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Regulation and function of the tissue-specific transcription factor HNF1α (LFB1) during *Xenopus* development

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ABSTRACT We review the data available on the structure, developmental appearance and embryonic regulation of the tissue-specific transcription factor HNF1α (LFB1) in *Xenopus*. The expression of the HNF1α gene starts early in embryogenesis shortly after mid-blastula transition and the protein accumulates in the region of the embryo where liver, pronephros and gut – tissues that contain HNF1α in the adult – are developing. The cofactor DCoH, known to stabilize dimer formation of HNF1α, is present as a maternal factor in the egg and has a partially distinct tissue distribution compared to HNF1α. This implies that DCoH does not only modulate HNF1α dimerization but may also cooperate with other transcription factors. By injecting HNF1α promoter CAT constructs into fertilized *Xenopus* eggs we obtained activation of the injected gene restricted to the region of the developing larvae expressing endogenous HNF1α. Deletion analysis allowed to define the OZ-element that is essential for embryonic activation. This element also occurs in other promoters activated at mid-blastula transition in the embryo and interacts with the maternal factor OZ-1. As the HNF1α promoter also contains functional binding sites for HNF4 and HNF1, we postulate that all these transcription factors contribute to the cascade leading to proper embryonic activation of the HNF1α gene.

KEY WORDS: *maternal transcription factor, DCoH, HNF4*

Introduction

A key question in embryology is how various cell types are generated during development of a multicellular organism from a single cell type, the egg. This basic process involves many different regulatory mechanisms on various cellular and molecular levels. One critical step in embryogenesis is the establishment of distinct regions containing tissue-specific transcription factors that initiate cell type specific gene transcription. To address this problem, we decided a few years ago to investigate the embryonic expression and regulation of a tissue-specific transcription factor that was considered to regulate a well defined set of genes. We have chosen the transcription factor HNF1α, also called HNF1 or LFB1 (Mendel and Crabtree, 1991), that was initially cloned from rat liver (Frain *et al.*, 1989) and that interacts with the promoters of a series of genes specifically expressed in the liver (Tronche and Yaniv, 1992). Later it turned out that this transcription factor is also present in other tissues, such as kidney and the intestinal tract (see below). However, in these tissues the potential HNF1α dependent genes are largely unknown (Tronche and Yaniv, 1992). As we expected that HNF1α might be activated very early in embryogenesis we have chosen the frog *Xenopus laevis* as an experimental system because early development can be analyzed much more easily in amphibians compared to mammals. We anticipated that the basic regulatory mechanisms would be conserved in vertebrates and therefore the results obtained with *Xenopus* embryos might be directly instructive for the events occurring in mammalian development.

Structure of the HNF1α gene and its protein

To allow a precise analysis of HNF1α in *Xenopus* embryogenesis, we started our project by cloning the cDNA and the genomic sequences of HNF1α from *Xenopus*. As the genome of *Xenopus laevis* is pseudo-tetraploid (Kobel and Du Pasquier, 1986), we were not surprised to find two closely related genes, i.e. XHNF1αa and XHNF1αb, (Bartkowski *et al.*, 1993). We do not believe that one of these genes encodes the homolog of the mammalian HNF1ß, another member of the HNF1α family which has the same DNA binding specificity, as this protein is considerably more divergent (Mendel and Crabtree, 1991; Tronche and Yaniv, 1992). In fact, the *Xenopus* HNF1ß has been recently cloned and shows extensive sequence homology to the mammalian homolog (Demartis *et al.*, 1994). Analyzing the exon/intron structure of the XHNF1α gene (Zapp *et al.*, 1993b) we observed that the protein coding sequence is encoded in 9 exons (Fig. 1). The first exon contains the amino terminal dimerization domain, exons 2, 3 and 4 encode the DNA binding

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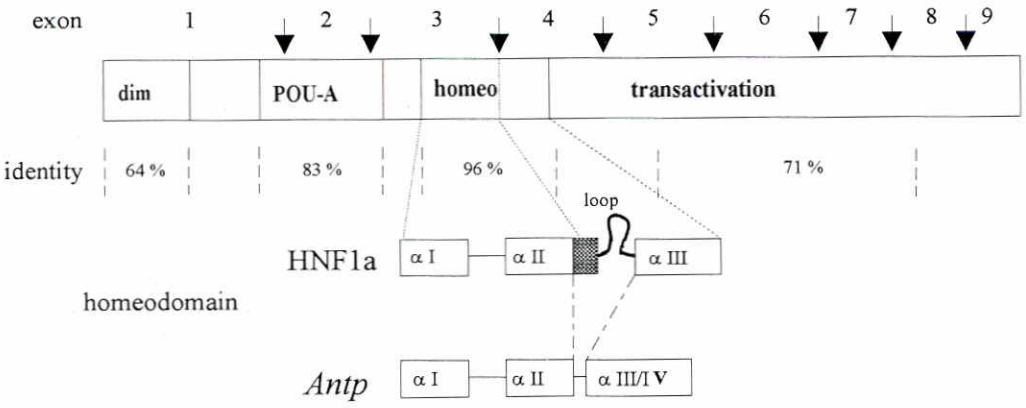


