行政院國家科學委員會專題研究計劃期中成果報告

早期發育階段豬胚表現其胚源性基因及時間的特異性 (二)

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一、中英文摘要：

本研究計畫旨在探討，源自豬隻發情配種後不同生理階段輸卵管上皮細胞之基因轉譯物，對於豬胚早期發育之調控機制。試驗首先針對體外培養系統中添加轉型生長因子-α(TGF-α)**，對豬之胚源性基因表現的影響情形；亦即將處於早期** 4-**細胞階段**(early 4-cell stage)**之豬胚，置於每** ml **培養液中含有** 20ng TGF-α**之培養條件下，經** 3 **小時之體外培養後，再抽取其** Total RNA **進行基因差異表現分析。試驗結果證明，此等早期豬胚中有二個基因** *pTIG-1* **及** *pTIG-2***，其表現分別有賴** TGF-α**之誘發，始克有濟；蓋豬胚之於培養液中未添加** TGF-α**而被培養者，毫無** *pTIG-1* **及** *pTIG-2* **之表現可言。進一步試驗乃針對** *pTIG-1* & *pTIG-2* **之完整** cDNA **進行選殖與定列，俾提供往後深入探討該等基因之分生特性，及其對於早期胚胎發育可能扮掩之功能角色；為謀順利達成此一目標，本研究首先乃將源自為數約** 100 **個處於** 4-**細胞階段之豬胚所抽取之** Total RNA**，成功構築於** Clontech® **之** λTriplEx2 **載體上，並證明由此所建立之** cDNA **基因庫，其力價達** $1X10^6$**；進一步選用** *pTIG-1* **中長度為** 194 bp **之基因片段為探針，已自前述建立之** cDNA **基因庫中，成功篩選獲得** *pTIG-1* cDNA **之選殖株及次選殖株。針對此等選殖株進行定序分析結果得，證明完整之** *pTIG-1* cDNA **序列全長為** 3280 bp**，可轉譯出** 500 **個胺基酸；刻除藉由表現載體製被備該基因之部份蛋白質抗原，俾供後續進行功能性分稀之使用外，並亦嘗試針對該基因進行其起動子序列之選殖與定序，俾深入瞭解其對豬早期胚發育之分子調控機制。**

Abstract:

In a previous study, we demonstrated the expression of transforming growth factor-α (TGF-α) gene in the epithelium of porcine oviductal at a time corresponding to 4-cell stage (Chang *et al.*, 2000). Here we report on the *pTIG-1* (porcine TGF-α induced gene-1) was induced in the porcine four cell embryo within 3 hours of TGF-α treatment (20 ng/ml) using mRNA differential display. By further RT-PCR using these specific primers, we obtain the *pTIG-1* mRNA was absent in *in vivo* developed early four cell embryos but was detected in eight cell and 16-cell up to blastocyst stage embryo *in vivo*. However, TGF-α treatment of in vitro cultured early four cell embryos resulted in the appearance of *pTIG-1* transcripts. In situ hybridization analysis demonstrated that the pTIG-1 was expressed late four cell embryos. Sequencing the full-length pTIG-1 cDNA clone identified one open reading frames of 3280 bp which encode 500 amino acids using cDNA library screen. Sequence computer

analysis display that the nucleotide sequence of the *pTIG-1* gene was homologous to the mouse Mo25 (mMo25) (Miyamoto *et al.*,1993). The predicated amino acid sequence revealed that the pTIG-1 might encode a Ca^{2+} binding protein like as mMo25. In this study, we further investigated the role of TGF-α on early embryogenesis using an *in vitro* culture system and mRNA differential display in pre-implantation embryos. Indeed, we identified a candidate gene, *pTIG-1*, the expression of which seemed to be modulated by TGF-α.

