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Prox1 對 Ff1b 於班馬魚 adrenal/interrenal 發育過程中之輔助調控(1/3)

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Homeoprotein Prox1 as a Downstream Target of Orphan Nuclear Receptor Ff1b in the Multiple Aspects of Organogenesis in Zebrafish, *Danio rerio*

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計畫中文摘要

Homeoprotein Prox1 (Drosophilla Prospero 在脊椎動物系統中之對應蛋白), 在胚胎發育過程中呈現快速變化之組織特異性表現. Prox1 並已在老鼠系統中證實其對於細胞分化及器官生成之重要性. 對於胚胎發育階段之神經幹細胞, 水晶體及網膜細胞, 肝細胞以及淋巴內皮細胞, Prox1 為一重要之分子標記. 本人於 2003 年發表之著作中亦闡述 Prox1 表達在胚胎時期斑馬魚之腎間腺(相當於哺乳類之腎上腺), 此為一新奇的發現. 並且, 在發育中的斑馬魚胚胎, Prox1 可作為孤獨核接受器(orphan nuclear receptor) 之共同調控蛋白(Liu et al, 2003). 在目前進行之計畫中, 我們初步發現 Prox1 不僅可作為孤獨核接受器 Ftz-F1 之共同調控蛋白, 並且其基因表達可能直接受 Ftz-F1 之調控. 在多處之器官生成過程中, Prox1 之表達集中侷限在已先行表達 Ftz-F1 之細胞群中. 譬如說: 多個斑馬魚 Ftz-F1 基因諸如 *ff1a, ff1b* 及 *ff1d*, 與 *prox1* 基因共同表達在胚胎時期之前神經板. 隨著前神經板之分化, *ff1a, ff1b* 及 *ff1d* 表達於不同之神經組織, 然而 Prox1 仍維持與不同之 *ff1* 基因, 在不同之神經組織中共同表達. *ff1* 之基因表達, 往往開始於器官發生之模型結構形成(pattern formation) 階段, 在時序上早於 Prox1 開始表達之時間. 與以上之基因表達實驗一致的是: 在去年由 Sanger Institute 及 Wellcome Trust 所發表之斑馬魚基因圖譜及序列中, 已顯示 *prox1* 基因之啟動子結構中, 有數個可能的 Ftz-F1 蛋白作用位點. 我們正著手進行在試管內及活體中, 檢驗這些可能的 Ftz-F1 蛋白作用位點, 是否真能被 Ftz-F1 所調控. 綜合以上發現, 我們認為 Ftz-F1 可能可以直接調控其共同調控蛋白(諸如 Prox1 蛋白)之基因表達. 此研究指出一種可能性: 即 Ftz-F1 藉其共同調控蛋白調節下游基因表達之路徑中, 極可能存在正迴饋或負迴饋之機制.

如上所題, *prox1* 之起動子可能被 Fzt-F1 直接調控, 再加上 *prox1* 基因本身擁有之動態的器官及組織特異性表達, 這些證據促使我們有系統的去分析 *prox1* 基因之啟動子如何在胚胎發育過程中被調控, 以致於其基因表達在空間與時間上產生快速的變化. 由於斑馬魚之基因體計畫採用隨機定序法(random sequencing), 無法對應至含特定基因片段之載體, 我們已藉由雜交法篩選得到含斑馬魚 *prox1* 基因及其啟動子區域之 BAC DNA 載體. 目前進行之實驗包括將不同長度或不同定點誘變之斑馬魚 *prox1* 啟動子片段殖入報導蛋白載體, 俾進行更多之基因表達研究. 我們將利用細胞株之瞬間轉染(transient transfection) 及斑馬魚胚胎之轉基因研究來探討 *prox1* 如何被上游基因所調控.

