• 系統編號	RN9607-3574
• 計畫中文名稱	生長休止基因 8(Gas8)生物學功能的探討
• 計畫英文名稱	Biological Functions of Growth Arrest-Specific Gene 8 (Gas8) (II)
• 主管機關	--
• 計畫編號	NSC94-2314-B038-011
• 執行機構	台北醫學大學泌尿科
• 本期期間	9408 ~ 9507
• 報告頁數	42 頁
• 使用語言	中文
• 研究人員	葉劭德 Yeh, Shauh-Der
• 中文關鍵字	--
• 英文關鍵字	--

• 中文摘要

生長休止基因 8 (Gas8) 最初是從生長休止的小鼠纖維母細胞株中以基因捕捉的方式所被選殖出來的，其蛋白質產物大量表現在生長休止的細胞中。在老鼠的睪丸中，Gas8 的表現量是受到睪丸發育的控制且主要表現在後減數分裂時期。利用酵母菌雙雜合系統來搜尋其在睪丸中的交互作用蛋白，發現數個蛋白質可能與 Gas8 有交互作用。在它們之中，結合蛋白一(Gas8-BP1)爲 Axin，其參與在 Wnt 訊號傳遞的路徑中。結合蛋白二(Gas8-BP2)爲 Meiosis Nuclearstructural protein，其功能尚不清楚。結合蛋白三(Gas8-BP3)爲一個新的蛋白(Musmusculus hypothetical protein)其功能尚需要研究。利用 GST pull down 和免疫沉澱法證明 Gas8 與 Gas8-BP3 之間確實有交互作用的存在。首先製備了能辨認 Axin 的抗體對 Gas8 的結合蛋白 Axin 進行定性分析。這抗體能夠辨認在小鼠纖維母細胞中的一個 110kDa 大小的蛋白，但是在睪丸中卻辨認到一個 68kDa 大小的蛋白。由於 Axin 在 Wnt 訊息傳遞路徑中是扮演一個巨大的支架蛋白，此結果暗示著在睪丸中 Axin 並不是扮演著其已知的角色。爲了要確認 Axin 在小鼠睪丸中表現位置，發現 Axin 在造精小管子的尾巴被內精偵測到，且在副睪中成熟精子的尾巴同樣可被 Axin 的抗體染到。爲了要更進一步的探索 Axin 的功能，利用 GST pull down assay 來測試 Axin 是否會和 Gas8 及另一個 Gas8 的交互蛋白 Gas8-BP3 作用。其結果顯示 Gas8、Gas8-BP3、Axin 會在同一個複合體中存在。利用 BLAST 程式將 Gas8-BP3 與資料庫進行比對發現在人類有一個與其相似度非常高的蛋白稱爲中心體蛋白 63(centrosomal protein 63, Cep63)。這兩個蛋白的相似度達 81.4%，相同度達 86.3%。運用免疫螢光染色技術發現 Gas8-BP3 及 Gas8 和一個已知的中心體蛋白 γ-tubulin 表現的位置一樣且明顯的在細胞核的周圍。接著利用蔗糖梯度來分離細胞中的複合體，發現 Gas8、Gas8-BP3、Axin 和中心體會在同一個分層中。最後，共同免疫沉澱顯示 Gas8 和 γ-tubulin 的確會在老鼠的睪丸中有交互作用並且可能調控中心體內 γ-tubulin 的功能。

- 英文摘要

Growth arrest-specific gene 8 (Gas8) was originally identified in NIH-3T3 cells and its mRNA and protein expressed predominantly in the testes. In adult mice, its expression is regulated during postmeiotic development of male gametocytes. Several Gas8 binding proteins were isolated by a yeast two-hybrid system in the mouse testis cDNA library. Gas8-BP1, also known as Axin, is involved in Wnt signaling pathway. Gas8-BP2 is a Meiosis Nuclear structural protein whose function is not understood. Gas8-BP3 is Mus musculus hypothetical protein, which is a novel protein and still has yet to be investigated. Studies using GST pull down and co-IP assay have shown that Gas8 and Gas8-BP3 can interact physically. To characterize Gas8-BP1, the antiboby against the C-terminus of Axin was generated. This antibody can recognize an 110kDa protein in NIH-3T3 cells, but only a 68kDa protein in testis. Immunohistochemical stain indicated that Axin was detected in the tail of mature spermatids. The flagella of spermatozoa in epididymis can also be stained with the Axin antibody. To future investigate possible functions of Axin, we performed the GST pull down assay. The results indicate that Gas8, Gas8-BP3, and Axin may function in the same complex. Using BLASTP, one human centrosomal protein, Cep63, was identified to be homologous with Gas-BP3. The two proteins exhibit 81.4% amino acid identity and 86.3% similarity. Gas8-BP3, Gas8, and gamma -tubulin, a well-known centrosome marker, were prominently labeled as a pair of small round dots near the nucleus. Gas8, Gas8-BP3, and Axin appeared in the same fractions in sucrose gradient with centrosomes. The result showed that Gas8, Gas8-BP3, and Axin were centrosomal components in adult mouse testis. Finally, in vivo physical association between Gas8 and gamma-tubulin was later analyzed by a coimmunoprecipitation assay. The experiment indicated that endogenous Gas8 was associated with gamma-tubulin and may modulate the function of gamma-tubulin at the centrosome.