二、緣由與目的：

處於早期發育階段之豬胚在體外被培養時，經常面臨無法發育超越 4-細胞期之難題；此一現象稱為豬胚之"培養障阻"。鑑於豬之受精卵自 1-細胞卵裂至 4-細胞階段係在其輸卵管內完成者，本研究室乃嘗試針對母豬在發情後不同生理階段，探討其輸卵管上皮細胞之蛋白質分泌模式及其基因之表現。初步結果證明，母豬自開始發情後第 1 日至第 3 日，其輸卵管上皮細胞之蛋白質分泌模式，確實頗有差異；且在該特定時段中，其輸卵管上皮細胞內有 13 個基因表現具時間特異性。其中細胞轉型因子-α (transformation growth factor-α,TGF-α)在輸卵管上皮細胞內表現之時間為 hCG 注射後 96 h，適與豬胚之 4-細胞發育障阻時間相吻合，本研究遂進一步深入探討 TGF-α與豬胚早期發育之關係。此等研究之完成，除有助於瞭解豬胚早期發育之分子調控外，對於未來建立豬胚之體外培養系統，及應用遺傳工程技術改善母豬生殖效率等，亦其有極大之助裨。

三、結果與討論：

有關生殖道上皮細胞所表現之各類生長因子，對於胚早期發育之重要性，可經由其在胚之體外培養系統添加與否，分別于以比較證明之。過去試驗針對豬胚體外培養系統中添加該轉型生長因子-α並探討其對胚源性基因表現之調控情形；結果發現添加 20ng/ml 的轉型生長因子-α 條件下對於 4-細胞期之豬胚在進行共培養 3 小時後，再抽取其 Total RNA 進行基因差異表現之分析，證實豬胚添加有轉型生長因子-α 被培養者，表現有一基因 *pTIG-1*，係有別豬胚之在未添加生長因子被培養者，此等結果已投稿於 Molecular Reproduction and Development 並被接受。試驗為求獲得完整之 *pTIG-1* cDNA 序列，以期探討該基因之生化功能及於早期胚胎發育所扮掩之角色，試驗遂先行建立豬 4-細胞階段之 cDNA 基因庫以供篩選 *pTIG-1* 之用；鄴經抽取 4-細胞豬胚 100 個之 Total RNA 後，進一步構築於 Clontech® 之 λTriplEx2 載體上並經測試力價達 $1X10^6$ 者，方可用於基因庫篩選之用。基因庫篩選係以 *pTIG-1* 中之 194 bp 為探針分批檢測 $1X10^5$ 個噬菌體菌斑後，分別獲得十個帶有完整 *pTIG-1* cDNA 者 (date no shown)，進一步將此等選殖株陸續次選殖及基因定序後，分析得知 *pTIG-1* 完整 cDNA 序列為 3280 bp (圖一)，並藉由軟體推測該序列可連續轉譯出 500 個胺基酸(圖二) ，而該 *pTIG-1* 基因進一步以 GCG 軟體比對後，證實其序列類似於小鼠早期胚之 *mo25* 基因 (89%) 者然，鑑於在小鼠之 *mo25* 基因序列中具有鈣離子結合區，顯示其功能涉及細胞訊息之傳導(圖三)，惟確實之生化功能則有待更進一步之試驗；此外，針對另一 TGF-α 所誘發表現之基因 *pTIG-2*，現已循同樣模式進行選殖中。

四、參考文獻：

1. Chang HS, Cheng Winston T.K., Choo KB. 2000. Identification of genes expressed in the epithelium of porcine oviduct at various stages of early embryonic

development. Molecular Reproduction and Development. (Accepted).

2. Miyamoto H, Matsushiro A, Nozaki M. 1993. Molecular cloning of a novel mRNA sequence expressed in cleavage stage mouse embryos. Molecular Reproduction and Development. 34:1-7.

