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Competition between transcription factors HNF1 and HNF3, and alternative cell-specific activation by DBP and C/EBP contribute to the regulation of the liver-specific aldolase B promoter

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ABSTRACT

The aldolase B proximal promoter is controlled by at least five elements spanning from −190 to −103 bp with respect to the start site of transcription. From 5′ to 3′, we found : a negative DE element, an activating C/EBP-DBP binding site, a CCAAT box binding NFY that seems to play a negative role, and an activating element consisting of two overlapping binding sites for HNF-1 and HNF-3. Cotransfection experiments of aldolase B/CAT constructs and of expression vectors for different transcription factors were carried out in human hepatoma Hep G2 cells. We found that DBP and HNF-1 are strong transactivators of the aldolase B promoter while C/EBP and vHNF-1 are only weak activators and HNF-3 alone does not modify such activity. Deletion of the distal negative element results in a similar transactivation by C/EBP and DBP, enhanced for the former and reduced for the latter. In hepatocytes in primary culture, the strong transactivator is C/EBP while DBP is essentially inactive. This tissue-specificity of C/EBP and DBP action could depend on interaction with tissue-specific proteins bound to a neigbouring site, probably DE. Finally, HNF3 behaves as a very strong anti-activator of the aldolase B promoter. It competitively antagonizes transactivation by HNF-1 and non-competitively transactivation by DBP. This negative effect of HNF-3 and tissue-specificity of the transactivation potential of DBP and C/EBP are unique features of the aldolase B promoter.

INTRODUCTION

Fructose 1,6 bisphosphate aldolase, an enzyme involved in both anaerobic glycolysis and gluconeogenesis, exists as the combination of three different isozymic subunits : A (ubiquitous and abundant in muscle), C (present in fetal and cancerous cells, abundant in brain) and B (specific to liver, kidney and small intestine) (1, 2). The concentration of the different mRNAs varies depending on tissues and developmental stages. In fetal hepatocytes, both aldolase A and B mRNAs are synthesized, while in newborn and adult hepatocytes aldolase B is the only gene expressed (3).

It has been well documented that tissue-specific expression, in particular in liver, is primarily determined at the level of transcription initiation (4). The correct expression of such tissue-specific genes is governed by a combination of cis-acting elements, some of them being recognized by ubiquitous factors while others bind more or less tissue-specific factors (5). Transient transfection assays in cultured cells as well as *in vitro* transcription experiments have shown that relatively short 5′ flanking segments upstream from the cap site are often involved in hepatocyte-specific expression. Liver-specific genes are also very frequently controlled by distal regulatory elements (6, 7, 8, 9, 10, 11). Several liver-specific or liver-enriched DNA binding proteins have been identified as factors controlling liver-specific transcription : HNF-1 (Hepatocyte Nuclear Factor1 (12), also referred to as HNF-1α (13), APF (14) and LFB-1 (15); vHNF-1 (variant HNF-1) (16), also referred to as HNF-1β (13); HNF-3 α, β, and γ (17, 18); HNF-4 (17); HNF-5 (19) C/EBP (CAAT/enhancer binding protein) (20) and related proteins DBP (albumin D-element Binding Protein) (21) and LAP (Liver Activating Protein) (22). These factors are thought to act by interacting with the transcription initiation complex, regardless of the localization of their binding site, whether it is in the vicinity of the cap site or in a remote position. In addition, tissue specificity can be achieved by a combination of positive and negative effects.

We have previously shown that 200 bp upstream from the transcription start site of the aldolase B gene were sufficient to confer weak, but tissue-specific expression on a reporter gene: such a construct was active in transiently transfected hepatocytes in primary culture and in hepatoma Hep G2 cells, but not in fibroblasts (23). However, a 2 kbp internal fragment located in the first intron was required for the aldolase B constructs to reach a high level of expression in liver-derived cells (23). The proximal promoter fragment has been shown to bear several binding sites

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for liver-enriched and ubiquitous transcription factors. The identified binding factors are, from 3′ to 5′, tissue-restricted factors HNF-1 and HNF-3, binding to overlapping sites; ubiquitous factor NFY, and perhaps other CCAAT binding proteins; liver enriched factor C/EBP, and probably related factors (24, 25).

