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Dominant and Redundant Functions of TFIID Involved in the Regulation of Hepatic Genes

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SUMMARY

To study the in vivo role of TFIID in the transcriptional regulation of hepatic genes, we generated mice with liver-specific disruption of the *TAF10* gene. Inactivation of TAF10 in hepatocytes resulted in the dissociation of TFIID into individual components. This correlated with the downregulation of most hepatocyte-specific genes during embryonic life and a defect in liver organogenesis. Unexpectedly, however, the transcription of less than 5% of active genes was affected by TAF10 inactivation and TFIID disassembly in adult liver. The extent of changes in transcription of the affected genes was dependent on the timing of their activation during liver development, relative to that of TAF10 inactivation. Furthermore, TFIID dissociation from promoters leads to the re-expression of several postnatally silenced hepatic genes. Promoter occupancy analyses, combined with expression profiling, demonstrate that TFIID is required for the initial activation or postnatal repression of genes, while it is dispensable for maintaining ongoing transcription.

INTRODUCTION

Accurate and efficient transcription of eukaryotic genes by RNA polymerase II requires the combined activities of several protein complexes assembled at promoter regions. These include sequence-specific DNA-binding proteins; general transcription factors TFIIA, TFIIB, TFIID, TFIIE, and TFIIH; the Mediator complex; and different coactivators (Orphanides et al., 1996; Lemon and Tjian, 2000). The TFIID complex composed of the TATA-binding protein (TBP) and 14 TBP-associated factors (TAFs) is considered a central component of the transcription apparatus, owing to its multiple molecular functions. First of all, individual TAFs can recognize core promoter elements, such as Inr and DPE, which, in conjunction with TBP binding to TATA element, nucleate the assembly of other general transcription factors into a functional preinitiation complex (PIC) (Butler and Kadonaga, 2002; Pugh, 2000). TAFs also provide interaction surfaces targeted by the activation domains of several transcription factors (Verrijzer and Tjian, 1996). In addition, the TAF1 component of TFIID possesses enzymatic activities, including histone acetyltransferase, kinase, and ubiquitin ligase, which contribute to the activation of a specific subset of genes (Dikstein et al., 1996; O'Brien and Tjian, 2000; Pham and Sauer, 2000). Recent studies have also demonstrated a direct role of TFIID in targeting acetylated or lysine 4 trimethylated histone 3, which provides a molecular link between certain nucleosome modifications and active transcription (Vermeulen et al., 2007). While the above studies establish a pivotal role of TFIID in integrating diverse molecular signals for the formation of functional PIC, the general requirement of this complex for the transcription of all genes has been challenged by several findings. For example, the results of a recent genome-wide study in IMR90 fibroblasts showed that, although there is a strong correlation between TAF1 occupancy and the transcriptional activity of the majority of Pol II transcribed genes, TAF1 is not recruited to a considerable number of active promoters (Kim et al., 2005). The lack of detection of TAF1 occupancy in this second group of genes could be due to weak signals that fall below the sensitivity of the chromatin immunoprecipitation (ChIP) on chip method or due to the involvement of either TAF1-less TFIID-like complexes or other factors that can replace TFIID function. The latter two scenarios are supported by several recent findings. The isolation of the TAF1 and TBP-free TAF-containing complex TFTC provided a first demonstration for the existence of multiple functional TFIID-like complexes (Wieczorek et al., 1998). Subsequently, the identification of tissue-specific TAF-containing TFIID complexes (Freiman et al., 2001; Pointud et al., 2003) or the specialized forms of TFIID in apoptotic cells (Bell et al., 2001) highlighted a remarkable structural and functional diversity of the TFIID complex and its active role in mediating tissue and signal-specific gene expression patterns. An additional layer of complexity comes from the discovery and functional characterization of TBP-related factors that can replace TFIID function. In vertebrates, two TBP homologs, TBP2/TRF3 and TLF/TRF2, have been described that are able to support Pol II-mediated transcription. A crucial function of TBP homologs in zygotic transcription and early embryonic development has been demonstrated by genetic studies in several organisms (Veenstra et al., 2000; Muller et al., 2001; Persengiev et al., 2003; Bartfai et al., 2004; Jacobi et al., 2007). The diversified function of the different TFIID-like complexes establishes the view that general transcription factors not only are involved in executing transcriptional activation

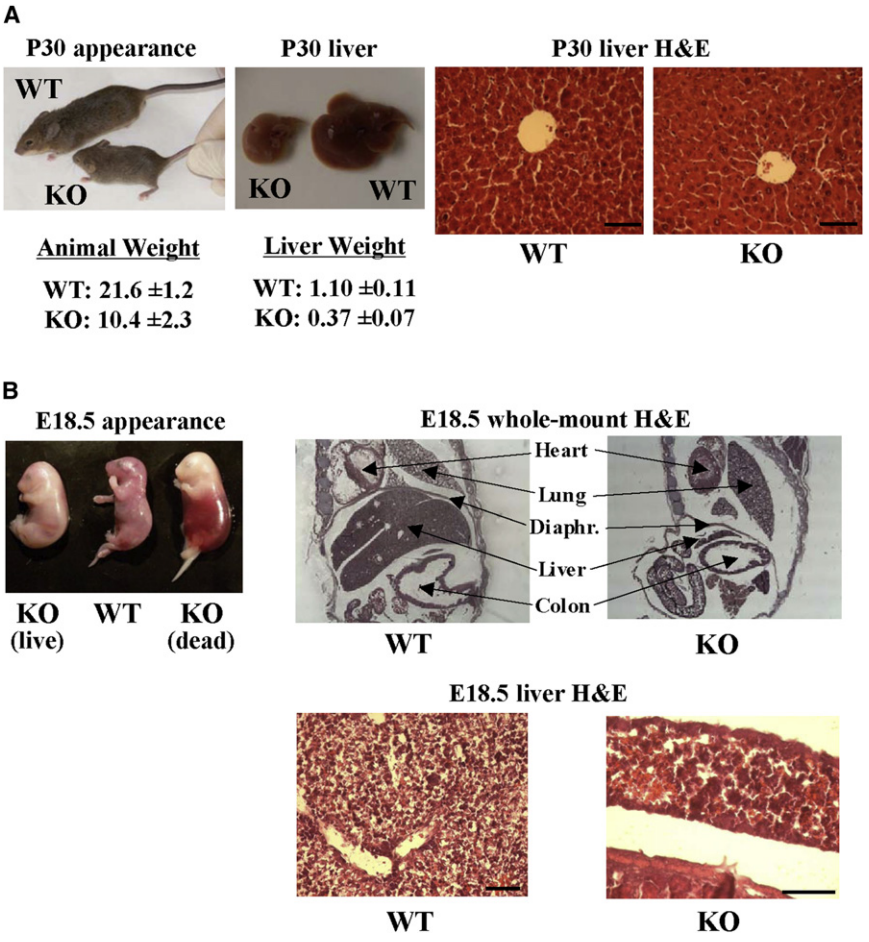

Figure 1. Phenotypes of Hepatocyte-Specific TAF10 KO Mice

(A) Representative pictures of individual animals and their livers and hematoxylin and eosin (H&E) staining of liver sections from postnatal day 30 (P30) wild-type (WT) and *TAF10*$^{lox/lox}$*-Alb-Cre* (KO) mice.

(B) Representative pictures of embryos at 18.5 day postcoitum (E18.5) and hematoxylin and eosin (H&E) staining of whole-mount embryos or liver sections from WT and *TAF10*$^{lox/lox}$*-Alfp-Cre* mice. Living embryos were distinguished by heartbeat. Scale bars, 100 μm.

determined by gene-specific activators but also are themselves gene-specific factors contributing to the generation of cell and pathway-specific expression patterns (Albright and Tjian, 2000; Bell and Tora, 1999; Muller et al., 2007). Perhaps the most striking evidence for this is the recent demonstration of a widespread loss of TFIID subunits during the terminal differentiation of myoblasts to myotubes and the replacement of TFIID by TAF3-containing TBP2/TRF3 complex on the promoters of muscle-specific genes (Deato and Tjian, 2007).