關鍵詞: Ftz-F1; Prox1; 斑馬魚

計畫英文摘要

Homeoprotein Prox1, the vertebrate counterpart of *Drosophilla* Prospero, has received much attention due to its dynamic tissue-specific expression patterns, as well as its essential roles in cell differentiation and organogenesis. In various species, it has been shown to be a molecular marker for neural stem cells, lens and retinal cells, liver tissue as well as lymphatic endothelial cells. My recent published work has further illustrated the novel expression of *prox1* at interrenal tissue (homologue of mammalian adrenocortical tissues), and its coregulating properties for orphan nuclear receptor Ftz-F1, in developing zebrafish (Liu *et al,* 2003). Some of our preliminary results in this ongoing project indicated that Prox1, as a coregulator for Ftz-F1s, could also be a downstream target gene of FtzF1 activities. In multiple aspects of organogenesis, *prox1* expression was shown to be restricted within the Ftz-F1-expressing cell populations. For instance, multiple *ff1* genes including *ff1a*, *ff1b* and *ff1d* were co-expressed with *prox1* in the embryonic anterior neural plate, and the co-localization of *prox1* with each respective *ff1* persists, during the process of differentiation. Various *ff1* genes start to be expressed from the early patterning stage of organ formation, with the timing preceding the onset of *prox1* expression. Consistent with the expression studies, the recently-completed zebrafish genome assembly (announced in 2003 by Sanger Institute and Wellcome Trust) revealed the existence of several putative Ftz-F1 responsive elements in the promoter region of zebrafish *prox1*. We are in the course of examining the Ftz-F1 responsiveness in several *in vitro* and *in vivo* systems. Together with my previous results, the current findings in this project interestingly point out that Ftz-F1 may be able to regulate the expression of its own coregulator proteins. It thus implied the existence of a feedback loop for the modulation of Ftz-F1-mediated gene expression.

The putative Ftz-F1 responsiveness of *prox1* mentioned above, along with its novel organ- and tissue-specific expression, further prompt us to explore how *prox1* promoter is spatially as well as temporally regulated in the developing zebrafish embryos. Due to the unavailability of individual clones from the random sequencing genome program, we have proceeded to obtain a zebrafish BAC clone containing the genomic DNA of *prox1* and its promoter region, through a hybridization screening. The ongoing efforts include the subcloning of zebrafish *prox1* BAC clone into various reporter constructs, in serial deletional and site-directed mutagenized forms, for expression studies. We aim to explore the regulatory properties of *prox1* promoter utilizing transient transfection as well as stable transgene approaches.

Key words: Ftz-F1; Prox1; zebrafish

本研究工作之背景及目的

The conserved expression patterns of Prox1 among various vertebrate species

Prox1 is a vertebrate homologue of *Drosophilla* transcription factor *prospero*. In the mouse, *Prox1* is expressed in many of the same tissues including young neurons of the subventricular region of the CNS, developing eye lens and retina, and heart (Oliver et al, 1993). Also, *Prox1* is an early specific marker for the developing liver and pancreas in the mammalian foregut endoderm (Burke & Oliver, 2002). In various species including murine and humans, Prox1 has been shown to be a very specific and reliable marker for lymphatic endothelial cells, and can be used to distinguish from blood vascular endothelial cells (Petrova et al, 2002; Wigle et al, 2002; Wigle and Oliver, 1999).

The functions of *Prox1* as revealed by *prox1* knock-out mice

Consistent with its expression patterns, *Prox1* has been shown to be required in the formation of developing organs or tissues including neural stem cells, lens and retina, liver and lymphatic system. The targeted disruption studies in mice demonstrated that Prox1, together with transcription factor Mash-1, delineate early steps in differentiation of neural stem cells (Torii et al, 1999). The inactivation of *Prox1* in mouse also result in defected lens fiber elongation and eventually hollow lens, suggesting that the progression of terminal fiber differentiation is dependent on *Prox1* activity during lens development (Wigle et al, 1999). While hepatocyte migration during liver development requires *Prox1* (Sosa-Pineda et al, 2000), the role of *Prox1* in pancreas development remains unclear.

