行政院國家科學委員會專題研究計畫期中報告

小鼠卵母細胞及早期胚發生時母源性及胚源性基因之表現及其扮演之生物功能 (1/3)

計畫編號: NSC90-2313-B-002-303-
90 年 8 月 1 日至 91 年 7 月 31 日
主持人: 鄭登貴 國立台灣大學 畜產學系

一、中文摘要

本研究旨在以小鼠為模式，探討母源性與胚源性基因對於早期胚發育之分子調控機制。試驗首先應用 mRNA 分示法 (mRNA differential display)，針對小鼠之成熟卵母細胞與處於早期發育階段 (2- ~ 8-細胞) 之小鼠胚，比較其對於母源性基因及胚源性基因之表現的差異性，再就彼等表現具有差異性之特定基因分別進行選殖及定序，並針對其上游序列進行測試分析，俾瞭解其表現之分子調控機制。試驗結果合計獲得 20 個表現具有生理階段特異性之基因片段，包括 3 個係源自母源性基因所表現者，蓋其轉錄物僅在成熟母細胞及 2-細胞階段之早期胚中可被測得；及 17 個係源自胚源性基因所表現者，蓋其轉錄物並未出現於成熟母細胞中，而需俟胚發育達 2- ~ 4-細胞甚或更後期之階段，始克被測得。在前述 3 個母源性基因之一，名為 *mOSG* (mouse oocyte specific gene)者，經完成其全長 cDNA 之選殖與定序結果，並證明係一長度為 875 bp，可轉譯出一個含有 105 胺基酸之蛋白質。在後續試驗中，更經由反轉錄聚合 連鎖反應 (RT-PCR)及免疫定位 (immuno-localization)技術之配合應用，證實 *mOSG* 之基因轉錄物確係僅呈現於成熟母細胞及 2-細胞階段之早期胚中者，惟其轉譯出之蛋白質則遲至 8-細胞階段始被降解完全。此外，配合 DNA 序列網羅策略(DNA sequencing shotgun strategy)與表現性序列標株 (expressional sequencing tag clone, EST-clone)之聯合應用，針對處於埋殖前發育階段之小鼠胚，嘗試篩選彼等表現具有發育階段特異性之胚源性鋅指蛋白 (zinc finger proteins)轉錄因子；結果並成功獲得一個名為 *mZFG-1* (mouse zinc finger gene-1)之鋅指蛋白基因，進一步針對該基因完成其全長 cDNA 之選殖、定序及其表現模式(expression profile)之鑑定。試驗結果證明該基因轉錄物全長為 1,338 bp；鑑於 *mZFG-1S* 之基因轉錄物僅當成熟卵母細胞完成受精作用後始克被表現，且表現量係隨著卵裂之進展而增加，而於到囊胚階段時其表現量復又轉趨減少，顯示該基因不僅確係屬於胚源性基因，且其表現頗與胚之發育階段有密切關係。

二、英文摘要

In this present study the expression of mouse maternal and zygotic genes was compared and identified using two strategies based on differential display and expression sequences tags (EST) against samples from freshly ovulated mouse oocytes and 8-cell embryos, respectively.

For mRNA differential display studies, a total 30 combinations of arbitrary with anchor primers were used and results appeared that of the 20 genes identified, 17 zygotic genes were found to be specifically expressed in embryos only if they had reached to 2- ~ 4-cells or even

at much advanced stages. Whereas, expression of the other three genes were characterized as the maternal ones found only in those ovulated oocytes before ferilization. One of the *mOSG* (mouse oocyte specific gene; Gene bank accession number: AF313913) cDNA was detected only in mouse oocytes and showed a high sequencing homology with the mouse *OM2a* and *OM2b*, and may be a novel member of this gene family. By further rapid amplification 5'-end (RACE) techniques, we obtained the full-length cDNA with 875 bp to encode 105 amino acids. RT-PCR and Northern blotting analyses demonstrated that the *mOSG* mRNA was expressed merely in the mouse ovary. Further localization of transcripts using *in situ* hybridization confirmed that expression of *mOSG* occurred only in matured oocytes in antrum follicles. On the other hand, using the mouse EST-based primer-sets for RT-PCR, the *mZFG-1* (Zinc finger protein-1) gene was identified and this gene was further confirmed being expressed specifically in mouse pre-implantation embryos (between 1-cell and blastocyst stages). Based on the fact that increase in amount of *mZFG-1* gene transcripts along the stages of embryos from 1-cell to 8-cells and subsequently followed by decrease in its transcription activity suggested *mZFG-1* gene may play an important role related to early development and/or early differentiation events.