In order to better understand the function of the core transcription machinery in the regulation of tissue-specific genes during development, we studied animal models in which TAF10, a key component of TFIID, was conditionally inactivated in embryonic or adult liver. We show that hepatocyte-specific inactivation of TAF10 leads to the dissociation of TFIID into individual components and to the downregulation of most hepatocyte-specific genes during embryonic life, with parallel defects in liver organogenesis. In contrast, transcription of the majority of the genes in the adult liver was not affected by TAF10 inactivation and TFIID disassembly. We demonstrate that, after the initial activation of genes, TFIID is dispensable for ongoing transcription and that, in addition to its pivotal role in the initial activation of hepatic genes, TFIID is also required for the developmental stage-specific repression of previously active genes. These data suggest that TFIID actively participates in the regulation of the temporal pattern of gene expression during liver development.

RESULTS

Conditional Inactivation of TAF10 in Embryonic and Adult Liver

We crossed *TAF10*^{loxP} mice (Mohan et al., 2003), in which exon 2 of the *TAF10* gene is flanked by loxP sites, with *Alfp-Cre* or *Alb-Cre* transgenic mice to obtain liver-specific TAF10-deficient mice in embryonic or adult stages, respectively. PCR analysis of genomic DNA prepared from hepatocytes revealed complete exon 2 excision at embryonic (E) day E15.5 in the livers of *TAF10*$^{lox/lox}$*/Alfp-Cre* mice and between postnatal (P) days P15 and P22 in the livers of *TAF10*$^{lox/lox}$*/Alb-Cre* mice (Figure S1 available online). Both animal models exhibited interesting phenotypes. Adult stage-specific TAF10KO mice displayed attenuated growth from the second week after birth, resulting in dwarfism with an about 50% reduced body weight at day P30 (Figure 1A). Parallel reduction in the size of different organs, including liver, was observed. Hematoxylin and eosin (H&E) staining of liver sections revealed no gross morphological alterations in TAF10KO mice, although some of the hepatocytes seemed somewhat enlarged (Figure 1A). Apart from the dwarf phenotype, most of the mice looked normal until day P30. Starting from days P34–P35, massive death occurred, and no animal survived day P38. Growth retardation and the reduced expression of several key metabolic genes (see below), which should result in progressive liver dysfunction, are likely to represent the underlying causes of the observed mortality. Therefore, we performed all of our experiments in adult mice at day P30, a time point when hepatocytes are still normal and devoid of TAF10 protein for at least 8–15 days.

Inactivation of TAF10 in day E15.5 resulted in a mixed phenotype. None of the embryos reached birth. At day E18.5, about half of the embryos were dead, with massive blood infiltration throughout the body. The other embryos had an anemic, pale appearance but were alive (Figure 1B). Macroscopic and histological analysis of the living embryos revealed that liver size

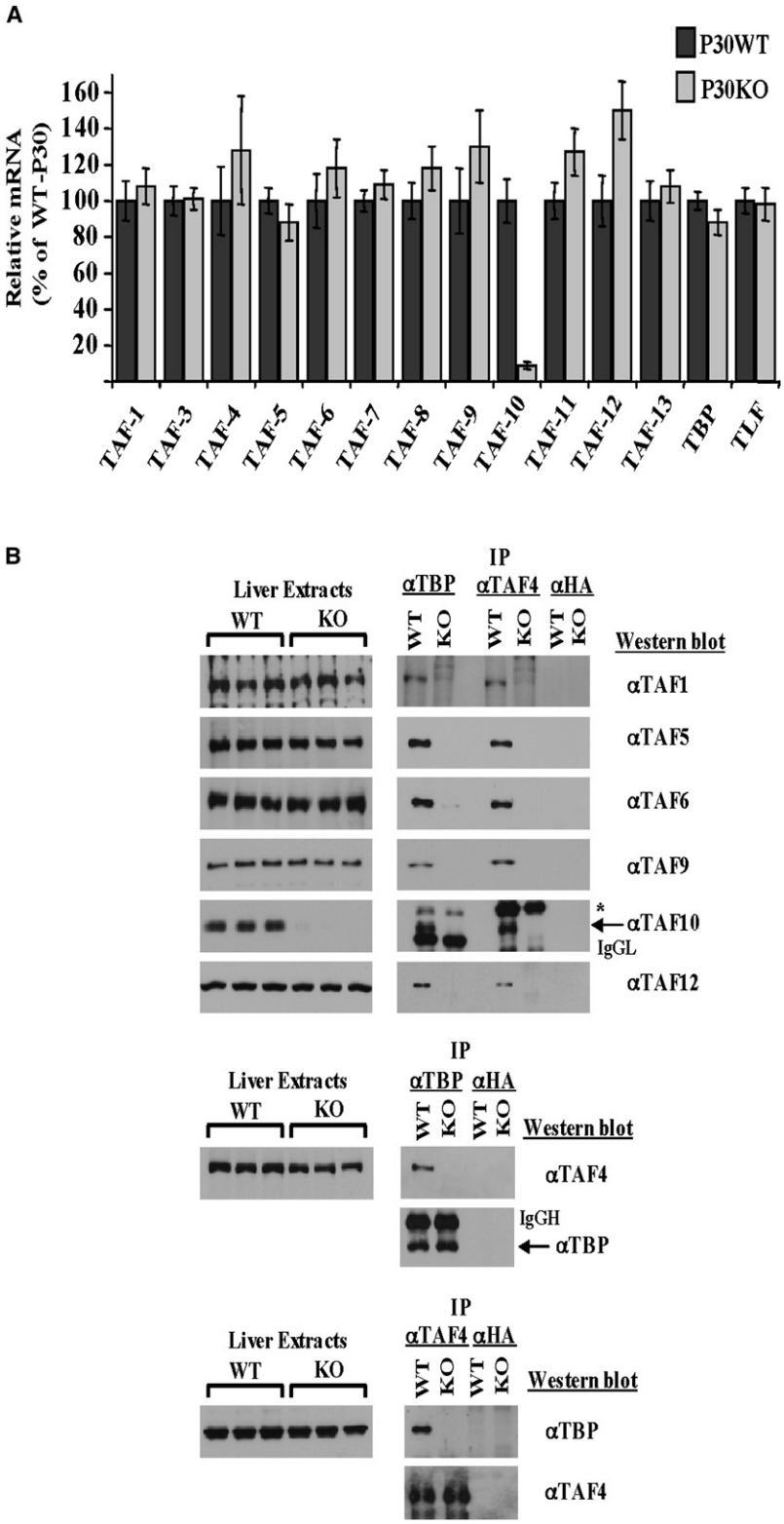

Figure 2. TAF10 Inactivation Does Not Affect the Expression of TFIID Subunits but Leads to the Disassembly of TFIID Complex

(A) Real-time PCR analysis was performed with total liver RNAs prepared from day P30 WT (dark bars) and *TAF10$^{lox/lox}$-Alb-Cre* (gray bars) male mice. The values obtained were normalized to GAPDH and are expressed as percentage of WT P30 data. Bars represent mean values and standard errors from RNA samples of ten individual mice.

(B) Nuclear extracts from the livers of three individual day P30 WT and *TAF10$^{lox/lox}$-Alb-Cre* mice were analyzed in western blot assays (left panel) or mixed and immunoprecipitated with either αTBP, αTAF4, or αHA (control) antibodies and subjected to western blot analysis (right panel) with the indicated antibodies.

was dramatically reduced, while the other organs seemed intact (Figure 1B). Cells stained with H&E in sections from the strip of liver tissue were mostly mononuclear cells or red blood cells not resembling to hepatocytes (Figure 1B), suggesting that, in the absence of TAF10, hepatocyte differentiation is blocked and other cell types accumulate in the liver. This notion was further confirmed by gene expression profiling (see below).

The TFIID Complex Is Disassembled in TAF10 KO Mice

Earlier work on F9 carcinoma cells indicated that TAF10 is required for the stability of TFIID (Mohan et al., 2003). To assess the presence or absence of TFIID complex formation in TAF10KO livers, we first tested the expression of its different subunits by real-time PCR and western blot analysis. As shown in Figure 2, no significant changes were detectable in mRNA or protein levels of any of the tested subunits in TAF10-deficient livers. Next, we immunoprecipitated TFIID complexes using antibodies against TBP or TAF4. We could detect all of the tested TAFs in wild-type liver-derived extracts immunoprecipitated either by αTBP or αTAF4, but not in extracts prepared from TAF10-deficient livers (Figure 2B). Similar results were obtained when the same experiment was performed at low stringency. These results demonstrate that TAF10 inactivation does not affect the expression of TFIID subunits but leads to the disassembly of TFIID. Thus, the liver-specific TAF10KO mouse is considered a good model for studying TFIID function, as it is devoid of assembled TFIID complexes.