Prox1 is not only a specific marker for lymphatic endothelial cells, but also a required regulator of the development of the lymphatic system (Wigle et al, 1999). *Prox1* is expressed in a subpopulation of endothelial cells that, after budding from veins, give rise to the mammalian lymphatic system. In *Prox1*-knock out mice, this budding becomes arrested and results in embryos without lymphatic vasculature (Wigel et al, 2002). Interestingly, overexpression of *Prox1* in human primary blood vascular endothelial cells was capable of inducing gene transcription specific for lymphatic endothelial cells, indicating a lymphatic endothelial reprogramming of vascular endothelial cells (Petrova el al, 2002). Therefore, these results suggested that a blood vascular phenotype is the default fate of budding embryonic venous endothelial cell; upon expression of *Prox1*, these budding cells adopt a lymphatic vasculature phenotype.

The gene regulation of *prox1* in zebrafish

Although the developmental functions of *Prox1* have been extensively investigated in the knockout mice system, its biochemical properties remain unclear. I have characterized the novel function of Prox1 as a nuclear receptor coregulator in zebrafish (Liu et al; 2003), which is suggested to occur in a tissue/organ specific manner. The relevance of this finding to the role of Prox1 in the mammalian system awaits further studies. Nevertheless, the tissue/organ specificity of Prox1 displayed in its expression pattern, phenotypic significance as well as biochemical role prompt us to study how its promoter is dynamically regulated in development.

最近研究成果及討論

The conserved and novel expressions of *prox1* in zebrafish

The expression patterns of *prox1* in zebrafish bear high degree of resemblance with those discovered in other vertebrates. Antibodies raised against human *Prox1* cross-react with zebrafish Prox1 and are capable of detecting developing tissues including prospective lens placode, a stripe along the forebrain-midbrain boundary, glial cells in the ventral hindbrain, maturing muscle pioneer cells and superficial muscle cells (Glasgow & Tomarev). In our recent study, additional sites of *prox1* expression were discovered, including liver and interrenal primordia (Fig. 1, unpublished data; Fig. 2, adapted from Liu et al, 2003), by using antisense *prox1* mRNA as probes. We have been able to demonstrate that *prox1* is co-localized with orphan nuclear receptor *ff1b* at the developing interrenal cells, with the onset of expression concurrent with the initiation of cell differentiation (Fig. 2). So far, the expression of *prox1* at zebrafish interrenal cells appear to be novel, and the conservatism of this adrenal/interrenal expression has not been discovered in other vertebrate species. The expression of *prox1* at liver is however conserved among the vertebrates.

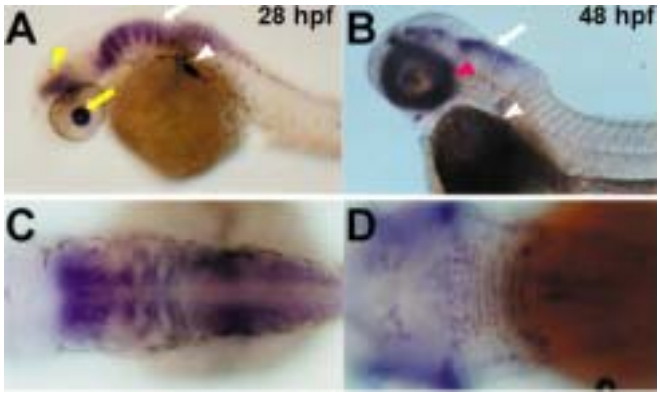


Fig. 1 The gene expression of *prox1* at 28 hour post-fertilization (hpf, panels A & C) and 48 hpf (panels B & D) of zebrafish embryogenesis, using antisense RNA as probes. Anterior is to the left in all panels. A and B show lateral views while C and D are dorsal top views. yellow arrowhead: stripe along fore-mid brain barrier; white arrow: segmented glial cells; white arrowhead: liver primordium; yellow arrow: lens; red arrowhead: retina. ***unpublished data***

