計畫編號：BM06-00
計畫名稱：探討哺乳類生殖系統的功能蛋白和醣質
計畫主持人：陳義雄
計畫摘要(中)：

哺乳類精子從副睪尾部 (caudal epididymis) 射出後，必須於生殖道適時適地修飾，才會有能力到達輸卵管和卵作用而授精。這個過程自 1950 年代就被認知，通稱獲能作用 (capacitation)。在自然狀況下於子宮腔道中發生。隨後，因為人工方法可於試管中誘導獲能。一些獲能關聯的生化事件 (capacitation-related biochemical events) 陸續被發現，但引發獲能的分子機理，至今仍然不清楚。十幾年前發現獲能會促進精子內一群蛋白的酪胺酸磷酸化 (tyrosine phosphorylation)。之後，這一個生化反應常作為獲能的最重要指標。研究這些蛋白的生理功能有其重要意義。以蛋白質體學的策略和方法鑑別這一類蛋白 (tyrosine-phosphorylated protein)，雖然有些進展，但引發這個生化反應所需激酶 (tyrosine kinase) 至今不明。在這一方面的研究，我的研究群首先發現了精子內的 CCCTC-binding nuclear factor (CTCF) 於獲能後會產生酪胺酸磷酸化；但在精子獲能前，則在絲胺酸/羥丁胺酸磷酸化。自從獲得拔尖計畫的研究補助 (執行期：2006 年 8 月至 2008 年 7 月)，我的研究團隊從 2006 年 10 月繼續了 CTCF 的研究。綜合過去一年來檢視 CTCF 於獲能引發的酪胺酸磷酸化，有無影響該轉錄因子對 17 個標的基因順序(target DNA sequences) 的結合，有了兩個重要的發現：(一) 不論被 CTCF 活化或抑制的基因，CTCF 被 tyrosine phosphorylation 後，結合增強；(二) CTCF 被 tyrosine phosphorylation 後，對甲基化 (methylation) 的標的基因，結合力加強。預期於不久的將來，可將這些結果具體化，發表於國際期刊。基於過去不錯的研究進展，希望往後 3 年 (執行期：2008 年 8 月至 2011 年 7 月) 可以繼續得到拔尖計劃探討三個子題：(a) 評估 tyrosine-phosphorylated CTCF 在受精卵發育時的表遺傳調控 (epigenetical regulation)；(b) 決定 CTCF 分子 (762 胺基酸) 內酪胺酸的磷酸化位置；(c)評估精子的 insulin growth factor receptor (IGFR) 或/和 epidermal growth factor receptor (EGFR) 的酪胺酸激酶 (tyrosine kinase) 活性是否引發獲能關聯的 protein tyrosine phosphorylation；(d)以 STZ-diabetic rat 為實驗動物評估其 IGF-1 system 是否失調而減低精子內和 capacitation 有關的 tyrosine phosphorylation。

計畫摘要(英)：

The fertile condition of mammalian spermatozoa is not a terminal condition but a transient one. Upon ejaculation, spermatozoa discharged from the caudal epididymis must undergo cell modification in the reproductive tract at the right place and on the right time before sperm-egg encounter. Generally, the sperm modifications proceeded in the female reproductive tract to acquire the fertile ability are collectively called “capacitation” that was stated in 1950’s. Of the biochemical events that have been found to associate with sperm capacitation, an increase in the tyrosine phosphorylation of a subset of spermatozoal protein has been discovered only one decade ago. Subsequently, a very effort has been made to identify these tyrosine-phosphorylated proteins that become a prerequisite in order to understand the significance of such a biochemical event in mammalian reproduction. On the other hand, it remains obscure about what kind of tyrosine kinase(s) is responsible for the phosphorylation. In this regard, we are the first to demonstrate recently the tyrosine-phosphorylated CTCF, a CCCTC-binding nuclear factor, in the capacitated mouse sperm. Further, we have made very effort to continue the study on CTCF after the receipt of an approval notice for a grant from Excellent Research Projects of National Taiwan University (ERPNTU) in Oct/2006 (executive period: Aug 1/2006 ~ July 31/2008). We examined the impact of capacitation-related tyrosine phosphorylation on the binding of CTCF to 17 target DNA sequences. We summarize our results from the last year’s study to manifest that: (a) the tyrosine phosphorylation enhances the binding of CTCF to the target sequence of both its activated genes, and its inactivated genes; (b) The binding ability of CTCF from capacitated sperm to any target DNA sequences enhances once they are methylated. We anticipate solidifying the data and publishing the paper in the near future. Here, we submit a research proposal to seek the support from ERPNTU (executive period: Aug 1/2008 ~ July 31/2011). Based on our promising progress, we are

planning to focus on three research aspects: (a) assessing the role of tyrosine-phosphorylated CTCF in the epigenetical regulation in the development of fertilized egg; (b) determining the tyrosine-phosphorylated site(s) in the CTCF molecule that contains 762 amino acid residues; (c) assessing whether the kinase activity of insulin growth factor receptor (IGFR) or/and epidermal growth factor receptor (EGFR) of spermatozoa is responsible for the capacitation-related tyrosine phosphorylation;(d) using STZ-diabetes rat as experimental animals to assess the deregulation of IGF-1 system in the suppression of tyrosine phosphorylation in the capacitated sperm.

