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Developmental Expression Pattern

C/EBPα and C/EBPβ are markers of early liver development

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ABSTRACT Pancreatic cells can be converted to hepatocytes by overexpression of C/EBPβ (Shen, C-N, Slack, J.M.W. and Tosh, D., 2000. Molecular basis of transdifferentiation of pancreas to liver. *Nature Cell Biology* 2: 879-887). This suggested that expression of one or more C/EBP factors may distinguish liver and pancreas in early development. We have now studied the early expression of C/EBPα and C/EBPβ in the mouse embryo and show that both are expressed exclusively in the early liver bud and not in the pancreatic buds. Their expression is identical to that of hepatocyte nuclear factor 4 (HNF4), another key hepatic transcription factor and alpha-fetoprotein (AFP), a differentiation product characteristic of immature hepatocytes. Both are complementary to the early expression of Pdx1, a key pancreatic transcription factor. These results are consistent with the idea that C/EBP factors are master regulators for liver development.

KEY WORDS: *C/EBP, HNF4, α-fetoprotein (AFP), Pdx1, liver development*

The liver develops from the ventral endoderm of the foregut at the 14-20 somite stage (Le Douarin, 1975). A hepatic bud is formed first; it then expands and proliferates into the septum transversum to form the embryonic liver (Zhao and Duncan, 2005). Much of our understanding of the molecular control of early liver development has come from a series of experiments from the Zaret laboratory. It has been shown in mouse that the ventral endoderm must express GATA-4 and FoxA2 (previously known as HNF3β) to be competent to undergo hepatic differentiation (Bossard and Zaret, 1998). Specification of the liver then may depend on the concerted action of members of the fibroblast growth factor family (FGFs), signalling from the adjacent cardiac mesoderm (Jung *et al.,* 1999) and of bone morphogenetic protein (BMP) signalling from the adjacent septum transversum (Rossi *et al.,* 2001). BMP2 signalling is also required for liver development in the chick, where it induces the expression of the homeobox protein Hex (Zhang *et al.,* 2004). Hex is the earliest identified marker for endoderm that will form liver (Bogue *et al.,* 2000, Thomas *et al.,* 1998). However, evidence for a role for mesodermal FGFs in chick is lacking (Zhang *et al.,* 2004). In the absence of FGF and BMP signals in the mouse, the anteroventral endoderm assumes a pancreatic fate (Rossi *et al.,* 2001). The initial specification of liver and pancreas may therefore depend on the responses to these signals from the mesoderm but the identity of the genes concerned is not yet certain. In the case of the pancreas it is known that the transcription factor Pdx1 plays a key role in specification (Jonsson *et al.,* 1994) whereas in liver, the transcription factors Foxa1 (HNF3α) and Foxa2 are required for initiation of development (Lee te al., 2005).

Previous work from our laboratory has suggested that members of the C/EBP family are important in distinguishing liver and pancreas (Tosh and Slack, 2002; Tosh *et al.,* 2002; Slack and Tosh, 2001; Shen *et al.,* 2000). Using the rat pancreatic cell line, AR42J-B13, we showed that transfection with *C/EBPβ* provokes hepatic differentiation, while transfection with a dominant negative inhibitor of C/EBPβ (the liver-enriched transcriptional inhibitory protein, LIP) will both inhibit glucocorticoid-induced formation of hepatocytes and also causes loss of the hepatic phenotype from cells maintained for a long periods in dexamethasone (Shen *et al.,* 2000, Kurash *et al.,* 2004, Burke *et al.,* 2006). C/EBPβ is also activated in organ cultures of embryonic pancreas following glucocorticoid treatment and its expression is associated with hepatic differentiation in this system (Shen *et al.,* 2000; Shen *et al.,* 2003).

C/EBPβ is a member of the CCAAT/enhancer binding protein (C/EBPs) family, which are basic-leucine zipper transcription factors that interact with CCAAT motifs present in numerous gene promoters. Six members have been reported and named α, β, γ, δ, ε and ζ (Lekstrom-Himes and Xanthopoulos, 1998). Although six genes exist, the number of C/EBP protein isoforms is greater due to alternate promoters, translational start sites, differential

Abbreviations used in this paper: AFP, alpha-fetoprotein; C/EBP, CCAAT/enhancer binding protein; HNF, hepatocyte nuclear factor.