Key words: mouse embryo, maternal gene, zygotic gene, gene expression.

二、緣由與目的

哺乳動物之胚胎發育處於早期未埋植階段時，已知在該階段之胚胎須歷經三次有關型態及基因等層次之轉換，俟轉換後該胚胎方具備埋植之條件；此等埋植前胚胎轉換就發生時間而言依序為：1. **八細胞階段前之母源性基因** (maternal genes) **與胚源性基因** (zygotic genes activation) **調控胚胎發育之基因轉換，此等母源性與胚源性之轉換時程係與體外培養障阻期相吻合，**2. **桑椹胚** (morula) **階段之型態緊縮** (compaction) **暨增加各分裂球** (blastomeres) **間之接觸面，**3. **囊胚** (blastocyte) **階段之分裂球分化為內細胞團** (inner cell mass) **及滋養外胚層** (trophectoderm) **二群細胞** (Piko and Clegg, 1982, Ram and Schultz, 1993; Henrion *et al*, 2000)。

以小鼠為例，已知發育中的卵母細胞於第一次停止於減數分裂之雙染色體期時，該卵母細胞會大量進行特定基因之轉錄及轉譯行為，此等鄴經轉錄、轉譯後所存在之訊息核醣核酸及蛋白質，已有實驗證實該轉錄、轉譯物足夠支撐受精後之胚胎發育至八細胞之所須；成熟卵已知均係處於第二次減數分裂之中期 (metaphase)**，相較於第一次停止者，該成熟卵已知並不進行新的轉錄行為，並且於先前大量表現之轉錄、轉譯物已逐漸降解，隨後當受精發生將誘發成熟卵完成減數分裂並進行雌雄原核** (pronucleus) **之融合，俟** 1-**細胞階段後期染色體重組完成則肇始新的轉錄、轉譯行為發生後，此階段新轉錄之產物即為胚源性基因者** (Schultz and Heyner, 1992; Wassarman and Kinloch, 1992)**；就小鼠母源性基因而言，經試驗證實俟** 2-**細胞階段前已降解** 90%(Bachvarova, 1992)**，而此等母源性蛋白質亦已知為卵成熟、受精及前幾次胚分裂所必須，但俟其降解則須胚源性基因之轉錄、轉譯物承接並調控胚胎發育**(Ram and Schultz, 1993)**。因此，體外培養發生障阻係取決於是否適時轉換調控胚胎發育之機轉。**