Fig.1

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CCAAAAATCTGGTTGCCATGAAAGAAATTCTGTATGGCACAAATGAAA
AAGAGCCTCAGACAGAAGCAGTAGCTCAACTTGCTCAAGAACTCTATA
ATAGTGGGCTCCTTAGCACCCTGGTAGCTGATTTACAGCTCATTGACT
TTGAGGGCAAAAAAGACGTGGCTCAAATTTTCAACAATATTCTCAGAA
GACAAATTGGTACGAGAACTCCTACTGTTGAATACATCTGCACCCAAC
AGAATATTTTGTTCATGTTATTGAAAGGGTATGAATCTCCAGAAATAG
CTCTAAATTGTGGAATAATGTTAAGAGAATGCATCAGACATGAACCAC
TTGCAAAAATCATTTTGTGGTCGGAACAGTTTTATGATTTCTTCAGAT
ATGTCGAAATGTCAACATTTGACATAGCTTCAGATGCATTTGCCACAT
TCAAGGATTTACTTACAAGACATAAATTGCTCAGTGCAGAATTTTTGG
AACAGCATTATGATAGATTTTTCAGTGAATATGAGAAGTTACTTCATT
CAGAAAATTATGTGACAAAAAGACAGTCACTGAAGCTTCTCGGTGAAC
TACTACTAGATAGACACAACTTCACAATTATGACAAAATACATCAGTA
AACCTGAGAACCTCAAATTAATGATGAACCTGCTGCGAGACAAAAGTC
GCAACATCCAGTTTGAGGCCTTTCACGTTTTTAAGGTGTTTGTAGCCA
ATCCTAACAAGACGCAGCCCATCCTAGACATCCTCCTCAAGAACCAGG
CCAAACTCATAGAGTTCCTCAGCAAGTTTCAGAACGACAGGACGGAGG
ATGAGCAGTTTAACGACGAGAAGACCTATTTAGTTAAACAGATCAGGG
ATTTGAAGAGACCAGCTCAGCAAGAAGCTTAATCTCCAATAAACATCT
ATGTTAAATCCAAATTCAGCATTTGCTGTTAGCTATTCAGCATCAGGC
ACTCTTATTGATTCATGAGGAACATTACTGCTAATCTGCTGTTAAGTG
AACGGTTTTTCATTTTACCCTTTTGTTTTTCAGTCCAGGTTGGAGATC
GTAGCTGCTGCTGCTTGCACACTAGGGCACATGTGGGCTTTCTCTTGA
TCTTTGTGTCATTTCAGAATTCAAAGACTGTGCTACGGGAGTTCTGAA
CATGGCTGGGTTCATGAAGGCAAATGTATGGATGAGAGTGTGGTTTAG
GAAAGAGGGCACTGATATCAGATTAGACCTATGTGTTTGCACCCATCT
TTGTTGGCGATCTGAGTGCAGTGTGGCAAGTGCACACCTGGCATCCCT
GCGTCAGATCGCGCACCTTCAGGTCGCGCACCTTCGCTGAAGGAAGAT
GACGCAGAGCTTTATCTGAAATCAGAGGGGAGCTATCCAAAATGGGAG
TTTGGGGGCAGCTAAAGTTGACATGCGAATAAATTGATACTGAAACTT
AGCAACTTCTTAAAAGTGTAAAGAAGCCTCATAAGATCATAAGGAAAA
TGTATATATGCTTTTCACAGCTTTCTAGAATTTTTTGACATTTGATTT
TCTTGAGACTTGTAAACCTGGATATGTTGAAGGGTATTTGTTAATTTT
ACTTTTCAAAGATACTTTAAAACAGTAGAGCTAGCAATGACACCTTGC
ATTTCATTTCAACACTGCTTCAAGGTTTCTTTTGTATATAATTCTTAG
AATGCTCATTTCTTTTAAATGGTTTAATTTGTACAGCAGAGGAATGTT
ATTGTAGTAGTATGTAACTATTACCTAATACTGAGTTTTTGCAAAAAA
