D101 Expression and Purification of Mouse Sulfated Glycoprotein-2 in *E. coli.*

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Sulfated glycoprotein-2 (SGP-2) was first recognized as the major secreted protein in cultured rat Sertoli cells. The secreted glycosylated SGP-2 is made of two different subunits whose molecular weights are 34 and 47 kDa measured by SDS-polyacrylamide electrophoresis under reducing condition. These two subunits are cotranslated from a single mRNA and glycosylated before proteolytic cleavage to give rise to the mature two subunits which are connected by disulfide bonds in mouse. We have previously reported cloning and DNA sequencing of the cDNA encoding SGP-2 from mouse. When the cDNA was expressed in *E. coli* using T7 promoter and polymerase system, the protein had 51 kDa in molecular weight.

In an attempt to purify SGP-2 from *E. coli*, we have used a glutathion-S transferase (GST) fusion vector system. The open reading frame of SGP-2 was fused to the 3' end of GST gene which is under inducible lac promoter. The expression of the fusion protein was identified by Western blot. It was found that when the fusion protein is expressed at 37°C, most of the protein was present in insoluble fraction. However, when it was induced at 25°C, significant fraction (30%) was soluble. We were able to purify the fusion protein in a single step using glutathion-sepharose affinity column. Cleavage of SGP-2 from the purified GST fusion protein using thrombin is in progress.

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Expression Pattern and a Possible Role of *myn* in Preimplantation Mouse Embryo Development.

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The c-*myc* protooncogene is involved in cellular proliferation and differentiation, and its biological function is executed by dimerization with heterodimeric partner, *myn*. In the present study, endogenous c-*myc* and *myn* gene expression in preimplantation embryos were determined by reverse transcription-polymerase chain reaction (RT-PCR). *myn* transcripts were constitutively expressed throughout embryonic stages with a slight increase only at blastocyst stage. In contrast to the *myn* expression pattern, c-*myc* gene transcripts were detected at 1-cell stage, then declined abruptly at 2-cell stage and increased gradually until blastocyst stage. To determine the functional role of *myn* in preimplantation embryo development, several anti-sense *myn* oligodeoxynucleotides, spanning either the second helix domain(Myn3; 20-mer), or the tail of zipper domain (Myn2; 20-mer), were microinjected into fertilized 1-cell embryos. Microinjection of Myn2 and Myn3 exhibited developmental deregulation at 8-cell-morula/blastocyst transition stage. Taken together, these results suggest that c-*myc*:*myn* heterodimer may play an important role in the mouse preimplantation embryogenesis.