計畫編號：BM06-01
計畫名稱：探討精子獲能引發 CTCF 和酪胺酸激酶受體 (tyrosine kinase receptor) 磷酸化的生殖意義
計畫主持人：陳義雄
計畫摘要(中)：

哺乳類精子從副睪尾部 (caudal epididymis) 射出後，必須於生殖道適時適地修飾，才會有能力到達輸卵管和卵作用而授精。這個過程自 1950 年代就被認知，通稱獲能作用 (capacitation)。在自然狀況下於子宮腔道中發生。隨後，因為人工方法可於試管中誘導獲能。一些獲能關聯的生化事件(capacitation-related biochemical events) 陸續被發現，但引發獲能的分子機理，至今仍然不清楚。十幾年前發現獲能會促進精子內一群蛋白的酪胺酸磷酸化 (tyrosine phosphorylation)。之後，這一個生化反應常作為獲能的最重要指標。研究這些蛋白的生理功能有其重要意義。以蛋白質體學的策略和方法鑑別這一類蛋白(tyrosine-phosphorylated protein)，雖然有些進展，但引發這個生化反應所需激酶 (tyrosine kinase) 至今不明。在這一方面的研究，我的研究群首先發現了精子內的 CCCTC-bindingnuclear factor (CTCF) 於獲能後會產生酪胺酸磷酸化；但在精子獲能前，則在絲胺酸/羥丁胺酸磷酸化。自從獲得拔尖計畫的研究補助 (執行期：2006 年 8 月至 2008 年 7 月)，我的研究團隊從 2006 年 10 月繼續了 CTCF 的研究。綜合過去一年來檢視 CTCF 於獲能引發的酪胺酸磷酸化，有無影響該轉錄因子對 17 個標的基因順序(target DNA sequences) 的結合，有了兩個重要的發現：(一) 不論被 CTCF 活化或抑制的基因，CTCF 被 tyrosine phosphorylation 後，結合增強；(二) CTCF 被 tyrosine

phosphorylation 後，對甲基化 (methylation) 的標的基因，結合力加強。預期於不久的將來，可將這些結果具體化，發表於國際期刊。基於過去不錯的研究進展，希望往後 3 年 (執行期：2008 年 8 月至 2011 年 7 月) 可以繼續得到拔尖計劃探討三個子題：(a) 評估 tyrosine-phosphorylated CTCF 在受精卵發育時的表遺傳調控 (epigenetical regulation)；(b) 決定 CTCF 分子 (762 胺基酸) 內酪胺酸的磷酸化位置；(c)評估精子的 insulin growth factor receptor (IGFR) 或/和 epidermal growth factor receptor (EGFR)的酪胺酸激酶 (tyrosine kinase) 活性是否引發獲能關聯的 protein tyrosine phosphorylation；(d) 以 STZ-diabetic rat 為實驗動物評估其 IGF-1 system 是否失調而減低精子內和 capacitation 有關的 tyrosine phosphorylation。

計畫摘要(英)：

The fertile condition of mammalian spermatozoa is not a terminal condition but a transient one. Upon ejaculation, spermatozoa discharged from the caudal epididymis must undergo cell modification in the reproductive tract at the right place and on the right time before sperm-egg encounter. Generally, the sperm modifications proceeded in the female reproductive tract to acquire the fertile ability are collectively called “capacitation” that was stated in 1950’s. Of the biochemical events that have been found to associate with sperm capacitation, an increase in the tyrosine phosphorylation of a subset of spermatozoal protein has been discovered only one decade ago. Subsequently, a very effort has been made to identify these tyrosine-phosphorylated proteins that become a prerequisite in order to understand the significance of such a biochemical event in mammalian reproduction. On the other hand, it remains obscure about what kind of tyrosine kinase(s) is responsible for the phosphorylation. In this regard, we are the first to demonstrate recently the tyrosine-phosphorylated CTCF, a CCCTC-binding nuclear factor, in the capacitated mouse sperm. Further, we have made very effort to continue the study on CTCF after the receipt of an approval notice for a grant from Excellent Research Projects of National Taiwan University (ERPNTU) in Oct/2006 (executive period: Aug 1/2006 ~ July 31/2008). We examined the impact of capacitation-related tyrosine phosphorylation on the binding of CTCF to 17 target DNA sequences. We summarize our

results from the last year’ s study to manifest that: (a) the tyrosine phosphorylation enhances the binding of CTCF to the target sequence of both its activated genes, and its inactivated genes; (b) The binding ability of CTCF from capacitated sperm to any target DNA sequences enhances once they are methylated. We anticipate solidifying the data and publishing the paper in the near future. Here, we submit a research proposal to seek the support from ERPNTU (executive period: Aug 1/2008 ~ July 31/2011). Based on our promising progress, we are planning to focus on three research aspects: (a) assessing the role of tyrosine-phosphorylated CTCF in the epigenetical regulation in the development of fertilized egg; (b) determining the tyrosine-phosphorylated site(s) in the CTCF molecule that contains 762 amino acid residues; (c) assessing whether the kinase activity of insulin growth factor receptor (IGFR) or/and epidermal growth factor receptor (EGFR) of spermatozoa is responsible for the capacitation-related tyrosine phosphorylation;(d) using STZ-diabetes rat as experimental animals to assess the deregulation of IGF-1 system in the suppression of tyrosine phosphorylation in the capacitated sperm.