Global mRNA Profiling Identifies Four Classes of Genes with Different Requirements of TFIID for Transcription

In order to obtain a global view of the genes affected by TAF10 inactivation and TFIID disassembly in hepatocytes, we performed transcript profiling using pools of RNAs prepared from E18.5 and P30 livers of wild-type and TAF10KO mice. Scatter plot analysis of the data shows

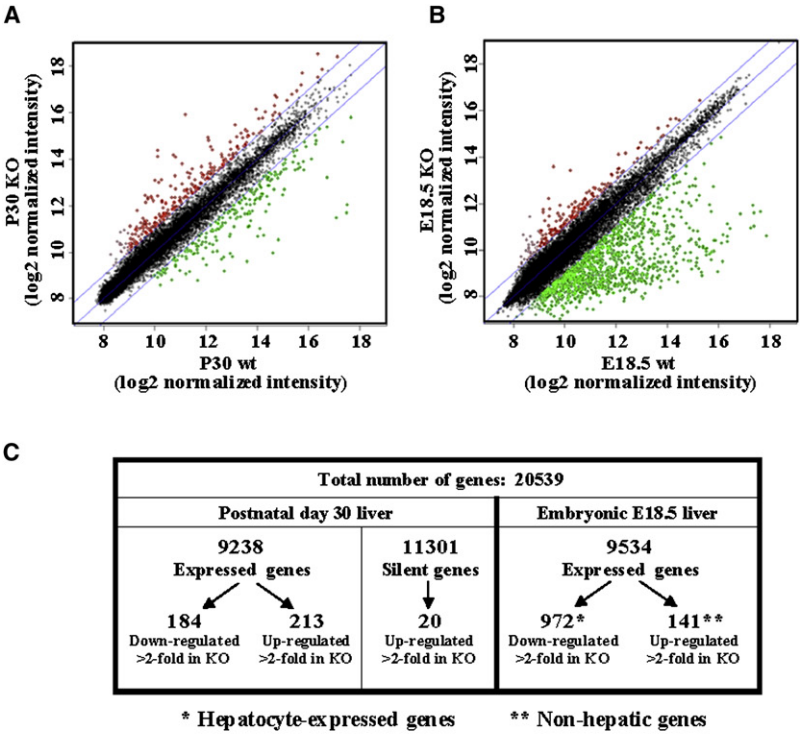

Figure 3. Global Gene Expression Profiling in Liver-Specific TAF10 KO Mice

(A) Scatter plots of normalized microarray hybridization signals obtained with liver RNA samples from day P30 WT versus *TAF10$^{lox/lox}$-Alb-Cre* mice.

(B) Scatter plots of normalized microarray hybridization signals obtained with liver RNA samples from day E18.5 WT versus *TAF10$^{lox/lox}$-Alfp-Cre* mice.

(C) Comparison of the number of genes affected by TAF10 inactivation in day P30 and day E18.5 livers. Transcripts with normalized intensities above 9 (log2) were considered "expressed genes," while those with normalized intensities below 9 (log2) were considered "silent genes." The number of genes changed >2-fold is shown for each group.

(D) Real-time PCR analysis for the indicated transcripts of hepatocyte-specific genes (category 1) was performed with total liver RNAs prepared from day P30 WT (dark bars) and *TAF10$^{lox/lox}$-Alb-Cre* (gray bars) male mice (upper panel) or day E18.5 WT (dark bars) and *TAF10$^{lox/lox}$-Alfp-Cre* (gray bars) embryos (lower panel). The values obtained were normalized to GAPDH and are expressed as percentage of wild-type P30 data. Bars represent mean values and standard errors from RNA samples of ten individual male adult mice or ten individual embryos.

major differences between embryonic and adult mouse livers with respect to the number of genes affected (Figures 3A and 3B). Using a cutoff value of 9 for relative spot intensity, we calculated the number of expressed genes and found that inactivation of TAF10 affected less than 5% of the expressed genes in adult livers and about 11% of those expressed in fetal livers (Figure 3C and Tables S1 and S2). Inspection of the list of annotated genes affected in embryonic TAF10KO liver strips suggested that the downregulated 972 transcripts correspond to genes specifically expressed in hepatocytes, while the majority of the 142 upregulated transcripts are characteristic to genes expressed in hemopoetic or other epithelial cell types, normally present in embryonic liver (Table S2). Apart from literature search, we also performed cross-comparison of gene expression levels in wild-type E18.5 and wild-type P30 livers, where hepatocytes correspond to about 40%–60% and 70%–80% of the total cell population, respectively (Kyrmizi et al., 2006). We found that the majority (91%) of the downregulated genes in E18.5 TAF10KO livers had equal or increased expression in wild-type adult versus wild-type embryonic liver, while an opposite relationship was evident for most of the upregulated genes (data not shown). We also confirmed the microarray data by analyzing the expression of ten widely used hepatocyte-specific marker genes with real-time PCR. As shown in Figure 3D, embryonic inactivation of TAF10 resulted in a dramatic decrease of all mRNA species examined. Furthermore, in immunostaining experiments, we observed a total absence of Albumin, HNF-4, and FoxA2 markers in cells populating E18.5 TAF10KO livers (Figure S3B). Interestingly, expression of most of the genes that were found downregulated in E18.5 TAF10KO livers, including the tested hepatic markers (Figures 3D and S3A), were not significantly affected in adult stage-specific TAF10KO mice. The widespread loss of expression of hepatocyte-specific genes, together with the morphological data of Figure 1, demonstrates that TAF10 inactivation in fetal life leads to major defects in liver organogenesis.

In contrast to fetal liver, a surprisingly small number of genes were affected in adult stage-specific TAF10KO livers. According to their expression ratio in wild-type E18.5 and wild-type P30

livers, we set up four gene categories. Genes in the first category are highly expressed in fetal liver and continue to be active postnatally. TAF10 inactivation did not significantly affect the transcription of these genes in adult (P30) mice. Within this category are the *Albumin*, *HNF-1*, *HNF-4*, *LRH-1*, *FoxA2*, *ApoA1*, *ApoB*, *ApoCIII*, *Transferrin*, and *Aldolase B* genes, whose unchanged full-length transcript levels were verified by real-time PCR analysis (Figures 3D, 4A, and S2). Moreover, no significant alterations in the mRNA levels of these genes could be detected in animals surviving day P35 (Figure 4A). Importantly, in vitro nuclear run-on assays and RNA pol II occupancy analysis at the coding regions revealed that these genes are actively transcribed in TAF10KO hepatocytes at a rate similar to that observed in wild-type cells (Figures S4 and S5).

Genes in the second and third categories are not expressed in E18.5 livers but are induced postnatally. The expression of these genes was negatively regulated by TAF10 inactivation in adult mice (Figures 4B and 4C). In the above two categories, we observed a different extent of downregulation in TAF10 KO livers, which directly correlated with the time of the initial activation of the genes compared to the timing of TAF10 inactivation. Initial activation of "category 2" genes, such as *Mup3*, *Cyp7B1*, *Slco1a1*, *CAR3*, and *Cyp2f2*, occurs between days P14 and P21, which coincides with the time of complete loss of TAF10 from the hepatocytes of TAF10KO mice (Figure 4B). These genes were not transcribed in P30 livers of TAF10KO mice. *Igf1*, *GhR*, *Otc*, *Fasn*, and *Cyp1A2* belong to category 3 genes, which are induced in early postnatal life, and their expression levels reach 35%–50% of the maximum at the time of TAF10 inactivation (Figure 4C). In P30 TAF10KO livers, we could detect moderate decreases (to 30%–50% of wild-type) of their corresponding mRNA levels (Figure 4C). These results demonstrate that TAF10 and TFIID are required for the initial (category 2) or further (category 3) activation of genes, the latter of which likely depends on new initiation events. On the other hand, TFIID is dispensable for the transcription of already active genes (category 1), which comprise the largest group in the adult liver.