Fig. 1. Structure of the HNF1α gene with its extended homeodomain. *The protein coding region of HNF1α is drawn with the various protein domains. The position of the introns is given by arrows and the exons are numbered (Zapp et al., 1993b). The amino acid identity between the Xenopus XHNF1αa protein and the rat protein in specific domains is given. Slightly different values are obtained using XHNF1αb in the comparison (Bartkowski et al., 1993). The composition of the homeodomain (homeo) of HNF1α is compared to the classical structure in the Antennapedia (Antp) protein as determined by nuclear magnetic resonance (Leiting et al., 1993) and by high resolution X-ray (Ceska et al., 1993). The helixes αI, αII and αIII between the two homeodomains are aligned. The 21 amino acid loop specific for the HNF1α homeodomain is located partially in helix αII (shaded area). Comparing the exon/intron structure of the Xenopus HNF1α with the structure found in the rat genome (Bach et al., 1992), it is clear that exon 5 of Xenopus is encoded in 2 exons in the rat (exon 5 and 6) whereas the Xenopus exons 8 and 9 correspond to the exon 9 of the rat. Furthermore it is notable that the chicken HNF1α gene contains ten exons by combining the introns specific for rat and Xenopus (Hörlein et al., 1993).*

domain, whereas all the other exons constitute the carboxy terminal transactivation domain. The DNA binding domain of HNF1α contains a POU-A related sequence and a so called extended homeodomain as typical features. The extended homeobox is characterized by a 21 amino acid loop inserted between helix II and helix III that is only found in the related protein HNF1ß but in none of the classical homeodomains (Frain *et al.*, 1989). Structural analysis of the rat protein has revealed that the 21 amino acid loop extrudes from the protein region contacting the DNA (Ceska *et al.*, 1993; Leiting *et al.*, 1993). In this context it is interesting that this 21 amino acid loop resides in exon 4 exactly at the border to exon 3. Therefore, it is most likely that this extra sequence has been introduced in a primordial gene by changing the position of the splicing site. As none of the homeodomains in other genes contains an intron at this position (see Bach *et al.*, 1992), it is clear that HNF1α constitutes a distinct member of the homeodomain proteins.

Comparing the primary structure of HNF1α between the *Xenopus* and rat protein (Fig. 1) the extended homeodomains and the POU-A related domain are the most conserved regions with 96% and 83% identity, respectively. Additional highly conserved regions are the activation domain (71% identity) as well as the dimerization domain (64% identity). Taken together the strong conservation of distinct regions of the HNF1α gene during vertebrate evolution reflects the strong requirement to maintain functional important structures. Surprisingly the genomic structure of HNF1α in *Xenopus* (Zapp *et al.*, 1993b), the chicken (Hörlein *et al.*, 1993) and the rat (Bach *et al.*, 1992) reveals in all three species an extraordinarily large first intron, exceeding 10 kb. Although the conservation of this feature indicates some functional relevance, we have no obvious explanation so far.

HP1, the promoter element interacting with HNF1α

We have previously identified in the *Xenopus* 68 kd albumin promoter the regulatory element HP1 that mediates liver specific activity in transfection experiments as well as in cell free transcription systems (Schorpp *et al.*, 1988). This HP1 element turned out to be a bona fide HNF1α binding site (Kugler *et al.*, 1988; Frain *et al.*, 1989). The fact that this regulatory element is the exclusive element common to the albumin promoters of *Xenopus* and mammals (Schorpp *et al.*, 1988) supports the important role of HNF1α in gene control. Binding sites for HNF1α have also been identified in many other promoters of genes expressed specifically in the liver (for review see Tronche and Yaniv, 1992). The HNF1α binding site of 13 bp has a palindromic structure (Fig. 2), although a perfect palindrome has never been observed so far in any promoter (Tronche and Yaniv, 1992). The site is recognized by a dimer of HNF1α (Mendel and Crabtree, 1991) as illustrated in Figure 2. In the case of *Xenopus* HNF1α we could show that recombinant XHNF1αa and XHNF1αb form heterodimers: in a gel retardation assay (shown in Fig. 3) XHNF1αbΔ, representing a truncated version of the normal protein, generates a complex with labeled HP1 (lane 1) moving faster than the full length XHNF1αa protein (lane 5), whereas an intermediary moving complex is seen in addition if XHNF1αbΔ and XHNF1αa are mixed in the binding assay. This documents heterodimerization between XHNF1αa and