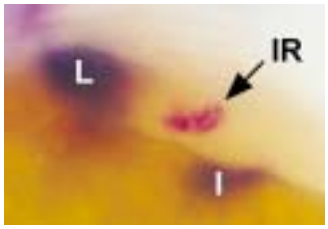


Fig 2. The colocalization of *prox1* (dark blue) and *ff1b* (red) in the developing interrenal primordium (IR). *Prox1* is also detected at liver (L) and intestinal (I) cells. The picture is a magnified lateral view of a 48 hpf embryo, with anterior to the left. Note that liver and intestinal primordia arise immediately above the yolk sac. ***adapted from Liu et al, 2003***.

A two-color fluorescence microscopy study revealed that *prox1* is majorly expressed at the inner zone of *ff1b*-expressing interrenal cells, while the transcripts are barely detectable at certain cells along the marginal zone (Fig.

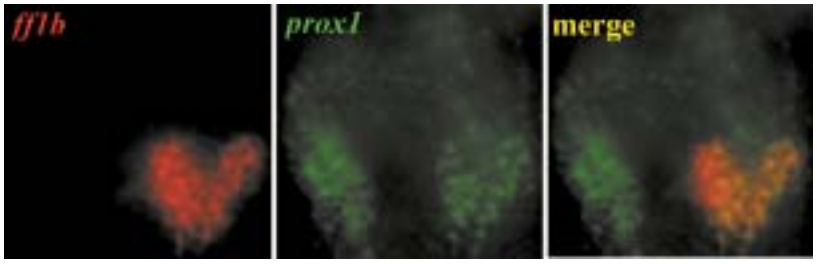


Fig. 3 The fluorescence microscopy showing the expression of *ff1b* (red) and *prox1* (green) transcripts in a 76 hpf embryo. Yellow indicates the merge signals of *ff1b* and *prox1* at interrenal cells. The embryo is oriented as ventral side facing the reader. Only the trunk region is shown, with anterior to the upper side. ***unpublished data***

3, unpublished data). The non-*prox1*-expressing interrenal cells may thus reflect a differential status of differentiation, from the *prox1*-expressing ones. Indeed, the knock-down of Prox1 protein by antisense-morpholino oligonucleotide approach led to reduced size and defected morphogenetic movements of interrenal primordium. The degree of "sprouting" of developing interrenal primordium displayed a significant decrease in *prox1* morphants (Fig. 4, unpublished data).

Fig. 4 The embryos injected with antisense morpholino oligo against *prox1* can be classified into either class A (panel B) or class B (panel C), according to the relative severities of phenotypic perturbations. The wild type control is shown in A.

So far, we have yet to display any evidence for the expression of *prox1*at lymphatic endothelial cells, using conventional *in situ* hybridization methods. The formation of lymphatic phenotype remains enigmatic in this newly-emerged model organism, although a secondary vascular system have been found to exist in fish, which was hypothesized to be an evolutionary predecessor of the lymphatic system (Vogel & Claviez, 1981; Steffensen & Lomholt, 1992). This secondary circulation functions presumably in skin respiration, osmoregulation and immune defense. We expect that the high detection sensitivity of transgenic reporters, such as green fluorescence or β-galactosidase, which are directed by *prox1* promoter would allow us to explore the plausible existence of lymphatic endothelial cells in zebrafish.

Prox1 is a coregulator for Ftz-F1

Zebrafish *ftz-f1* gene, *ff1b*, has been identified to be a homologue of mammalian SF-1, and the earliest known molecular marker for teleost interrenal development (Chai et al., 2000; Chai et al., 2003). The expression of *ff1b* precedes the onset of steroidogenic identity during the ontogeny of the interrenal. Disruption of the *in vivo* gene activity of *ff1b* by antisense morpholino knockdown produced larval morphological phenotypes that are suggestive of impaired interrenal function, implicating a direct involvement of *ff1b* in zebrafish interrenal development. These results from zebrafish are highly reminiscent of findings from mouse SF-1 knockout studies, in which SF-1 null mice demonstrated a complete lack of adrenal development (Luo et al., 1994; Sadovsky et al., 1995; Shinoda et al., 1995).