計畫編號：BM06-02

計畫名稱：睪丸發育過程轉穀胺醯氨酶摧化之 polyamine 修飾所扮演的角色

計畫主持人：張震東

計畫摘要(中)：

轉穀胺醯氨酶摧化之反應基本上是以酵素 Cys 的 thiol group 去攻打 Gln 上的 carbonyl group，NH3 離開，形成 Thioester bond。而此 Thioester bond 可能接受另一蛋白質上 Lys 的 ε-amino group 攻擊形成 Isopeptide bond 而酵素離開。此 Thioester bond 亦可接受另一個一級氨、水分子或者醇類的攻擊。因此此酵素作用的反應為 Transadmidation; 造成蛋白質之聚合、Amine incorporation 造成 polyamines 共價結合上蛋白質(polyamine conjugation) 或 Esterification 造成 Gln 轉換成酸酯、Deamidation 造成 Gln 轉換成 Glu、及 Isopeptide cleavage 造成聚合蛋白質的分開。其主要功能在於腫瘤形成及轉移、凝血、瘍口癒口、調控細胞自戕死亡、神經傳導物質釋放、訊息傳導及細胞間質形成等。轉穀胺醯氨酶之功能必需透過受質之鑑

定以及 Transamidation 造成之影響才得以瞭解。本實驗室以新穎方法來純化及鑑定轉穀胺醯氨酶之受質，活化小鼠(>10 周齡)之肝臟及睪丸其轉穀胺醯氨酶酵素活性，並純化出受質，通過質譜分析鑑定受質的身分，最後以免疫轉漬法以確認受質之真正身分。在超過一百個鑑定受質中，大概屬下列性質蛋白；細胞骨架及其調節蛋白、Chaperones 及 Co-chaperones、內質網蛋白、細胞 Detoxification 用蛋白、蛋白質轉譯調節蛋白等，大多是與細胞壓力反應有關之蛋白。本計畫擬以小鼠睪丸為研究對象，調查 tTG 受質經 polyamine conjugation 造成之影響。假設 polyamine conjugation 造成之影響有：酵素之活化或去活化、蛋白質交互作用能力改變、及細胞分佈改變，計對上述可能影響我們設計實驗來逐一探討。實驗項目包括：建立研究 polyamine-conjugation 之方法學, 自小鼠睪丸純化 polyamine-binding proteins , 檢查 in vitro polyamine-conjugation 對特定酵素功能之影響, 建立研究 tTG 及 polyamine-conjugation 於睪丸功能之動物模式; 一是在小鼠青春期前給予 tTG 抑制劑(Cystamine),觀察其對小鼠睪丸發育之影響, 另一是於小鼠青春期前後施以化療藥物 Dorxorubicin 造成對睪丸的氧化壓力,並探討 tTG 及 polyamine-conjugation 在此的作用.

計畫摘要(英)：

The roles of polyamine-conjugation catalyzed by transglutaminase in mouse testis development

Transglutaminases (TG) are Ca2+-dependent enzymes which catalyze a post-translational modification of proteins. The enzyme reaction leads to the formation of an isopeptide bond either within or between polypeptide chains. The γ-glutamyl-ε-lysine crosslinks are formed between the γ-carboxamide group of peptide-bound glutamine residues and the ε-amino group of peptide-bound lysine residues. Polyamines can replace lysine residue in the transamidation reaction in vitro and in vivo. In order to understand the physiological functions of tissue transglutaminase (tTG or TG2), one needs to identify the acyl donor and the acyl acceptor substrates in the transamidation reaction. To this end, we have identified over 70 potential transglutaminase substrates from mouse liver extract and over 100 potential substrates from mouse testis extract by substrate purification and tandem Mass Spectrometry analysis. Five groups of substrates were disclosed; cytoskeleton proteins,

proteins involved in ER stress response, molecular chaperones, proteins involved in redox regulation and proteins involved in stress-induced translation arrest. Most of the tTG substrates are intracellular proteins with functions related to cellular response to stress. Importantly, many of the tTG substrates have not been reported.

計畫編號：BM06-03
計畫名稱：合成卵子表面醣結構的醣轉化酶及其表現機制的研究
計畫主持人：余榮熾
計畫摘要(中)：

本研究計畫的目標，要證明並鑑定負責建構卵子表面醣結構的專一性醣轉化酶，同時進一步的闡明這些醣轉化酶在卵巢，特別是在卵子形成過程中，的表現機制。

初步的授精作用經由精子結合於卵子上的 zon pellucida (ZP)，此結合已證明需要特定的醣結構。絕大部分連結於 ZP 的醣結構，帶有 type 2 LacNAc（Gal 1-4GlcNAc）醣鏈結構。許多這些修飾過後的尾端醣結構，已被證明和精卵的辨識結合有關。另外，type1 LacNAc（Gal 1-3GlcNAc）醣鏈結構也被發現存在於 ZP 之上。另外，最近發現 ZP 上,存在有相當量的 Sda 醣抗原結構(NeuAc 2-3[GalNAc 1-4]Gal 1-4）。Sda 結構已知僅分布於人類少數幾個器官，例如大腸、胃、腎臟、及紅血球。最特別的是，它們的表現呈現 onco-developmentally regulated 的情形。Sda 醣抗原結構存在於 ZP 上，令人十分好奇它可能的扮演的功能。

雖然這些表現於卵子 ZP 上的醣結構多已被解出；但其負責生成的醣轉化酶，仍然有待進一步闡明。這些可能參與合成的醣轉化酶包括 -1,4-galactosyltransferase ， -1,3-N-acetylglucosaminyl-transferase ， -1,3-galactosyltransferase ， 和 Sda -1,4-N-acetylgalactosaminyltransferase。這些醣轉化酶大都是包含了數個同源基因的醣轉化酶家族。

另外，這些醣鏈另可形成所謂 I branching（Gal 1-4GlcNAc 1-3[Gal 1-4GlcNAc 1-6]Gal 1-4 GlcNAc）的分支結構。I branching 的形成，將使表面醣抗原結構與其相對應的辨識結合分子的 valency 大幅增加，具有重要的生理意義。I branching 由 I -1,6-N-acetylglucosaminyltransferase 負責生成。

