Analysis of Jpk and its interactome during murine embryogenesis

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Analysis of Jpk and its interactome during murine embryogenesis

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감사의 글

처음에 발생학 실험실에 들어왔을 땐 2년이라는 시간이 길고 많은 것을 할 수 있는 시간이라고 생각했는데 시간은 빨리 지나가고 아쉬움이 많이 남는 것 같아요.
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우리 실험실 동료들에게도 많이 감사 합니다.
유병기 선생님! 실험실에서 일들이 생길 때 마다 손수 챙겨 주셔서 감사합니다. 그리고 우리 실험실의 맏형 박성도 선생님!! 우선 박박사 되신 것 축하드려요. 처음 단백질 실험을 가르쳐 주시고 좋은 주제로 일을 할 수 있게 배려해 주셔서 감사합니다.
실험실의 큰언니!! 양혜원·정현주 선생님!! 힘들 때 마다 상담해주시고 세상에 어떠한 길들이 있는지 알려 주신 두 큰언니 고마워요*^^* Thank you~~~~
처음 실험실에 들어와 아무것도 모르는 제가 하나하나 친절하게 가르쳐주신 권윤정 선생님!! 고민이 있을 때마다 친절하게 들어주시고 좋은 방법을 찾아주신 선생님께 너무 감사 드립니다. 또 샘이 있어서 플룻 1년 동안 끈기 있게 할 수 있었던 것 같아요. 고마워요
공경아 선생님!! 첫 사수 언니!! 실수 많고 어리버리한 제게 실험은 어떻게

해야 하는지 가르쳐 주셔서 감사합니다.
실험실 동기 이자 친구인 진주 진희! LG challenger 도전했을 때가 생각난다. 내 든든한 힘이 되어 주고 젊은 열정을 느끼게 해준 진희! 선의의 경쟁자이자 좋은 조언자가 되어준 진주! 너희가 있어서 정말 행복한 실험실 생활을 할 수 있었던 것 같아. 고맙다.
때론 동료로서 때론 친구처럼 힘들 때 마다 이야기 들어주고 묵묵히 힘이 되어준 이은영 선생님! 샘이 있어서 실험실 생활에 따뜻함을 느낄 수 있었어요. 사회에서 보면 우리 언니동생하자!
함께 있은 지 오랜 시간은 아니지만 마지막까지 즐겁게 실험을 할 수 있도록 도와준 이미희 선생님 김은신 선생님! 그 말투와 재치 잊지 못할 것 같아요... 정명섭 선생님! 실험은 쉬 엄 쉬 엄하면서 스트레스 받지 말았음 좋겠어요. 힘내요!! ..... 군생활 잘하고 있을 정오, 병원생활 잘하고 있을 오샘!! 재미있는 생활을 할 수 있게 도와줘서 고마워요~~~

제 정신적 지주이자 인생의 한걸음 한걸음을 내디딜 때마다 길을 밝혀 주시는 김신덕 교수님!! 항상 고맙다는 말만 반복하게 되네요. 사랑해요~*^^*
미향, 준란, 정은 언니!! 우리 학교의 빛이 되어주는 언니들이 있어서 한결 사회에 나가는 두려움을 덜었어요. 선배 동기 언니들이 있어서 힘이 많이 되었답니다. 고마워요~~~
이제는 다른 길로 각자의 길을 걸으면서 힘이 되어주는 나의 친구!
영임 미화 진아 승연 미현 숙현!
성질 낼 때 마다 받아줘서 고맙다!! 덕분에 졸업한다~~~ㅋㅋㅋ
석사과정을 마칠 수 있게 도와준 식구들!! 아빠 엄마 언니 동생!!
논문 쓰는 동안 짜증내는 것 받아주느라 힘들었죠? 고마워요! 사랑해요~~~~이제 논문도 썼으니 효도 해야지*^^*

내 사랑하는 모든 분들께 감사 드립니다.
훌륭한 과학자가 되도록 노력하겠습니다~~~.

2005-06-28 **김혜선 올림**

CONTENTS

List of Figures

List of Tables

ABSTRACT

Analysis of Jpk and its interactome during murine embryogenesis

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Jopock (Jpk) was originally isolated as an associating factor of position-specific regulatory element located in the upstream region of *Hoxa-7*, which is a member of *Hox* genes and plays an important role as transcriptional factors during anteroposterior axial pattern formation by being expressed at a specific time and position during embryogenesis. In mammals, *Jpk* previously triggered apoptosis by promoting the production of ROS and increasing *Bax* and *Bad*. During development, the apoptosis plays important roles; controlling the number of cells, sculpturing by deleting certain structure and eliminating the injured or misplaced

cells.

