

吳郭魚第四型肝細胞核因子a型之選殖與分析

許志堅、黃尉東

E-mail: 9510667@mail.dyu.edu.tw

ABSTRACT

Hepatocyte nuclear factor (HNF)-4 α is a liver-enriched transcription factor and belongs to the highly conserved member of the nuclear receptor superfamily. HNF-4 α together with other factors play a key role in the tissue specific expression of a large number of genes involved in lipid and glucose metabolism. However, its function in fish is still poorly understood. In our previous study, RNAs and proteins of HNF-1 α , HNF-1 β and HNF-3 α are detected in the liver, ovary, and testis of tilapia (*Oreochromis mossambicus*). And the expression of HNF-3 α in the gonads could be induced by 17 β -estradiol and hydrocortisone in vitro. Besides, HNF-4 binding site on the promoter region of HNF-3 α gene has also been found. However, there are only limited literatures dealing with HNFs in bony fish. The roles and their relation of HNFs in the physiology of fish remain to be explored. Here we report on the first cloning of full-length cDNA and protein localization of HNF-4 α from a tilapia (*O. mossambicus*). A total of 2,031 bp of tilapia HNF-4 α has been cloned and its deduced amino acid sequence of the coding region (340 amino acids) of tilapia HNF-4 α has a 89% identity with that of zebrafish, over 84% with mammals (human, bovine, rat, and mouse), 81% with chicken, and 79% with *Xenopus*. RT-PCR detected HNF-4 α in liver, kidney, intestine and stomach, and the identity of the PCR fragments was confirmed by sequencing analysis and PCR hybridization. Its relative expression ratio was higher in the liver than in intestine and kidney, and lowest expression ratio was observed in stomach. The same result happened in both genders. Western blotting and immunohistochemical localization also detected HNF-4 α protein in the tissues mentioned before. Expression of HNF-4 α in the tilapia suggests that multi-HNFs may form a cascade to regulate physiology in the bony fish.

Keywords : 吳郭魚 ; 第四型肝細胞核因子a型 ; 消化器官

Table of Contents

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|----|------|-----|-----|-----|------|----|------|---|----|----|-----|-----|---|-----|----|----|-----|-----|----|---|-----|----|----|---|-----|------------------|---|-----|-----------|---|-------|--------------|---|-------|-----------------|---|-----|--------------------|---|-------|-------------------------|---|-------|------------------------|---|-----|------|----|-----|-------|----|-----|------|----|-------|------|----|-----|------|----|-------|--------------------------------------|----|---------|---------------------|----|---------|------------------|----|---------|--|----|---------|------------------------|----|---------|--------------|----|---------|------|----|---------|--|----|---------|------------|----|---------|-------------------------|----|-------|--------------------------------|----|-------|------------------------|----|---------|------|----|---------|----------------|----|---------|---------|----|-------|---------------------------|----|---------|--------