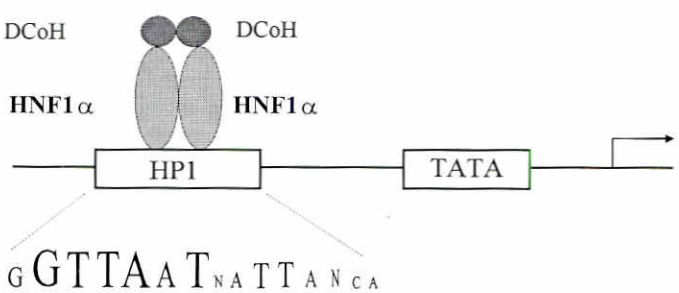


Fig. 2. The interaction of HNF1α with the HPI element. *The tetramer consisting of HNF1α and DCoH dimer is shown as bound to the regulatory element HP 1 upstream of the TATA-box and the transcription start site (arrow). The consensus shown for HPI is taken from Tronche and Yaniv (1992). The size of the letter reflects the importance of each residue.*

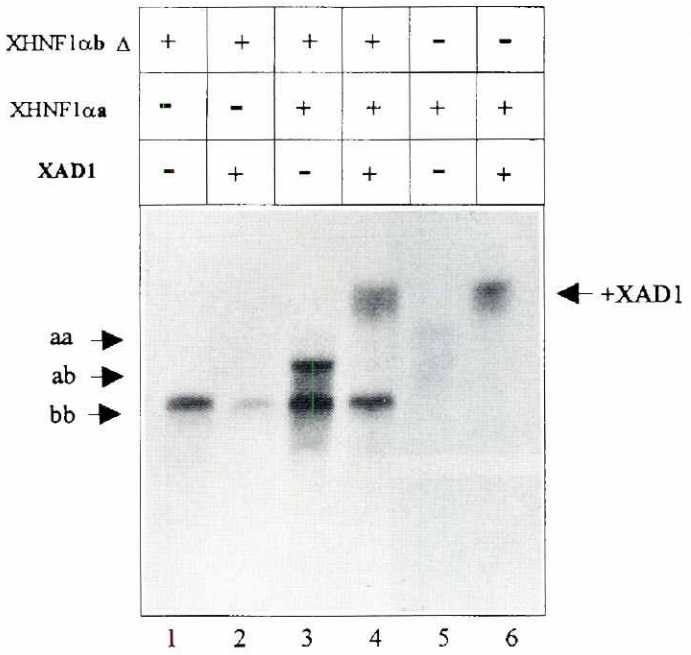


Fig. 3. XHNF1αa and XHNF1αb form heterodimers. *A gel retardation assay using the labeled HPI oligonucleotide was performed with the in vitro translation products XHNF1αb and XHNF1αa made in reticulocyte lysates with mRNA encoding a truncated version of XHNF1αb (aa 1-410) and the full-length XHNF1αa, respectively (Bartkowski et al., 1993). The addition of the monoclonal antibody XAD1 is indicated. The complexes containing aa and bb homodimers as well as the ab heterodimer are marked. The free HP1 oligonucleotide has left the bottom of the gel.*

XHNF1αb. Further support is given by the upshifts obtained by adding the monoclonal antibody XAD1 that recognizes the a form and thus exclusively reacts with the heterodimer ab (compare lanes 3 and 4) and the homodimer aa (compare lanes 3 and 4 as well as 5 and 6). We assume that such heterodimers do occur also *in vivo* but we doubt that they have significant different properties as the a and b protein are very similar and most likely the result of the pseudo-tetraploidy of *Xenopus laevis* (Kobel and Du Pasquier, 1986). In mammals heterodimerization between HNF1α and HNF1ß has previously been well documented (Tronche and Yaniv, 1992) and in this situation the heterodimers might have distinct properties, as HNF1α and HNF1ß have quite different activation domains (Mendel and Crabtree, 1991; Tronche and Yaniv, 1992). In *Xenopus* an HNF1ß homolog has recently been identified (LFB3 in Demartis *et al.*, 1994), however formation of heterodimers of XHNF1ß with XHNF1αa and XHNF1αb has not been analyzed yet.