In this study, we tried to identify the interactome of Jpk using GST-pull down assay to explore the function of Jpk during embryogenesis. As a result, the candidates of Jpk interactome in F9 cells and *E.coli* were categorized; 1) protein modification and degradation (TraP, endopeptidase, PPlase and VCAM1); 2) ROS regulation (pheneyl proionate dioxygease, GST, VCAM1 and renin2); 3) signal transduction and metabolism (putative amidase, OmpR, cytoskeratin KRT2-6HF, and Grb14); 4) protein synthesis (EF-tu and 40S ribosomal protein S2). VCAM1 and Grb14 of these proteins are known to participate in glucose regulatory pathway and renin acts in mesonephric tubule, pancreatic primodium and hepatic primordium during embryogenesis. Especially VCAM1 and PPlase are translocated to plasmamembrane by passing through endoplasmic reticulum (ER), and renin2 is secreted through ER.

Furthermore, fluorescence microscopy, used to study the location of Jpk in mammalian, showed that the Jpk protein, which was fused into the EGFP, was localized into the ER. In addition, deletion analysis revealed that the TM domain is essential for membranous localization.

To examine the expression pattern of Jpk in ER, we analyzed the Jpk transcripts under ER-stress condition. When MCF7 and F9 cells were treated with the low concentration of ER-stress inducer, DTT or EGTA, the expression of *Jpk* was upregulated at the transcriptional level like that of *Grp78*, a molecular chaperone well known to be overexpressed under ER-stress condition. In the presence of high concentration of ER stress inducer, cells death has induced and *Jpk* transcripts were strongly expressed. These results altogether indicate that the ER-stress upregulated the expression of *Jpk* and the excess stress induces the ER-stress induced apoptosis and the concomitant expression of *Jpk*.

In conclusion, Jpk, which is located in ER, is regulated by ER stress, and it is estimated that Jpk interact with Grb14 and VCAM1 relevant to glucose regulation pathway, considering that it was controlled by glucose. In addition, it is inferred that Jpk gives support to transport Renin2, which is a secretory protein by passing through ER and associates with the development of kidney during embryogenesis, from ER to golgi.

Key words : Cell death, ER stress, F9 cells, GST pull down system, Hox, interactome, Jpk, Proteomics, RT-PCR, subcellular localization

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Ⅰ. INTRODUCTION

Hox genes, a family of developmental regulatory genes, control many aspects of morphogenesis and cell differentiation in mammals.[1-3] The term "*Hox*" is derived from homeosis, which is a pattern where one body part develops as a likeness of another.[1-3] Every Hox protein contains a well-conserved domain (homeodomain) which composed of 61 amino acid residues that appear to recognize and interact with a cognate cis-acting regulatory element near the flanking regions of the down stream

genes controlled as a unit by the specific homeotic protein.[3]

Jpk, which was originally isolated as an associating factor of position-specific regulatory element located in the upstream region of *Hoxa-7*, [3-5] was inferred that it plays an important role as transcriptional factors during anteroposterior (AP) pattern formation.

Jpk recently has been suggested to be one of the selenoproteins, SelS[6,7] which contains selenocystein insertion sequence (SECIS) in the 3' untranslated region (UTR) that is necessary for decoding the stop codon, TGA. Moreover it has revealed that *SelS* mRNA was regulated by insulin stimulation[7] in adipose tissue in type 2 diabetic subject and increased by glucose deprivation and ER stress in HepG2 cells.[8] This protein was known to retrograde the unfolded/misfolded proteins in ER to cytoplasm for ER-associated protein degradation (ERAD) pathway of unfolded protein response.[9]

In previous study, *Jpk* has shown to induce apoptotic cell death in prokaryotic and eukaryotic cells.[10,11] In mammals, Jpk triggered apoptosis by promoting the production of ROS and EGFP-fused Jpk was shown to be mainly expressed in the

endoplasmic reticulum (ER) in F9 murine embryonic teratocar cinoma cells.[4]

During the development, the apoptosis, programmed cell death, is important to control the number of cells, sculpture by deleting a certain structure and eliminate the injured or misplaced cells.[4] Apoptosis is induced by 'extrinsic pathway', which stimulate the plasma membrane death receptors, or by 'intrinsic pathway', which is stimulated by perturbation of the intracellular homeostasis.[12] The cytoplasmic organelles such as mitochondria, ER and others received the various stresses induced by these stimuli, which ultimately lead to apoptosis.[12,13]

In this study, we identified the candidates of Jpk interacting proteins in embryo and F9 cells using GST-pull down system to understand the function of Jpk. Moreover we analyzed the localization of Jpk using EGFP-tagged Jpk constructs in COS7 cells (monkey kidney cell line) and the expression of *Jpk* under the ER stress mediated apoptosis condition.

Ⅱ. MATERIALS AND METHODS

1. Cell culture and transient transfection.

F9 (murine embryonic teratocarcinoma cell line), MCF-7 (human breast cancer cell line) and COS7 (monkey kidney cell line) cells were maintained in Dulbecco's modified Eagle's medium (DMEM: Gibco BRL, Carsbad, CA) containing 10% fetal bovine serum (JBI, Seoul, Korea), 100 μg/ml penicillin, and 100 μg/ml streptomycin (Gibco BRL) at 37°C in an incubator with 5% CO_2.