Our results concerning the genes belonging to the fourth category are of particular interest. In wild-type animals, Afp, Akr1b7, Igfbp1, H19, and Igf2 are highly expressed in the fetal liver and silenced postnatally to barely detectable levels at days P14–P21 (Figure 4D). Inactivation of TAF10 leads to re-expression of these genes in adult hepatocytes (Figure 4D), suggesting that TFIID may play a repressive role in their postnatal regulation.

Gene-Specific Differential Effects of TFIID Disassembly on the Association of Pol II and General Transcription Factors with Promoters

In order to gain mechanistic insights into how TAF10 inactivation-mediated TFIID disassembly leads to differential transcriptional responses, we performed ChIP assays. ChIP data obtained in category 1 genes, such as *Albumin*, *HNF-4*, *ApoCIII*, and *FoxA2*, whose expression was unaltered by TAF10 inactivation, showed that all of the tested TAFs, as well as TBP, TFIIB, Mediator, and Pol II, occupied these promoters in wild-type E18.5 and P30 livers (Figure 5A). An exception to the above rule was the lack of recruitment of TAF11/TAF13 pair on FoxA2 promoter, which points to the involvement of a TFIID complex with altered subunit composition. In P30 TAF10KO livers, RNA pol II, Med1, Med6, and TFIIB occupancy was not significantly affected, while ChIP signals for all of the TFIID components were reduced to background levels (Figure 5A). The recruitment of other components of the PIC, such as DNA-binding factors (HNF-4α, C/EBPα, FoxA2) or CBP/p300 to FoxA2 promoter and the histone 3 acetylation or lysine 4 trimethylation levels, were not significantly changed in TAF10KO livers (Figure 5B). Due to limitations of the ChIP technique, one cannot entirely exclude the possibility of transient formation of unstable complexes or the involvement of so far unidentified complexes containing other components of TFIID, which were not included in our ChIP studies (TAF2, TAF3, TAF7, or TAF8). However, the differences observed with ten different TFIID subunits provide a strong indication that the active state of the promoters could be sustained without the stable association of the canonical TFIID on already active genes. The possibility of a switch from TFIID to other known complexes that can replace its function was excluded by the following observations. First, we were unable to detect any TBP2/TRF3 expression in wild-type or TAF10KO fetal and adult livers by real-time PCR (Figure S7) or western blot analysis, even at low stringency conditions (data not shown). Second, the occupancy of TLF/TRF2, which is expressed in hepatocytes (Figure 2), was investigated by ChIP but gave negative results on the promoters of all gene categories in all conditions (Figure S6).

On the promoters of *Mup3*, *Cyp7B1*, *Slco1a1*, and *Car3* genes, TFIID, including TBP and all tested TAFs, was detected only in wild-type P30 livers, except for Mup3 promoter, where TAF11/TAF13 pair was absent (Figure 6A). Pol II, Med1, Med6, and TFIIB occupancy was also evident, consistent with the active transcription of the genes at this stage. None of the TFIID subunits, TFIIB, Mediator components, or RNA pol II occupied the above promoters in wild-type E18.5 embryonic livers and TAF10KO P30 adult livers. Recruitment of C/EBPα onto the Mup3 promoter was detectable in embryonic stages when the gene is inactive, and its association with the promoter was not affected in P30 TAF10KO livers. HNF-4α, CBP/p300 occupancy, H3 acetylation, or H3K4 trimethylation could only be detected in wild-type P30 livers (Figure 6B). Together with the data showing that, in fetal livers, these genes are yet to be activated, while in TAF10KO P30 livers, they fail to be activated (Figure 4B), the above results suggest that TFIID is required for the initial assembly of the Pol II machinery on these promoters.

We observed an interesting promoter occupancy pattern on category 4 genes, which are silenced during early postnatal periods of liver development. In wild-type fetal livers, TAFs, TBP, TFIIB, Mediator, and Pol II were readily detected on Afp, Akr1b7, and Igfbp1 promoters, which is in agreement with the high-level expression of these genes at the embryonic stages (Figure 7A). As expected, RNA pol II and Mediator subunits were absent from the promoters in wild-type P30 livers, where these genes are not transcribed. Interestingly, however, TBP and TAFs and, in the case of Igfbp1 promoter, TFIIB as well, remained associated with the regulatory regions at this stage (Figure 7A). In P30 TAF10KO livers, Pol II, Mediator, and TFIIB ChIP signals reappeared in all promoters, which correlates with the reactivation of these genes. TBP remained associated with

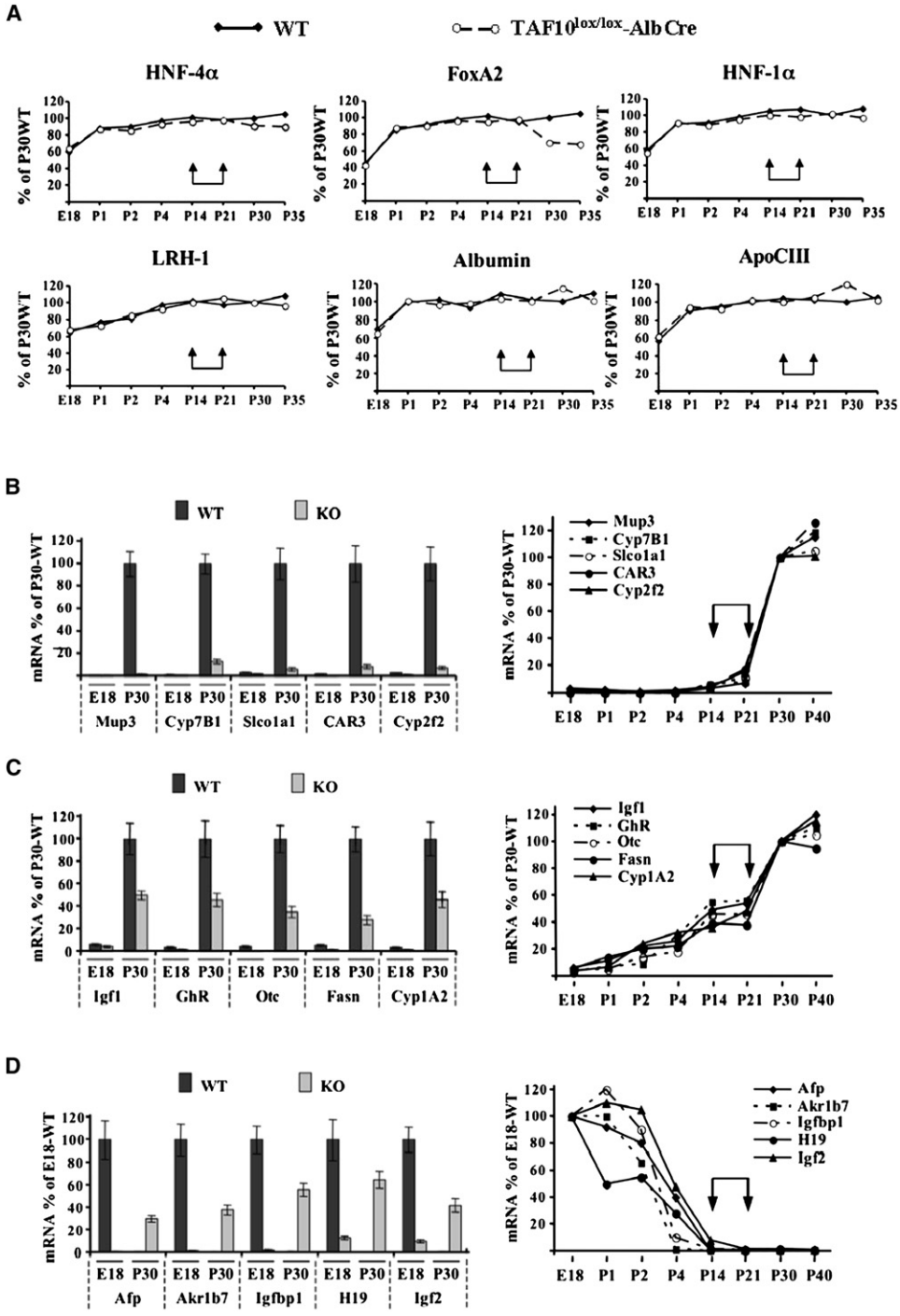

Figure 4. Effects of TAF10 Inactivation on the Transcription of Hepatic Genes

(A) Time course analysis of category 1 gene transcripts during postnatal liver development in WT (solid line) and *TAF10$^{lox/lox}$-Alb-Cre* mice (dashed line). Arrows indicate the time of complete loss of TAF10 from individual animals (P15 to P22).