DCoH, a cofactor of HNF1α

In mammals a cofactor, DCoH, that is involved in dimerization of HNF1α as well as of HNF1ß has been identified (Mendel *et al.*, 1991b). As this cofactor enhances the transactivation potential of HNF1α in transfection experiments considerably we searched for the corresponding factor in *Xenopus*. Using the rat cDNA as a probe we cloned the *Xenopus* homolog of the mammalian DCoH (Pogge v. Strandmann and Ryffel, 1995). DCoH of *Xenopus* is identical in size to DCoH in mammals and the 104 amino acid protein reveals a 85% identity between *Xenopus* and rat. Furthermore we have observed in transfection experiments that *Xenopus* and rat DCoH are equally efficient in stimulating the transactivation potential of HNF1α either derived from *Xenopus* or rat. This increased transactivation reflects the interaction of HNF1α with DCoH as seen in a gel retardation assay using the HNF1α binding site HP1 as radioactive probe (Fig. 4). Clearly DCoH cannot bind to HP1 on its own (lane 2) whereas a recombinant *Xenopus* HNF1α generates a complex with HP1 (lane 3) that can be supershifted by the monoclonal antibody XAD1 (lane 4). Incubating HNF1α and XDCoH simultaneously with HP1 leads to a more prominent complex (lane 6) that migrates slightly slower due to the binding of DCoH. The presence of HNF1α and DCoH in the complex with HP1 is proven by the supershift mediated by the monoclonal antibody XAD1 against HNF1α (lanes 7 and 13) and the DCoH specific polyclonal antiserum (lane 14). Our experiments and the previous data obtained in mammals (Mendel *et al.*, 1991b) suggest that HNF1α and DCoH form a tetrameric structure with each component in duplicate as illustrated in Figure 2. This tetramer also exists *in vivo*, as HNF1α and DCoH specific antibodies react with the complex formed by incubating *Xenopus* liver extract with HP1 (Pogge v. Strandmann and Ryffel, 1995).

Tissue distribution of HNF1α and DCoH

To determine the tissue distribution of HNF1α and DCoH we used specific antibodies raised against the recombinant proteins. The immunohistochemistry shown in Figure 5 documents the nuclear localization of HNF1α in the liver and intestine of adult *Xenopus*. In the liver the hepatocytes as well as the biliary duct cells are positive for HNF1α whereas the blood cells lack HNF1α (compare panel a and c). In the intestine only the nuclei

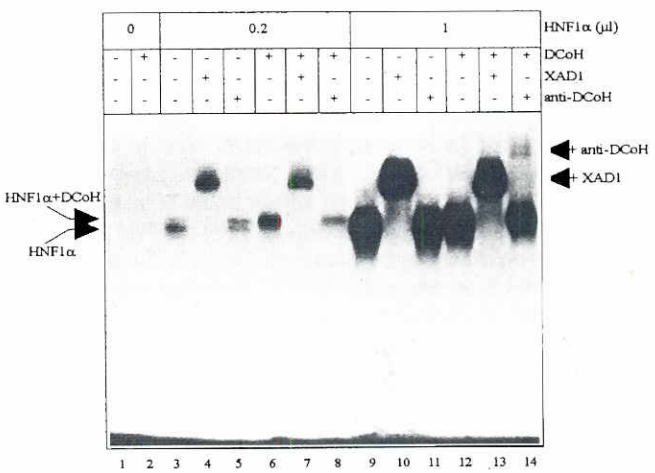


Fig. 4. Interaction of XDCoH and HNF1α. *Using the labeled HPI oligonucleotide, gel retardation assays were made with 0.2 or 1 µl HNF1α (XHNF1αa) and 5 µl Xenopus DCoH translated in a reticulocyte lysate. The addition of the monoclonal antibody XADI specific for HNF1α (Bartkowski et al., 1993) and the polyclonal rabbit antiserum against DCoH (Pogge v. Strandmann and Ryffel, 1995) is given. Complexes containing HNF1α and DCoH and the corresponding complexes with the antibody XAD1 (+XAD1) or the DCoH antiserum (+anti-DCoH) are marked with arrows.*