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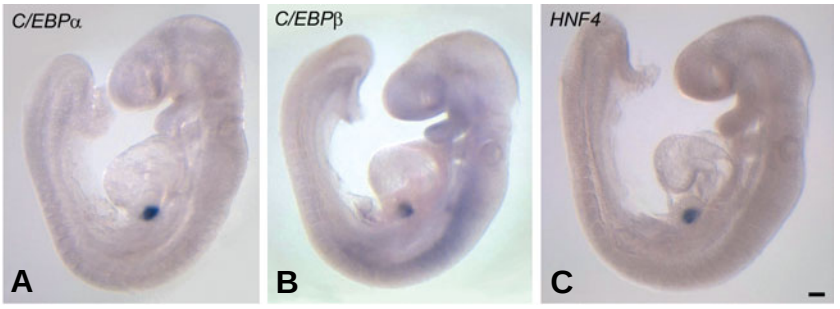

Fig. 1. Wholemount *in situ* hybridisation staining of *C/EBPα, C/EBPβ* and *HNF4* in E9.5 mouse embryos. (A) C/EBPα. **(B)** C/EBPβ. **(C)**HNF4. *Scale bar, 200 μm.*

splicing and regulated proteolysis. Members of the family are expressed in liver, hematopoietic and adipocyte tissues of the adult (Lekstrom-Himes and Xanthopoulos, 1998). Expression of C/EBPs has been examined during mouse and zebrafish development (Birkenmeier *et al.,* 1989, Shiojiri *et al.,* 2004., Lyons *et al.,* 2001). Initially mice were shown to express C/EBPα in developing intestinal tissue from E17.5d (Birkenmeier *et al.,* 1989). Subsequently, using antibody staining, Shiojiri *et al.,* demonstrated C/EBPα is expressed in the E9.5d mouse endoderm in the region of the developing liver. Because the authors showed no parallel expression of any other liver or pancreas-specific markers it is difficult to know exactly which endodermal organs express the C/EBP factors. More convincing hepatic C/EBPα immunostaining was observed from E10.5d and the onset of C/EBPβ expression was between E13.5 and E14.5d. C/EBPα expression was later suppressed in biliary cell precursors suggesting this transcription factor may be involved in the distinction between hepatocyte and biliary cell differentiation (Shiojiri *et al.,* 2004). In zebrafish, C/EBPα and β were expressed in a subset of hematopoietic cells, in the liver and regions of the gastrointestinal tract (Lyons *et al.,* 2001).

Results and Discussion

Although the transdifferentiation work suggests that these factors may distinguish the early specification of liver and pancreas, a critical condition for this hypothesis is that at least one of the C/EBP genes is actually expressed in the early liver bud and is not expressed in the early pancreatic buds, an issue which has not been addressed by the previous studies. In the present work, we have determined by *in situ* hybridisation the expression patterns during embryonic development of the genes *C/EBPα* and *C/EBPβ*. We have compared *C/EBPα* and *C/EBPβ* expression to a well characterised liver transcription factor, *HNF4* and to an early liver differentiation product, α-fetoprotein (AFP).

We find that *C/EBPα* and *C/EBPβ* are absent in E8.5d embryos but expression of both *C/EBP* factors and of HNF4, is first apparent at E9.5. Wholemount *in situs* show clearly that the only location for expression at this stage is the early liver bud (Fig. 1). Sense probes were negative (data not shown). At E10.5 cryosections show patchy expression within the early liver bud, with some positive and some negative cells (Fig. 2). This is to be expected because at this stage the hepatic cells are invading the septum transversum mesenchyme and the liver rudiment contains a high proportion of mesenchymal cells. By E11.5 expression in the liver is becoming more uniform as the hepatocytes outgrow the mesenchyme. The level of C/EBP factors expressed in the hepatoblasts remains significantly higher than in surrounding tissues (Fig. 3). At this stage, HNF4 (but not C/EBPα or β) is also expressed at a low level in the duodenum. Comparison of the immunostaining profiles for AFP and Pdx1 show that Pdx1 is expressed in the pancreas and duodenum but not in the liver. We continued to follow expression in the liver for the next three days and the situation remains similar with an exact correspondence of expression of *C/EBPα* and *β* with that of *HNF4* and AFP. Figure 4 shows the situation at E13.5 which is representative of the period. This mouse expression pattern is different from that of the zebrafish which shows duodenal as well as hepatic expression of the C/EBPs (Lyons *et al.,* 2001)