The goal of this paper was to clarify the role the different binding sites and cognate transcription factors play in the activity of the aldolase B gene proximal promoter. We have demonstrated that HNF-1 and either DBP in hepatoma cells or C/EBP in hepatocytes in primary culture, are major transactivators. In contrast, HNF-3 behaves as a strong inhibitor counteracting competitively the action of HNF-1 and non-competitively the action of DBP. Tissue-specificity of C/EBP and DBP action as well as competition between HNF-1 as a transactivator and HNF-3 as a transinhibitor seem to be unique features of the aldolase B gene promoter as compared to other liver-specific gene promoters described so far.

MATERIALS AND METHODS

Cell culture and transient transfections

Monolayers of human hepatoma (Hep G2) cells were maintained in Dubellcco's modified medium (DMEN) supplemented with 10% (vol/vol) fetal calf serum, 1 μM L-triiodothyronin, 1 μM dexamethasone, 10 μM insulin. Cells were plated at a density of 10^6 per 28 cm^2 dish. Hepatocytes were isolated from adult rats liver fasted for 72 h, they were then maintained in chemically defined medium 199, supplemented with 10% (vol/vol) fetal calf serum, 10 mM lactate, 20 mM glucose, 1 μM L-triiodothyronin, 1 μM dexamethasone, 10 μM insulin (23). Cells were plated at a density of 1.5×10^6 per 28 cm^2 dish.

Transfection was carried out by the calcium phosphate method (26). Each dish received a total of 10 μg of DNA including 1.5 μg of the pRSV luciferase standardization plasmid that was used to monitor variations in transfection efficiency. Calcium phosphate precipitates were removed 18 h after their addition, the cells were fed fresh medium, cultured further for 40 h (Hep G2) or 68 h (hepatocytes in primary culture) and harvested for chloramphenicol acetyltransferase (CAT) assay (27) and luciferase assay (28) as described.

Construction of vectors

The series of constructs containing the 5′ deletions of the aldolase B promoter was prepared using several different 5′ primers and a common 3′ primer in the polymerase chaine reaction (PCR) to generate various segments of the promoter which were subcloned into previously described PeCAT (23). Each 5′ primer contained an added Kpn1 site and the 3′ primer an added Sst1 site.

The constructs containing a 6 bp substitution (bp -132 to -126 or -119 to -113) into the full length aldolase B promoter were obtained by a second step of subcloning of the appropriate PCR fragment, at the Kpn1 sites of the -126 or -113 constructs.

All the constructs were checked by DNA sequencing.

RESULTS

Contribution of the different protein binding sites to the aldolase B promoter efficiency in hepatoma Hep G2 cells

We have previously mapped five protein binding elements along 110 bp of the aldolase B promoter. Some of the binding proteins were identified as C/EBP or related proteins: NFY; HNF-1, HNF-3 (Fig.1). Progressive deletion of these binding elements was performed and constructs containing the CAT gene directed by the deleted promoters were transiently transfected into hepatoma Hep G2 cells.

Deletion of the distal element (DE) between nt -190 and -170, binding a not yet characterized factor, led to a 5- to 6-fold increase in CAT activity in Hep G2 cells, suggesting an inhibitory effect for the factor binding to this site. In transfected fibroblasts,