(B) (Left) Real-time PCR analysis of Mup3, Cyp7B1, Slco1a1, CAR3, and Cyp2f2 transcripts (category 2) was performed with total liver RNAs prepared from day P30 WT (dark bars) and *TAF10$^{lox/lox}$-Alb-Cre* (gray bars) male mice or day E18.5 WT (dark bars) and *TAF10$^{lox/lox}$-Alfp-Cre* (gray bars) embryos. The values obtained were normalized to GAPDH and are expressed as percentage of WT P30 data. Bars represent mean values and standard errors from RNA samples of ten individual male adult mice or ten individual embryos. (Right) Total liver RNAs from five to ten animals were prepared and pooled from WT mice at day E18.5 and the indicated days after birth (P). Real-time PCR values are expressed as percentages of the data obtained with day P30 samples. Arrows indicate the time of complete loss of TAF10 from individual animals (P15 to P22).

the promoters, while all TAFs were absent in Afp and Igfbp1 promoters. In the case of Akr1b7 promoter, TAF9, TAF11, TAF12, and TAF13 could still be detected in P30 TAF10KO livers, pointing to the retention of a partial or aberrant TFIID complex (Figure 7A). These data suggest that certain TAFs within the TFIID complex participate in the mechanism of postnatal silencing of the category 4 genes. More detailed analysis of transcription factors occupying the Afp promoter revealed a TFIID-dependent exchange of activator and repressor complexes. In agreement with previous reports (Nguyen et al., 2005), we found that the SBE/p53RE located at the −850 nt region of the Afp gene is occupied by FoxA2 activator in embryonic liver, which is replaced by p53 and Sin3a repressor proteins in postnatal stages (Figure 7B). Changes in CBP/p300 occupancy, H3 acetylation, and H3K4 trimethylation levels correlated with the transition from active to repressed state of the gene (Figure 7B). We could not detect any H3K9 dimethylation signal in wild-type P30 livers, indicating that, at least at this developmental period, heterochromatinization of the Afp gene does not take place. Importantly, we observed a dissociation of p53/Sin3a complex and a reassociation of FoxA2/CBP proteins with the upstream region in P30 TAF10KO hepatocytes, which coincided with the reappearance of active histone modifications (Figure 7B). These results provide a mechanistic explanation for the reactivation of the Afp gene in TAF10KO mice and demonstrate an active role of core promoter-bound TFIID in the selective association of regulators with upstream sequences.

DISCUSSION

TFIID is a key constituent of the transcription apparatus in the majority of active genes (Kim et al., 2005; Huisinga and Pugh, 2004). The main findings of this study indicate that TFIID function is restricted to the initial phase of PIC assembly, while it is dispensable at subsequent stages, when stable PIC has already been formed on the promoters of transcriptionally active genes. In addition, our study reveals an additional function of TFIID by demonstrating its importance in postnatal silencing of a number of hepatic genes.

Liver-Specific TAF10 KO Mice: A Model for Studying TFIID Function

TAF10 is one of the 14 TAFs within the TFIID complex. In TAF10 null F9 carcinoma cells, TFIID is disintegrated (Mohan et al., 2003), and the cells are blocked in G1 phase of the cell cycle and undergo apoptosis due to the deregulated expression of several cell-cycle regulators (Metzger et al., 1999). In agreement with its effect on genes regulating cell-cycle progression, *TAF10*$^{-/-}$ mice display an early embryonic lethal phenotype at the blastocyst stage due to the death of pluripotent cells in the inner cell mass (Mohan et al., 2003). On the other hand, studies on the above models provided some indication for differential sensitivity to TAF10 inactivation between proliferating and differentiated cells: TAF10 null F9 carcinoma cells differentiated by retinoic acid treatment or trophoblast cells in *TAF10*$^{-/-}$ blastocysts were viable (Metzger et al., 1999; Mohan et al., 2003). More direct evidence for differential TAF10 sensitivity came from studies on skin-specific conditional TAF10 knockout mice. Ablation of TAF10 in fetal keratinocytes induced serious defects in the granular and cornified cell layers, which impaired skin barrier function (Indra et al., 2005). In contrast, no detectable phenotype was observed when TAF10 inactivation was induced in adult differentiated keratinocytes. The inherent technical difficulties of performing biochemical analysis with skin tissue, which contains many different cell types, and the early lethality of the standard TAF10 knockout mice precluded in-depth studies on TAF10 function.

In our first animal model, we inactivated TAF10 in embryonic liver (*TAF10*$^{lox/lox}$*-Alfp-Cre* mice) at a developmental stage when proliferating hepatoblasts differentiate into biliary epithelium and hepatocyte lineage (Zaret, 2002). Liver-specific TAF10 null embryos displayed a phenotype with widespread loss of expression of hepatocyte-specific genes and defects in hepatocyte differentiation and proliferation, culminating in the failure of liver organogenesis. Because TAF10 is an integral component of TFIID complex, inactivation of hepatocyte-specific genes in TAF10 KO embryonic hepatocytes is in agreement with the well-known transcription-activating function of TFIID. However, one cannot exclude the possibility that TAF10 inactivation may have affected only a small subset of genes required for proper hepatocyte proliferation or differentiation, and, thus, the observed reduction of hepatic transcripts could be a consequence of the associated loss of hepatocytes from the liver tissue.

In order to circumvent the above problems, we inactivated TAF10 in adult liver dominated by a single cell type (hepatocytes) in G0 nonproliferating phase. Contrary to embryos, when TAF10 is inactivated in adult stage (days P15–P22), liver architecture and hepatocyte morphology remained normal for a considerable length of time, although the animals displayed a dwarf phenotype. The observed dwarfism is attributed to the significant downregulation of growth hormone receptor (*GhR*) and insulin-like growth factor 1 (*Igf1*) genes since similar changes of their expression in other animal models have been directly linked to attenuated growth phenotype (Lee et al., 1998).

Importantly, our biochemical assays showed that inactivation of TAF10 at the adult stage results in a complete disassembly of TFIID complex into individual subunits. The lack of occupancy by other TFIID subunits on several individual promoters suggests that virtually no residual canonical TFIID complex exists in the cells, which could have been missed by our biochemical detection approach. These results also rule out the presence of known TFIID-like TAF-containing complexes (e.g., TFTC) in TAF10-deficient hepatocytes. Taken together, *TAF10*$^{lox/lox}$*-Alb-Cre* mouse provides an animal model with morphologically normal hepatocytes, which lack assembled TFIID-type complexes.

TFIID Is Not Required for the Maintenance of Ongoing Transcription of Hepatic Genes

One of the most surprising findings of this study is the very small number of genes whose transcript levels were affected in the

(C) Real-time PCR analysis as in (A) for Igf1, GhR, Otc, Fasn, and Cyp1A2 transcripts (category 3).
(D) Real-time PCR analysis as in (A) for Afp, Akr1b7, Igfbp1, H19, and Igf2 transcripts (category 4). Here, values are expressed as percentage of E18 WT data.