C/EBPs are already known to regulate several important liver genes (Friedman *et al.,* 1989, Umek *et al.,* 1991). These include both regulatory transcription factors, such as HNF4 and differentiation products, such as albumin (Bossard *et al.,* 1997). Albumin and α-fetoprotein start to be transcribed at E9.5 (Jochheim *et al.,* 2004). The timing is similar to C/EBPα expression suggesting the transcription factor may regulate albumin and α-fetoprotein expression. Evidence from mouse knockouts shows that loss of function mutants of either *C/EBPα* or *C/EBPβ* are viable with slight liver defects (Wang *et al.,* 1995; Screpanti *et al.,* 1995; Liu *et al.,* 1998) but the *C/EBPα* knockout mice die soon after birth due to hypoglycaemia (Wang *et al.,* 1995). Two separate studies have generated conditional deletions of C/EBPα demonstrating a role for C/EBPα in postnatal mice. The first knockout showed impaired glucose tolerance and hyperammonemia due to reduced glucokinase and carbamoylphosphate synthetase expression

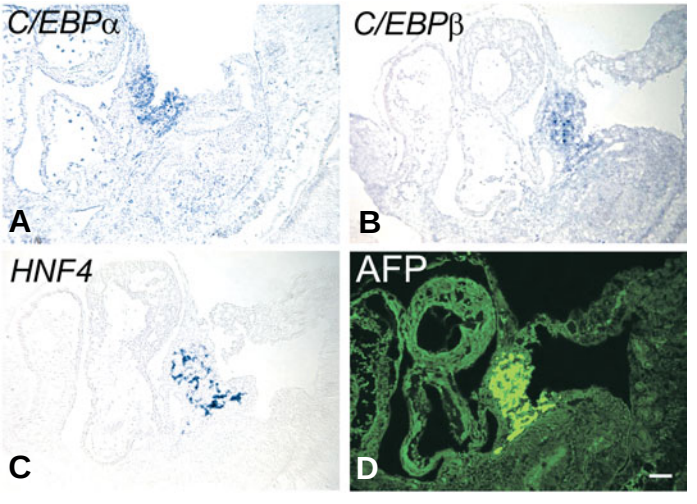

Fig. 2. Expression of *C/EBPα, C/EBPβ* and *HNF4* in E10.5 mouse embryos. *Neighbouring cryosections were processed for* in situ *hybridisation or immunostaining.* **(A)** In situ *of* C/EBPα. **(B)** In situ *of* C/EBPβ. **(C)** In situ *of* HNF4. **(D)** *Immunostain of α-fetoprotein (AFP) showing the region of the developing liver. Scale bar, 100 μm.*