|----|---------|--------|----|---------|---------------|----|-----------|---------------------------|----|-----------|-------------------------|----|---------|-------|----|---------|----|----|---------|-------|----|-------|--------------------------------|----|---------|------------|----|---------|----------|----|-------|------|----|-----|----|----|-----|---|----|-----|----------------------|----|-------|------------|----|-------|----------|----|-------|--------|----|-------|---------|----|-----|----|----|-----|----|----|------|----|-----|------------------------------|----|-------------------------|----|--|----|---|----|---|----|--|----|--------------------------------------|----|-------------------------------------|----|---------------------------------------|----|--------------------------------------|----|---------------------------------------|----|---------------------------------------|----|---|----|---------------------------|----|---------------------------|----|--|----|--|----|--------------------------------------|----|---------------------------------------|----|------------------------------------|----|-----------------------------|----|
| 目錄 | 封面內頁 | 簽名頁 | 授權書 | iii | 中文摘要 | iv | 英文摘要 | v | 致謝 | 目錄 | vii | 圖目錄 | x | 表目錄 | xi | 附錄 | xii | 第一章 | 前言 | 1 | 第二章 | 文獻 | 檢討 | 3 | 2.1 | 肝細胞核因子 (HNFs) 簡介 | 3 | 2.2 | 第四型肝細胞核因子 | 4 | 2.2.1 | 第四型肝細胞核因子家族成 | 4 | 2.2.2 | 第四型肝細胞核因子之結構與功能 | 5 | 2.3 | 第四型肝細胞核因子 α | 8 | 2.3.1 | 第四型肝細胞核因子 α 恆洵岑略 | 8 | 2.3.2 | 第四型肝細胞核因子 α 恆洵孝 | 9 | 2.4 | 研究目的 | 13 | 第三章 | 材料與方法 | 15 | 3.1 | 試驗材料 | 15 | 3.1.1 | 試驗動物 | 15 | 3.2 | 試驗方法 | 15 | 3.2.1 | 吳郭魚HNF4- α 宸穉]序列之選殖 (cloning) | 15 | 3.2.1.1 | 吳郭魚肝臟核糖核酸 (RNA) 之抽取 | 16 | 3.2.1.2 | 互補DNA (cDNA) 之合成 | 16 | 3.2.1.3 | 吳郭魚HNF-4 α 恆 ' 驢]partial) cDNA序列之選殖 | 17 | 3.2.1.4 | 聚合 α 連鎖反應 (PCR) | 18 | 3.2.1.5 | RT-PCR產物電泳分析 | 18 | 3.2.1.6 | 膠體萃取 | 19 | 3.2.1.7 | 連接反應與轉型作用 (ligation reaction and transformation) | 19 | 3.2.1.8 | 少量質體之抽取與定序 | 20 | 3.2.1.9 | 選殖HNF-4 α 恆? 嬌 C | 21 | 3.2.2 | RT-PC觀察HNF-4 α 恆韓U組織之表現 | 21 | 3.2.3 | 南方點墨轉漬 (Southern blot) | 22 | 3.2.3.1 | 膠體轉印 | 22 | 3.2.3.2 | 探針 (probe) 之製備 | 23 | 3.2.3.3 | 雜合反應與壓片 | 23 | 3.2.4 | 西方點墨轉漬 (Western Blotting) | 23 | 3.2.4.1 | 蛋白質之萃取 | 24 | 3.2.4.2 | 蛋白質之定量 | 24 | 3.2.4.3 | SDS-PAGE膠體之配製 | 25 | 3.2.4.3.1 | 分離凝膠 (separating gel) 之配製 | 25 | 3.2.4.3.2 | 堆積凝膠 (stacking gel) 之配製 | 25 | 3.2.4.4 | 電泳之進行 | 26 | 3.2.4.5 | 轉印 | 26 | 3.2.4.6 | 雜合與偵測 | 27 | 3.2.5 | 免疫組織化學法 (Immunohistochemistry) | 27 | 3.2.5.1 | 組織之石蠟包埋及切片 | 28 | 3.2.5.2 | 免疫組織化學分析 | 28 | 3.2.6 | 統計分析 | 29 | 第四章 | 結果 | 30 | 4.1 | 第四型肝細胞核因子 α 恆活]HNF-4 α 恆^序列選殖 | 31 | 4.2 | HNF-4 α 恆 C分析 | 32 | 4.2.1 | 利用RT-PCR觀察 | 34 | 4.2.2 | PCR-雜合反應 | 34 | 4.2.3 | 西方點墨轉漬 | 35 | 4.2.4 | 免疫組織化學法 | 35 | 第五章 | 討論 | 37 | 第六章 | 結論 | 43 | 參考文獻 | 86 | 圖目錄 | 圖一、肝細胞核因子家族間之調控路徑及其上、下游之相關因子 | 44 | 圖二、各物種間第四型肝細胞核因子基因序列之比對 | 45 | 圖三、PCR選殖出吳郭魚第四型肝細胞核因子 α 恆洵坐互q序列 | 47 | 圖四、PCR選殖出5' 吳郭魚第四型肝細胞核因子 α 恆洵壯 C | 48 | 圖五、PCR選殖出3' 吳郭魚第四型肝細胞核因子 α 恆洵壯 C | 49 | 圖六、吳郭魚之第四型肝細胞核因子 α 恆洵壯 C及其RT-PCR引子設計之位置 | 50 | 圖七、各物種間第四型肝細胞核因子 α 恆皇i基酸序列之比對 | 54 | 圖八、吳郭魚第四型肝細胞核因子 α 