過去十年來，本研究室即專注於研究人類的醣轉化酶。我們研究

的醣轉化酶，如 fucosyltransferase、Sda -1,4-N-acetylgalactosaminyltransferase、I -1,6-N-acetylglucosaminyltransferase 等，參與表現於細胞膜上重要醣結構訊息分子的生合成。我們延伸此方面的經驗與知識，來研究負責合成卵子 ZP 上醣結構的醣轉化酶基因。此子計畫希望完成下列研究：（一）闡明建構 ZP 上 type 1 及 type 2 醣鏈的醣轉化酶基因。（二）證明負責 ZP 上 Sda 醣結構的 Sda 4GalNAcT 基因結構。（三）I 醣鏈分支在卵子表面醣結構形成的機制。（四）建立負責 ZP 醣結構生合成的醣轉化酶基因，在卵巢及卵子生成過程中的表現圖譜與機制。

計畫摘要(英)：

It has been demonstrated that the gamete interactions are mediated by the binding of lectin-like sperm proteins with carbohydrate moieties on the zona pellucida (ZP) of eggs. Although the structures of the cglyco-epitopes on ZP have been demonstrated, the identities of the glycosyltransferases that responsible for the formation of these glyco-structures have never been elucidated. The goal of this project is to demonstrate the identities of the glycosyltransferases involved in the synthesis of the glyco-structures on ZP of eggs, and to further elucidate the expression profiles and mechanisms of these glycosyltransferases in ovary and during oogenesis.

Murine sperm initiate fertilization by binding to the outer covering of the egg that known as murine zona pellucida (mZP). This binding is thought to require the interaction of O-glycans linked to a specific mZP glycoprotein (mZP3) with the egg-binding proteins on the sperm plasma membrane. The majority of the O-glycans that are linked to mZP3 has been shown to contain core type 2 sequences terminated with sialic acid, including LacNAc (Gal 1-4GlcNAc), LacdiNAc ([GalNAc 1-4GlcNAc]2), Gal 1-3Gal, and Sda antigen (NeuAc 2-3[GalNAc 1-4]Gal 1-4). Core type 1 (Gal 1-3GlcNAc) O-glycans are also present on the glyco-structures of ZP3.

The glycosyltransferases responsible for the formation of these glyco-structures may include -1,4-galactosyltransferase, -1,3-N-acetylglucosaminyltransferase, -1,3-galactosyltransferase, and Sda -1,4-N-acetylgalactosaminyltransferase. Most of these are known

to comprise a family of homologous glycosyltransferases.

In addition, these core type 1 and type 2 can be further modified to form branching structure, the I branching (Gal 1-4GlcNAc 1-3[Gal 1-4GlcNAc 1-6]Gal 1-4GlcNAc). The formation of the I branching structure leads to the multiple valency of the terminal epitopes built on the branched glyco-chains. The formation of the I branching structure is determined by the activity of I -1,6-N-acetylglucosaminyltransferase.

During the past ten years, our laboratory has focused on the investigation of the human glycosyltransferases participating in the syntheses of several critical glyco-structures expressing on cell surfaces. We will extend our expertise to investigate the identities of the glycosyltransferase genes that involve in the formation of important glyco-structures on ZP of eggs. The specific aims of this component project are to accomplish the following investigation: a) Elucidation of the specific glycosyltransferases gene identities that involve in the synthesis and branching of type 1 and type 2 glyco-structures on ZP. b) Identification of the Sda 4GalNAcT gene structure responsible for the expression of the Sda glyco-epitope on ZP. c) Elucidation of the molecular basis of I branching formation on the glyco-structures of eggs. d) Profiling the expression patterns and investigating the expression mechanisms of the glycosyltransferase genes responsible for modifying ZP glyco-structures in ovary and during oogenesis.

計畫編號：BM06-04

計畫名稱：mp29 基因轉殖鼠是否可以挽救 Atm 基因剔除鼠的精蟲發育不全的缺陷？

計畫主持人：張茂山

計畫摘要(中)：

為了避免在細胞分裂時錯誤的核苷酸配對遺傳給下一代子細胞，同時為了維持 DNA 的完整性防止細胞不正常分裂，真核細胞發展出了縝密的偵測與修補系統，如果正常細胞 DNA 的突變嚴重至難以修補，甚至會啟動細胞凋亡機制來防止不正常細胞有進一步癌化的可能。當 DNA 受到紫外光、輻射線或化療藥物傷害時，會啟動偵測系統 ATM/ATR、Chk1/Chk2 蛋白質磷酸酶對下游受質(如 Cdc25, p53,

and E2F)進行磷酸化作用，藉已停止或延長細胞週期運轉來等待 DNA 被修復或直接活化細胞凋亡機制。天生缺乏 ATM 基因表現的病人在臨床上顯示出神經系統衰退、免疫能力缺乏、對放射線或化療藥物極度敏感有發展成癌症的可能性，這些病人的生殖細胞因有缺陷的減數分裂重組反應因而導致不孕。在 ATM 基因剔除鼠的生殖細胞也看到了有缺陷的減數分裂細胞，伴隨著染色體斷裂的現象，同樣地，ATM 基因剔除鼠也有精卵發育不全的現象。