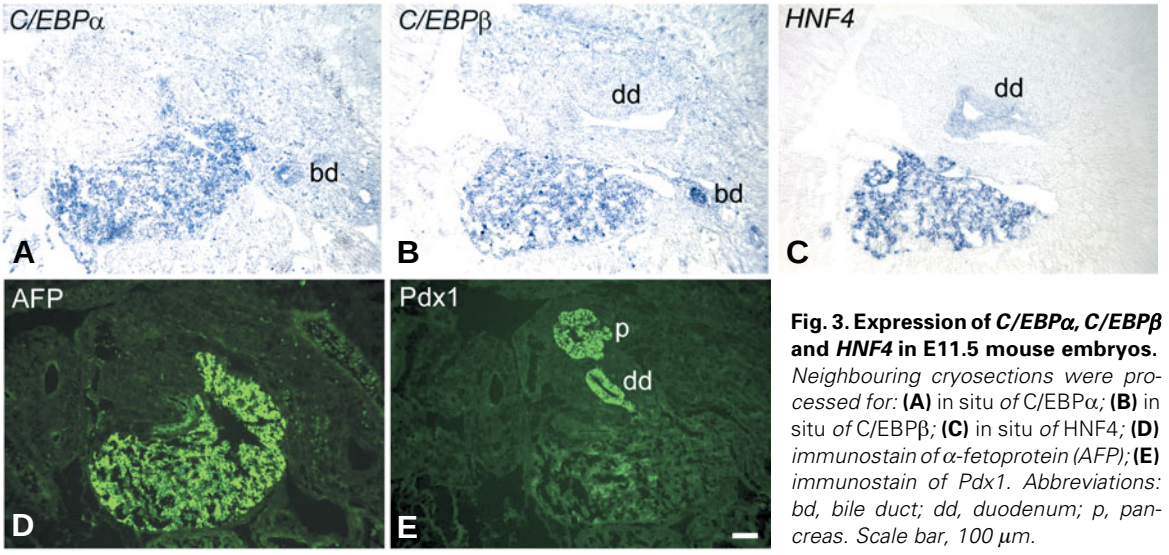

Fig. 3. Expression of *C/EBPα, C/EBPβ* and *HNF4* in E11.5 mouse embryos. *Neighbouring cryosections were processed for:* **(A)** in situ *of* C/EBPα*;* **(B)** in situ *of* C/EBPβ*;* **(C)** in situ *of* HNF4*;* **(D)** *immunostain of α-fetoprotein (AFP);* **(E)** *immunostain of Pdx1. Abbreviations: bd, bile duct; dd, duodenum; p, pancreas. Scale bar, 100 μm.*

(Inoue *et al.,* 2004). The second knockout model demonstrated hypoglycaemia and fatty, steatotic liver (Yang *et al.,* 2005). In addition *C/EBPβ* has been shown to be needed for liver regeneration (Diehl, 1998). In *Drosophila,* there is a single C/EBP homologue which is expressed in the lining of the gut. Loss of function mutants are embryonic lethal (Rørth and Montell, 1992), so we suspect that simultaneous loss of all of the C/EBP family in the mouse would have a more dramatic effect on liver development than the loss of just one.

The present study has added the critical expression pattern evidence to the previous data from gain and loss of function. The combined evidence is consistent with a role for the *C/EBP* genes as key regulators of liver development.

Experimental Procedures

Mice and isolation of embryos

Animal husbandry and embryo isolation were carried out in accordance with Home Office regulations. Stage specific embryos were isolated from timed matings with CD1 mice, based on the observation of a copulatory plug representing embryonic day 0.5 (E0.5).

RNA probe synthesis for in situ *hybridisation*

Digoxigenin (DIG)-labelled antisense and sense riboprobes were transcribed via the T7/SP6 RNA transcription system (Roche Diagnostics) according to manufacturer's guidelines. The RNA probe sizes were as follows: the HNF4α sequence was 707 bp corresponding to position 328-1035 bp of the cDNA; the C/EBPα was 439 bp and located in the region corresponding to 960-1399 bp of the cDNA sequence; the C/EBPβ probe was 1500 bp and represented the full length cDNA sequence. *In situ* hybridization experiments were performed at least six times. For each experiment, at least four embryos from each developmental stage were used. Typical results are shown.

Tissue preparation

Embryos were dissected and washed several times in sterile PBT (phosphate-buffered saline containing 0.1% Tween 20). Following fixation in 4% PFA at 4ºC for 2 hours (E9.5) or overnight (E10.5-14.5) all embryos were washed three times in sterile PBT and processed for whole-mount *in situ* hybridisation or cryosectioning. For whole-mount *in situ* hybridisation E9.5 and E10.5 embryos were dehydrated through a series of washes containing 25%, 50% or 75% methanol (in PBT) followed by a final wash and storage in 100% methanol at -20ºC. Alternatively, E10.5-E14.5 embryos were cryopreserved overnight in 30% sucrose in PBS, embedded in OCT media and stored at -80ºC.

Whole mount in situ *hybridisation*

Embryos were rehydrated through a series of graded methanol (75%, 50% and 25% in PBT) and treated for 1hour in 6% H_2O_2 to quench endogenous peroxidases. Whole mount *in situ* hybridisation was performed as described previously (Burke and Oliver, 2002) after which embryos were cleared through a series of 20%, 50% and 80% glycerol for photography.