The $2x10^5$ COS7 cells were split on the 22 mm x 22 mm slide glass, coated 0.1% gelatin. The plasmids were transfected into cells using a Lipofectamine Plus^{TM} Agent (Invitrogen, Carlsbad, CA) according to manufacturer's instructions and incubated at 37 ℃ in an incubator with 5 % CO_2 for 20 hours.

2. Preparation of total protein extract

When F9 cells reached about 90% confluency, they were washed in 1x PBS and then harvested. Then cells were lysed with high salt buffer containing 50 mM HEPES, pH7.0, 500 mM

NaCl, 1% NP-40, 1 μg/ml Aprotinin and 100 μg/ml PMSF and RIPA buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 1 mM sodium vanadate, 10 mM sodium fluoride, 10 mM sodium pyrophsphate, 1 mM PMSF, 10 ㎍/ml aprotinin, 10 ㎍/ml leupeptin and 1% NP-40).

3. GST-pull down method

Jpk was cloned into the expression vector pGEX-4T1, and transfered into BL21(DE3).[10] Fusion proteins were expressed by induction with isopropylthio-ß-galactoside (IPTG) as suggested by the supplier of the GST fusion system (Amersham Pharmacia Biotech). The bacteria were collected by centrifugation at 12,000 rpm for 30 minutes, and lysed by sonication. Fusion proteins were purified from the lysates using the reduced glutathion sepharose. Bacterial cell lysates were incubated for 3 hours at 4°C with the glutathione sepharose 4B bead (SIGMA, Louis, MO, USA) to immobilize the fusion proteins (GST-Jpk) and then washed with 1x PBS at 3 times. In order to isolate the Jpk interacting proteins, F9 cell

lysates were incubated with GST-Jpk fusion protein (100 μg of beads, 50% slurry) at 4°C and washed to 1x PBS 3 times (Figure 1).[14,15]

4. SDS-PAGE gel electrophoresis and western analysis

Proteins were denatured with 5x sample buffer (60 mM Tris-HCl (pH6.8), 25% glycerol, 2% SDS, 14.4 mM 2-mercaptoethaol, and 0.1% bromophenol blue) at 100°C for 5 minutes. Samples were electrophoresed on a 12.5% polyacrylamide gel and then transferred to nitrocellulose membrane using Bio-rad kit (Hercules, CA) at 30V, 4°C for overnight. This membrane was exposed to the primary goat anti-rabbit GST and second antibody for 1hour at room temperature, and then washed with 1x PBS. After detecting GST with an ECL solution (Amersham Pharmacia), the membrane was exposed into the X-ray film (AGPA cop., Belgium).

5. Gel electrophoresis and MALDI-TOF analysis

To separate proteins interacting with GST-Jpk interactome

from embryo, a 5~20% gradient gel of 13 cm x 13 cm size was used. After commassie staining the specific bands were excised from the gel and analyzed by MALDI-TOF TOF.

To identify the interactome of Jpk in F9 cells, we performed the proteomics. the first-dimensional gel separation was carried out with 7 cm pH3-10 IPG strips (Bio-rad) and proteins were treated with lysis buffer containing 7 M Urea, 2 M thiourea, 4% HAPS, 1% DTT, 2% IPG buffer (Amersham Pharmacia Piscataway, NJ, USA) and 0.01% bromophenol blue (BPB). IPG strip was rehydrated in the protein lysis solution in reswelling tray (Amersham Pharmacia) and electrophoresed using multiphorⅡelectrophoresis unit (Amersham Pharmacia). After completing the first-dimension isoelectric focusing, IEF strips were equilibrated for 15 minutes in 1.5 M Tris-HCl (pH8.8), 6 M urea, 30% glycerol, 2% SDS, 50 mM DTT, and 0.01% BPB, and then transferred to SDS-polyacrylamide gel for the second-dimension electrophoresis. The gels were stained with Bio-safe coomassie brilliant blue (Bio-rad, Hercules, California, USA) by shaking overnight. The spots

appeared on the gel compared with those of control, were selected and analyzed using MALDI-TOF.

To analyze these proteins, we used the research program called Profound (http://prowl.rockefeller.edu/profpund_bin/Webpro Found.exe) as well as Mascot program (http://www.matrixscience.com/home.html).

6. Computer analysis

Amino acid sequences of Jpk (Jopock) proteins were analyzed using DAS-TM prediction server (http://www.sbc.su.se/~miklos/DAS) and CBS prediction server (http://www.cbs.dtu.dk/services). The secondary structure of Jpk was predicted using 3D-PSSM server (http://www.sbg.bio.ic.ac.uk/).