本研究有鑑於此，乃以小鼠為模式嘗試

探討彼等母源性及胚源性基因之表現情形，並針對此等基因之表現如何影響小鼠早期胚胎發育及分化；冀對哺乳動物早期胚胎發育及分化之分子調控機制能有更深入詳實之瞭解。

三、結果與討論

本研究室成功克服微量胚胎核醣核酸萃取之瓶頸，順利接續應用 mRNA differential display 及 Expression sequencing tags 兩種分析方式，進行探討小鼠母源性基因與胚源性之表現差異行為，並進一步得知，該等差異性基因之屬性及於早期發育階段所扮演之生物功能。在本計畫第一年試驗中除成功利用 30 組 mRNA differential display 引子組，進行比較分析經超級排卵所收集之早期 1-細胞及 8-細胞小鼠胚中基因表現差異性(Fig. 1)外，並進一步將基因選殖且加以核酸定序及進行基因庫比對；結果合計選殖獲得為 20 個具有階段表現差異性之母源性或胚源性基因(Table 1)。按 mRNA differential display 的原理每組引子可比較 150-250 個基因之估算，及哺乳動物不同細胞於不同生理階段皆約表現 15,000 個基因而計，吾等推測母源性及胚源性基因之差異應有 100-200 個之譜。此外，本研究亦利用 www.ncbi.nlm.nih.gov 之 EST 基因庫，於電腦比對確認其中四個可能具有階段表現差異性之基因，遂再進一步以 Nested RT-PCR 逐一測試前述 20 個基因，俾確定該等基因於早期胚胎發育階段 (1-cell, 2-cell, 4-cell, 8-cell, and morula) 之表現模式。Fig. 2 乃前述表現差異性測試結果之二個典型例證，分別為卵源性基因-*Mosg* (mouse oocyte specific gene) 及胚源性基因-*mZFG-1* (mouse zinc finger gene-1) 者(Fig. 3)，分別為 875 bp 及 1,338 bp 之 cDNA. 其中 *Mosg* 已經 *in situ* hybridization 證實係特定表現於成熟卵母細胞(Fig. 4)，惟進一步於細胞內之分佈則有待未來分析之。職是之故，本計畫下一年度之工作重點，除針對前述具表現差異性之基因，在細胞內及組織間表現特性之完整分佈情形，詳加深入探討外，並將進一步瞭解此等基因，在特定細胞內所扮演之生化功能。

四、參考文獻

1. Bachvarova, R.F. 1992. A maternal tail of poly(A): the long and the short of it. Cell 69(6):895-897.
2. Henrion, G., J.P. Renard, P. Chesne, J.F. Oudin, D. Maniey, A. Brunet, H.B. Osborne, and V. Duranthon. 2000. Differential regulation of the translation and the stability of two maternal transcripts in preimplantation rabbit embryos. Mol. Reprod. Dev. 56:12-25.
3. Piko, L. and K.B. Clegg. 1982. Quantitative changes in total RNA, total poly(A), and ribosomes in early mouse embryos. Dev. Biol. 89:362-378.
4. Ram, P.T. and R.M. Schultz. 1993. Reporter gene expression in G2 of the 1-cell mouse embryo. Dev. Biol. 156:552-556.
5. Schultz, G.A. and S. Heyner. 1992. Gene expression in pre-implantation mammalian embryos. Mutat. Res. 296:17-31.
6. Wassarman, P.M. and R.A. Kinloch. 1992. Gene expression during oogenesis in mice. Mutat. Res. 296:3-15.

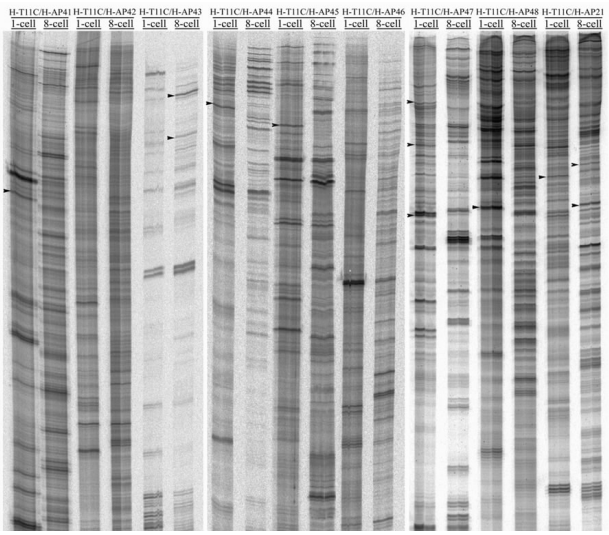

Fig.1. Differential display of mouse maternal and zygotic RNA with primers 3'-T11MC and 5'-APs. Duplicate PCR reactions were prepared from each reverse transcription reaction and were run in adjacent acrylamide gel (6%) lanes. Arrowheads indicate examples of conserved cDNA bands selected due to stage-specific expression. Use a razor blade to cut the bands and elute the fragments from the dried gel, add 100 ul ddH_2O, and heat at 90 ℃ for 10 min. Spin for 5 min to pellet, and remove the supernatant to fresh tube. Precipitate and 5 ul eluted fragment for re-amplification in 50 ul reaction tube.