In situ *hybridisation on sections*

For *in situ* hybridisation, cryosections (10 μm) were obtained using a Leica CM1850 cryostat. Sections were fixed for 10 min in 4% PFA and

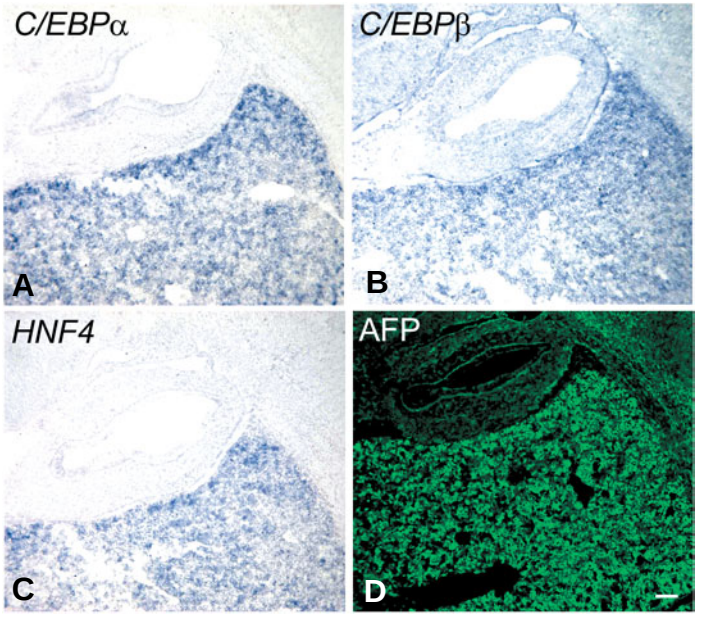

Fig. 4. Expression of *C/EBPα, C/EBPβ* and *HNF4* in E13.5 mouse embryos. *Neighbouring cryosections were processed for:* **(A)** in situ *of* C/EBPα*;* **(B)** in situ *of* C/EBPβ*;* **(C)** in situ *of* HNF4 *and* **(D)** *immunostain of α-fetoprotein (AFP). Scale bar, 100 μm.*

washed three times in PBS for 3 min each. The sections were then treated with 1 μg/ml proteinase K in 50 mM Tris pH 7.5, 5 mM EDTA for 2-6 min according to the embryonic stage (2 min for E10.5, 3 min for E11.5, 4 min for E12.5, 5 min for E13.5 and 6 min for E14.5). Following proteinase K treatment, the sections were fixed again in 4% PFA for 10 min and then washed 3 min in PBS. The sections were washed a total of three times. Sections were acetylated in a buffer containing 1.36% triethanolamine, 0.18% HCl and 0.25% acetic anhydride for 10 min and washed three times in PBS for 5 min each. Prehybridisation was carried out in a 5 x SSC humidified chamber at room temperature for 2 hours in hybridisation buffer containing 50% formamide, 5 x SSC (pH4.5), 50 μg/ml yeast RNA, 1% SDS and 50 μg/ml heparin. Probes were diluted to 0.5 μg/ml in hybridisation buffer, heated to 80ºC for 5 min and cooled for 5 min at room temperature. Hybridisation was carried out overnight at 72ºC in a 50% formamide/5 x SSC humidified chamber.

Posthybridisation, the sections were washed as follows; 3 hours at 72ºC in 0.2x SSC for 3 hours, three times 5 min each in 0.2 x SSC at room temperature and once in solution 1 containing 0.1 M Tris (pH7.5), 0.15 M NaCl and 0.24 mg/ml levamisole for 5 min. Sections were blocked for 1 hour in blocking solution containing 2% heat inactivated fetal calf serum and 2 % blocking reagent (Roche) in maleic acid buffer (100 mM maleic acid and 150 mM NaCl, pH7.5) and incubated overnight with an anti-DIG alkaline phosphatase antibody (1:5000 dilution in blocking solution) at 4ºC in a humidified chamber.

Excess antibody was removed by washing three times 5 min each in solution 1 and sections equilibrated by washing three times 10 min each in solution 2 containing 0.1 M Tris pH9.5, 0.1 M NaCl, 50 mM $MgCl_2$, 1% Tween-20. Sections were incubated with NBT/BCIP substrate (diluted to 20 μl/ml in solution 2) for 6 hours – 3 days. The reaction was stopped with 50 mM Tris pH8.0, 5 mM EDTA and the sections mounted in Gel Mount (Biomeda) prior to photography.