7. Constructs of EGFP fusion proteins

The plasmid pJpk-EGFP was constructed from pEGFP-Jpk[5] To clone pJpk-EGFP, pEGFP-Jpk[5] was digested with *Apa* I and this fragment was inserted into *Apa* I site of pGEM-7zf vector. This construct was digested with *Sal* I and *Kpn* I and then was

inserted between the *Xho* I and *Kpn* I sites of pEGFP-N1 vector so that Jpk was fused in frame to the N-terminus of GFP. To construct pJpk(1-92)-EGFP was constructed that pGEM-Jpk was digested with *sal* I and *Bgl* Ⅱ and then the small fragment was isolated and inserted into the *Xho* I and *Bam*H I site of pEGFP-N1 vector. In the same manner, pJpk(1-134)-EGFP was constructed: pGEM-Jpk was digested with *Sal* I and *Stu* I and then inserted in between the *Xho* I and *Bam*H I . To construct pJpk(89-182)-EGFP, the *Eco*R I site of pEGFP-N1 were digested and cohesive ends were filled in with Klenow fragment. The large fragment was isolated and digested with *Kpn* I . The pJpk-EGFP was digested with *Pvu* Ⅱ and *Kpn* I , and then ligated with this large fragments. To clone pJpk(122-182)-EGFP, pJpk-EGFP was digested with *Sph* I and then inserted into the *Sph* I site of pGEM-3zf to make pGEM-Jpk(122-182). And then pGEM-Jpk(122-182) was digested again with *Hin*d Ⅲand *Bam*H I and inserted into the same sites of pEGFP-N1.

8. Fluorescence microscopy analysis

Twenty hours after transfection, specific staining of the ER and mitochondria were achieved by incubation of the cells for 30 minutes at 37℃ with 200 nM ER-Tracker ™ Blue-White DPX (Molecular probes, Eugene, OR) and 50 nM MitoTracker ™ (Molecular probes), prior to fixation with 4% paraformaldehyde. Cells were analyzed by Image analyzer fluorescent microscope and fluorescent images were acquired by using an Image analyzer (Oylmpus corp. Melville, NY) and analyzed by Meta-Morpho program.

9. ER stress induction in F9 and MCF7cells

One day before treatment, MCF7 cells were split and incubated in the complete DMEM medium on six-well plates. Cells at approximately 70~80% confluency were washed in 1x phosphate-buffered saline (PBS) and incubated with serum free medium. For 24 hours, the samples were changed to serum free media with ER stress inducer such as dithiothreitol (DTT) and ethylene glycol-bis(b-aminoethyl)-N,N,N,N-tetraacetic acid (EGTA) for indicated concentrations (0.001, 0.01, 0.1, 0.5, 1 and 10 mM). F9 cells were split in growth

medium in 100 mm dish plates. Cells at approximately 50~60% confluency were washed in PBS and incubated with complete media containing ER stress agents for indicated concentration 0, 1, 2, 3, 4 and 5 mM of DTT for 24 hours.

10. Cell viability analysis.

After cells were harvested using 1x Trypsin and suspend gently in DMEM medium, sample 100 ㎕ was mixed in 4% Trypan blue 100 ㎕. The number of cells was counted by the hematocytometer.

11. Isolation of RNA and RT-PCR analysis

Cells were harvested at 1,500 rpm for 5 minutes. Total RNA was isolated using RNAzolB (TEL-TEST.INC, Friendswood, Texas, USA) according to the manufacturer's instruction.

The purity and amount of RNA were assessed by spectrophotometric measurement at 260 and 280 nm using UV-spectrometer (Amersham Biosciences, UK). Total RNA (3 ㎍) was reverse transcripted to cDNA at 37℃ for 60 minutes in a volume of 22 ㎕ containing the following reagents;

0.5 mM dNTP, 10 mM DTT, 0.5 mg Oligo (dT), 5x buffer, Reverse Transcriptase and RNase Inhibitor (Poschem. INC, Korea). ß-actin, Jpk, GRP78, and CHOP-10 transcripts were quantified using the PCR method with appropriate primers *ß-actin*, 5'-CATGTTTGAGACCTTCAACAC-3' (forward) and 5'-GCCATCTCCTGCTCGAAGTCT-3' (reverse); *Jpk* (mouse) 5'-CCATGGATCGTGATGAGG-3' (forward) and 5'-GTGAAAGT GTGCGTAAGGC-3' (reverse), *Jpk/SelS* (human), 5`-CATGG AACGCCAAGAGGAG-3' (forward) and 5'-ACAAACCCCATC AACTGTCC-3' (reverse); *Hoxa-7*, 5'-CGGGCTTATACAA TGTCAAC-3' (forward) and 5'-CTGGCTCTCATCTTTATG CT-3' (reverse); *GRP78*, 5'-CTCGAATTCCAAAGATTCAG-3' (forward) and 5'-TAC CAAGTGTAAGGGGACAC-3' (reverse); *CHOP-10*, 5'-CTTCATACACCACCACACCT-3' (forward) and 5'-AAGCACTTTACTGGACATGG-3' (reverse). PCR reaction was performed with the following conditions; 95℃, 5 minutes; 95℃, 1 minutes; the appropriate annealing temperature of each primer (*ß-actin*, 55℃; *Jpk*, 53℃; *SelS*, 53℃; *Hoxa-7*, 55℃; *GRP78*, 45℃; and *CHOP-10*, 50℃), 1 minutes; 72℃, 1 minutes

(repeated for 25 cycles); and 72℃, 10 minutes using Thermal cycler (Perkin Elmer, Wellwsley, MQ, USA).