恆洵坐G級結構預測圖 | 56 | 圖九、吳郭魚第四型肝細胞核因子 α 恆洵坐T維立體結構預測圖 | 58 | 圖十、各物種間第四型肝細胞核因子 α 恆皇i基酸序列之比對 | 59 | 圖十一、各物種間第四型肝細胞核因子 α 恆皇i基酸序列之比對 | 60 | 圖十二、各物種間第四型肝細胞核因子 α 恆皇i基酸序列之比對 | 61 | 圖十三、各物種間第四型肝細胞核因子 α 恆皇i基酸序列之雙聚體/配位體結合區比對 | 62 | 圖十四、吳郭魚雄魚之RT-PCR及PCR-雜合反應 | 63 | 圖十五、吳郭魚雌魚之RT-PCR及PCR-雜合反應 | 65 | 圖十六、吳郭魚雄魚之第四型肝細胞核因子 α 恆泵莖颯氏I法分析結果 | 67 | 圖十七、吳郭魚雌魚之第四型肝細胞核因子 α 恆泵莖颯氏I法分析結果 | 68 | 圖十八、第四型肝細胞核因子 α 恆洵駢z道上皮細胞核之表現 | 69 | 圖十九、第四型肝細胞核因子 α 恆洵駢 p管上皮細胞核之表現 | 70 | 圖二十、第四型肝細胞核因子 α 恆洵駢x臟細胞核之表現 | 71 | 圖二十一、第四型肝細胞核因子 α 恆洵駢 | 71 |
|----|------|-----|-----|-----|------|----|------|---|----|----|-----|-----|---|-----|----|----|-----|-----|----|---|-----|----|----|---|-----|------------------|---|-----|-----------|---|-------|--------------|---|-------|-----------------|---|-----|--------------------|---|-------|-------------------------|---|-------|------------------------|---|-----|------|----|-----|-------|----|-----|------|----|-------|------|----|-----|------|----|-------|--------------------------------------|----|---------|---------------------|----|---------|------------------|----|---------|--|----|---------|------------------------|----|---------|--------------|----|---------|------|----|---------|--|----|---------|------------|----|---------|-------------------------|----|-------|--------------------------------|----|-------|------------------------|----|---------|------|----|---------|----------------|----|---------|---------|----|-------|---------------------------|----|---------|--------|----|---------|--------|----|---------|---------------|----|-----------|---------------------------|----|-----------|-------------------------|----|---------|-------|----|---------|----|----|---------|-------|----|-------|--------------------------------|----|---------|------------|----|---------|----------|----|-------|------|----|-----|----|----|-----|---|----|-----|----------------------|----|-------|------------|----|-------|----------|----|-------|--------|----|-------|---------|----|-----|----|----|-----|----|----|------|----|-----|------------------------------|----|-------------------------|----|--|----|---|----|---|----|--|----|--------------------------------------|----|-------------------------------------|----|---------------------------------------|----|--------------------------------------|----|---------------------------------------|----|---------------------------------------|----|---|----|---------------------------|----|---------------------------|----|--|----|--|----|--------------------------------------|----|---------------------------------------|----|------------------------------------|----|-----------------------------|----|

胞核因子 β 悅洗飄G上皮細胞核之表現 72 圖二十二、第四型肝細胞核因子 β 悅洗駢 β B膽及鰓之表現 73 圖二十三、第四型肝細胞核因子 β 悅洗鞠菱式B肌肉、睪丸及卵巢之表現 74 表目錄表一、選殖吳郭魚HNF-4 β 桶互q退化性引子之序列 76 表二、選殖吳郭魚HNF-4 β 掙廢搗M一性引子之序列 77 表三、選殖吳郭魚HNF-4 β 搗瑯搗M一性引子之序列 78 表四、HNF-4 β 桶刪T-PCR專一性引子 79 表五、吳郭魚HNF-4 β 宸穡]與其他物種間之序列比對 80 表六、各物種間第四型肝細胞核因子 β 琮皇i基酸序列之比對 81 表七、各物種間第四型肝細胞核因子 β 蚰皇i基酸序列之比對 82 附錄 附錄一、各物種間HNF-4 β 啞i基酸序列比對之相似度及相同度百分比 83 附錄二、各物種間HNF-4 β 啞i基酸DNA結合區之相似度及相同度百分比 84 附錄三、各物種間HNF-4 β 啞i基酸序列雙聚體/配位體結合區之相似度及相同度百分比 85