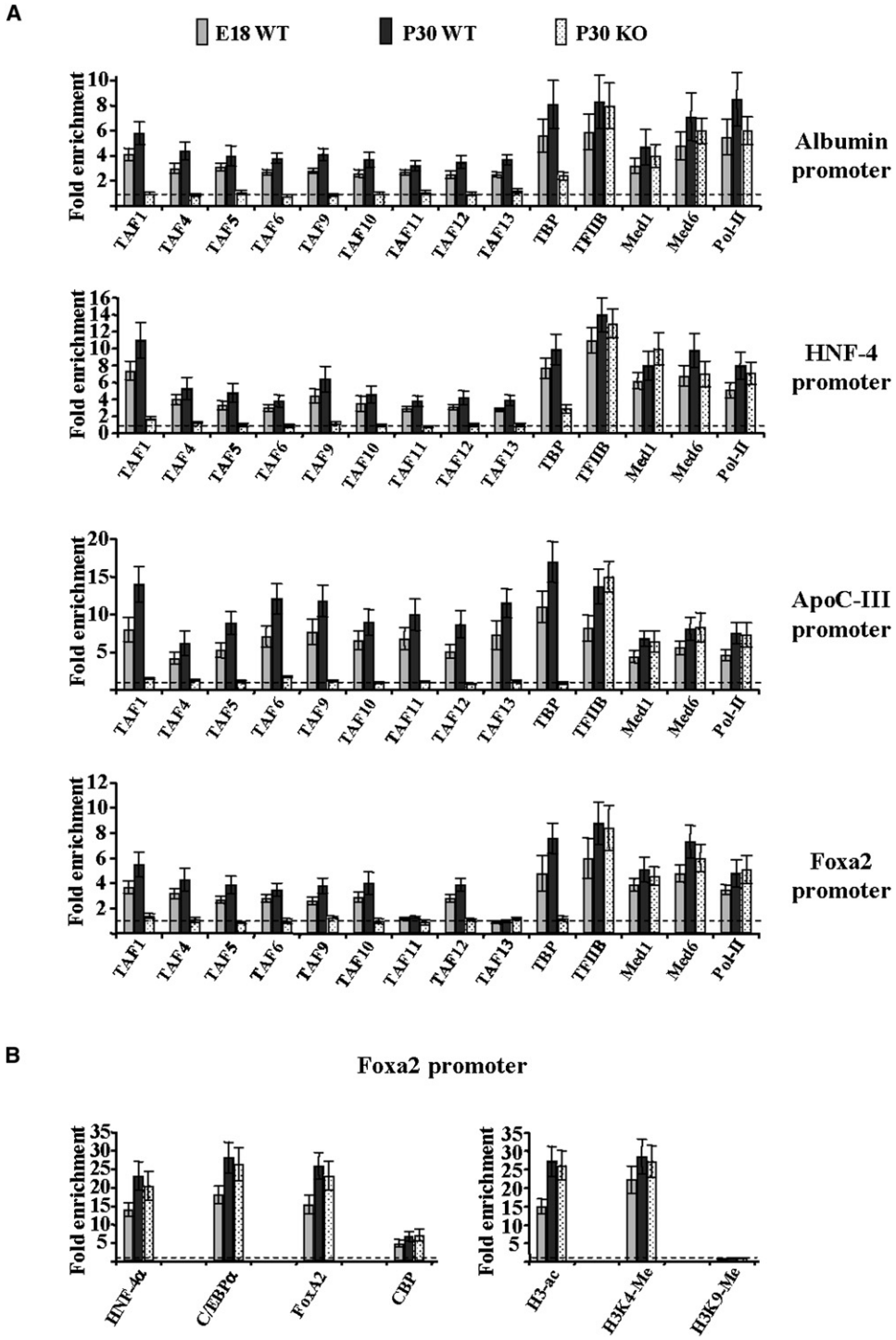

Figure 5. Transcription Factor Occupancy in the Promoter Regions of Category 1 Genes

(A and B) Chromatin immunoprecipitation assays with antibodies against TAFs, TBP, TFIIB, Med1, Med6, and RNA pol II or HNF-4a, C/EBPa, FoxA2, CBP, acetylated H3 tails (H3ac), trimethylated lysine 4 H3 tails (H3K4-Me), and dimethylated lysine 9 H3 tails (H3K9-Me) were performed in crosslinked chromatin prepared from the livers of WT embryos at day E18.5 (gray bars), WT adult animals at day P30 (dark bars), and *TAF10*$^{lox/lox}$*-Alb-Cre* mice at day P30 (dotted bars). The data from qPCR reactions with primers amplifying the promoter regions of the indicated genes were normalized to input and expressed as fold enrichment over those obtained with control antibody (αHA), which were set at 1 (dashed horizontal line). Scale bars show mean values and standard errors from three independent experiments.

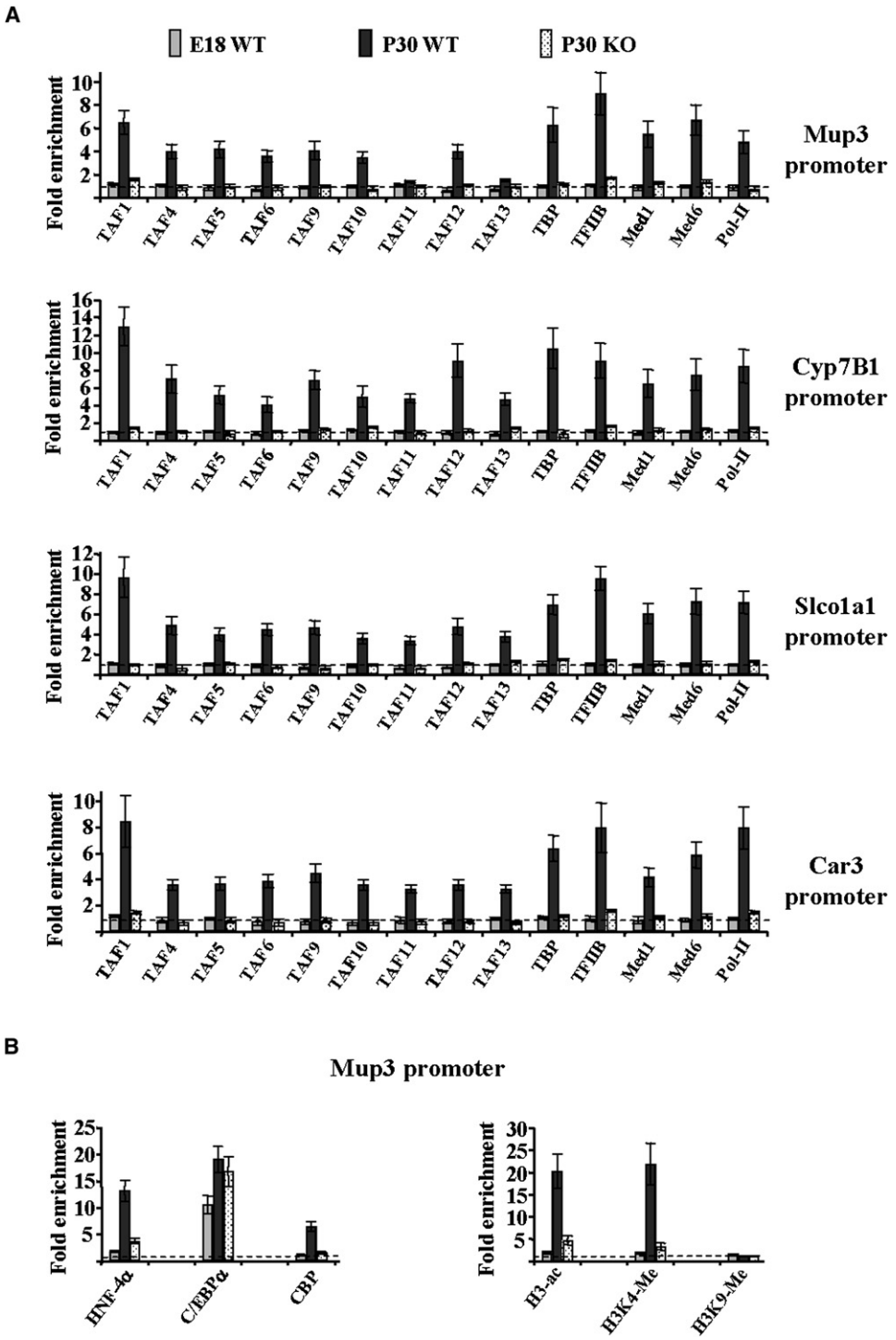

Figure 6. Transcription Factor Occupancy in the Promoter Regions of Category 2 Genes

(A and B) Chromatin immunoprecipitation assays analyzing the Mup3, Cyp7B1, Slco1a1, and Car3 promoters. The data are presented as in the legend of Figure 5.

livers of *TAF10*$^{lox/lox}$*-Alb-Cre* mice. One plausible explanation for this observation is that TFIID function could be replaced in the majority of the promoters by TBP-related factors, such as TBP2/TRF3 or TLF/TRF2, and associated proteins. Such switching of core promoter complexes from TFIID to TRF3/TAF3 complex on myogenic genes during skeletal muscle differentiation has been described recently (Deato and Tjian, 2007). A similar type of exchange between TFIID and TLF/TRF2, which is able