Ⅲ. RESULTS

1. The analysis of Jpk interactome in Embryo and F9 cells.

A. The interactome of Jpk in vivo (Embryo)

Jpk, which was isolated as an transacting transcriptional factor of *Hoxa-7*, [3-5] presumed that it plays an important role during AP axial pattern formation by being expressed at a specific position during gastrulation.[3] Thus, in order to analyze the function of Jpk during embryogenesis, we tried to identify the Jpk-interacting proteins in the specific stage and position of murine embryo where *Jpk* was strongly expressed. Jpk interactome means the proteins interacting with Jpk which of study is essential to understand the capability of Jpk protein.

First, in order to detect the stage and position that Jpk were strongly induced, spatio-temporal expression pattern of *Jpk* was analyzed using murine embryos from day 10.5 to 16.5 post coitum (p.c.) (Figure 2) through RT-PCR. Using embryos of day 11.5 p.c., which is start to form liver during organogenesis, special expression pattern of *Jpk* was analyed after dissecting the embryo into 7 parts along the AP axis (H1, H2, M1, M2, M3,

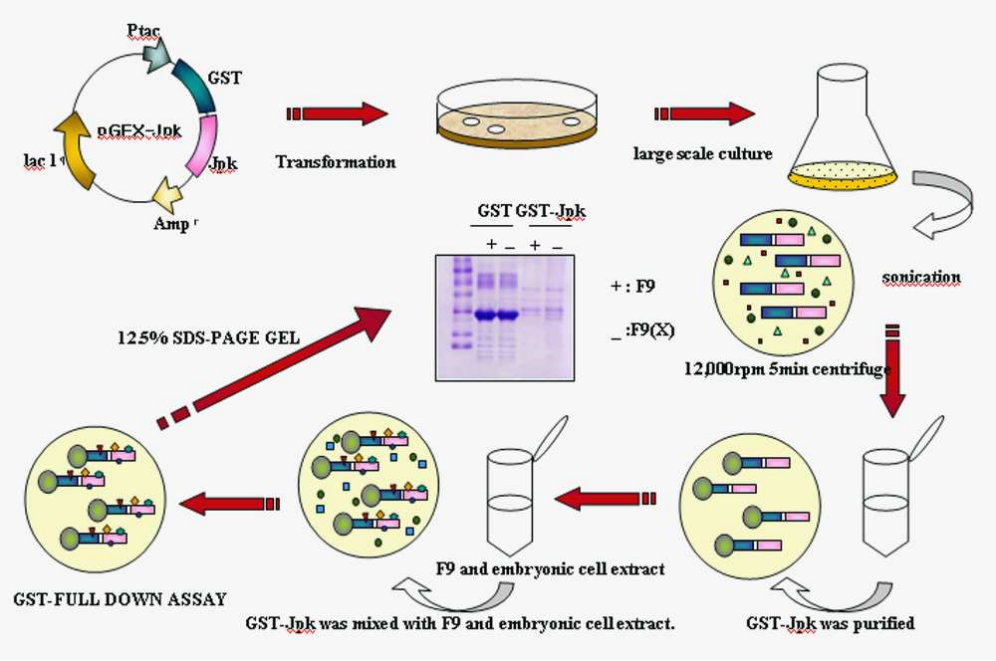

Figure 1. Diagram to show the GST-pull down method.