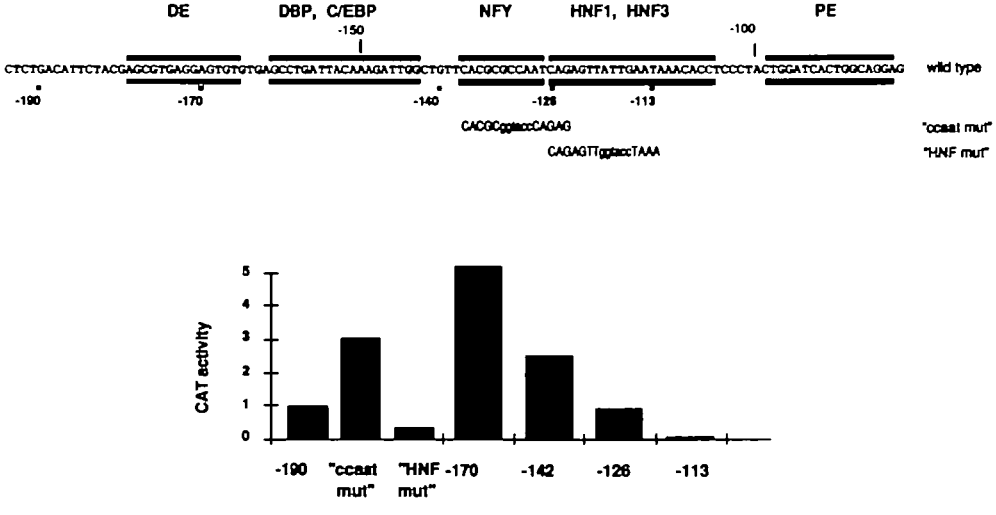

Figure 1. Activity of various mutants of the aldolase B promoter in Hep G2 cells. The different footprints and, when determined, cognate binding proteins are schematized in the upper part of the figure, together with the sequence of the promoter from nt -194 to -82. The linker mutations are shown under this sequence, changed nucleotides being represented in lower-case letters. The positions are indicated in bp with respect to the cap site. The lower part of the figure shows CAT activity of different ald B/CAT constructs: deletion mutants (shaded rectangles) or 'linker' mutants hatched rectangles). The 5′ extremity of the deletion mutants is indicated. Hep G2 cells were cotransfected with 7.5 μg of each ald B/CAT construct and 2.5 μg of plasmid RSV luciferase. The CAT activity was normalized with respect to the luciferase activity in the same cell lysates. The results presented are the mean of at least 3 independent measurements for each construct.

however, CAT activity generated by this construct remained very low, indicating that this negative DE element is probably not directly involved in tissue-specificity of the promoter (not shown). The next deleted sequence bears the binding site for C/EBP and related proteins. The resulting −140 bp promoter was 2-fold less active than the −170 bp promoter. Deletion of the NFY binding site led to a further 2-to 3-fold reduction of the aldolase B promoter activity, while deletion of the region up to nt −113, removing the binding sites for factors HNF-1 and HNF-3, resulted in an essentially inactive promoter. In order to assess the importance of these NFY and HNF-1/HNF-3 sites by themselves in the aldolase B promoter activity, we performed a six bp substitution in the 'CCAAT box' (nt −132 to −127) as well as in the HNF site (nt −119 to −114) of the −190 bp promoter fragment. The resulting 'CCAAT mut' construct was 2-fold more active than the intact construct while the 'HNF mut' construct was 4 fold less active.

These results indicate that the −190/−103 bp fragment of the aldolase B promoter analyzed in cultured hepatoma cells consists of two positive elements, i.e. binding sites for C/EBP and related proteins and for HNF-1 and HNF-3, one negative distal element (DE) and a NFY binding site that, in the context of the intact −190 bp promoter, could also play a negative role.

Transactivity of different DNA binding factors on the expresssion of the aldolase B gene

The results of transient expression in Hep G2 cells of different aldolase B construct deletion mutants can be biased with respect to the *in vivo* situation because some transcription factors, especially tissue-specific factors, can be less abundant in cultured cells, in particular in hepatoma cell lines, than in liver (20). An alternative approach to test the importance of specific transcription factors for the activity of a promoter is to evaluate the ability of these factors to transactivate the promoter in cotransfection experiments. Therefore, we cotransfected Hep G2 cells with the −190 aldolase B construct and various amounts of either C/EBP, DBP, HNF-1, vHNF-1 or HNF-3α expression vectors.

As shown on Fig. 2 and 3, over-expression of DBP stimulated transcriptional activity of the aldolase B promoter about 30 fold, while over-expression of C/EBP had a weak influence only. The very low transactivation observed using the C/EBP expression vector is striking, since bacterially produced purified C/EBP protein was previously shown to bind to the aldolase B promoter from nt −162 to nt −140 (24). This inefficiency of the C/EBP expression vector was specific to the aldolase B promoter in the context of the Hep G2 cell line since, under the same conditions, the albumin promoter activity was strongly enhanced in these cells (not shown). In addition, we found that in hepatocytes in primary culture, the respective effects of C/EBP and DBP on transactivation of the aldolase B promoter were the opposite of those in Hep G2 cells: C/EBP transactivated the construct while DBP was inefficient (Fig. 4).

The maximal transactivation was obtained with the HNF-1 expression vector (a 80 fold stimulation at the highest plasmid concentration used) while vHNF-1 expresion was much less efficient (5-fold stimulation at the most).