我們先前的研究顯示人類 p29 蛋白屬於與染色質結合的蛋白質，細胞若轉殖 p29 的短鏈 siRNA 會造成進行 DNA 複製的細胞減少，利用紫外光照射已轉殖 p29 短鏈 siRNA 的細胞，結果會降低 ATM 與 Chk1 的磷酸化，推測缺少 p29 蛋白的細胞無法完成 DNA 複製的先期準備工作，因而減少 ATM 與 Chk1 的磷酸化，顯示 p29 與 ATM 的活化有關；我們與國家動物中心合作已建立 mp29 基因轉殖鼠，為了探討 mp29 基因轉殖鼠是否能補救 ATM 基因剔除鼠不孕的現象，我們將 mp29 基因轉殖鼠與 ATM 基因剔除鼠交配，觀察子代精虫發育情形並研究其可能機制。

計畫摘要(英)：

Maintenance of genomic integrity and protection against harmful mutagenic effects of DNA damage rely on DNA damage response machinery, a complex network of signalling and effector pathways that coordinate cell cycle checkpoints with DNA repair and cell death mechanisms. In response to DNA lesions, the signal transducing kinases, ATM (ataxia telangiectasia-mutated), ATR(ataxia telangiectasia and Rad3-related), Chk1, and Chk2, phosphorylate downstream checkpoint effector proteins, such as Cdc25, p53, and E2F, to regulate cellular responses and ensure error-free DNA replication. In Atm-deficient mice, male and female gametogenesis is severely disrupted as early as leptonema of prophase I with significant chromosome fragmentation, and apoptotic degeneration, indicating recombination defects during meiosis.

Our previous data demonstrated that the phosphorylation of ATM kinase at S1981 was suppressed in p29-depleted HeLa cells with UV irradiation (Cancer Res. 66, 8484-8491, 2006), suggesting a functional relevance between p29 and ATM. Recently, we generated mp29 transgenic mice and preliminary results showed that these mp29

overexpressed mice are less sensitive to UV irradiation compared with wild-type mice. Here, we would like to investigate whether mp29 overexpression could rescue the prophase-I arrest characteristic of Atm-deficient spermatocytes.

計畫編號：BM06-05
計畫名稱：胎盤發育的分子機制 - 缺氧及磷酸化調控 GCMa 活性之探討
計畫主持人：陳宏文
計畫摘要(中)：

GCMa 為胎盤發育所必需的胎盤轉錄因子。具體而言，GCMa 調節 syncytin 基因的表現而產生一融合蛋白質調控胎盤細胞的融合形成一多核滋養葉融合層細胞。滋養葉融合層細胞為母體與胎兒之間氣體及養分交換所必需，而且進行細胞凋零以及藉由細胞之間的融合而補充。因此，在妊娠期間 GCMa 活性的調節對維持滋養葉融合層細胞的結構及功能的完整性極為重要。在之前的研究中我們曾證明 GCMa 的活性可經由乙醯化，泛素化及 sumoylation 等不同後轉譯修飾而調節。GCMa 乙醯化可防止 GCMa 泛素化因而增加胎盤內的 GCMa 活性。反之，GCMa sumoylation 會抑制 GCMa 的 DNA 結合能力因而降低胎盤內的 GCMa 活性。

子癇先兆症影響 6-10%的妊娠且是導致胎兒出生前後發病及死亡的主要原因，其特徵為胎盤發育缺陷造成胎盤組織缺氧。在之前的研究中我們也發現相對於同妊娠週數的正常胎盤，GCMa 蛋白質在子癇先兆症的胎盤中有較低的表現量。我們最近的初步研究結果顯示缺氧狀態下會促進 GCMa 蛋白質降解。另一方面，F-box 蛋白質 FBW2 辨識磷酸化的 GCMa 而與之結合，並促進 GCMa 泛素化及接受 26S proteasome 降解。我們的初步研究結果也已發現在 GCMa 蛋白質上影響 FBW2 促進 GCMa 泛素化的重要 serine 及 threonine 氨基酸。為了進一步瞭解 GCMa 活性的調節機制，本研究計劃將探討(I) GCMa 磷酸化促進 FBW2 控制 GCMa 泛素化的機制及(II)缺氧狀態促進 GCMa 蛋白質降解的機制。本研究計劃得到的結果將有助於瞭解磷酸化如何在胎盤發育時調節 GCMa 的活性以及在子癇先兆症致病機制上缺氧狀態如何促進 GCMa 蛋白質的降解，而且有助開啟治療子癇先兆症的新途徑。

計畫摘要(英)：

GCMa is a placental transcription factor essential for placental development. Specifically, GCMa regulates expression of syncytin gene, which encodes a fusogenic protein mediating cell-cell fusion for syncytiotrophoblast formation in the human placenta. Syncytiotrophoblasts, which are essential for maternal-fetal exchange of gas and nutrient, undergo apoptosis and are replenished by cell-cell fusion. Therefore, regulation of GCMa activity is imperative to maintain the structural and functional integrity of syncytiotrophoblast during pregnancy. We have previously demonstrated that GCMa activity can be regulated by different post-translational modifications including acetylation, ubiquitination, and sumoylation. Acetylation of GCMa prevents GCMa from ubiquitination and thereby increases the GCMa activity in placenta, whereas sumoylation suppresses the DNA-binding activity of GCMa and thereby decreases the GCMa activity in placenta.

Pre-eclampsia, which is featured by defective placentation and placental hypoxia, affects 6-10% of pregnancies and is a leading cause of perinatal morbidity and mortality. In our previous studies, we have also demonstrated that the GCMa protein level in pre-eclamptic placentas is decreased compared with the gestation age-matched normal placentas. Recently, our preliminary results have shown that degradation of GCMa is enhanced under hypoxic conditions. On the other hand, the F-box protein, FBW2, interacts with GCMa in a phosphorylation-dependent manner and mediates GCMa ubiquitination and degradation by the 26S proteasome. Our preliminary results have also identified key serine and threonine residues in GCMa for FBW2-mediated GCMa ubiquitination. To further our understanding of regulation of GCMa activity, this proposal intends to investigate (I) mechanism of GCMa phosphorylation for FBW2-mediated ubiquitination and (II) mechanism of hypoxia-induced GCMa degradation. Results from these studies will help us to understand how phosphorylation regulates GCMa activity during placental development and how hypoxia regulates GCMa activity in pathogenesis of pre-eclampsia and may open a new avenue for medical

intervention of pre-eclampsia.