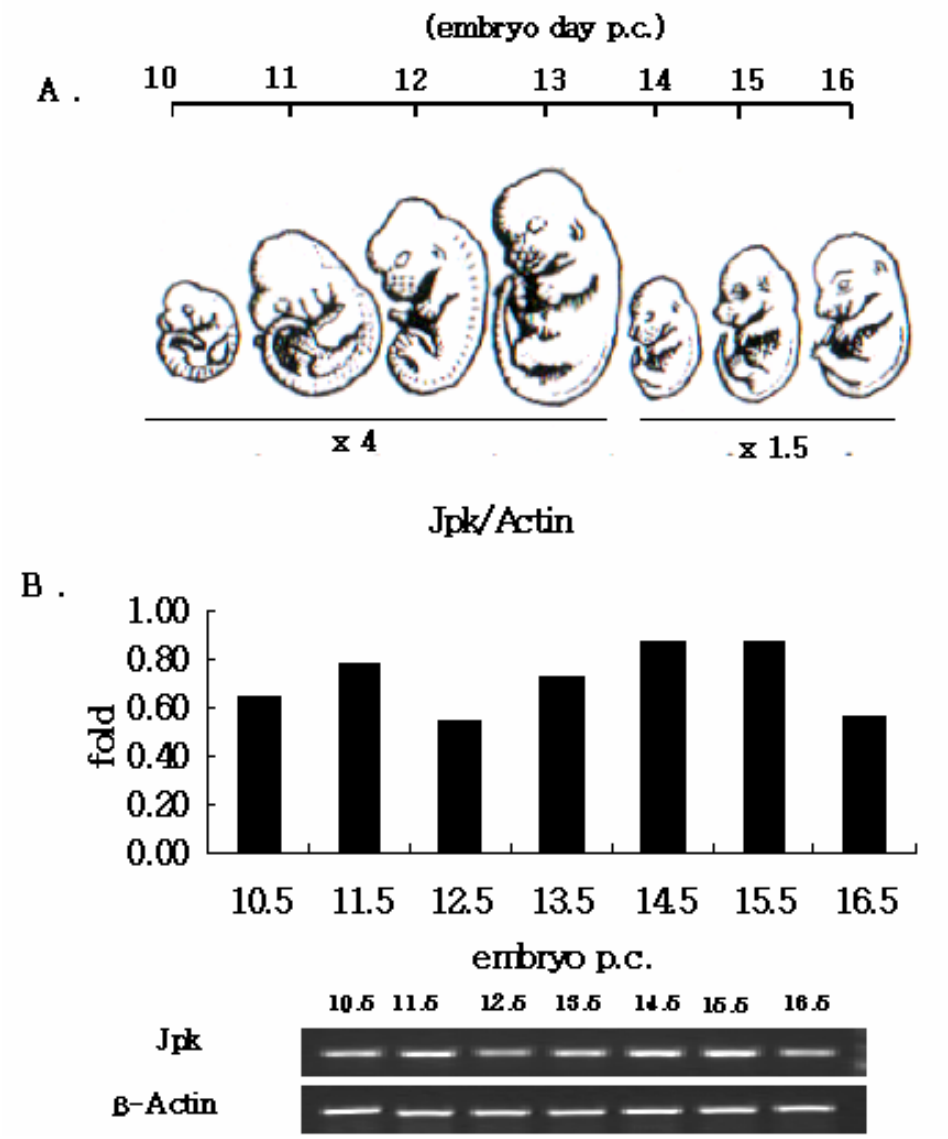

Figure 2. Temporal expression pattern of Jpk during murine embryogenesis; (A) diagram of murine embryo from 10.5 to 16.5 day p.c. (B) expression pattern of Jpk during embryogenesis. Total RNA was extracted from mouse embryos of day 10.5 through 16.5 p.c., and the expression of Jpk was analyzed using RT-PCR.

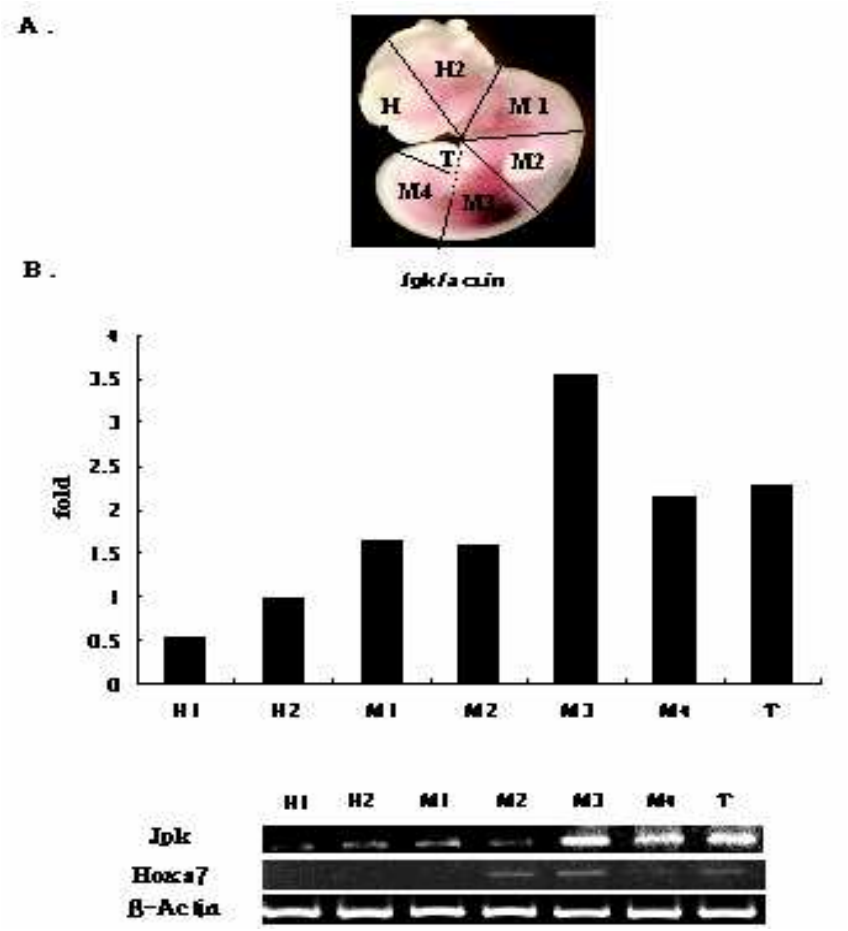

Figure 3. Spacial expression pattern of Jpk in murine embryo of day 11.5 p.c. (A) expression pattern of *Hoxa-7* (B) expression pattern of Jpk. Total RNA was extracted from each segment (H1, H2, M1, M2, M3, M4, and T) divided along the AP axis of mouse embryo of day 11.5 p.c. and expression level of *Jpk* as well as *Hoxa-7* was analyzed by RT-PCR.