HNF-3 over-expression did not modify the basal activity of the 190 bp aldolase B promoter, even at the highest expression vector concentration used (Fig. 2 and 3). HNF-1 and DBP are, therefore, major transactivators of the aldolase B promoter in Hep G2 cells. It is noticeable that DBP activation seems to reach a plateau at high concentrations of expression vector, while HNF-1 activation continues to increase. In contrast, vHNF-1 and C/EBP are only weak activators, while HNF-3 was essentially inactive on the −190 aldolase B promoter basal activity, even at high expression vector concentrations. These results were unexpected since both C/EBP and HNF-3 have been reported to be transactivators of liver-specific promoters (20, 18, 29, 30) and both are able to bind to the aldolase B promoter, as established by footprinting and band shift experiments (24). This prompted us to examine the effect of these factors in combination on aldolase B promoter activity.

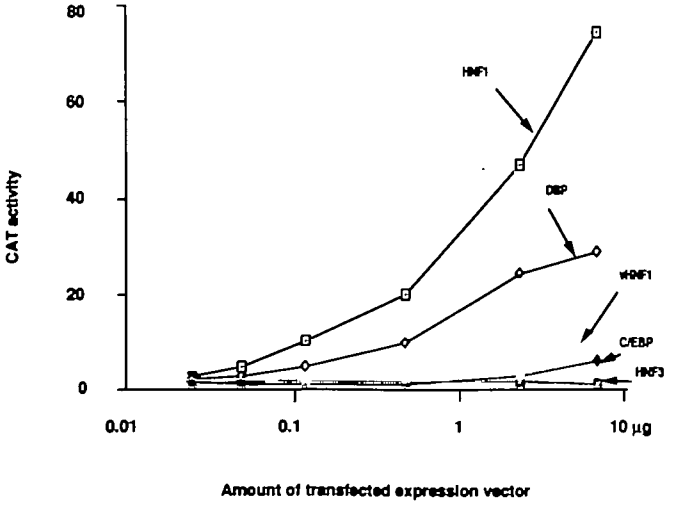

Figure 2. Transactivity of various transcription factors on the −190 ald B/CAT construct. Hep G2 cells were cotransfected with 7.5 µg of the −190 ald B/CAT construct and with variable concentrations of expression vectors ····◆···· MSV-C/EBP, —◇— CMV-DBP, —▣— RSV-HNF1, ····■···· RSV-vHNF1, ····□···· RSV-HNF3. The CAT activity was normalized with respect to the luciferase activity in the same cell lysate. The results presented are the mean of at least 3 independent measurements for each point.

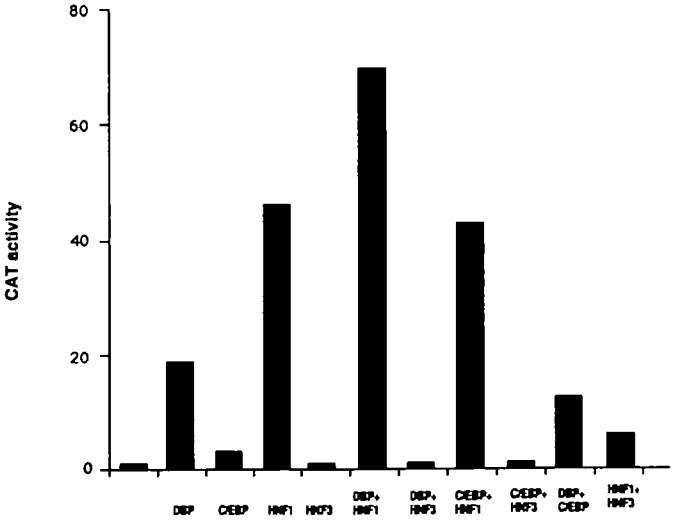

Figure 3. Combinatorial effect of various transacting factors on the activity of the −190 ald B/CAT construct. Hep G2 cells were cotransfected with 7.5 µg of the −190 ald B/CAT construct and 2.5 µg of each tested expression vector.

Additive transactivation by DBP and HNF-1 and antagonist effect of HNF-3

Figure 3 shows that transactivation of the −190 bp aldolase B promoter by DBP and HNF-1 together was additive. In contrast, the C/EBP expression vector partially antagonized transactivation by DBP while it was without any effect on HNF-1-dependent transactivation. This result suggests that, in spite of its very low transactivation potential in Hep G2 cells, C/EBP is able to compete with DBP for DNA binding.

