders, S. L., Romier, C., Kirschner, D., Weil, P. A., Tora, L., and Davidson, I. (2001) *Mol. Cell. Biol.* **21,** 1841–1853
18. Grant, P. A., Schieltz, D., Pray-Grant, M. G., Steger, D. J., Reese, J. C., Yates III, J. R., and Workman, J. L. (1998) *Cell* **94,** 45–53
19. Maity, S., and de Crombrugghe, B. (1998) *Trends Biochem. Sci.* **23,** 174–178
20. Mantovani, R. (1998) *Nucleic Acids Res.* **26,** 1135–1143
21. Mantovani, R. (1999) *Gene* **239,** 15–27
22. Bell, B., and Tora, L. (1999) *Exp. Cell Res.* **246,** 11–19
23. Green, M. R. (2000) *Trends Biochem. Sci.* **25,** 59–63
24. Timmers, H. T. M., and Sharp, P. A. (1991) *Genes Dev.* **5,** 1946–1956
25. Dikstein, R., Zhou, S., and Tjian, R. (1996) *Cell* **87,** 137–146
26. Jacq, X., Brou, C., Lutz, Y., Davidson, I., Chambon, P., and Tora, L. (1994) *Cell* **79,** 107–117
27. Wieczorek, E., Brand, M., Jacq, X., and Tora, L. (1998) *Nature* **393,** 187–191
28. Martinez, E., Kundu, T. K., Fu J., and Roeder, R. G. (1998) *J. Biol. Chem.* **273,** 23781–23785
29. Ogryzko, V. V., Kotani, T., Zhang, X., Schiltz, R. L., Howard, T., Yang, X.-J., Howard, B. H., Qin, J., and Nakatani, Y. (1998) *Cell* **94,** 35–44
30. Brand, M., Yamamoto, K., Staub, A., and Tora, L. (1999) *J. Biol. Chem.* **274,** 18285–18289
31. Lavigne, A. C., Gangloff, Y. G., Carr, L., Mengus, G., Birck, C., Poch, O., Romier, C., Moras, D., and Davidson, I. (1999) *Mol. Cell. Biol.* **19,** 5050–5060
32. Yamit-Hezi, A., and Dikstein, R. (1998) *EMBO J.* **17,** 5161–5169
33. Perletti, L., Dantonel, J.-C., and Davidson, I. (1999) *J. Biol. Chem.* **274,** 15301–15304
34. Apone, L. M., Virbasius, C. A., Holstege, F. C., Wang, J., Young, R. A., and Green, M. R. (1998) *Mol. Cell* **2,** 2653–2661
35. Michel, B., Komarnitsky, P., and Buratowski, S. (1998) *Mol. Cell* **2,** 663–673
36. Moqtaderi, Z., Keaveney, M., and Struhl, K. (1998) *Mol. Cell* **2,** 675–682
37. Natarjan, K., Jackson, B. M., Rhee, E., and Hinnebusch, A. G. (1998) *Mol. Cell* **2,** 683–692
38. Apone, L. M., Virbasius, C. A., Reese, J. C., and Green, M. R. (1998) *Genes Dev.* **10,** 2368–2380
39. Shen, W.-C., and Green, M. R. (1997) *Cell* **90,** 615–624
40. Walker, S. S., Shen, W.-C., Reese, J. C., Apone, L. M., and Green, M. R. (1997) *Cell* **90,** 607–614
41. Bellorini, M., Lee, D.-K., Dantonel, J.-C., Zemzoumi, K., Roeder, R. G., Tora, L., and Mantovani, R. (1997) *Nucleic Acids Res.* **25,** 2174–2181
42. Coustry, F., Sinha, S., Maity, S. N., and deCrombrugghe, B. (1998) *Biochem. J.* **331,** 291–297
43. Hoey, T., Weinzierl, R. O. J., Gill., G., Chen, J.-L., Dynlacht, B. D., and Tjian, R. (1993) *Cell* **72,** 247–260
44. Gill, G., Pascal, E., Tseng, Z. H., and Tjian, R. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 192–196
45. Ferreri, K., Gill, G., and Montminy, M. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 1210–1213