CAATGAATGCTCATATGTAATTGAAATACTTCAGATCACATGAAAATG
CTGATTTAACATTTAAGTATCACAGCATTAAAAGAAAAAGAAAGTAAA
CCAGTCCTTTGTATTCAGTTACCTAATGGGGTGCCATCAATAAGCTGC
GATACAGCCCTGGAGCTCAGTCAGCCACACCTTCCTGCATCCTATTGG
CCTTATTCATTTTAAATGAGTTAATGAATCTGCCAGATCTGTGAATGA
TAGAGATTATGCTAAATTAATGCTGATTCTTTGTGTGTGTGGGAAATC
TCTGTAGAGCACCTTTTCTTTCTTAGACTAAGTAACCCAGTACAATAG
TTGTGAACTGAATAATTAAAACTTTGGCTTCTCTTAGGAAAAGACGAC
TTCCTAGTCATAGGTGTCCTATGGGGAAATTTATTTTTTTTAATGTCC
TGTTCCTTAATGCTGCAAATTATCAGTATTTATAAAGTAACTGATTTT
GCACCACTTTTTTGTTACTGTGACCACGGCAGAACAATGTCTTCTAGA
CTATATCTATGTAAAGTTATTAGAATGGTATCTGTTCATTTTAGTGAT
ATGAAGATCACAACTAACAACTGACAAATCAGAGTTTGCCAGTTCAAA
TTCAGCATGGCTGCAGCTGATTAAGAAATTGATATGATTATTCTTTGC
TAGCCTCTCTTACTAATGGAATTATATACTGGCCAGTAAAATGGGCCT
CCCAATTGCTGTTTCAGCAGGTTTTAAACCTTCAGGAACACCAGTTAG
GAAAATAGCTCCAGAAAATATAGATATATTTTATTTTTATTAAAATGG
CAGTCTACATCATAATTGGCATTTCTCAAGACTGTCTTTACCAGAATC
TGTGTGAAATAAGGCAATCTAGTCTCCTTGAAAAAAAAATCTCTTGGA
TGTTTAGGAAGGAAGACTTGGCCGTGATGTGGTGTCCTGGCTTTGTGG
TGTAGTGCTGTGTGTATGGAGTTAGTGTAAAAACATGGATTACACCAA
GTGGAAGAAACGTCTTCTTGCCAAGCTCATTCTTAGAACTTACACATC
TAGAACAGCTTCCACTTTGGCAGTGAGGTCGTAGCCTTTTAGGTGGAA
GAAGTGAGGGTGCAGCGTGTCAGACACAACATTCATGTTACTCTTACA
TTGGAATCTGAAGGTAGTTCAGACTTCAAGCTTAACGAGGTCATAAGG
AAAATGTATATATGCTTTTCACAGCTTTCTAGAATTTTTTGGACATTT
GGTTTTTCTTGAGACTCGTAAACCTGGAATATGTTGAAGGGTATTTGT
TCATCTTACTTTTTGAAGGTATTTTAAAACAGTAGAGCTAGCAACGAC
ACCTCGCATTTCATTTCAACGATGCTTACAGGTTTCTTTTGTATATAA
TTCTTAGAATGCTCATTTCTTTTAAATGATTTAATTTGTACAGCAGAG
GAATGTTATTGTATTAGTATGTAACTATTACCTAATAAAGAGTTTTTG
CAAAAAAAAAAAAAAA--3280 bp
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Fig. 1. The *pTIG-1* sequence of the full-length cDNA was obtained by cDNA library screening. Nucleotide sequence of the mouse *mZFG-1L* cDNA contain one cytoplasm polyadenylation signal (gray region) AATAAA, as well as a poly-A tail.