The more dramatic effect was that of HNF-3 whose overexpression totally suppressed DBP-dependent transactivation and strongly reduced HNF-1-dependent transactivation.

In order to better define the type of interaction occurring between the different transcription factors binding to the aldolase B promoter, we next performed experiments using increasing amounts of one of the expression vectors with a constant amount of the other.

Figure 5 confirms the additivity of the transactivations produced by DBP and HNF-1 at different HNF-1 concentrations.

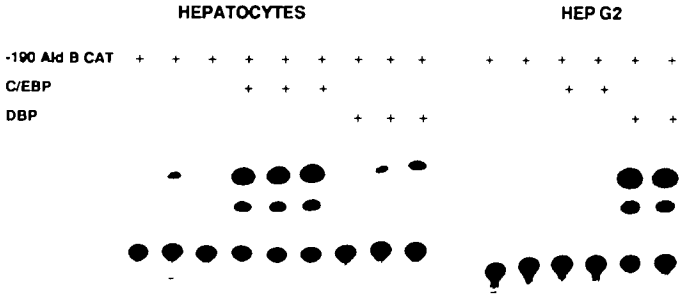

Figure 4. Transactivation of the −190 ald B/CAT construct by C/EBP and DBP expression vectors in hepatoma Hep G2 cells and hepatocyes in primary culture. Hep G2 cells or hepatocytes in primary culture were cotransfected with 7.5 μg of the −190 ald B/CAT construct and 2.5 μg of either MSV-C/EBP plasmid or CMV-DBP plasmid. The experiment was performed in triplicate, each individual CAT assay being shown on the figure.

HNF-3 suppressed DBP-activation, even at the lowest concentration of HNF-3-expression vector used. HNF-3 also suppressed the HNF-1 transactivation, but HNF-3-dependent inhibition could be partly abolished by high amounts of HNF-1 expression vector. These results are fully consistent with HNF-1 and HNF-3 binding to overlapping, mutually exclusive sites, therefore competing for binding to the aldolase B promoter, while the DBP binding site is distinct. It seems, then, that binding of HNF-3 instead of HNF-1 to the aldolase B promoter impairs transactivation by DBP. This blockade could be overcome by displacing HNF-3 with excess HNF-1.

Influence of the negative elements of the aldolase B promoter on the response to transacting factors

We next investigated the role of negative elements, that is to say the DE element and the NFY binding site, in the control of the aldolase B promoter by the different transacting fators studied here.

Transient cotransfection experiments similar to those previously described were performed using the −170 aldolase B, DE deleted construct, and the results were compared to those obtained with the −190 bp aldolase B promoter Fig. 6. Removal of the DE negative element did not impair DBP and HNF-1 transactivation, but reduced the level of activation: DBP-dependent and HNF-1-dependent transactivation were stimulated 28- and 36-fold respectively for the −190 bp construct but only 7- and 17-fold for the −170 construct. In contrast, C/EBP, which was a weak transactivator on the −190 bp aldolase B construct (a 3-fold maximal stimulation), was slightly more efficient on the DE-deleted construct whose CAT activity was stimulated 5 fold in the presence of the C/EBP expression vector. Finally, HNF-3 suppression of transactivation by DBP and HNF-1 persisted with the −170 bp construct.

We also performed these transactivation experiments with the 'CCAAT mut' construct whose CCAAT binding site for NFY had been eliminated (Fig. 6). This mutant responded very well to DBP and HNF-1, poorly to C/EBP and remained sensitive to HNF-3 antagonism to DBP and HNF-1-dependent activation.