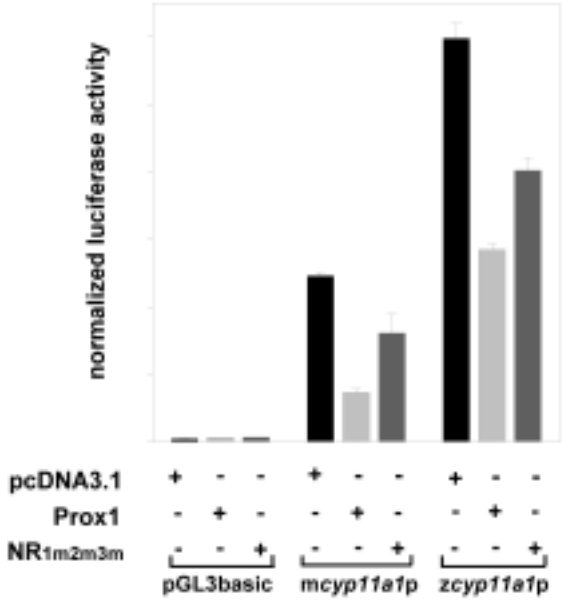


Fig. 5 Prox1 represses the promoter activities of mouse and zebrafish *cyp11a1* in adrenocortical Y1 cells. ***unpublished data.***

Interestingly, we have discovered the physical molecular interactions between Prox1 and various forms of Ftz-F1, including Ff1b, *in vitro* as well as *in vivo* (Liu et al, 2003). In the developing embryos, Prox1 is capable of

down-regulating Ftz-F1-mediated transactivations. The coregulating abilities of Prox1 have been examined, through both deletional- and site-directed mutagenesis, to be determined by three NR box motifs (LXXLL) present on Prox1 protein structure. Our studies have thus imposed a new biochemical function to Prox1, beyond its properties as a homeobox-binding protein. However, the repertoire of gene expressions modulated through Prox1 coregulation of Ftz-F1 remains unclear till date.

In Y1 cells, which are derived from adrenalcortical tumor, Prox1 overexpression is capable of significantly down-regulating the promoter activities directed by either mouse or zebrafish *cyp11a1*. Prox1 site-directed mutants with amino acid substitutions at nuclear receptor (NR) boxes, and thus lost the physical binding abilities to NR, could partially revert the repression as it was overexpressed in place of wild-type Prox1. I thus speculate that at least part of the repressing effects of Prox1 upon *cyp11a1* promoter was resulted from the physical interactions of Prox1 with Ftz-F1, which should be confirmed later by chromatin immunoprecipitation method. The results would help us to understand whether the repression was mediated through a primary or secondary pathway.

<u>*prox1* gene expression being a direct target of Ftz-F1: preliminary evidences</u>

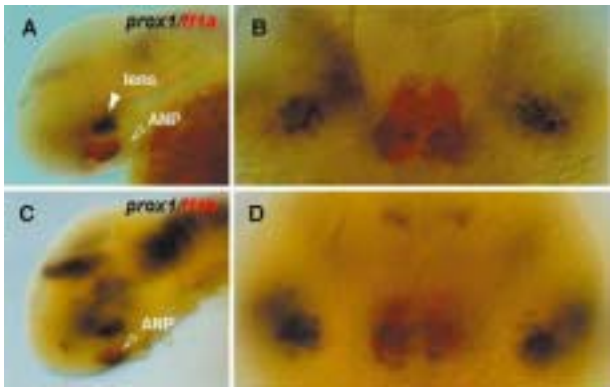
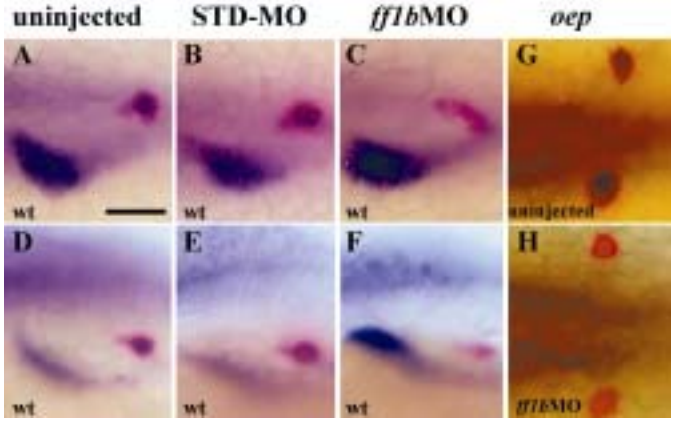