REFERENCES

- 邵廣昭, 1996. 台灣常見魚貝類圖說(下). 台灣省漁業局. pp. 125-146. 王水良、房建中和傅繼梁。(2003)。肝富集轉錄因子及其相互作用研究進展。國外醫學遺傳學分冊。26(1):20-25. Argyrokastritis, A., Kamakari, S., Kapsetaki, M., Kritis, A., Talianidis, I. and Moschonas, N. K. (1997). Human hepatocyte nuclear factor-4 (hHNF-4) gene maps to 20q12-q13.1 between PLCG1 and D20S17. *Hum Genet*, 99(2), 233-236. Au-Fliegner, M., Helmer, E., Casasova, J., Raaka, B. and Samuels, H. (1993). The conserved ninth C-terminal heptad in thyroid hormone and retinoic acid receptors mediates diverse responses by affecting heterodimer but not homodimer formation. *Mol Cell Biol*, 13(9), 5725-5737. Bogan, A. A., Cohen, F. E. and Scanlan, T. S. (1998). Natural ligands of nuclear receptors have conserved volumes. *Nature Struct*, 5(10), 679-681. Bourguet, W., Vivat, V., Wurtz, J.-M., Chambon, P., Gronemeyer, H. and Moras, D. (2000). Crystal structure of a heterodimeric complex of RAR and RXR ligand-binding domains. *Mol Cell*, 5(2), 289-298. Carew, J. A., Pollak, E. S., Lopaciuk, S., and Bauer, K. A. (2000). A new mutation in the HNF4 binding region of the factor VII promoter in a patient with severe factor VII deficiency. *Blood*, 96(13), 4370-4372. Cereghini, S., Yaniv, M. and Cortese, R. (1990). Hepatocyte dedifferentiation and extinction is accompanied by a block in the synthesis of mRNA coding for the transcription factor HNF1/LFB1. *Embo J*, 9(7), 2257-2263. Chartier, F. L., Bossu, J. P., Laudet, V., Fruchart, J. C. and Laine, B. (1994). Cloning and sequencing of cDNAs encoding the human hepatocyte nuclear factor 4 indicate the presence of two isoforms in human liver. *Gene*, 147(2), 269-272. Chen, K. J., Chao, H. K., Hsiao, K. J., and Su, T. S. (2002). Identification and characterization of a novel liver-specific enhancer of the human phenylalanine hydroxylase gene. *Hum Genet*, 110(3), 235-243. Chen, M., Hieng, S., Qian, X., Costa, R. and Ou, J. H. (1994). Regulation of hepatitis B virus EN1 enhancer activity by hepatocyte-enriched transcription factor HNF3. *Virology*, 205(1), 127-132. Dhe-Paganon, S., Duda, K., Iwamoto, M., Chi, Y. I. and Shoelson, S. E. (2002). Crystal structure of the HNF4 alpha ligand binding domain in complex with endogenous fatty acid ligand. *J Biol Chem*, 277(41), 37973-37976. Drewes, T., Senkel, S., Holewa, B. and Ryffel, G. U. (1996). Human hepatocyte nuclear factor 4 isoforms are encoded by distinct and differentially expressed genes. *Mol Cell Biol*, 16(3), 925-931. Duncan, S. A., Manova, K., Chen, W. S., Hoodless, P., Weinstein, D. C., Bachvarova, R. F. (1994). Expression of transcription factor HNF-4 in the extraembryonic endoderm, gut, and nephrogenic tissue of the developing mouse embryo: HNF-4 is a marker for primary endoderm in the implanting blastocyst. *Proc Natl Acad Sci U S A*, 91(16), 7598-7602. Duncan, S. A., Nagy, A. and Chan, W. (1997). Murine gastrulation requires HNF-4 regulated gene expression in the visceral endoderm: tetraploid rescue of Hnf-4(-/-) embryos. *Development*, 124(2), 279-287. Egea, P. F., Mitschler, A., Rochel, N., Ruff, M., Chambon, P. and Moras, D. (2000). Crystal structure of the human RXR ligand-binding domain bound to its natural ligand: 9-cis retinoic acid. *EMBO J*, 19(11), 2592-2601. Forman, B. M., Yan, C.