As shown in Figure 3, *Jpk* was strongly detected in M3 portion. Moreover *Hoxc-8* and *Hoxa-7* appear to be expressed strongly in M3 part as well (Figure 3). As a result, it's been supposed that the expression of Jpk is depending on position and influenced the Hoxa7 expression.

To identify the interacting proteins of Jpk in murine embryo, we tried to perform the GST-pull down assay using GST-Jpk(C), which make to delete the transmembrane of Jpk to product soluble Jpk protein. After purifying large amount of GST-Jpk(C) proteins, they were incubated with various conditions (0.5, 1 and 1.5 mg) of total proteins extracted from the M3 part of day 11.5 p.c. murine embryo. Using 5-20% gradient gel, interacting proteins were resided (Figure 4) and the bands appeared only in the extract containing sample, not in control (Figure 15B), isolated and analyzed to be unknown protein (B) and GST α chain (C), respectively.

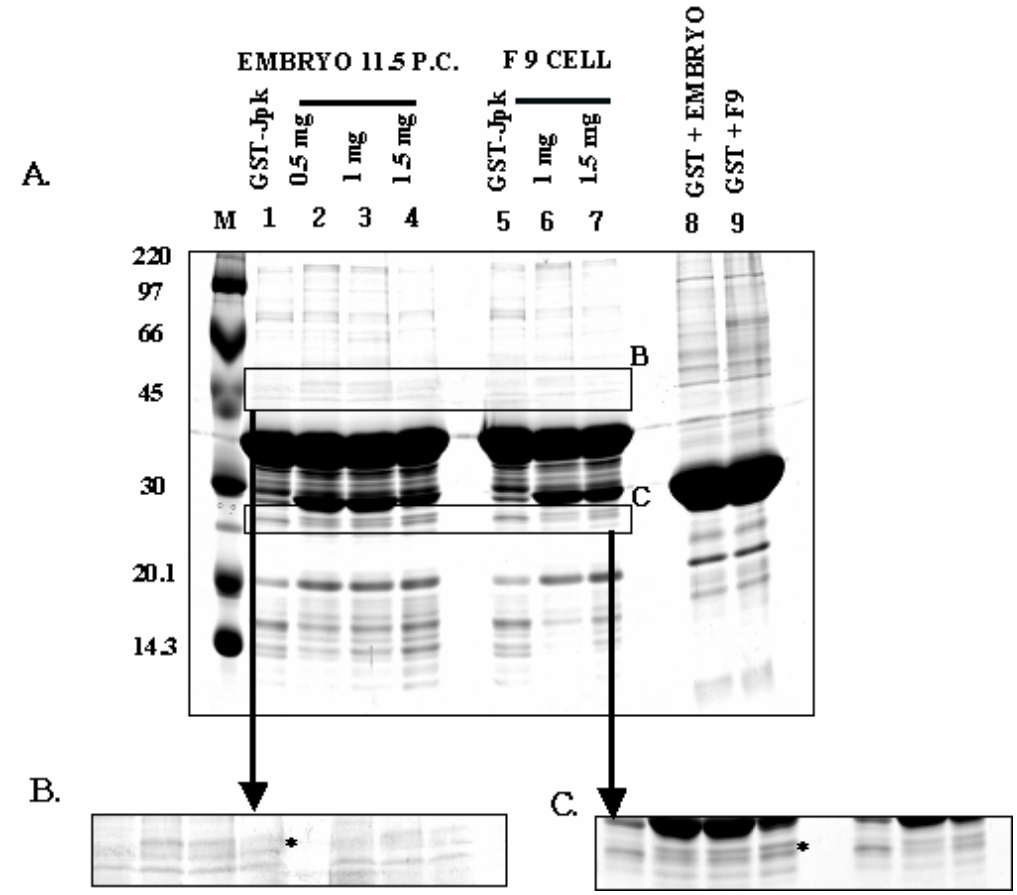

Figure 4. Analysis of Jpk interactome in embryo as well as in F9 cells using 5~20% gradient gel. Total Jpk interacting proteins purified through GST-pull down method were analyzed on a 5~20% gradient gel. The bands detected only in the extract containing samples were marked with * in B and C.

B. Jpk interactome in vitro (F9 cells).

It was difficult to analyse the interactome of Jpk, because the M3 part of murine embryo 11.5 p.c. retains various cell mixtures. Thus we tried to isolate the interacting proteins of Jpk in F9 cells, murine embryonic teratoca cinoma cell.