Immunohistochemistry

Cryosections were washed for 10 min in 1x PBS to remove OCT embedding media then blocked for 30 min in blocking buffer containing 10% normal goat serum (Vector Labs) and 0.5% BSA (British Biocell) in PBS. Prior to antibody incubations, cryosections were washed for 5 min in PBS. Anti rabbit-AFP (DAKO) was diluted 1:100 in blocking buffer containing 1% normal goat serum and 0.5% BSA in PBS. Similarly, anti-rabbit Pdx1 (A generous gift from Professor Chris Wright, Vanderbilt University) was diluted to 1:1000 using the same blocking buffer as described above. Both antibodies were allowed to incubate overnight at 4ºC. Sections were washed three times (10 minutes each) in PBS prior to and following incubation with anti-rabbit FITC (Vector Labs) for 3 h at room temperature then mounted with Gelmount media (Biomeda). Anti-rabbit FITC was diluted 1:100 in blocking buffer containing 1% normal goat serum and 0.5% BSA in PBS.

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References

BIRKENMEIER, E.H., GWYNN, B., HORWARD, S., JERRY, J., GORDON, J.L., LANDSCHULZ, W.H. and McKINGHT, S.L. (1989). Tissue-specific expression, developmental regulation and genetic mapping of the gene encoding CCAAT/enhancer binding protein. *Genes and Dev*. 3: 1146-1156.

BOGUE, C.W., GANEA, G.R., STURM, E. IANUCCI, R. and JACOBS, H.C. (2000). Hex expression suggests a role in the development and function of organs derived from foregut endoderm. *Dev. Dyn*. 219: 84-89.

BOSSARD, P. and ZARET, K.S. (1998). GATA transcription factors as potentiators of gut endoderm differentiation. *Development* 125: 4909-4917.

BOSSARD, P., MCPHERSON, C.E. and ZARET, K.S. (1997). In vivo footprinting with limiting amounts of embryo tissues: A role for C/EBP beta in early hepatic development. *Methods – A companion to Methods in Enzymology* 11: 180-188.

BURKE, Z.D., SHEN, C-N., RALPHS, K and TOSH, D. (2006). Characterization of liver function in transdifferentiated hepatocytes. *J. Cell. Physiol*. 206:147-159.

BURKE, Z. and OLIVER, G. (2002). Prox1 is an early specific marker for the developing liver and pancreas in the mammalian foregut endoderm. *Mech. Dev*. 118:147-155.

DARLINGTON, G.J., ROSS, S.E. and MACDOUGALD, O.A.,(1998). The role of C/EBP genes in adipocyte differentiation. *J. Biol. Chem.* 273: 30057-30060.

DIEHL, A. M. (1998). Role of CCAAT/Enhancer binding proteins in regulation of liver regenerative growth. *J. Biol. Chem*. 273: 30843-40846.

FRIEDMAN A.D., LANDSCHULZ W.H. and McKNIGHT, S.L. (1989). CCAAT enhancer binding-protein activates the promoter of the serum-albumin gene in cultured hepatoma-cells. *Genes Dev*. 3: 1314-1322.

INOUE, Y., INOUE, J., LAMBERT, G., YIM, S. H. and GONZALEZ, F.J. (2004). Disruption of hepatic C/EBPa results in impaired glucose tolerance and age-dependent hepatosteatosis. *J. Biol. Chem.* 279: 44740-44748.

JOCHHEIM, A., HILLEMANN, T., KANIA, G., SCHARF, J., ATTARAN, M., MANNS, M.P., WOBUS, A. and OTT, S. (2004). Quantitative gene expression profiling reveals a fetal hepatic phenotype of murine ES-derived hepatocytes. *Int. J. Dev. Biol.* 48: 23-29.

JONSSON J, CARLSSON L, EDLUND T and EDLUND H. (1994). Insulin Promoter Factor 1 is required for pancreas development in mice. *Nature* 371: 606-609.

JUNG, J.N., ZHENG, M.H., GOLDFARB, M. and ZARET, K.S. (1999). Initiation of mammalian liver development from endoderm by fibroblast growth factors. *Science* 284: 1998-2003.

KURASH, J.K., SHEN, C-N. and TOSH, D. (2004). Induction and regulation of acute phase proteins in transdifferentiated hepatocytes. *Exp Cell Res*. 292: 342-358.

LE DOUARIN, N.M. (1975). An experimental analysis of liver development. *Med. Biol.* 53: 427-455.

LEE, C.S., FRIEDMAN, J.R., FULMER, J.T. and KAESTNER, K.H. (2005). The initiation of liver development is dependent on Foxa transcription factors. *Nature* 435: 944-947.

LEKSTROM-HIMES, J. and XANTHOPOULOS, K.G. (1998). Biological activity of the CCAAT/enhancer binding protein family of transcription factors. *J. Biol. Chem*. 273: 28545-28548.