-R., Au, M., Casanova, J., Ghysdal, J. and Samuels, H. H. (1998). A domain containing leucine-zipper-like motifs mediate novel in vivo interactions between the thyroid hormone and retinoic acid receptors. *Mol Endocrinol*, 3(10), 1610-1626. Furuta, H., Iwasaki, N., Oda, N., Hinokio, Y., Horikawa, Y., Yamagata, K. (1997). Organization and partial sequence of the hepatocyte nuclear factor-4 alpha/MODY1 gene and identification of a missense mutation, R127W, in a Japanese family with MODY. *Diabetes*, 46(10), 1652-1657. Green, V. J., Kokkotou, E. and Ladias, J. A. (1998). Critical structural elements and multitarget protein interactions of the transcriptional activator AF-1 of hepatocyte nuclear factor 4. *J Biol Chem*, 273(45), 29950-29957. Griffo, G., Hamon-Benais, C., Angrand, P. O., Fox, M., West, L., Lecoq, O. (1993). HNF4 and HNF1 as well as a panel of hepatic functions are extinguished and reexpressed in parallel in chromosomally reduced rat hepatoma-human fibroblast hybrids. *J Cell Biol*, 121(4), 887-898. Hadzopoulou-Cladaras, M., Kistanova, E., Evagelopoulou, C., Zeng, S., Cladaras, C. and Ladias, J. A. (1997). Functional domains of the nuclear receptor hepatocyte nuclear factor 4. *J Biol Chem*, 272(1), 539-550. Hall, R. K., Sladek, F. M. and Granner, D. K. (1995). The orphan receptors COUP-TF and HNF-4 serve as accessory factors required for induction of phosphoenolpyruvate carboxykinase gene transcription by glucocorticoids. *Proc Natl Acad Sci U S A*, 92(2), 412-416. Harish, S., Khanam, T., Mani, S. and Rangarajan, P. (2001). Transcriptional activation by hepatocyte nuclear factor-4 in a cell-free system derived from rat liver nuclei. *Nucleic Acids Res*, 29(5), 1047-1053. Hata, S., Inoue, T., Kosuga, K., Nakashima, T., Tsukamoto, T. and Osumi, T. (1995). Identification of two splice isoforms of mRNA for mouse hepatocyte nuclear factor 4 (HNF-4). *Biochim Biophys Acta*, 1260(1), 55-61. Hata, S., Tsukamoto, T. and Osumi, T. (1992). A novel isoform of rat hepatocyte nuclear factor 4 (HNF-4). *Biochim Biophys Acta*, 1131(2), 211-213. Hattersley, A. T. (1998). Maturity-onset diabetes of the young: clinical heterogeneity explained by genetic heterogeneity. *Diabet Med*, 15(1), 15-24. Hayashi, Y., Wang, W., Ninomiya, T., Ohta, K. and Itoh, H. (1999). Liver enriched transcription factor and differentiation of hepatocellular carcinoma. *Mol. Pathol.* 52 : 19-24. Hayhurst, G. P., Lee, Y. H., Lambert, G., Ward, J. M. and Gonzalez, F. J. (2001). Hepatocyte nuclear factor 4alpha (nuclear receptor 2A1) is essential for maintenance of hepatic gene expression and lipid homeostasis. *Mol Cell Biol*, 21(4), 1393-1403. Hertz, R., Magenheimer, J., Berman, I. and Bar-Tana, J. (1998). Fatty acyl-CoA thioesters are ligands of hepatic nuclear factor-4alpha.