Fig. 6 The co-localization of *prox1* (dark blue stain) and *ff1a* (A, B; red stain) or *ff1b* (C, D; red stain) at the anterior neural plate (ANP). A and C are ventral views with anterior to the left, while B and D are frontal views. Embryos are at the stages of either 28 hpf (A, B) or 30 hpf (C, D). white arrowhead: lens; blank arrowhead: ANP. ***unpublished data***

The colocalization of *prox1* with *ftz-f1*, in various aspects of organogenesis, follows the same trend that *ftz-f1* expression precedes the occurrence of *prox1* transcripts. And in some cases, *prox1* displays a more restricted expression pattern than that of *ftz-f1*, in the same organ or tissue. For example, two *ftz-f1* forms, *ff1a* and *ff1b*, are co-localized at the anterior neural plate (ANP) from about 24 hpf onwards till 30 hpf. The *prox1* transcription initiated at ANP from approximately 28 hpf, demarcating a restricted anterior subset of *ff1a*/*ff1b* expressing cells (see Fig. 5 below, unpublished data). Similar co-localizing relationships between *ftz-f1* and *prox1* have also been demonstrated in developing liver and interrenal tissue (Liu et al, 2003), indicating that Prox1 could act downstream of Ftz-F1 in development.



With a later onset of expression than *ftz-f1* in morphogenesis, *prox1* transcripts could be specifically downregulated while the levels of Ftz-F1 protein are inhibited *in vivo*. Previously we have shown the absence of *prox1* expression at interrenal cells, while Ff1b translation was knocked-down by antisense morpholino

Fig. 7. *ff1b*-MO perturbs *prox1* expression at the interrenal but not liver. Either (B, E) STD-MO or (C, F) *ff1b*-MO was injected into *wt* and (G, H) *oep* embryos at 1-cell stage (0.8 pmole per embryo). Uninjected and injected embryos were fixed at 31 hpf and *in-situ* hybridization was carried out using *prox1* (blue) and *ff1b* (red) as probes. The apparently strong liver *prox1* staining in (C, F) *ff1b*-MO injected embryo resulted from our effort to highlight the thin layer of *ff1b*-expression cells. Scale bar represents 50 µM. ***adapted from Liu et al, 2003.***

oligos (see Fig. 6 below, adapted from Liu et al, 2003). In this case, *prox1* expressions at other non-*ff1b*-expressing tissues, such as liver and ANP, remained unperturbed. Taken together, it is hypothesized that *prox1* could be a direct downstream target of Ftz-F1 proteins. Hence we expect the presence of Ftz-F1 responsive elements upon the promoter structure of *prox1*.

The promoter and genomic structures of *prox1*: putative presence of Ftz-F1 responsive elements