Table 1. Isolation and identification of genes differentially expressed in mouse during early embryogenesis.

Clone no.	Size (nt)	Poly (A) signal	Character and nucleotide homology
MAG1	574	AATAAA	EST: BE380895, 522/522 nt (100%)
MAG2	584	AATAAA	Mouse EST: AW546958, 562/562 nt (100%)
ZAG1	494	AATAAA	Mouse U2 small nuclear ribonucleoprotein, AF230356, 362/362 nt (100%)
ZAG2	335	AATAAA	Mouse Sui1 mRNA, AF129888, 255/261 nt (97%)
ZAG3	311	AATAAA	No candidate matched
ZAG4	622	AATAAA	Human CGI-65 protein, AF151823, 201/226 nt (88%)
ZAG5	587	AATAAA	Mouse translocase Tim23 mRNA, AB021122, 570/574 nt (99%)
ZAG6	308	AATAAA	No candidate matched
ZAG7	486	AATAAA	Human splicing factor 3b, subunit 1, NM_012433, 153/163 nt (93%)
ZAG8	272	TTTATT	Mouse EST: AV374359, 209/216 nt (96%)
ZAG10	371	AATAAA	Mouse DNA Polymerase Gamma, AI429670, 304/304 nt (100%)
ZAG11	309	ATTAAA	Mouse NIPI-like protein NIPIL(A3) mRNA, MMU67328, 267/297 nt (89%)
ZAG12	335	AATAAA	Mouse ribosomal protein L35a, MMY16430, 316/316 nt (100%)
ZAG13	290	AATAAA	Mouse ribosomal protein L37a (Rpl37a), NM_009084, 268/268 nt (100%)
ZAG14	361	AATAAA	Mouse ribosomal protein L3, GI: 7305440, 361/361 nt (100%)
ZAG15	478	Unknown	EST: 8832512, 478/478 nt (100%)
ZAG16	408	AATAAA	Mouse calmodulin synthesis (CaM), GI: 192364, 408/408 nt (100%)
ZAG17	580	AATAAA	Mouse Rac GTPase-activating protein, AF079974, 580/580 nt (100%)
ZAG18	378	Unknown	EST: AU046182, 378/378 nt (100%)
ZAG19	320	AATAAA	Mouse ATP synthase subunit F, H+ transporting, GI: 7949004, 320/320 nt (100%)

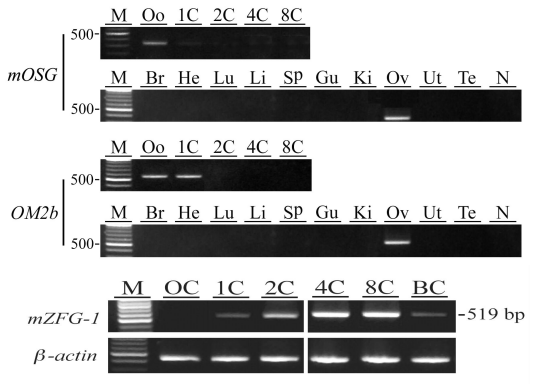

Fig. 2. Temporal expression of *Mosg* (MG3 or C44M1), *Om2b* and *mZFG-1* during pre-implantation and adult tissues. A, RT-PCR analysis of MG3 (C44M1) RNA expression from various mouse pre-implantation embryos and adult tissues using specific primers. PCR products are visualized by EtBr staining during agarose gel electrophoresis.