Fig.2

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pTGI-1:aggtcataaggaaaatgtatatatgctttt-cacagctttctagaatttttgacatttg 69
       |||||||||| ||| |||||||||||||| |||||||||||||| ||| |||||||||
mMo25 :aggtcataag-aaagtgtatatatgctttttcacagctttctagaactttgtgacatttg 1772
pTGI-1:atttttcttgagactcgtaaacctggaatatgttgaagggtatttgttcatcttacttttt 129
       ||||| ||||| ||| |||||||||| || |||||||||| ||| | || ||||||||
mMo25 :atttt-cttgaaacttgtaaacctgga-taggttgaagggtgttt--taattttacttttt 1828
pTGI-1:tgaaggtattttaaaacagtagagctagcaacgacacctcgcatttcatttcaacgatgc 189
       |||||| |||||||||||||||  ||| | ||||||  ||||||||||| |||
mMo25 :-gaaggtgttttaaaacagtagagcatgcagcaacaccttatgtttcatttcaacactgc 1887
pTGI-1:ttacaggtttcttttgtatataattcttagaatgctcatttctttttaaatgatttaattt 249
       |||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||
mMo25 :ttacaggtttcttttgtatataattcttagaatgctcatttctttttaaatcgtttaattt 1947
pTGI-1:gtacagcagaggaatgttattgtattagtatgtaactattacct 293
       ||||||||||||||||||||||| ||||||||||||||||||
mMo25 :gtacagcagaggaatgttattgtagtagtatgtaactattacct 1991
```

Fig. 2. Sequence homology analysis of the cloned *pTIG-1* sequence and the mouse Mo25 (mMo25) gene. The homology search using BLASTN and BLASTX programs in the nucleic acid and protein database. The BLASTN program was used to search GenBank and EMBL databases and a partial sequence *mMo25* to share 89% identify with the 3'-ubtranslational region of *pTIG-1* in 284 bp that contain calcium binding sequence.

Fig. 3

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HGCSGKARHFZZKSRKGYRRSFQKSGCHERNSVWHKZKRASDR
SSSSTCSRTLZZWAPZHPGSZFTAHZLZGQKRRGSNFQQYSQK
TNWYENSYCZIHLHPTEYFVHVIERVZISRNSSKLWNNVKRMH
QTZTTCKNHFVVGTVLZFLQICRNVNIZHSFRCICHIQGFTYK
TZIAQCRIFGTALZZIFQZIZEVTSFRKLCDKKTVTEASRZTT
TRZTQLHNYDKIHQZTZEPQINDEPAARQKSQHPVZGLSRFZG
VCSQSZQDAAHPRHPPQEPGQTHRVPQQVSERQDGGZAVZRRE
DLFSZTDQGFEETSSARSLISNKHLCZIQIQHLLLAIQHQALL
LIHEEHYCZSAVKZTVFHFTLLFFSPGWRSZLLLLAHZGTCGL
SLDLCVISEFKDCATGVLNMAGFMKANVWMRVWFRKEGTDIRL
DLCVCTHLCWRSECSVASAHLASLRQIAHLQVAHLRZRKMTQS
FIZNQRGAIQNGSLGAAKVDMRINZYZNLATSZKCKEASZDHK
ENVYMLFTAFZNFLTFDFLETCKPGYVEGYLLILLFKDTLKQZ
SZQZHLAFHFNTASRFLLYIILRMLISFKWFNLYSRGMLLZZY
VTITZYZVFAKNNECSYVIEILQITZKCZFNIZVSQHZKKKKV
NQSFVFSYLMGCHQZAAIQPWSSVSHTFLHPIGLIHFKZVNES
ARSVNDRDYAKLMLILCVCGKSLZSTFSFLDZVTQYNSCELNN
ZNFGFSZEKTTSZSZVSYGEIYFFZCPVPZCCKLSVFIKZLIL
HHFFVTVTTAEQCLLDYIYVKLLEWYLFILVIZRSQLTTDKSE
FASSNSAWLQLIKKLIZLFFASLSYZWNYILASKMGLPIAVSA
GFKPSGTPVRKIAPENIDIFYFYZNGSLHHNWHFSRLSLPESV
ZNKAIZSPZKKNLLDVZEGRLGRDVVSWLCGVVLCVWSZCKNM
DYTKWKKRLLAKLILRTYTSRTASTLAVRSZPFRWKKZGCSVS
DTTFMLLLHWNLKVVQTSWVLFLSKTMZKHLSLKCCIZSYDHL
IYSYYQDYYTGSGFCVIKVZFYIRQLLNVIFNPZKSGDVYICZ
QCHESIFFLLRKSEFLIRISGWPCNLNZNKILEKQKKKKK
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Fig. 3. The pTIG-1 sequence of the full-length peptide acids