5′-GCTTTAAAGAGCCGATTGACAACAACAGTGAACTTGAGAACCTATAATGTCCTAACATAACTTTTTTTAAACTACAAAA
AGCCTCTTAATAAGAACATGTTTTTTTTCTGAACCTAAGGTGCTACCATTGATCAACCTTTATTTTTATAAACGTAGGCTTA
TACTTAGCATCTCACTTTCACTGCCACTACAGCTTGACGCGTTACTTCATCATCATCATCGTTTCATGACGTGAGAGTCAGA
TAGACATCAAAGCTCTAGTTTACGCGCACATAAGATTCAAAGATTTGCCGACATATACGTAAAGTCGGTTTTAGATGTTTAT
TGTCAAAATGAATGCCACGCACGTGGTTGTTGATAAAAATATTTTTGGTTTGATGATCAGTGTATCACTGTGCTCCTACTAA
AGTGATGCGACCAGGGTTTGGTGGAGACAATATACATATGATAGCATAATTTTCAAAGGTTTTTATGACTTGTCGAAACTAA
TCTGAAATTTCTTAACTAACAAAATTTTAAAATAATTTTTAAATGTTTCAGAAGTTTTTTTTTTTTTTTTTTTTTTGTTT
GTTTTTTGGAGTTGTAATGCTTTCCAGGGCTCTTTAAGTTGGCTGAGTGAGGGTTCTTGGGTTTTTAAATGTTTAAAACTAC
TATTTCCTCGAACTTGCTGGTGGGCACATACATGTTTAAGCTATTTAACGCTTCAACTGCGTTATCCGATTTATGTTCACAT
GAGTGTGATTGAATTAGTTTAATTTTCTTACTTTGACAACTTTTTCGGCTTTAAGCTGAACGGCTTTAAATATTTGGCGAGA
AGAGGTGCGCATAGGACGCAGTGTCCGTATGAGGTGAAAGGCATCCAAAACGAACAGATTTTGTTGCCATTTTGTCTTGATT
TGTCAGATGTCTATATTTTGAGACAGCTAAAGCAAGTTGTCGCTGTATTTTATACCCTAAATGAAAACCACTGACAACCTTA
ATCGGTGCGTCTTGAAGAACAATTAAAGTTATGTCTCACAAGGTGCATGTCTCAGAATTTTTTTTCTAATAATAACGACTGT
TAACAACTCTTTATCATGTAGCCGTACTGATGATAATAAATGCACTGTAACATTATATTATTACACGTATATTTTATCAAAA
ACTTTTATATTTGTATGTTCTGGAGAAGTAGTTGCGTTAATTACTATATGCTTTGCAAAACTGTGCACCTTAGTCATGCGTG
AGATGATGTGCCTTTTTAATGTGCGTTACATTCCCAATTAGCAAAGCTAGATATTAGCTATGTTTTTTTTTTCTAACGTGCA
TGGTGAGCTCAAACTTTTTAAAATGTGATTTAAGATTAGGCTAATAACGTTGACCGATCTCCTTCAACAAACTTGCAAAACG
TGCAATTAGCGTTTATTACACTGCATTACACACAAAAAGGAGTTATCTTTGAATTCAATTCATTAACCCTGAATTTAATTTG
TGTGGTGTATTATACTTGTTGTATAATTTTAATTACATGGGAAGTAGGCAGTTAATCTTTAAAAGATAATATTGAGTGTCTT
TATTTGCTAAGTGTCTTTGCAAATATCAACTGTAGCTGTTTCTTTGGCAAATGGTACATCGACACTTACCAAAGTGAACAAA
GCCTGCAAGTTGGCTGTGTGAGCACAATAGTGGCAGGACATGTCTAGCTAAACACAGCAATTTGATTTTTAAATGGGGTCTT
ACATGAGAATTAGACTTTTGGCATGCAGCCTTTTGGTTGATTATTTAATAAATGTTGTAATGACAACTTTGTAACTGACTTT
AATACAAATACATTTTAAGCTTTTTGTCAGTTTCTTTTAAAATACTGTAGGCCTAGCATTTTAAGCATTTTTAATCTTGTGT
GATGTTGTCTAAGACATTAGCCTGTTCAAATAGCATTTAGAATTGGGCACAAGAAACTTGGAGACATATAAAGAAAATTATT
CATCAGTCCTTTCTTTCTGGCTAGGTTCCCAGGGTTCTCGAGCTGTGCCCAGCTGACAGGCTTTCGAAGATGGCACAATAAC
AGTTCCAGTGATG
Met

Fig. 8 The putative promoter region of *prox1* gene. The predicted untranslated region and translation start site are highlighted by blue and red fonts respectively. The three putative Ftz-F1 responsive elements predicted by TESS program were shadowed as orange boxes. The putative TATA box is underlined.