Nature, 392(6675), 512-516. Holewa, B., Zapp, D., Drewes, T., Senkel, S. and Ryffel, G. U. (1997). HNF4beta, a new gene of the HNF4 family with distinct activation and expression profiles in oogenesis and embryogenesis of *Xenopus laevis*. *Mol Cell Biol*, 17(2), 687-694. Huang, W. T., Gong, H. Y., Lin, C. J., Weng, C. F., Chen, M. H. and Wu, J. L. (2001). Hepatocyte nuclear factors-1alpha, -1beta, and -3beta expressed in the gonad of tilapia (*Oreochromis mossambicus*). *Biochem Biophys Res Commun*, 288(4), 833-840. Huang, W. T., Yu, H. C., Yang, H. C., Lin, C. J., Gong, H. Y., Weng, C. F. and Wu, J. L. (2004). Studies on the expression and localization of hepatocyte nuclear factor (HNFs) in gonads of tilapia (*Oreochromis mossambicus*). In "Proc. for Comparative Endocrinology", Nara, Japan. P-054. Imamura, K. and Tanaka, T. (1982). Pyruvate kinase isozymes from rat. *Methods Enzymol*, 90, 150-165. Jiang, G., Nepomuceno, L., Hopkins, K. and Sladek, F. M. (1995). Exclusive homodimerization of the orphan receptor hepatocyte nuclear factor 4 defines a new subclass of nuclear receptors. *Mol Cell Biol*, 15(9), 5131-5143. Ktistaki, E. and Talianidis, I. (1997). Chicken ovalbumin upstream promoter transcription factors act as auxiliary cofactors for hepatocyte nuclear factor 4 and enhance hepatic gene expression. *Mol Cell Biol*, 17(5), 2790-2797. Kuo, C. J., Conley, P. B., Chen, L., Sladek, F. M., Darnell, J. E., Jr. and Crabtree, G. R. (1992). A transcriptional hierarchy involved in mammalian cell-type specification. *Nature*, 355(6359), 457-461. Ladas, J. A., Hadzopoulou-Cladaras, M., Kardassis, D., Cardot, P., Cheng, J., Zannis, V. (1992). Transcriptional regulation of human apolipoprotein genes ApoB, ApoCIII, and ApoAII by members of the steroid hormone receptor superfamily HNF-4, ARP-1, EAR-2, and EAR-3. *J Biol Chem*, 267(22), 15849-15860. Lannoy, V. J., Decaux, J. F., Pierreux, C. E., Lemaigre, F. P., and Rousseau, G. G. (2002). Liver glucokinase gene expression is controlled by the onecut transcription factor hepatocyte nuclear factor-6. *Diabetologia*, 45(8), 1136-1141. Lee, J., Gulick, T. and Moore, D. (1992). Thyroid hormone receptor dimerization function maps to a conserved subregion of the ligand binding domain. *Mol Endocrinol*, 6(11), 1867-1873. Lees, J. A., Fawell, S. E., White, R. and Parker, M. G. (1990). A 22-amino-acid peptide restores DNA-binding activity to dimerization-defective mutants of the estrogen receptor. *Mol Cell Biol*, 10(10), 5529-5531. Leng, X., Blanco, J., Tsai, S. Y., Ozato, K., O'Malley, B. W. and Tsai, M. J. (1995). Mouse retinoid X receptor contains a separable ligand-binding and transactivation domain in its E region. *Mol Cell Biol*, 15(1), 255-263. Li, J., Ning, G. and Duncan, S. A. (2000). Mammalian hepatocyte differentiation requires the transcription factor HNF-4alpha. *Genes Dev*, 14(4), 464-474. Lindner, T., Gragnoli, C., Furuta, H., Cockburn, B. N., Petzold, C., Rietzsch, H. (1997). Hepatic function in a family with a nonsense mutation (R154X) in the hepatocyte nuclear factor-4alpha/MODY1 gene. *J Clin Invest*, 100(6), 1400-1405. Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P. and Evans, R. M. (1995). The nuclear receptor superfamily: the second decade. *Cell*, 83(6), 835-839. Mietus-Snyder, M., Sladek, F. M., Ginsburg, G. S., Kuo, C. F., Ladas, J. A., Darnell, J. E., Jr. (1992). Antagonism between apolipoprotein A1 regulatory protein 1, Ear3/COUP-TF, and hepatocyte nuclear factor 4 modulates apolipoprotein CIII gene expression in liver and intestinal cells. *Mol Cell Biol*, 12(4), 1708-1718. Miura, N. and Tanaka, K. (1993). Analysis of the rat hepatocyte nuclear factor (HNF) 1 