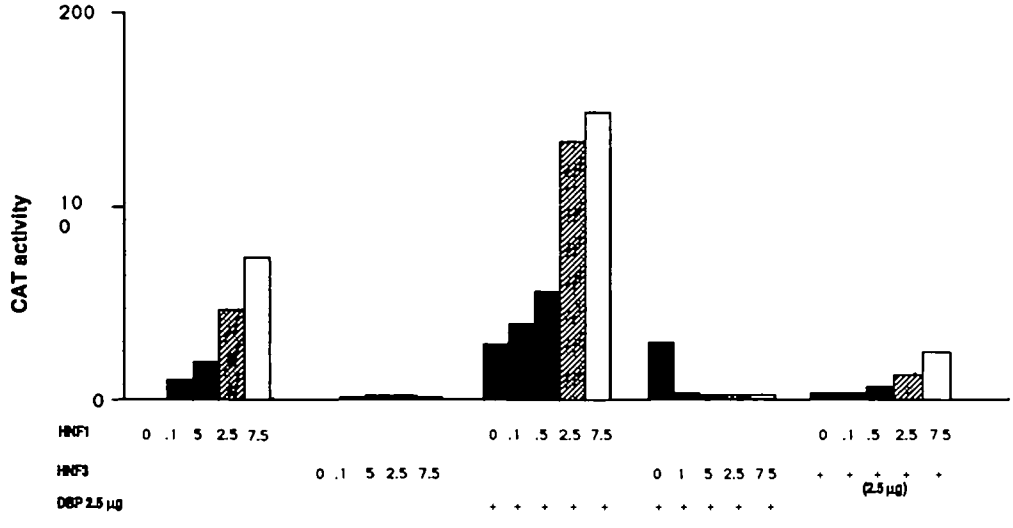

Figure 5. Quantitative analysis of the combinatorial effect of HNF1/DBP, DBP/HNF3 and HNF1/HNF3 on the activity of the −190 ald B/CAT construct. Hep G2 cells were cotransfected with 7.5 μg of the −190 ald B/CAT construct and the indicated amount of HNF1, HNF3 and (or) DBP expression vectors.

DISCUSSION

The aldolase B gene is exclusively expressed in hepatocytes, proximal tubular cells of the kidney and enterocytes and under dietary and hormonal transcriptional regulation: it is stimulated by glucose and insulin and inhibited by cyclic AMP (31). At least two regulatory regions are involved in the expression of this gene, in both cultured cells (23) and in transgenic mice (unpublished results): an activating region located inside the 1st intron and a proximal tissue-specific promoter studied in detail in this paper (23). This proximal promoter consists of at least five distinct elements, between nucleotides −190 and −103 with respect to the transcription start site whose interplay and interactions with transcription factors are unique. This makes the aldolase B gene a powerful and exciting model for investigating the possible role of various DNA binding proteins in the control of the activity of tissue-specific promoters.

A 'D-like' element can confer positive responsiveness to either DBP in hepatoma cells or C/EBP in hepatocytes and functionally interferes with a contiguous distal element