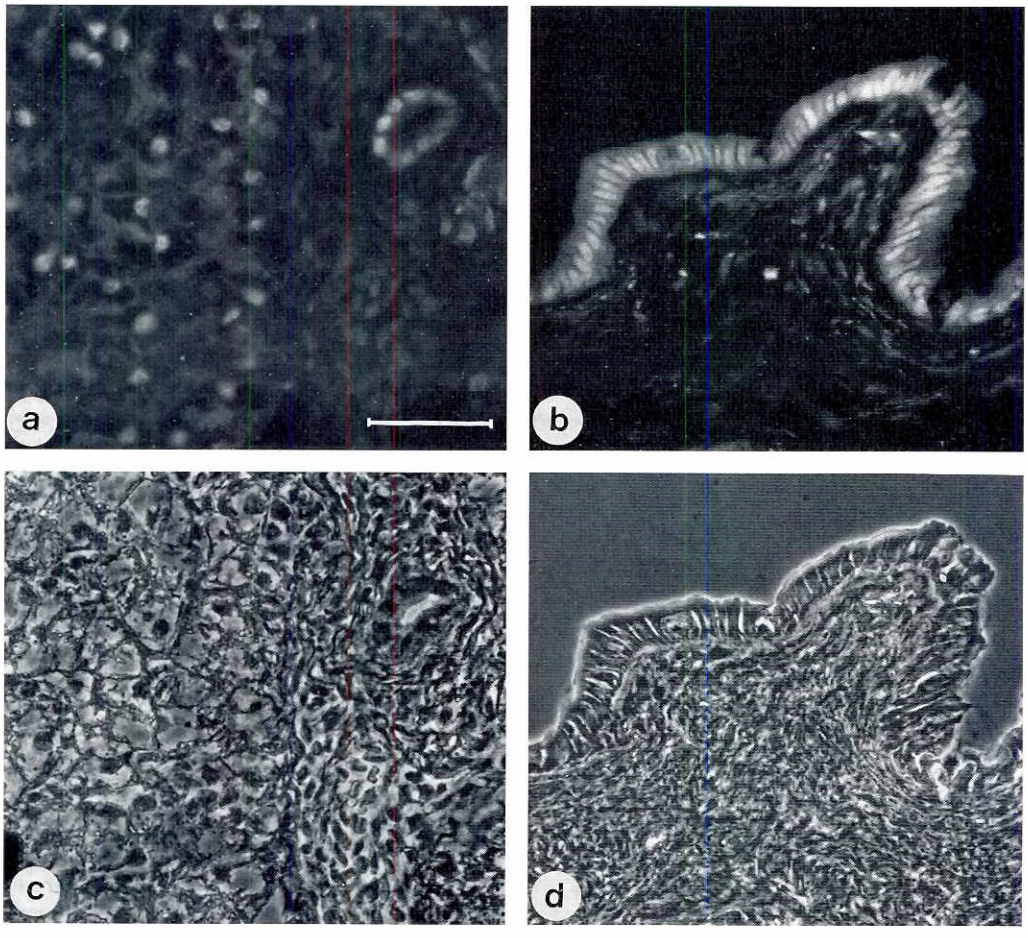


Fig. 5. Immunostaining of liver and gut sections of adult *Xenopus* to locate HNF1α. *Cryostat sections of the liver* **(a)** *and the gut* **(b)** *were immunostained with the HNF1α specific monoclonal antibody XAD5 (Bartkowski et al., 1993) as first antibody and the Cy3-conjugated rat anti-mouse IgG as second antibody. The corresponding phase contrast pictures are in panel c and d. The magnification in all panels is the same; bar, 100 μm.*

of the epithelial cells contain HNF1α (compare panels b and d). Using immunohistochemistry, Western blots and gel retardation assays we compared the tissue distribution of HNF1α and DCoH in adult *Xenopus*. Table I shows that in liver and kidney both HNF1α and DCoH are strongly present whereas in the intestine and stomach HNF1α is as abundant as in the liver but DCoH is absent or detectable only in trace amounts. Low amounts of DCoH are also found in the lung where no HNF1α can be identified. Other tissues investigated lack both HNF1α and DCoH. Immunostaining of *Xenopus* larvae reveals HNF1α and DCoH in the pronephros, liver and gut (Table 1), but most surprisingly the eyes of the larvae contain high levels of DCoH that is located in the nuclei of the pigmented epithelium but they lack HNF1α (Pogge v. Strandmann and Ryffel, 1995).

These results show that three different types of tissues can be identified: some contain HNF1α and DCoH together whereas others express either HNF1α or DCoH. From this differential distribution we conclude that DCoH can contribute only in some tissues to the effects mediated by HNF1α. In tissues expressing DCoH but lacking HNF1α we assume that the cofactor cooperates with other transcription factors. In the lung the cooperation partner might be HNF1ß, which is known to be present in the mammalian lung (Mendel *et al.*, 1991a). However, in the pigmented epithelium of the eye we expect a distinct transcription factor since in gel retardation assays with HP1 no binding of HNF1α or HNF1ß can be seen in this region of the embryo (Fig. 7A).

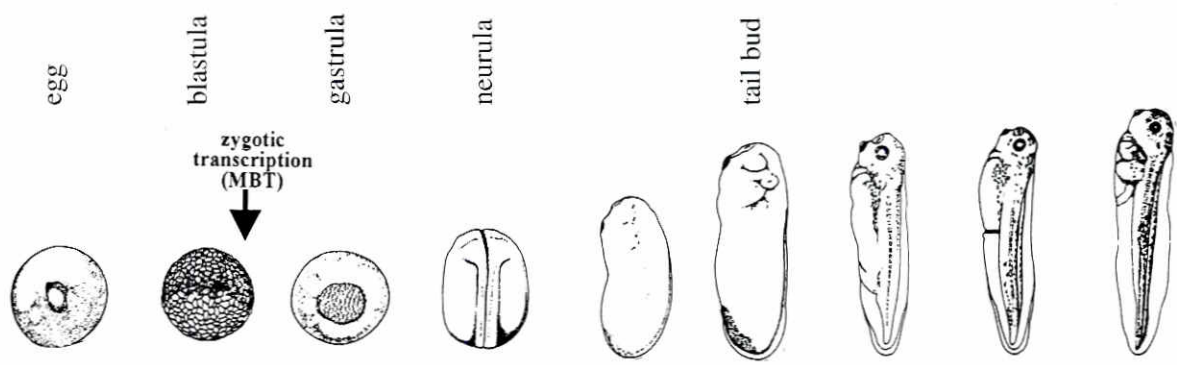
The developmental regulation of HNF1α and DCoH