計畫編號：BM06-06
計畫名稱：miRNA 和 tristetraprolin 家族蛋白作為人類卵巢腫瘤抑制因子的分子機轉
計畫主持人：張瀞仁
計畫摘要(中)：

卵巢癌的發生率雖不居首位，但因其不易早期發現，死亡率卻是婦科癌症中最高的。最普遍的卵巢上皮細胞癌主要發生於四十歲以後的婦女，目前認為不斷的荷爾蒙刺激(如濾泡刺激素與黃體生成素)可能是卵巢癌發生的風險因子，不過實際的卵巢癌發生原因尚不清楚。microRNA(miRNA)是細胞中不會製造出蛋白質的小片段雙股 RNA，會與特定的訊息 RNA 的 3’ 非轉譯區結合，進而造成目標訊息 RNA 的降解或抑制其轉譯成蛋白質；目前已知許多 miRNA 參與在細胞的生長與凋亡、發育的過程、或與疾病的發生(如癌症)有關。另外有一類 RNA 結合蛋白 tristetraprolin (TTP)家族可結合在多 AU 序列的訊息 RNA 的 3’ 非轉譯區，降低 RNA 的半衰期來調控基因的表現，並有報告指出 TTP 與 miRNA 有交互作用。最近分析人類卵巢癌中所有 miRNA 的表現，顯示有些 miRNA 的表現較正常組織多、有些則較少，其中 miRNA-125b1,-140,-145,和 199a 在卵巢癌中有顯著減少的現象；TTP 家族的三成員在人類卵巢癌的表現亦減少，推測這些 miRNAs 和 TTP 家族可能扮演腫瘤抑制因子的角色，當其減少時即引發腫瘤的生成，但其在人類卵巢癌中的生物功能與作用機制尚未被研究。因為 miRNAs 和 TTP 家族都是作用在訊息 RNA 的 3’ 非轉譯區來進行基因表現的後轉錄調控，我們推測它們可能透過同時作用在相同的訊息 RNA 上、調節其表現，而參與在人類卵巢腫瘤的形成或轉移的過程。首先，我們將在人類卵巢癌細胞中個別大量表現 miRNA-125b1, -140, -145, 和 199a 和 TTP 家族，檢測細胞生長、凋亡、貼附、入侵能力的變化，以了解這些 miRNAs 和 TTP 家族的生物功能；接著利用 DNA 微矩陣和蛋白質二維電泳分析，找出在這些 miRNAs 和 TTP 家族大量表現時，表現受影響的基因，挑選同時受到 miRNAs 及 TTP 家族影響而減少表現，且功能與細胞週期、或細胞凋亡、或腫瘤轉移有關係的基因，做進一步的研究。將這些 miRNA 和 TTP 家族的目標 RNA 的 3’ 非轉譯區選殖出，接在報告基因之後，經轉染實驗，偵測此報告基因所表現的酵素活性，可否受 miRNA 和 TTP 家族存在的影

響；同時也會確認這些目標 RNA 的 3’非轉譯區與 TTP 家族蛋白的交互作用；再以 RNA 干擾技術降低這些目標 RNA 的表現，觀測細胞的生長、凋亡、貼附、入侵能力的變化。最後再偵測這些目標 RNA 的表現可否受到促性腺素的影響。綜合我們的研究，將了解在人類卵巢癌中減少的 miRNA 和 TTP 家族的功能角色，與其參與腫瘤的生成或轉移的分子機轉，將對卵巢癌的治療提供重要的資訊。

計畫摘要(英)：

Ovarian cancer is the sixth most common cancer and the fifth cause of cancer-related deaths among women worldwide. Ovarian epithelial cancer accounts for most of ovarian cancers, which etiology is not clear. Some reports suggested that exposure to excess gonadotropind is a possible risk factor for ovarian cancer. Micro RNAs (miRNAs) are nonprotein-coding RNAs that function as posttranscriptional gene regulators. They can target to 3’ UTR of specific mRNAs and trigger mRNA turnover or translation repression, depending on the degree of complementarity between miRNA and the target mRNA. Each miRNA can control multiple mRNAs expression to regulate cell growth, apoptosis and differentiation. Recently, miRNA microarray technologies have been used to identify several miRNAs that were deregulated in human ovarian cancer. Some of them including miR-199a, miR-140, miR-125b1 and miR-145 were significantly down-regulated. On the other hand, the AU-rich element (ARE)-binding proteins, tristetraprolin (TTP) family, also were downregulated in ovarian cancer. But their biological function is not investigated. Because both miRNAs and TTP family can bind to mRNA 3’ UTR and play as post-transcriptional regulators, we propose that they may downregulate the expression of the same mRNAs cooperatively and function as tumor suppressors. At first, we will ectopically express the miR-125b1, -140, -145, -199a and TTP family in human ovarian cancer cells and examine the cell growth, apoptosis, adhesion and invasion properties to clarify their biological activity. Secondly, the mRNA targets which expression is downregulated by miRNAs and TTP family are to be identified by using cDNA microarray and 2-D gel analysis. According to the gene ontology analysis, the cell cycle-, apoptosis- or invasion-related regulators will be chosen among

these target mRNAs for further study. Thirdly, the 3’ UTR of target-mRNAs are to be cloned and the detailed molecular mechanism of miRNA- and TTP-mediated posttranscriptional regulation will be demonstrated. When these target mRNAs are knockdowned by siRNAs, the ovarian cancer cells may be reversed into near normal cells, which represent decreased proliferation, apoptosis or poor invasion activity. Finally, we will check whether gonadrotropins treatment will affect the expression of target mRNAs of miR-125b1, -140, -145, -199a and TTP family. This study will shed light on the functional role of miRNAs and TTP family in the tumorigenesis and metastasis of human ovarian cancer.