LIU S, CRONIGER C, REN JM, POLI V, HANSON RW and FRIEDMAN JE. (1998). Targeted disruption of C/EBP beta gene results in hypoglycaemia and impaired glucagon-stimulated glucose production. *Diabetes* 47: A295-A295 Suppl. 1.

LYONS, S.E., SHUE, B.C., LEI, L., OATES, A.C., ZON, L.I. and LIU, P.P. (2001). Molecular cloning, genetic mapping and expression analysis of four zebrafish c/ebp genes. *Gene* 281: 43-51.

ROSSI, J.M., DUNN, N.R., HOGAN, B.L.M. and ZARET, K.S. (2001). Distinct mesodermal signals, including BMPs from the septum transversum mesenchyme, are required in combination for hepatogenesis from the endoderm. *Genes Dev* 15: 1998-2009.

RØRTH, P. and MONTELL, D.J. (1992). Drosophila C/EBP: a tissue-specific DNA-binding protein required for embryonic development. *Genes Dev.* 6: 2299-2311.

SCREPANTI I, ROMANI L, MUSIANI P, MODESTI A, FATTORI E, LAZZARO D, SELLITTO C, SCARPA S, BELLAVIA D, LATTANZIO G, BISTONI, F, FRATI, L., CORTESE, R., GULINO, A., CILIBERTO, G., COSTANTINI, F. and POLI, V. (1995). Lymphoproliferative disorder and imbalanced T-helper response in C/EBP-beta-deficient mice. *EMBO J.* 14: 1932-1941.

SHEN, C-N, SLACK, J.M.W. and TOSH, D. (2000). Molecular basis of transdifferentiation of pancreas to liver. *Nature Cell Biology* 2: 879-887.

SHEN, C-N., SECKL, J.R., SLACK, J.M.W. and TOSH, D. (2003). Glucocorticoids suppress beta cell development and induce hepatic metaplasia in embryonic pancreas. *Biochem. J.* 375: 41-50.

SHIOJIRI, N., TAKESHITA, K., YAMASAKI, H. and IWATA, T. (2004). Suppression of C/EBPα expression in biliary cell differentiation from hepatoblasts during mouse liver development. *J. Hepatol*. 41:790-798.

SLACK, J.M.W. and TOSH, D. (2001). Transdifferentiation and metaplasia – switching of cell types. *Curr. Opin. in Genet. Dev*. 11: 581-586.

THOMAS, P.Q., BROWN, A and BEDDINGTON, R.S.P. (1998). Hex: a homeobox gene revealing pre-implantation asymmetry in the mouse embryo and an early

transient marker of endothelial cell precursors. *Development* 125: 85-94.

TOSH, D., SHEN, C-N and SLACK, J.M.W. (2002). Differentiated properties of hepatocytes induced from pancreatic cells. *Hepatology* 36: 534-543.

TOSH, D. and SLACK, J.M.W. (2002). How cells change their phenotype. *Nature Reviews Molecular Cell Biology.* 3: 187-194.

UMEK R.M., FRIEDMAN A.D. and McKNIGHT, S.L. (1991). CCAAT-enhancer binding-protein - a component of a differentiation switch. *Science* 251: 288-292

WANG, N.D., FINEGOLD, M.J., BRADLEY, A., OU, C.N., ABDELSAYED, S.V., WILDE, M.D., TAYLOR, L.R., WILSON, D.R. and DARLINGTON, G.J. (1995). Impaired Energy Homeostasis in C/EBP-alpha knockout mice. *Science* 269: 1108-1112.

YANG, J., CRONIGER, C.M., LEKSTROM-HIMES, J., ZHANG, P., FENYUS, F, TENEN, D.G., DARLINGTON, D.J. and HANSON, R.W. (2005). Metabolic response of mice to a postnatal ablation of CCAAT/Enhancer-binding protein α. *J. Biol. Chem.* 280: 38689-38699.

ZHANG, W., YATSKIEVYCH, T.A., BAKER, R.K. and ANIN, P.B. (2004). Regulation of Hex gene expression and initial stages of avian hepatogenesis by Bmp and Fgf signalling. *Dev. Biol*. 268: 312-326.

ZHAO, R. and DUNCAN, S.A. (2005). Embryonic development of the liver. *Hepatology* 41: 956-967.

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