Using an RNase protection assay we detected HNF1α mRNA shortly after mid-blastula transition, the time of first zygotic gene transcription, and the level of the mRNA gradually increases by approximately tenfold up to the tail bud stage (Fig. 6). The HNF1α protein could be detected in Western blots at later stages compared to the RNA and the level increases approximately tenfold between the larval stages 35 and 42 (Fig. 6). As the delay between the first appearance of HNF1α mRNA and the corresponding protein may just reflect the higher sensitivity of the RNA assay compared to the Western blot we cannot exclude that lower amounts of HNF1α are present at earlier time points and thus HNF1α might influence events in organogenesis. Investigating the appearance of the cofactor DCoH we were surprised to find DCoH as a maternal protein in the fertilized egg (Pogge v. Strandmann and Ryffel, 1995). Since HNF1α (Bartkowksi *et al.*, 1993) and at least on the RNA level also HNF1ß (Demartis *et al.*, 1994) are both absent at this early stage we postulate another maternal transcription factor cooperating with maternal DCoH.

As HNF1α is considered to be a main stimulatory factor for genes expressed in the liver (Tronche and Yaniv, 1992) we have determined the developmental appearance of albumin transcripts. As Figure 6 summarizes albumin mRNA appears after HNF1α as one would expect for a gene induced by HNF1α during embryogenesis.

Regulatory elements and factors involved in embryonic activation of HNF1α

As HNF1α transcripts appear at mid-blastula transition, we wondered which factors in the embryo are responsible for activation of HNF1α gene transcription. To get an insight into this cascade, we injected HNF1α promoter CAT constructs into fertilized *Xenopus* eggs and compared the activation of these injected genes with the endogenous gene. As we know that in the hatched larvae HNF1α can be detected in a gel retardation assay only in the middle section of the larvae (Fig. 7A) that contains pronephros, liver and gut, we dissected the larvae developing from the injected eggs into a head, middle and tail segment for measuring CAT activity. In Figure 7B an example of such an analysis is given: obviously the injected HNF1α promoter construct is mostly active in the middle part of the embryo. As a CAT reporter with the SV40 promoter and enhancer lacks this restricted activity (Zapp *et al.*, 1993a), we conclude that the HNF1α promoter contains regulatory elements that allow the proper temporal and spatial activation in the embryo. Using various deletion constructs of the HNF1α promoter we defined the minimal sequence required for correct embryonic activation (Zapp *et al.*, 1993a). Most surprisingly we also obtained correct embryonic activation of a reporter construct driven by the HNF1α promoter of the rat gene (Zapp *et al.*, 1993a). Thus the promoter elements mediating activation of the HNF1α gene during embryogenesis have been conserved between *Xenopus* and mammals. In Figure 8 the structural features of the *Xenopus* HNF1α promoter required for embryonic activation are given. At the 5' end we identified an OZ element that is essential for activation whereas at the 3' end the transcriptional start site is needed. The OZ element has previously been identified in similar experiments as regulatory sequence for embryonic activation of the *Xenopus* N-CAM and GS17 promoter (Ovsenek *et al.*, 1992; Krieg *et al.*, 1993). In a gel retardation assay shown in Fig. 9 we established that the retarded complex generated with the labeled OZ element from the HNF1α promoter (lane 1) is competed by the unlabeled OZ element from the N-CAM promoter (OZ-N-CAM, lanes 2 to 4) as well as with the unlabeled OZ-HNF1α oligonucleotide (lanes 11 to 13), whereas the unrelated oligonucleotides b1wt (Kugler *et al.*, 1990) and HPI (Schorpp *et al.*, 1988) yield no competition (lanes 5 to 10). In identical binding experiments the presence of an OZ element was also proven in the promoters of the rat and mouse HNF1α and the murine γ cristallin gene (see Zapp *et al.*, 1993a for sequence). Comparing various OZ elements we identify the sequence CCNCTCTC as the core consensus (Zapp *et al.*, 1993a). In gel retardation experiments Krieg's group has provided evidence that the factor OZ-1, binding the OZ element, is a maternal factor and that the binding activity to the OZ element accumulates during oogenesis (Ovsenek *et al.*, 1992). As OZ elements are found in several promoters that are activated early in *Xenopus* embryogenesis but that have completely different spatial activation profile in the embryo, we assume that



stage	**1**	**8**	**11**	**20**	**24**	**26**	**35**	**40**	**42**
HNF1α mRNA	-	-	+	+	+	+	++	++	++
HNF1α protein	-	-	-	-	-	-	+	+	++
DCoH protein	+	+	+	+	++	++	++	++	++
albumin mRNA	-	-	-	-	-	-	-	+	+

Fig. 6. Developmental appearance of HNF1α mRNA, HNF1α protein, DCoH protein and albumin mRNA. *The amount of HNF1α mRNA and HNF1α protein at various stages of development are given as determined in Bartkowski et al. (1993). The amount of DCoH is taken from Pogge v. Strandmann and Ryffel (1995) and the albumin mRNA was quantified by RNase protection analysis (unpublished data). + refers to low amounts and ++ indicates an approximately 10-fold higher amount.*