Zebrafish *prox1* locus has been determined by Postlethwait, J. *et al* to be on linkage group 17 (ZMAP). The 5'-flanking region of *prox1* contained three putative Ftz-F1 responsive elements (FRE), as predicted by Transcription Element Search System (TESS), among the 2 kb genomic sequence preceding the translation initiation site (see table below). Thus, it is of interest to investigate whether these putative FREs are capable of physically interacting with Ftz-F1, including Ff1b. As the mapping efforts of zebrafish genome have been completed through random-sequencing strategies, and individual clones are thus unavailable, we have gone on to obtain a BAC clone containing *prox1* gene and its 5'-flanking region, through a hybridization-based screening. Sequencing efforts have been undertaken to verify the genomic DNA contents of the BAC clone.

For analyzing the Ftz-F1 responsiveness, various lengths or site-directed mutants of *prox1* promoter will be fused to luciferase reporters and subsequently introduced into adrenocortical cells through transient transfections. Alternatively, *prox1* promoter constructs and various Ftz-F1 genes can be either co-transfected into non-adrenal cells or coinjected into zebrafish embryos for the subsequent quantitative reporter assays.

Perspectives

The gene expression of *prox1*, as a tissue- and cell-type specific homeobox gene, could be regulated in a highly organized and coordinated fashion. Our current findings suggested that Ff1b could regulate the expression of *prox1* in organogenesis in a direct manner. However, the dynamic expression of *prox1* in the non-*ff1b*-expressing tissues implied that FREs could be important, but not sufficient for controlling the tissue-restrictiveness of *prox1* expression. Hence, to understand the molecular mechanisms that control the tissue-specific developmental expressions of zebrafish *prox1*, cis- as well as trans- factors upon the isolated *prox1* promoter will be systematically analyzed, within various temporal as well as spatial compartments.

As a platform for unraveling the mechanism that is involved in the dynamic tissue-specific expressions of *prox1*, the isolated *prox1* promoter will be fused to fluorescent as well as colorimetric reporters. The *prox1* promoter-reporter constructs will then be injected into one-cell embryos of zefrafish. Subsequently, the transgenic expressions of live fish will be observed *in situ* through microscopic methods. We expect to obtain more histological details from this transgenic approach, as compared with the previous *in situ* hybridization methods, in defining the gene expressions of *prox1*.

In the near future, we will also set out to screen stable lines harboring transgenic *prox1* gene, from the F1 offsprings. We will compare how various lengths or site-directed mutants of *prox1* promoter will differentially contribute to the tissue-specific expression patterns. Furthermore, the generated transgenic zebrafish lines are expected to serve as powerful tools in exploring specific processes of organogenesis, especially in the developing retinal and lens cells, glial cells, liver, adrenocortical tissues and slow muscle pioneers. We are particularly interested to establish this transgenic system as a model for dissecting molecular mechanisms

or pathways, in the etiology of liver as well as adrenocortical diseases.

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計畫成果自評

The current results of this project have provided interesting evidences concerning the auto-regulation mechanisms of nuclear receptor coregulation events. The well-orchestrated processes could take place in specific aspects of organogenesis such as the formation of adrenal/interrenal gland. The findings in this project will bear high potential to be published, for extending current knowledge in the fields of nuclear receptor biology, molecular endocrinology as well as developmental biology.

As this project has been my first one after coming back to Taiwan from overseas, the grant support and the implementation process have helped me tremendously in establishing the in-house systems for biochemistry, molecular biology and developmental biology studies. It is no doubt that the coming second year of this project will prove to be even more fruitful with plenty of significant findings coming on the way.