gene promoter: synergistic activation by HNF4 and HNF1 proteins. *Nucleic Acids Res*, 21(16), 3731-3736. Morrissey, E. E., Tang, Z., Sigrist, K., Lu, M. M., Jiang, F., Ip, H. S. (1998). GATA6 regulates HNF4 and is required for differentiation of visceral endoderm in the mouse embryo. *Genes Dev*, 12(22), 3579-3590. Nakhei, H., Lingott, A., Lemm, I., and Ryffel, G. U. (1998). An alternative splice variant of the tissue specific transcription factor HNF4alpha predominates in undifferentiated murine cell types. *Nucleic Acids Res*, 26(2), 497-504. Navas, M. A., Vaisse, C., Boger, S., Heimesaat, M., Kollee, L. A. and Stoffel, M. (2000). The human HNF-3 genes: cloning, partial sequence and mutation screening in patients with impaired glucose homeostasis. *Hum Hered*, 50 : 370-81. Nishiyama, C., Hi, R., Osada, S. and Osumi, T. (1998). Functional interactions between nuclear receptors recognizing a common sequence element, the direct repeat motif spaced by one nucleotide (DR-1). *J Biochem (Tokyo)*, 123(6), 1174-1179. Nolten, L. A., Steenbergh, P. H. and Sussenbach, J. S. (1996). The hepatocyte nuclear factor 3beta stimulates the transcription of the human insulin-like growth factor I gene in a direct and indirect manner. *J Biol Chem*, 271(50), 31846-31854. Odom, D. T., Zizlsperger, N., Gordon, D. B., Bell, G. W., Rinaldi, N. J., Murray, H. L. (2004). Control of pancreas and liver gene expression by HNF transcription factors. *Science*, 303(5662), 1378-1381. Palamarchuk, A. Y., Kavsan, V. M., Sussenbach, J. S. and Holthuisen, P. E. (1999). The chum salmon IGF-II gene promoter is activated by hepatocyte nuclear factor 3beta. *FEBS Lett*, 446(2-3), 251-255. Peiler, G., Bockmann, B., Nakhei, H. and Ryffel, G. U. (2000). Inhibitor of the tissue-specific transcription factor HNF4, a potential regulator in early *Xenopus* development. *Mol Cell Biol*, 20(23), 8676-8683. Perlmann, T., Umesono, K., Rangarajan, P. N., Forman, B. M., and Evans, R. M. (1996). Two distinct dimerization interfaces differentially modulate target gene specificity of nuclear hormone receptors. *Mol Endocrinol*, 10(8), 958-966. Pogge v Strandmann, E., Senkel, S. and Ryffel, G. U. (2000). Ectopic pigmentation in *Xenopus* in response to DCoH/PCD, the cofactor of HNF1 transcription factor/pterin-4alpha-carbinolamine dehydratase. *Mech Dev*, 91(1-2), 53-60. Rosen, E., Beninghof, E. and Koenig, R. (1993). Dimerization interfaces of thyroid hormone, retinoic acid, vitamin D and retinoid X receptors. *J Biol Chem*, 268(16), 11534-11541. Ruse, M. D., Jr., Privalsky, M. L. and Sladek, F. M. (2002). Competitive cofactor recruitment by orphan receptor hepatocyte nuclear factor 4alpha1: modulation by the F domain. *Mol Cell Biol*, 22(6), 1626-1638. Schrem, H., Klemmner, J. and Borlak, J. (2002). Liver-enriched transcription factors in liver function and development. Part I: the hepatocyte nuclear factor network and liver-specific gene expression. *Pharmacol Rev*, 54(1), 129-158. Shih, D. Q., Dansky, H. M., Fleisher, M., Assmann, G., Fajans, S. S. and Stoffel, M. (2000). Genotype/phenotype relationships in HNF-4alpha/MODY1: haploinsufficiency is associated with reduced apolipoprotein (AII), apolipoprotein (CIII), lipoprotein(a), and triglyceride levels. *Diabetes*, 49(5), 832-837. Sladek, F. M. (1994). Orphan receptor HNF-4 and liver-specific gene expression. *Receptor*, 4(1), 64. Sladek, F. M. and Seidel, S. D. (2000). Hepatocyte nuclear factor 4? In *Nuclear Receptors and Genetic Diseases* (Burris, T. P. and McCabe, E., eds), Academic Press, London. In the press. Sladek, F. M., Zhong, W. M., Lai, E. and Darnell, J. E., Jr. (1990). Liver-enriched transcription factor HNF-4 is a novel member of the steroid hormone receptor superfamily. *Genes Dev*, 4(12B), 2353-2365. Sladek, R. and Giguere, V. (2000). Orphan nuclear