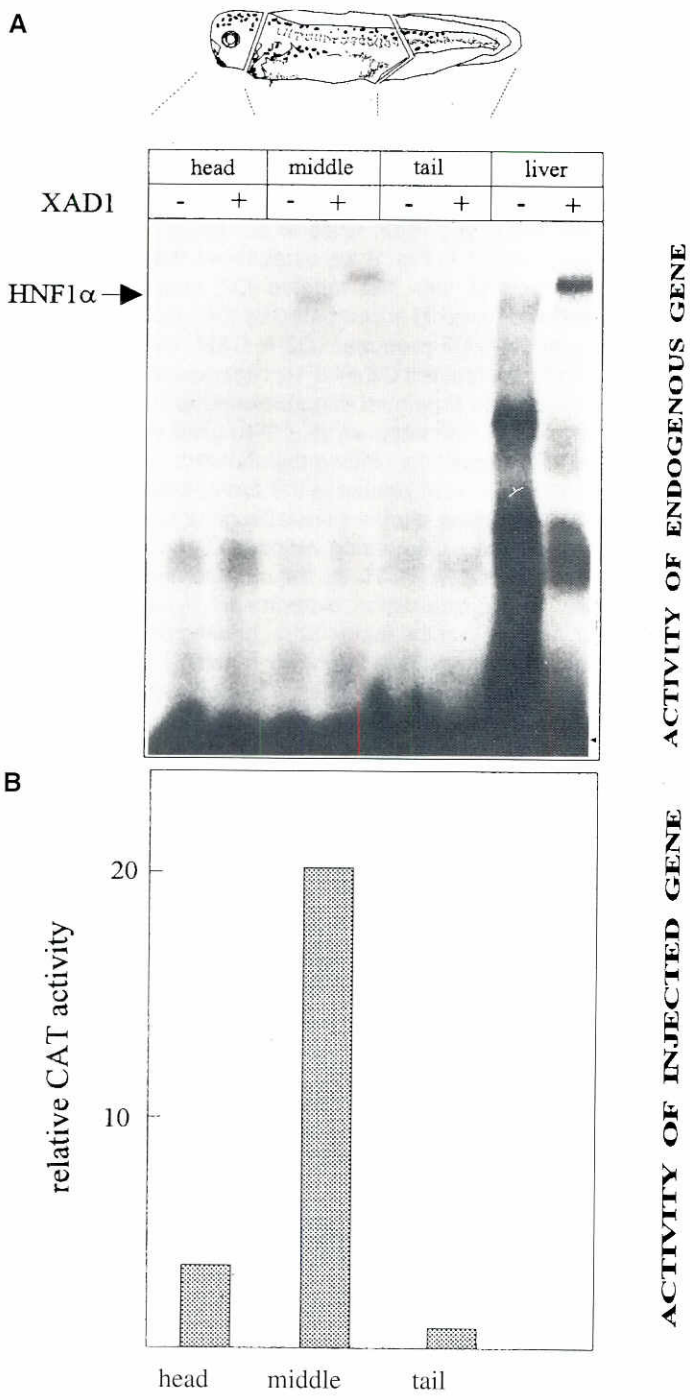


Fig. 7. Correct temporal and spatial expression of HNF1α promoter constructs injected into fertilized *Xenopus* eggs. (A) *Activation of the endogenous HNF1α gene: A Xenopus larvae at stage 38 was dissected into a head, middle and tail section and the presence of HNF1α in protein extracts derived from these pieces was determined in gel retardation assays using labeled HPI. The HNF1α specific antibody XAD1 was added as indicated. At the right a liver extract was used as a standard.* **(B)** *Activity of the HNF1α promoter construct in larvae derived from injected eggs. The relative CAT activity in the head, middle and tail section is given. The promoter construct used contains the sequence from -886 to -46 upstream of the translation start codon. The data are taken from Figure 5A in Zapp et al., 1993a.*

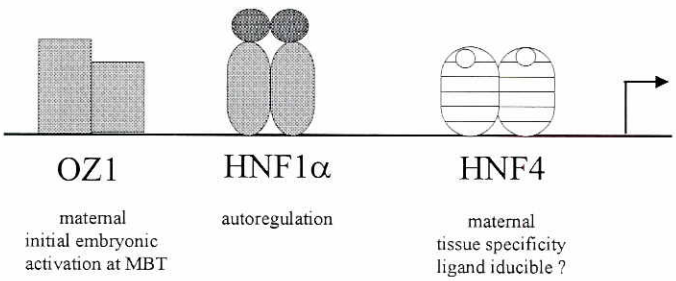


Fig. 8. Regulatory elements and factors involved in embryonic activation of the HNF1α promoter. *Schematic drawing of the HNF1α promoter with the binding sites for OZ1, HNF1α and HNF4. For details see text.*