計畫編號：BM06-07

計畫名稱：穀氨醯胺轉置酶(Transglutaminase)在卵泡促素與轉型生長因子 beta 調控卵巢顆粒細胞功能與卵巢血管新生之角色

計畫主持人：李明亭

計畫摘要(中)：

正常卵巢功能是成功生殖之要件。腦下腺分泌的卵泡促素(FSH)是調控空腔卵泡成長發育之主控者，而卵巢內之乙型轉型生長因子(TGF　)可促進 FSH 所誘發卵巢顆粒細胞增生及分化包括類固醇生成(增進黃體酮和雌性素的生成)與基質分解蛋白酶(MMP)之分泌，藉此而影響細胞與其微環境之交互作用。

穀氨醯胺轉置酶(TG)是一具有多重功能且可促使分子產生交聯之蛋白酶。在多種 TG 中，TG2 是一特殊成員；它普遍存在於各種組織細胞，且同時具有交聯-依賴性以及獨立性之生物功能。TG2 藉由與 fibronectin 和 integrin 之結合而成為一細胞黏附媒介者，同時 TG2 亦具有 G protein 功能可調節 G protein 藕合受體(GPCR)之訊息傳遞。文獻報導顯示在多種細胞，TGF　可增進 TG2 表現。另外，TG 活性存在於性腺；且在雄性睪丸細胞，活化 TG 可穩定 FSH 與其受體之結合以及增進 FSH 所誘發 cAMP 含量。FSH 受體亦屬一 GPCR 成員。這些研究顯示 TG2 可作為一 FSH R 以及一調控 FSH 訊息傳遞之 G protein。

血管新生(Angiogenesis)在卵泡發育過程扮演重要生理角色。文獻報導顯示 FSH 可增進卵巢顆粒細胞表現促進血管新生之因子-血管內皮細胞生長因子(VEGF)。在卵泡發育過程，VEGF 與其受體在健康卵泡中之表現增加。另外，VEGF 可藉由 autocrine 方式 增進卵巢顆

粒細胞之存活率。 文獻報導亦顯示在血管內皮細胞，TGF 1 可增進 VEGF 與其受體的表現。TG2 亦表現於血管內皮細胞，而且抑制 VEGF 受體活性導致 TG 活性減低並抑制類似微血管結構之形成。TG2 因此被認為具有維護血管壁完整性之重要功能。

計畫摘要(英)：

Normal ovarian function is critical for successful reproduction. Pituitary follicle-stimulating hormone (FSH) is the major regulator of growth and development of antral follicles. And local ovarian factor, transforming growth factor (TGF) plays an important role in facilitating FSH-induced proliferation and differentiation of ovarian granulosa cells including steroidogenesis and secretion of matrix metalloproteinases (MMPs) involved in modulating matrix remodeling, and hence the interactions between cells and their microenvironment.

Transglutaminases are crosslinking enzymes with pleiotropic functions. Among the transglutaminases, tissue transglutaminase is unique in its ubiquitous expression, and exhibition of crosslinking activity-dependent and -independent biological functions. Tissue transglutaminase could act as a cell adhesion mediator through interaction with fibronectin and integrin, and as a G protein in regulating G protein-coupled receptor signaling. Transglutaminase activity is present in the gonads; however its existence in ovarian granulosa cells has not been reported. And TGF has been reported to upregulate cell surface tissue transglutaminase in several cell types. Further, activation of transglutaminase is involved in stabilizing FSH and its receptor complexes, and FSH-induced cAMP production in testicular cells. In addition, tissue transglutaminase could act as a G protein in regulating G protein-coupled receptor signaling. FSH receptor also belongs to the superfamily of G protein-coupled receptors. These studies suggest that transglutaminase may act as a coreceptor for FSH receptors and/or a G protein modulating FSH signaling.

Angiogenesis plays an important role during ovarian follicular development. It is reported that FSH stimulates the expression of a pro-angiogenic factor, vascular endothelial growth factor (VEGF) in ovarian granulosa cells. The expression of VEGF and its receptors are

increased in healthy follicles throughout ovarian follicular development. Additionally, VEGF may act through autocrine manner to enhance the survival of granulosa cells. Also, TGF is reported to increase the expression of VEGF and its receptor in endothelial cells. Tissue transglutaminase is also expressed in vascular endothelial cells. And inhibition of VEGF receptors results in reduced tissue transglutaminase activity and suppression of capillary-like structure formation. Tissue transglutaminase is believed to be critical for the integrity of the vessel wall.