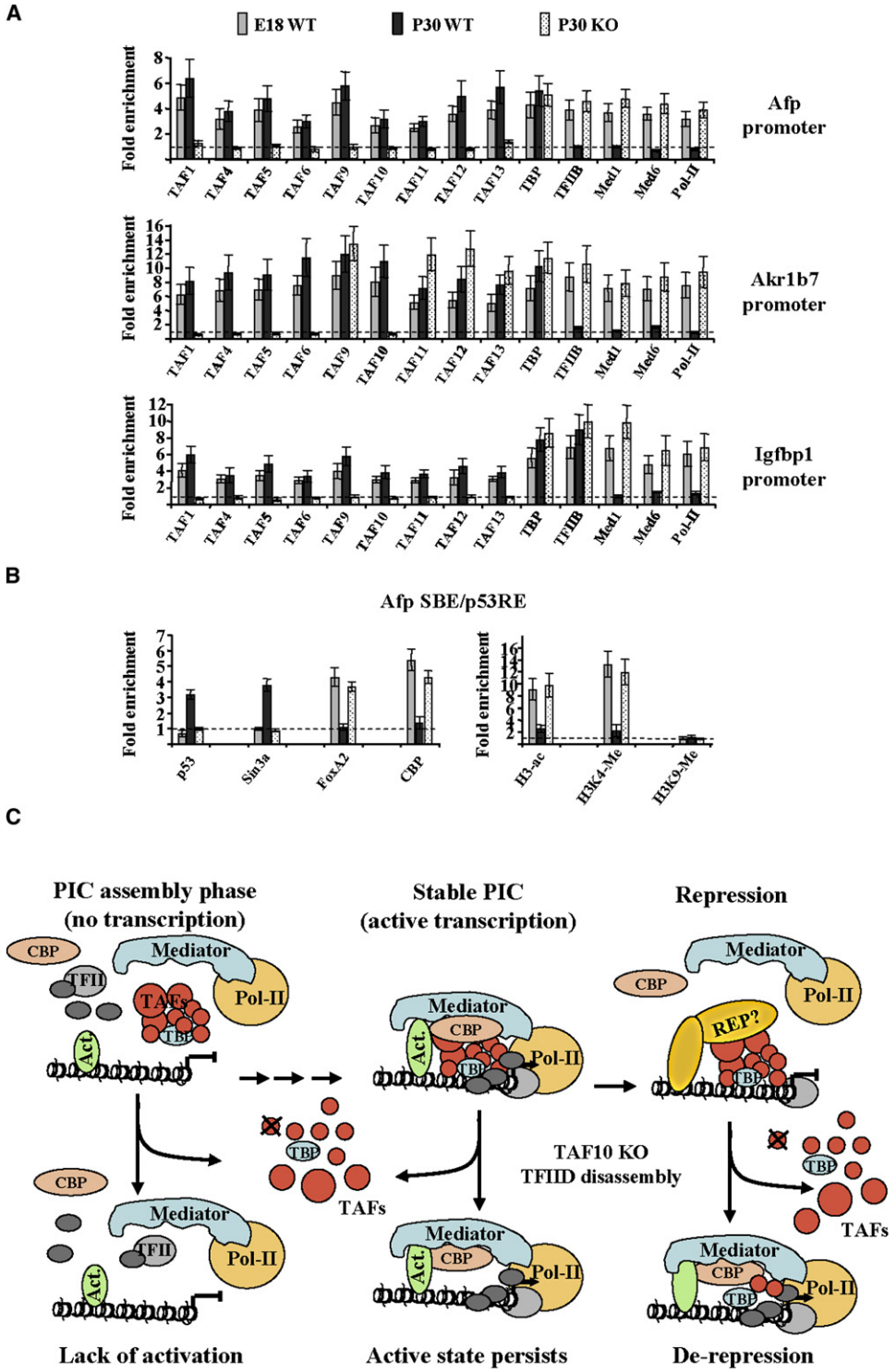

Figure 7. Transcription Factor Occupancy in the Promoter Regions of Category 4 Genes

(A and B) Data from chromatin immunoprecipitation assays analyzing the promoter occupancy of Afp, Akr1b7, and Igfbp1 genes are shown. The data are presented as in the legend of Figure 5.

(C) Schematic presentation of a model integrating the promoter occupancy profiles and the transcription states of the studied genes.

to drive transcription of specific genes, could also be considered. Both of the above possibilities are excluded by our findings, which showed that TBP2/TRF3 is not expressed in the liver and that TLF/TRF2 was not recruited to the promoters of studied genes.

A clue for the observed differential effects arose when we investigated the timing of the activation of genes during development, relative to the timing of TAF10 inactivation. The vast majority of hepatocyte-expressed genes are highly active long before the third postnatal week, reaching high expression levels in late embryonic stages. The genes whose transcript levels did not significantly change in adult TAF10KO mice belong to this category. Importantly, chromatin IP assays in TAF10KO livers revealed a complete dissociation of TFIID subunits from the promoters of four selected genes of this group, while DNA-binding factors, coactivators, or other general transcription factors, such as TFIIB and Mediator, were retained. RNA pol II was also observed on the promoters, consistent with the high transcript levels of these genes in the livers of 30-day-old (and also 35-day-old) TAF10 KO mice. As depicted in the scheme of Figure 7C, the results indicate that, once stable PIC is formed, TFIID is dispensable for transcription, and other PIC components can sustain a promoter architecture that allows efficient RNA pol II recruitment and transcription.

In a previous study, we demonstrated a progressive build-up of transcription factors on the promoters of target genes during liver development (Kyrmizi et al., 2006). The increasing number of factors occupying the genes during development had little correlation with increased rate of transcription but, rather, had an outcome of formation of more stable PICs that were less sensitive to the loss of one key regulator (Kyrmizi et al., 2006). Here, we demonstrate that the highly complex promoter architecture, formed on tissue-specific genes in terminally differentiated cells, can sustain transcription for a significant length of time even in the absence of the key core promoter complex TFIID. Multiple biological roles of this phenomenon can be envisioned. For example, reduced activity of the transcription apparatus in response to a variety of extracellular signals that influence the function of individual components may lead to global effects on a large repertoire of genes whose expression characterizes the identity of a given cell type. The ability of highly complex PICs to support transcription in the absence of one or more of its components may provide advantages to ensure reduced responses to such stimuli and prevent potential global changes that could alter the phenotype of the cell.

TFIID Is Required for the Initial Activation of Hepatic Genes

During the examination of the microarray data, we also noticed that, among the 184 downregulated genes in the livers of TAF10-deficient adult mice, the majority of the liver-specific genes were not expressed in wild-type E18.5 livers. The initial activation of the most affected genes, such as *MUP3*, *Cyp7B1*, *Slco1a1*, *Car3*, and *Cyp2f2*, coincided with the time of TAF10 inactivation (days P14–P21). Transcription of these genes was very low—close to detection limit—in TAF10KO mice. We also failed to detect recruitment of RNA pol II and other factors to their promoters in TAF10KO livers, suggesting that, without TFIID, active preinitiation complex is not formed on these genes. These results demonstrate that the above genes failed to get activated in TAF10KO mice and support the mechanistic model (Figure 7C) according to which TFIID is absolutely required for the assembly of the transcription apparatus at the initial phase of PIC formation.

About two-thirds of the downregulated transcripts decreased less dramatically (to 30%–50% of wild-type) in TAF10KO livers. Our analysis of five selected liver-specific genes in this category revealed that their initial activation occurs during the first week after birth and their transcript levels reach 40%–50% of the maximum at the time of TAF10 inactivation. Gradual increases of mRNA levels in a given cell population may be a result of the fact that genes are not activated in all cells or both alleles in the same cell at the same developmental time. Therefore, the lower extent of TAF10 inactivation-mediated changes in this category of genes may be explained by the lack of their further activation coming from new initiation events.