OZ elements mediate only the initial general embryonic activation at mid-blastula transition and that distinct regulatory elements are involved in later stages to maintain the activity in specific parts of the developing embryo. Thus a gene such as GS17 that is activated only transiently at the gastrula stage (Krieg and Melton, 1986) may get inactive whereas other genes, i.e. HNF1α and N-CAM genes, maintain their activity due to other factors. Clearly, the factors taking over the function of OZ-1 must be distinct between the HNF1α and NCAM genes, as the spatial activation of these two genes is very different in the embryo. In the case of the HNF1α promoter we could recently show that the binding sites of HNF4 and HNF1α are crucial regulatory elements for correct embryonic activation (Holewa *et al.*, 1996). The HNF4 binding site that has been conserved between the *Xenopus* and rat HNF1α promoter (Zapp *et al.*, 1993a) is recognized by the transcription factor HNF4, a member of the steroid nuclear receptor superfamily (Sladek, 1993). Most interestingly this transcription factor has a very similar tissue distribution compared to HNF1α in the adult (Sladek, 1993). More significantly using antibodies specific for HNF4 we could demonstrate that HNF4 is a maternal transcription factor in the

TABLE 1

TISSUE DISTRIBUTION OF HNF1α AND XDCoH

Adult tissues	HNF1α	XDCoH
liver	+	+
kidney	+	+
intestine	+	-
stomach	+	(+)
lung	-	(+)
blood cells	-	-
heart	-	-
muscle	-	-
testis	-	-
brain	-	-
Larval tissues		
pronephros	+	+
liver	+	+
gut	+	+
eye	-	+

The data are taken from Bartkowski *et al.* (1993), Pogge v. Strandmann and Ryffel (1995) and Weber, Holewa, Jones and Ryffel (submitted). The abundance is indicated by + (abundant), (+) (more than 10-times less) and - (absent).

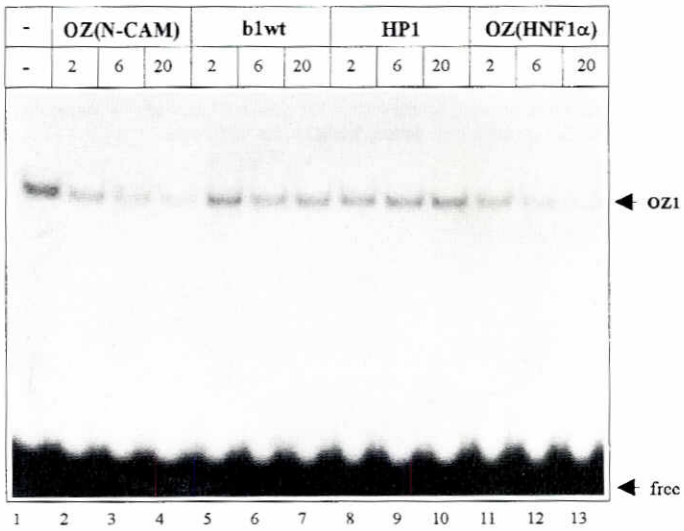


Fig. 9. The OZ element is a common regulatory element in genes activated early in *Xenopus* embryogenesis. *A gel retardation assay was made with the labeled OZ element from the HNF1α promoter. Various unlabeled oligonucleotides were added in the amounts (ng) as indicated.*

Xenopus egg and thus is a component that can initiate an embryonic transcriptional cascade (Holewa *et al.*, 1996). Clearly this regulatory cascade in embryogenesis has been conserved between *Drosophila* and vertebrates, as the HNF4 homolog of *Drosophila* has also been identified as a maternal factor (Zhong *et al.*, 1993). HNF4 is a member of the nuclear receptor superfamily with a conserved ligand binding domain. Although a potential ligand has not been identified so far, such a ligand might be a crucial component in regulating HNF4 function during embryogenesis.

Concerning the HNF1α binding sites in the *Xenopus* HNF1α promoter it seems possible that they establish some autoregulatory loop, as soon as HNF1α has accumulated in the embryo. But it should also be considered that HNF1ß might act through these sites. In fact, in mammals the appearance of HNF1ß precedes in the embryo the accumulation of HNF1α (De Simone *et al.*, 1991).

Based on our data we propose that embryonic HNF1α activation occurs in three main steps as summarized in Figure 8. Initially the maternal factor OZ-1 activates transcription of the HNF1α gene at mid-blastula transition. In a second phase the activation of the HNF1α promoter is taken over by HNF4, a second maternal transcription factor, whereas in a third phase the HNF1α promoter is regulated by transcription factors that are derived from zygotically expressed genes. These genes are HNF1α itself that acts through an autoregulatory loop, the related transcription factor HNF1ß as well as HNF4 that gets also transcribed after mid-blastula transition (Holewa *et al.*, 1996).

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