計畫編號：BM06-08
計畫名稱：斑馬魚含 ZPdomain 蛋白之功能性蛋白質體分析
計畫主持人：黃銓珍
計畫摘要(中)：

許多細胞外蛋白含有 ZP domain，這些 ZP domain 會相互作用，對細胞外膜進行 polymerization 十分重要。動物的卵膜就是由含 ZP domain 的蛋白質所組成，這些蛋白質負責精蟲與卵子之辨識作用與阻止多重精子之受精作用。在脊椎動物含 ZP domain 的蛋白質可分為四類，分別為 ZP1 (ZPB) 、 ZP2 (ZPA) 、 ZP3 (ZPC)和 ZPX。其中 ZP1、ZP2 和 ZP3 存在於哺乳動物的卵膜，而 ZPX 則存在於兩棲類鳥類和魚類。在哺乳動物 ZP3 是精蟲與卵子結合之主要受體，而 ZP2 則與產生 acrosome 反應後之精蟲結合，阻止多重精子之受精作用。目前為止，魚類沒有 ZP2，雖然過去曾有報導在鯉魚和斑馬魚發現 ZP2，但新的命名認為那些都是哺乳動物的 ZP1 (ZPB)相似基因。

本計畫擬表現六種斑馬魚含 ZP domain 的蛋白質並進行性質鑑定。其中 ZPB 和 ZPC 相當於哺乳動物的 ZP1 和 ZP3，另外三種則與 ZPd 相關，第六種則為 ZPX。在第一年，我們將利用 COS-1 細胞表達 ZPC 或者 ZPC 與 ZPB 以及 ZPC 與 ZPX，探討這些 ZP domain 是否會相互作用，並且分泌至細胞外膜進行 polymerization。

在第二年，我們將繼續利用 COS-1 細胞表達 ZPC 或者 ZPC 與 ZPB 以及 ZPC 與 ZPX，探討這些 ZP domain 分泌至細胞外膜後是否具有精子結合能力。另外 ZPC 與 ZPB 蛋白上醣類結構是否與精子結合能力有關，我們也將利用 site-directed mutagenesis 將 Asn-X-S/T 醣鏈位置上之 Asn 置換為 Asp 並進行基因表達。

在第三年，我們將探討另外三種與 ZPd 相關含 ZP domain 的蛋白

質。首先我們將利用 whole-mount in situ hybridization 研究他們在胚胎發育時期之基因表現情形，接著設計相關之 morpholino oligonucleotide (MO) ，並將 MO 注射入一至二個細胞期之胚胎，觀察是否產生明顯之外觀改變，從而研究其相關功能。

雖然已有許多含 ZP domain 的蛋白質自魚類選殖出來，但是有關 ZPB ZPC 和 ZPX 如何分泌至細胞外膜，並組成卵膜之機制，目前並不清楚。本計畫將利用 COS-1 細胞表達 ZPC 或者 ZPC 與 ZPB 以及 ZPC 與 ZPX，探討這些 ZP domain 是否會相互作用並且分泌至細胞外膜進行 polymerization，以及是否具有精子結合能力。以上研究將有助於卵膜組成機制之了解與可能精子結合部位之探討。此外，初步結果顯示三種 ZPd 蛋白表現於內耳與側線神經，其功能可能與聽覺和平衡有關。

計畫摘要(英)：

Many extracellular proteins contain Zona Pellucida (ZP) domains, which play important roles for polymerization. Some of these proteins constitute the extracellular coat of animal eggs. They are responsible for egg/sperm recognition as well as for the block of polyspermy. In vertebrate, the ZP proteins can be divided into four groups: ZP1 (ZPB), ZP2 (ZPA), ZP3 (ZPC), and ZPX. While ZP1, ZP2, and ZP3 are present in the mammalian zona pellucida, a novel isoform, ZPX, has recently been identified in Xenopus, chicken, and fish. In mouse, there are three ZP proteins, while there are four in human and rat. ZP3 is the primary sperm receptor and ZP2 is the secondary ligand binding to acrosome-reacted sperm. To date, no zp gene that corresponds to the mammalian zp2 (zpb) genes has been found in fish. In common carp and zebrafish, the published zp2 genes are phylogenetically related to the mammalian zp1 (zpb) genes.

In this proposal, we will express and characterize six ZP domain-containing proteins. Among them, two (ZPB and ZPC) are homologous to mammalian ZP1 and ZP3, while three are related to ZPd. In addition, the nonmammalian ZPX will be analyzed. The first aim of this study is to examine the possibility of reconstruction of egg-envelope matrix in vitro and to clarify the roles of each ZP glycoprotein in the matrix architecture of zebrafish egg-envelope. We will examine whether

ZPC can bind specifically to the other components, ZPB and/or ZPX, in vitro, and whether it accumulates around the cells secreting the other ZP protein in a cell culture system (monkey kidney COS-1 cells).

In Aim2, the recombinant ZPB in the presence or absence of recombinant ZPC will be examined for sperm binding activity. The carbohydrate moieties of the rZPB and rZPC will be further analyzed by site-directed mutagenesis to replace Asn with Asp at specific N-glycosylation sites. These mutant proteins will be assayed for sperm binding.

In Aim 3, we will determine the spatiotemporal expression patterns of four ZPd transcripts. The information on expression pattern would facilitate subsequent design of functional analyses. Then, we will utilize the morpholino (anti-sense) strategy to study the in vivo function of endogenous ZPd proteins.

Although several ZP-domain proteins have been cloned from different fish species, the role of ZPB, ZPC and ZPX in the matrix structure of zebrafish egg-envelope remains unclear. Through enforced expression of ZPC with or without ZPB and/or ZPX in COS-1 cells, we not only can examine the polymerization, but also can assay the sperm binding to the artificial zona matrix. Moreover, our preliminary data about three ZPd proteins indicated that they were expressed in the inner ear and the neuromast of zebrafish lateral line, respectively. These suggest that other ZP-domain proteins may play important roles in the hearing and balance. Taken together, our studies will provide further information about biochemical and functional properties of ZP domain proteins not only in the formation of egg membrane, but also in the regulation of hearing and balance.