Mosg

agtgtgccttgggggtggggcttgggggtggagagacaggaaagcagacagaaggcagcattta
gaagaaggtgtagccagtctgaagatcttttgcagggaagggtgATGATGTCTCAACTTCACAT
ATGTTATGGCCCTGTTTGTGAGATGCAACATCACCTTCCATCTTTCCTGGACCCCCTGGAGTTT
GTGATTGTGTACCTAGAGACCTGGGTCTACAAAGGATTGTTTGgtgagcatggtgggaacttag
acccctttgagagtaagctggccaaacacggtctggcaattgctaccaaagtcccagaatttcct
taatgcagcatctcttagaaagatagaggagccatcagctctgtctaggtggaccccacacaag
tccccttttttcttcaagtcttaagattgcctctcgtatcttaactgttctcttcttggggatt
attgctgctgctactgctggctaagcatccttttcccttcccagATTTGAGGCCGGAACTATTG
CTATACTTTTTGAATATGCACGTTCTTATTACATCTGATGGTGATTTAAGTGAAACAGTCCTGT
ACATTTATGGATCCAAAGGGATTAGGATCTTTGCAATGTTTGTGATTGTGTCGCTAGCTTGCAA
TCTCCGGACCAAGCGAAATCGAGgtaaacggcttcatgtaccttggtacaggatattttattcc
tttgtgggggtgggaggggcctgaaaagttggcttttcctaccacttgtgttccccctagtta
gttgcactgcctaaggcccagggcagagagttctgaaaactaccacccgatctaactactccaa
ggtgtgggttctggcttccgaggaagaccagcacgatgcagctgggaaagttgactgtgtggtt
gactcagatggcgaggatctagtctgcttctgctttccagGTGTGGCGTTGAGTGCCTAGgtag
cgcctctctagaggaacggattctatccaatggatccctggtccatcacttgggctcaccgatt
gtgcatttcagctgtgagcctgagggccagaagcctcaaggtcaaccgggaactggttaaaaat
gtttacctacccaggtaccctctccattaccacaagcagaaactggagaggcaaagcatagcag
ccagtgtttaattaagccctccagaaaattctgatacaggcccaagtccagaatcattcctgga
aactcacctctgcaagcatgtacctgaccagccgttttctctttagccagagcctgggccagag
gcctgcaaagccctgtgtggtggaaccggaggtacttcatttaagaggacgcctgtccaggacc
cgttgttcagatgtcctttgccataatgttaaccttggaacagccttgcctgtggagaagccgg
cttgaccatgatgccaagttccagatctttttgtgccaggacaggtggggaggggtttttacca
gtgttcttaactatactgtgatttttattattgccatgtccagcttttaagtggggttttaacg
gttttataataaataaaagtttcaggcagaaaaaaaaaaa