Element D of the albumin gene binds members of a family of liver-enriched proteins termed bZIP factors, including C/EBP, DBP and LAP (22, 20, 21). C/EBP and LAP are part of a subfamily that forms homo-and heterodimers (32); in contrast, DBP does not form heterodimers (33). All three factors are transactivators of the albumin gene. We have previously determined that a DNA element of the aldolase B promoter, from nt −162 to −140, was protected against DNAse 1 digestion in the presence of both liver nuclear extracts and recombinant C/EBP protein. This protection could be abolished by excess of an oligonucleotide reproducing the albumin D-element (24). Here, we demonstrate that DBP is a potent transactivator of the aldolase B promoter in hepatoma Hep G2 cells but is inefficient in hepatocytes in primary culture, while C/EBP is very active in these latter cells, but not in the former. In contrast, both transactivators are active on the albumin promoter in Hep G2 cells (20, 34 and our personal results). Although a poor activator of the aldolase B promoter in Hep G2 cells, C/EBP is able to bind to its cognate element in these cells since it partly antagonized DBP-dependent transactivation, while this antagonism cannot occur through DBP-C/EBP heterodimerization. In addition, transactivation by C/EBP increases when an immediate upstream negative element, termed DE, is removed. This is highly significant since, in the absence of any transactivators, the basal promoter activity is already stimulated 6 fold by DE deletion. In constrast, DE deletion slightly reduced DBP response. These results suggest that tissue-specificity of the DBP and C/EBP action is not due to tissue-dependent, exclusive binding of these factors to their target, but rather to different activating properties of bound factors, depending on the cell context. This influence of the cell context on transactivation ability of C/EBP and DBP might, in principle, be ascribed to: (i) tissue-specific, post-translational modifications; (ii) tissue-specific heterodimerization of the transcription factors; (iii) elective interaction of C/EBP and DPB with tissue-specific factors bound to neighbouring sites. The first two hypotheses cannot be excluded but are not sufficient to account for the observed C/EBP and DBP specificity of action, since this latter is specific to the aldolase B promoter. In addition, heterodimerization partners, well described for C/EBP (35), are not yet known for DBP. The third hypothesis is especially appealing since it is in line with the demonstrated cross-talk between the negative DE element and the DBP/C/EBP binding site. One of the effects of DBP could be to overcome DE-mediated promoter repression, C/EBP being inactive. Therefore, in the absence of DE, transactivation by C/EBP becomes relatively similar to that by DBP (5 and 7 fold stimulation, respectively). Although the nature of the DE-binding protein(s) is unknown to date, preliminary investigations have indicated that footprints at the DE site could be different depending on the tissue, which might explain differential interactions with factors bound to the contiguous C/EBP/DBP binding site (our unpublished results). Milos and Zaret have recently emphasized the importance of a precise positioning of C/EBP next to another factor, NFY in their case (36). A NFY site is also contiguous to the C/EBP/DBP binding site in the aldolase B promoter, but since this factor is ubiquitous, it is unlikely to explain tissue-specificity of the C/EBP and DBP action. In addition, selective mutation of the aldolase B NFY site not only does not reduce promoter activity (in fact, the 190 bp promoter mutated on the NFY site is more active than the wild promoter), but also does not impair transactivation by recombinant DBP and does not significantly increase responsiveness to C/EBP. Whatever the mechanism, the specific activity in different tissues of transcription factors binding to the same sites throws light on one of the possible roles of this apparent redundancy: it could provide different functional arrangements of factors in cells whose transacting factor distribution is very different from each other, and thus permit a promoter to be active in different cell contexts. Other exemples of tissue-dependence of the relative activity of two transcription factors binding to the same site have been reported, for instance for vHNF-1 and HNF-1 (37, 38). In no case, however, were the differences in the efficiency of these factors in different tissues as pronounced as reported here for DBP and C/EBP.

HNF-3 competitively antagonizes the activation by HNF-1 and noncompetitively the activation by DBP

HNF-1 is the first transcription factor involved in the activity of liver-specific promoters to have been well characterized (14, 39). It plays a major role in tissue-specific expression of numerous

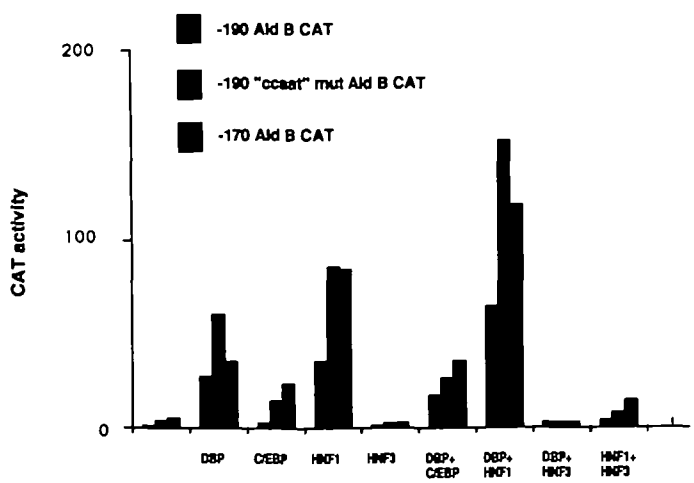

Figure 6. Effect of various transcription factors, alone and combined, on the activity of aldolase B promoter mutants with enhanced activity. The −170 ald B/CAT construct is lacking element DE while the 'CCAAT mut' ald B/CAT construct contains a KpnI restriction site in place nucleotides −132 to −126, inside the NFY binding site (see Fig. 1). The experimental conditions are the same as in Fig. 3.

genes active in the liver and small intestine (14, 17, 12, 40, 38, 41, 42, 43). HNF-1 is a homeo-POU protein (44) synthesized in tissues of endodermal origin (liver, small intestine, pancreas, yolk sack), but also in kidney (45). In this organ and in fetal endodermal tissues, HNF-1 is present together with vHNF-1, a related protein with a highly conserved homeo-POU domain (13). In some dedifferentiated hepatoma cells, vHNF-1 seems to be present in the absence of HNF-1 (46). However, vHNF-1 seems also to be a transcription activator of HNF-1-dependent promoters (46), although to a lesser extent.