Involvement of TFIID in the Mechanism of Postnatal Silencing of Hepatic Genes

The general transcription factor complex TFIID is one of the most studied components of the transcription apparatus. Its functions in the transcription process have always been linked to activation (Orphanides et al., 1996; Dorris and Struhl, 2000). Hence, it was surprising to see in our microarray list that, in adult TAF10KO mice, the transcript levels of several (233) genes were increased. An obvious explanation for this observation would be that loss of TFIID would indirectly affect the transcription of these genes. Inspection of the hepatocyte-specific genes within this list and comparison of their expression levels in wild-type E18.5 and adult P30 livers indicated that the majority of them are silenced postnatally. A detailed analysis of the five most affected liver-specific genes (*Afp*, *Akr1b7*, *Igfbp1*, *H19*, and *Igf2*) showed that, during postnatal liver development, the mRNA levels of these genes are gradually decreased, reaching essentially undetectable levels at days 14–21. In a good correlation with the postnatal repression of these genes in wild-type P30 livers, RNA pol II, Mediator, and TFIIB could not be detected on their promoters. Interestingly, however, TFIID components remained associated with the promoters during the repressed state. What could be the role of TFIID on an inactive promoter? One possibility is that TFIID occupancy serves a "bookmarking" role that keeps the regulatory regions competent for reactivation, analogous to that observed during mitosis in HeLa cells (Christova and Oelgeschlager, 2002). According to this scenario, TFIID would be inert with respect to transcriptional activity but would play an architectural role that prevents compaction of the underlying chromatin. This possibility gains some support from the reactivation potential of *Afp* and *H19* genes in hepatocellular carcinomas (Spear, 1999; Ariel et al., 1998).

A more likely scenario is that TFIID cooperates with repressor proteins for efficient silencing of the genes. Our findings directly support this hypothesis: in TAF10KO mice, TAFs dissociated from Afp, Akr1b7, and Igfbp1 promoters, which coincided with the rerecruitment of Mediator and RNA pol II. This new promoter configuration, which also included TAF-less TBP, correlated with the re-expression of the corresponding transcripts. The fact that

we observed such activation on silent genes that were active in an earlier developmental period points to a derepression mechanism depicted in Figure 7C.

Role of TFIID in the Crosstalk between Core Promoter and Upstream Regulatory Regions

With the exception of *Afp*, the mechanism of postnatal repression of the category 4 genes is poorly understood. Association of p53 with the distal repressor region of the *Afp* gene facilitates the recruitment of mSin3A-containing corepressor complex and displaces the main activator, FoxA2 (Nguyen et al., 2005). Independent in vitro studies also demonstrated that the NcoR component of the corepressor complex can interact with TAF9 and can lock it into a nonfunctional conformation that is not conductive for transcription (Muscat et al., 1998). It has also been reported that p53 itself can directly interact with TAF9 and TAF1 (Buschmann et al., 2001; Li et al., 2007). Thus, we speculated that TAFs may play an active role in the postnatal repression of *Afp* gene by providing interaction surfaces to repressor proteins. Dissociation of TAFs from the promoter in TAF10-deficient hepatocytes could destabilize the repressor complex and allow the reassociation of Mediator and RNA pol II with it. The results in this paper confirm the above hypothesis. We observed a dissociation of the p53/Sin3a repressor proteins and their concomitant replacement by FoxA2 and CBP activators at the upstream regulatory region when TFIID binding to the core promoter was eliminated. On the other hand, the opposite switch (from activator to repressor complexes) occurring during normal development does not seem to be regulated by TFIID since it was associated with the promoter in both E18.5 and postnatal livers. Therefore, we propose that the involvement of TFIID in the crosstalk between the two regulatory regions is a characteristic for the repressed state of the Afp gene. While open questions still remain, our demonstration that TFIID is required for transcriptional repression of specific genes provides an example for an additional function of this complex, which contributes to the regulation of cell type-specific gene expression patterns. In this regard, we note that, in the recent genome-wide occupancy study conducted in IMR90 fibroblasts, TAF1 (and presumably TFIID) was also found in the promoters of about 4.5% of inactive genes (Kim et al., 2005). Association of TFIID with such a high number of inactive promoters implies that the repressive function described here may not be restricted to hepatocytes but could also operate in other cell types.

EXPERIMENTAL PROCEDURES

Liver-Specific TAF10 KO Mice and Histological Analysis

$TAF10^{LoxP}$ mice containing floxed exon 2 allele of the *TAF10* gene (Mohan et al., 2003) were backcrossed to CBA-CAxC57Bl/10 background and maintained in grouped cages in a temperature-controlled virus-free facility on a 12 hr/12 hr light/dark cycle. $TAF\text{-}10^{LoxP}$ mice were crossed with *Alfp-Cre* and *Alb-Cre* transgenic mice (Kyrmizi et al., 2006) to obtain inactivation of *TAF10* gene in embryonic and adult hepatocytes, respectively. For histological analysis, livers were fixed in 4% paraformaldehyde and embedded in paraffin, and sections (6–8 μm thick) were used for staining with H&E. For immunostaining, frozen liver sections were fixed in 2% formalin; blocked with 1% BSA in PBS, followed by incubation with αTAF10, αTAF1, αHNF-4, αFoxA2, or αAlbumin primary; and Alexa Fluor 568-labeled (Molecular Probes) secondary antibodies as described (Ktistaki and Talianidis, 1997).

RNA Analysis and Expression Profiling

Total RNA was prepared by the guanidinium isothiocyanate extraction method and, after digestion with DNase I, was further purified by using the RNeasy kit from QIAGEN. Reverse transcription and quantitative real-time PCR assays were performed as described previously (Kyrmizi et al., 2006) with primer sets shown in Table S3.

For microarray analysis, two RNA pools were prepared from the livers of E18.5 days wild-type mice, E18.5 days $TAF10^{lox/lox}$-*Alfp-Cre* (embryonic KO) mice, P30 days wild-type mice, and P30 days $TAF10^{lox/lox}$-*Alb-Cre* (adult KO) mice. Each pool consisted of equal amounts of RNA samples from 20 embryos or from 5 adult male animals, respectively. cRNA synthesis, hybridizations of dye-swap replicates to UMC M. musculus 35K oligo Array Version 1.0, and data analysis are described in the Supplemental Experimental Procedures. The data can be accessed at http://www.ebi.ac.uk/arrayexpress/ (experiment code E-IMBB-Mm-1).

Extract Preparation and Coimmunoprecipitation Assays

Liver tissue was minced to small pieces in a buffer containing 0.32 M sucrose, 15 mM HEPES (pH 7.9), 60 mM KCl, 2 mM EDTA, 0.5 mM EGTA, 0.5% BSA, 0.5 mM spermidine, 0.15 mM spermine, and 0.5 mM DTT, supplemented with protease inhibitor cocktail (Roche). After ten strokes of homogenization with Teflon pestle, the nuclei were layered over equal volume of a buffer containing 30% sucrose, 15 mM HEPES (pH 7.9), 60 mM KCL, 2 mM EDTA, 0.5 mM EGTA, 0.5 mM spermidine, 0.15 mM spermine, and 0.5 mM DTT and centrifuged for 15 min at 3000 rpm. Pelleted nuclei were washed with PBS; resuspended in 50 mM Tris (pH 7.5), 1% NP40, 0.25% deoxycholate, 400 mM NaCl, 1 mM EDTA, 10% glycerol, and protease inhibitor cocktail (Roche); and incubated at 4°C for 20 min with constant agitation. After centrifugation at 18,000 rpm, the extracts were used for immunoprecipitation and western blot analysis as described (Kouskouti et al., 2004). The sources of the antibodies used are described in the Supplemental Experimental Procedures.

Chromatin Immunoprecipitation Assays

Chromatin immunoprecipitation assays were performed as described previously (Kyrmizi et al., 2006). The antibodies and primer sets used for chromatin immunoprecipitations and quantitative PCR are described in the Supplemental Experimental Procedures and Table S3. All of the amplification data were first normalized to input and expressed as fold enrichment over the values obtained with control antibody (anti-HA tag). The latter control values ranged between 0.1% and 0.14% of the input in the reactions amplifying the different promoter regions.

SUPPLEMENTAL DATA

The Supplemental Data include Supplemental Experimental Procedures, seven figures, and three tables and can be found with this article online at http://www.molecule.org/cgi/content/full/31/4/531/DC1/.

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