mZFG-1

GCAAAGACCTGGAAAAGCTGCTGAGGGACAGGAAACGCCACAACTGGgtaagcagagcctcagc
tatgtcctctgggaaggacggacagctgtgggggacccgggagcagaacctgtagaggatcttg
cccttaaccagtggctctcgaggggagagttttgcttcccaagagataaacagcaatgtcttgg
gggcttttatggatttatttcgtctttgtgaaggtcacacagaaactatttagtagccaaggga
ttgtatagatcagaaacagtgcaaactaacaggccaatgaccagttcacagagtcaaaagcaca
caaactctggggtgtttttgttttgttttgacagttttgagctttgatgttggtgctgggaa
ttgaatccaggtgttctggatagcagcctgtgctcttaatctctgagctatctctctttgttgt
tgttgtttttaatctgcatgtacctcatgatgcccttggaggccagaggaggacatcaggtccc
cttggaactagagttgcagttatgaggcaccatgttggtgcaggtcagtgaacctgaacagtga
acaaggagcagtgggtactcctaatctttccaactttgctgccccagactccgtttattgagac
agggtctaaggtagctcaggctggtctccaactcacttgtagctgagtatgaccttgtggacca
acccatgctcaggaagatagatttgcaagtagtaagcctagctgggctgctaaatcagaacaga
agtgagagtgcttgctgggaaaactatatccaggctcttacatggggtctgatcctgactccct
ttcttcccacaTCCATAATCTACTCCCAAGGACAGGCACACCTCCTTCGGCATCCCAGCGTTGG
GAAGGCTGAAGCCGCAGAGGATAAATGGGGACATACGGACTTCTCCCAGGAGCACCTATCAAAT
GAGAGCGAGGAGTCTCTCAATAGAGGCCAGGCCAGCCGGGAGCTACAGAACCTCTCTGAGACCG
AAGAGCCATCCACCAGCCAGGAAGAGGGCATTTTGCTGGGAGTGATTCCTGAGAGGAGACAGCC
AGACTACCTGAGACCTGAGATGAGCCCAGGGAGTGACTCAGTTCCTGACTTGGAAGAGGCAGAG
GCATCTGTGTTTGTTGGTCAGGACCCTCTTCCAGCCCTGGGTCCCGCTGGTTCTCTTGGAGTAA
AGGGTGCCGTGCAGCCCCAAGAAGACACTGTTGTGGATGCTGTACCATCGTTCACCCATATTCT
GGAAAGAGATCTAGCCTTGAACAGAGATCTTCAGAGTCTTTCTGGTTTCAACTTACCCACTTCC
CAAGGAGTGGCGTCCTATATGGGCAATACAGAAGATGGGCTAGAGGCTGCTAATCCCGAGCCTG
CTAACCCACAGCCTGAGAAGCAAGTTGACAGTCTTGCTGGCCAGGCTCGGTTTCAGTGTACTGA
ATGCAAGAAGAGCTTTCTTTACAAGTCCCGGTTTGACCTCCACCAGAGGTCACACACAGGAGAG
AGACCCTTCAAGTGCATCTTGTGCAACAAGGCCTTTGTTCAGTCCTCCGACCTGAGAGTCCACC
AGCGTGTCCACACAGGGGAGAAGCCTTACATGTGTGAAGTCTGTGGGATGGAGTTTGCCCATGG
GTCCACCCTGCAGGGCCACAGTCGAGTCCACACGAAGGAGAAGCCTTTTGTGTGTAAGGATTGT
GGGCAACGCTTCTGCCACAAGGGCAACCTCAATGTCCATTTCCGCATCCATTGCAACTTGAGAC

CCTATGTCTGTAAGAAGTGTAACAAGACTTTCCGACAGCAGGGGACTTGGAAGCGACACATGAA
AACCCACCTGAGAAAGAGAAAGGTGTCTGAGTAATTCTGGATCCAGTTTTTTCCCCTAGAAAGA
CTGAGATTGAAGTCTTTTCTCAGAGAAATGGAGGTTGGGTTGCCCCTATTCTGAGAGTCTCATC
CCTTCATGGTCATTGTATAGAGTATTCATTGGTGGTCTGTTTCACTATGCCCCCTGGGTTTTTA
TTTTTGAAATAGGGTTAGAACATGTATACTAAAAGTTAAATGTTTTAAATTAAATTTTCATTTA
AGTTAAAAAAAAAAAAAAAAAA

Fig. 3. Illustration of the structure of the *Mosg* (MG3 or C44M1) and *mZFG-1* genes.

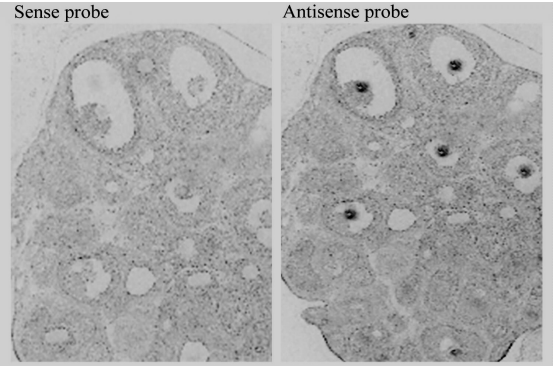

Fig. 4. Spatial expression pattern of *Mosg* gene during mouse oogenesis. *In situ* hybridization of mouse adult ovary with ^{35}S-labeled *Mosg* riboprobes. Antisense probe, *Mosg* is strongly expressed in the oocytes of mature oocytes in antrum follicles. Sections probed with either of the sense transcripts gave no detectable signals.