HNF-3 was first described as a transcription factor of the α1 antitrypsin and transthyretin gene promoters (47), and then shown to be involved in the expresssion of other tissue-specific genes. There are, in fact, at least three HNF-3 genes (α, β and γ) encoding related proteins with similar binding specificities and transcription activities, at least with the experimental systems used to test them (18). Various combination of HNF-3 isoforms are synthesized approximately in the same tissues as HNF-1, with the important exception of kidney, which is absolutely devoid of HNF-3 binding activity (48). HNF-3 belongs to a new class of binding factors, different from HNF-1 and vHNF-1 and that also includes the *Drosophila* developement gene *forkhead* (18).

Our previous investigations have demonstrated that both HNF-1 and HNF-3 could bind to the aldolase B promoter, between nt −126 and −104, on overlapping sites. The DNAse1 footprint obtained on this region with liver nuclear extracts could be displaced by a HNF-3 oligonucleotide in excess, but not by a HNF-1 oligonucleotide (24). The HNF-1/HNF-3 binding site is contiguous to a NFY binding site centered on a GCCAAT motif (nt −132 to −127).

In the present study we demonstrate that mutation of this CCAAT motif results in an activated promoter while its deletion up to position −126 slightly reduces its activity. Both constructs fully respond to HNF-1-dependent transactivation. We can hypothesize that a partial steric hindrance exists between binding of NFY and of a major transactivator on the contiguous, downstream site. This hindrance might be abolished by mutation of the CCAAT box, perhaps allowing better cooperation between this proximal transactivator site and the C/EBP/DBP binding site discussed above. A previously published result supports this interpretation: displacement of HNF3 by excess of a specific oligonucleotide in *in vitro* DNAse1 footprinting experiments modifies occupancy of the C/EBP/DBP binding site (24). Such effect could obviously not be observed with the −126 aldolase B construct.

The next question concerns the respective roles of HNF-1 and HNF-3 in controlling aldolase B gene transcription. Our results are unambiguous: HNF-1 is a very strong transactivator but HNF-3 is not; since both compete for binding to overlapping sites, HNF-3 counteracts HNF-1-dependent transactivation. HNF-3 also blocks transactivation by DBP, which signifies that this transactivation can only be exerted on a HNF-1-charged promoter, and not on a HNF-3-charged promoter. As expected, since the binding sites for DBP and HNF-3 are different, HNF-3 is a non-competitive inhibitor of DBP-dependent transactivation.

Since *in vitro*, in the presence of liver nuclear extract, HNF-3 seems to be more tightly bound to the promoter than HNF-1, the same might exist in transfected Hep G2 cells containing high amounts of both HNF-1 and HNF-3 (47 and our unpublished results). This could explain why the proximal aldolase B promoter by itself is strikingly weak in spite of bearing sites for efficient transactivators (C/EBP/DBP, NFY, HNF-1), as does the albumin promoter (49): it would be on negative constraint due to binding of HNF-3 instead of HNF-1. Cotransfection with a HNF-1 expression vector results in the synthesis of very important amounts of HNF-1 that competitively displace HNF-3, then HNF1 stimulates activity of the promoter by its own transactivating effect and by allowing DBP-dependent transactivation. *In vivo*, and in cultured cells in the presence of the intronic activator, activity of the aldolase B promoter is strong. This suggests that under these conditions supplementary interplay between the regulatory regions facilitates access of HNF-1 to its binding site.

The proximal tubular cells of the kidney strongly express the aldolase B gene (50 and Levrat et al. personal communication). Aldolase B mRNA is even more abundant in total kidney than in liver (31). It can be hypothesized that the absence of HNF-3 in kidney contributes to this high level of expression. However, vHNF-1 is abundant in this organ and should be able to compete with HNF-1 for binding to their common site. Since vHNF-1 is a considerably weaker transactivator than HNF-1, this could result in limitation of expression of the aldolase B gene in the absence of HNF-3.

Our data demonstrate for the first time that HNF-3 can, in some cases, behave as a repressor of the expression of a liver-specific gene. However, similar examples where the same factor is, depending on cell and promoter contexts, an activator or an inhibitor have been well documented (51).

In conclusion, our analysis of the proximal aldolase B promoter has further clarified the subtle interactions between factors controlling liver-specific genes and thrown light on some new functions of these factors.

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