46. Tanese, N., Saluja, D., Vassallo, M. F., Chen, J. L., and Admon, A. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 13611–13616
47. Nakajima, T., Uchida, C., Anderson, S. F., Parvin, J. D., and Montminy, M. (1997) *Genes Dev.* **11,** 738–747
48. Felinski, E. A., and Quinn, P. G. (1999) *J. Biol. Chem.* **274,** 11672–11678
49. Landsberger, N., and Wollfe, A. P. (1995) *Mol. Cell. Biol.* **15,** 6013–6024
50. Duan, Z., Stamatoyannopoulos, G., and Li, Q. (2001) *Mol. Cell. Biol.* **21,** 3083–3095
51. Viville, S., Jongeneel, V., Koch, W., Mantovani, R., Benoist, C., and Mathis, D. (1991) *J. Immunol.* **146,** 3211–3217
52. Bellorini, M., Dantonel, J. C., Yoon, J.-B., Roeder, R. G., Tora, L., and Mantovani, R. (1996) *Mol. Cell. Biol.* **16,** 503–512
53. Mach, B., Steimle, V., Martinez-Soria, E., and Reith, W. (1996) *Annu. Rev. Immunol.* **14,** 301–331
54. Caretti, G., Motta, M. C., and Mantovani, R. (1999) *Mol. Cell. Biol.* **19,** 8591–8603
55. Mantovani, R., Pessara, U., Tronche, F., Li, X. Y., Knapp, A. M., Pasquali, J. L., Benoist, C., and Mathis, D. (1992) *EMBO J.* **11,** 3315–3322
56. Di Silvio, A., Imbriano, C., and Mantovani, R. (1999) *Nucleic Acids Res.* **27,** 2578–2584
57. Liebermann, P. M., and Berk, A. J. (1994) *Genes Dev.* **8,** 995–1006
58. Mantovani, R., Li, X.-Y., Pessara, U., Hoof van Huijsduijnen, R., Benoist, C., and Mathis, D. (1994) *J. Biol. Chem.* **269,** 20340–20346
59. May, M., Mengus, G., Lavigne, A.-C., Chambon, P., and Davidson, I. (1996) *EMBO J.* **15,** 393–3104
60. Caron, C., Mengus, G., Dubrowskaya, V., Roisin, A., Davidson, I., and Jalinot, P. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 3662–3667
61. Saluja, D., Vassallo, M. F., and Tanese, N. (1998) *Mol. Cell Biol.* **18,** 5734–5743
62. Martin, J., Halenbeck, R., and Kauffman, J. (1999) *Mol. Cell. Biol.* **19,** 5548–5556
63. Burke, T. W., and Kadonaga, J. T. (1997) *Genes Dev.* **11,** 3020–3031
64. Chalkley, G. E., and Verrijzer, C. P. (1999) *EMBO J.* **18,** 4835–4845
65. Gusmaroli, G., Tonelli, C., and Mantovani, R. (2001) *Gene* (*Amst.*) **264,** 173–185
66. Bell, B., Scheer, E., and Tora, L. (2001) *Mol. Cell* **8,** 591–600
67. Farmer, G., Colgan, J., Nakatani, Y., Manley, J. L., and Prives, C. (1996) *Mol. Cell. Biol.* **16,** 4295–4304
68. Thut, C. J., Chen, J. L., Klemm, R., and Tjian, R. (1995) *Science* **267,** 100–104
69. Fontes, J. D., Kanazawa, S., Jean, D., and Peterlin, M. (1999) *Mol. Cell. Biol.* **19,** 941–947
70. Krestovali, A., Agalioti, T., Spilianakis, C., Tzortzakaki, E., Merika, M., and Papamatheakis, J. (1998) *Mol. Cell. Biol.* **18,** 6777–6783
71. Harton, L. A., and Ting, J. P.-Y. (2000) *Mol. Cell. Biol.* **20,** 6185–6194
72. Li, Q., Herrler, M., Landsberger, N., Kaludov, N., Ogryzko, V. V., Nakatani, Y., and Wollfe, A. P. (1998) *EMBO J.* **17,** 6300–6315