receptors: an emerging family of metabolic regulators. *Adv Pharmacol*, 47, 23-87. Sourdive, D. J., Transy, C., Garbay, S. and Yaniv, M. (1997). The bifunctional DCOH protein binds to HNF1 independently of its 4-alpha-carbinolamine dehydratase activity. *Nucleic Acids Res*, 25(8), 1476-1484. Soutoglou, E., Katrakili, N. and Talianidis, I. (2000). Acetylation regulates transcription factor activity at multiple levels. *Mol Cell*, 5(4), 745-751. Stoffel, M. and Duncan, S. A. (1997). The maturity-onset diabetes of the young (MODY1) transcription factor HNF4alpha regulates expression of genes required for glucose transport and metabolism. *Proc Natl Acad Sci U S A*, 94(24), 13209-13214. Taraviras, S., Monaghan, A. P., Schutz, G. and Kelsey, G. (1994). Characterization of the mouse HNF-4 gene and its expression during mouse embryogenesis. *Mech Dev*, 48(2), 67-79. Tian, J. M. and Schibler, U. (1991). Tissue-specific expression of the gene encoding hepatocyte nuclear factor 1 may involve hepatocyte nuclear factor 4. *Genes Dev*, 5(12A), 2225-2234. Torres-Padilla, M. E., Fougere-Deschatrette, C. and Weiss, M. C. (2001). Expression of HNF4alpha isoforms in mouse liver development is regulated by sequential promoter usage and constitutive 3' end splicing. *Mech Dev*, 109(2), 183-193. Torres-Padilla, M. E., Sladek, F. M. and Weiss, M. C. (2002). Developmentally regulated N-terminal variants of the nuclear receptor hepatocyte nuclear factor 4alpha mediate multiple interactions through coactivator and corepressor-histone deacetylase complexes. *J Biol Chem*, 277(47), 44677-44687. Torres-Padilla, M. E. and Weiss, M. C. (2003). Effects of interactions of hepatocyte nuclear factor 4alpha isoforms with coactivators and corepressors are promoter-specific. *FEBS Lett*, 539(1-3), 19-23. Whitfield, G. K., Hsieh, J. C., Nakajima, S., Macdonald, P. N., Thompson, P. D., Jurutka, P. W., Haussler, C. A. and Haussler, M. R. (1995). A highly conserved region in the hormone-binding domain of the human vitamin D receptor contains residues vital for transcriptional activation. *Mol Endocrinol*, 9(9), 1166-1179. Weber, A., Marie, J., Cottreau, D., Simon, M. P., Besmond, C., Dreyfus, J. C. and Kahn, A. (1984). Dietary control of aldolase B and L-type pyruvate kinase mRNAs in rat. Study of translational activity and hybridization with cloned cDNA probes. *J Biol Chem*, 259(3), 1798-1802. Woolf, A. S. (2000). Diabetes, genes, and kidney development. *Kidney Int*, 57(3), 1202-1203. Yamagata, K., Furuta, H., Oda, N., Kaisaki, P. J., Menzel, S., Cox, N. J. (1996). Mutations in the hepatocyte nuclear factor-4alpha gene in maturity-onset diabetes of the young (MODY1). *Nature*, 384(6608), 458-460. Yanai, K., Hirota, K., Taniguchi-Yanai, K., Shigematsu, Y., Shimamoto, Y., Saito, T. (1999). Regulated expression of human angiotensinogen gene by hepatocyte nuclear factor 4 and chicken ovalbumin upstream promoter-transcription factor. *J Biol Chem*, 274(49), 34605-34612. Yoshida, E., Aratani, S., Itou, H., Miyagishi, M., Takiguchi, M., Osumu, T. (1997). Functional association between CBP and HNF4 in trans-activation. *Biochem Biophys Res Commun*, 241(3), 664-669. Zhong, W., Sladek, F. M. and Darnell, J. E., Jr. (1993). The expression pattern of a Drosophila homolog to the mouse transcription factor HNF-4 suggests a determinative role in gut formation. *Embo J*, 12(2), 537-544. Zhong, W., Mirkovitch, J. and Darnell, J. E., Jr. (1994). Tissue-specific regulation of mouse hepatocyte nuclear factor 4 expression. *Mol Cell Biol*, 14(11), 7276-7284.