First of all, to obtain large amount of Jpk proteins, pGEX-Jpk which contains a full sequence of Jpk and a tac promoter inducible by IPTG was used. To optimize the concentration of IPTG inducer and the induction time, pGEX-Jpk was grown in 2x YT medium until it approached to 1.6 at O.D. 600 nm, then IPTG was added at the various concentrations such as 0, 0.1, 0.5, 1 and 10 mM and cultured for 30 minutes. The IPTG concentration for protein overexpression was the best at 1 mM IPTG. Using 1 mM IPTG concentration, optimal time of induction was tested; 0, 10, 30, 60 and 120 minutes (Figure 5A and B). The optimum condition for induction of GST-Jpk protein was turned out to be 1mM IPTG and 60 minutes.

A large quantity of GST-Jpk protein was obtained on above condition and purified by sephadex GST beads.

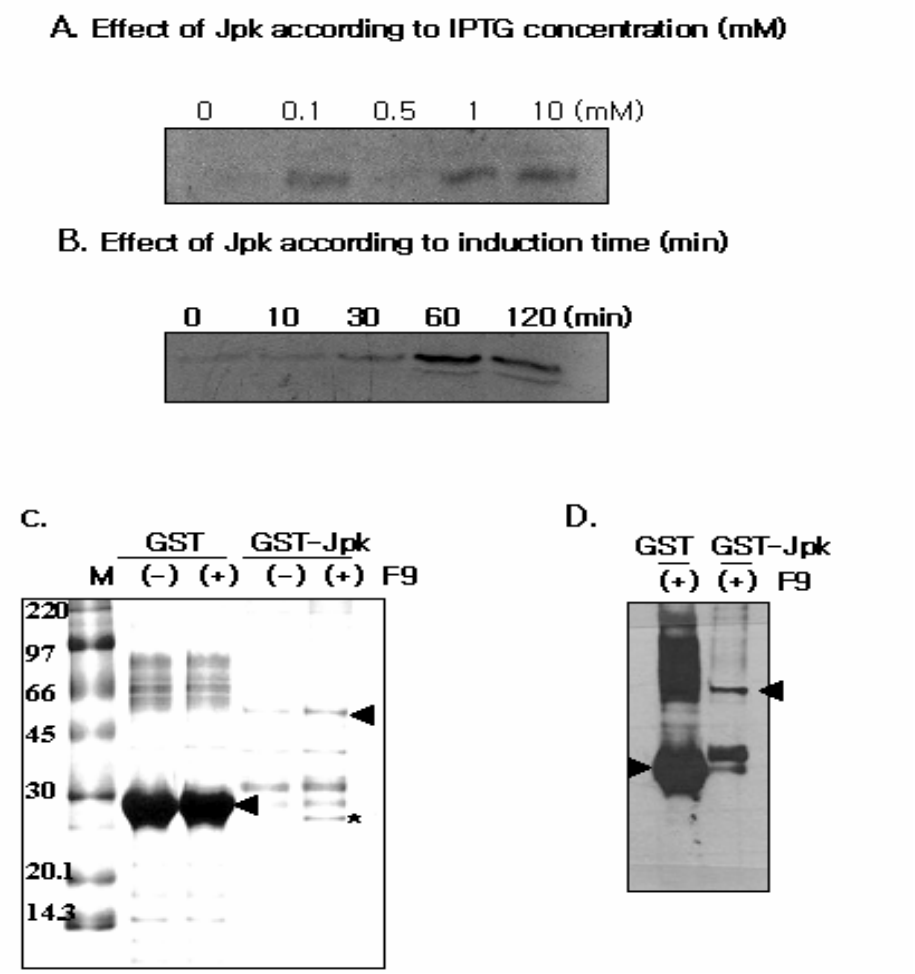

Figure 5. SDS-PAGE gel and western analysis. (A) Expression of Jpk in the presence of various concentration of IPTG and (B) induction time (minutes). The optimum condition for protein overexpression is 1 mM IPTG and and 1 hour induction time. (C) The interactome of GST and GST-Jpk in F9 cells. Short arrows (▶) indicate GST and GST-Jpk. The asterisk (*) indicate the interacting protein of Jpk. (D) The sizes of GST and GST-Jpk were analyzed using GST-antibody.

After incubating GST-Jpk protein with F9 total protein extracts, Jpk interacting proteins were analyzed on the SDS-PAGE gel (Figure 5). Through western blot analysis using GST-antibody, GST and GST-Jpk proteins were confirmed to be 26 kDa and 48 kDa respectively (Figure 5C and D).

In order to identify the interacting proteins with Jpk, we used proteomics analysis and MALDI-TOF. Red circles indicate bacterial origin of GST-Jpk interactome in Figure 6A and C, and Jpk interacting proteins of F9 cell in Fig 6 B and D. The 20 spots on 2D gel were isolated and identified as described in the materials and methods. Various proteins such as VCAM1, Grb14, cytokeratin, PPlase, renin 2 and 40S ribosomal protein S2 in F9 cells, and OmpR, amidase, endopeptidase, TraP and phenylpropionate dioxygenase in *E.coli* (Table 1 and 2). From these results, Jpk interactome seemed to be involved in: 1) protein modification and degradation, 2) ROS regulation, 3) protein synthesis and 4) signal transduction and metabolism (Table 3).

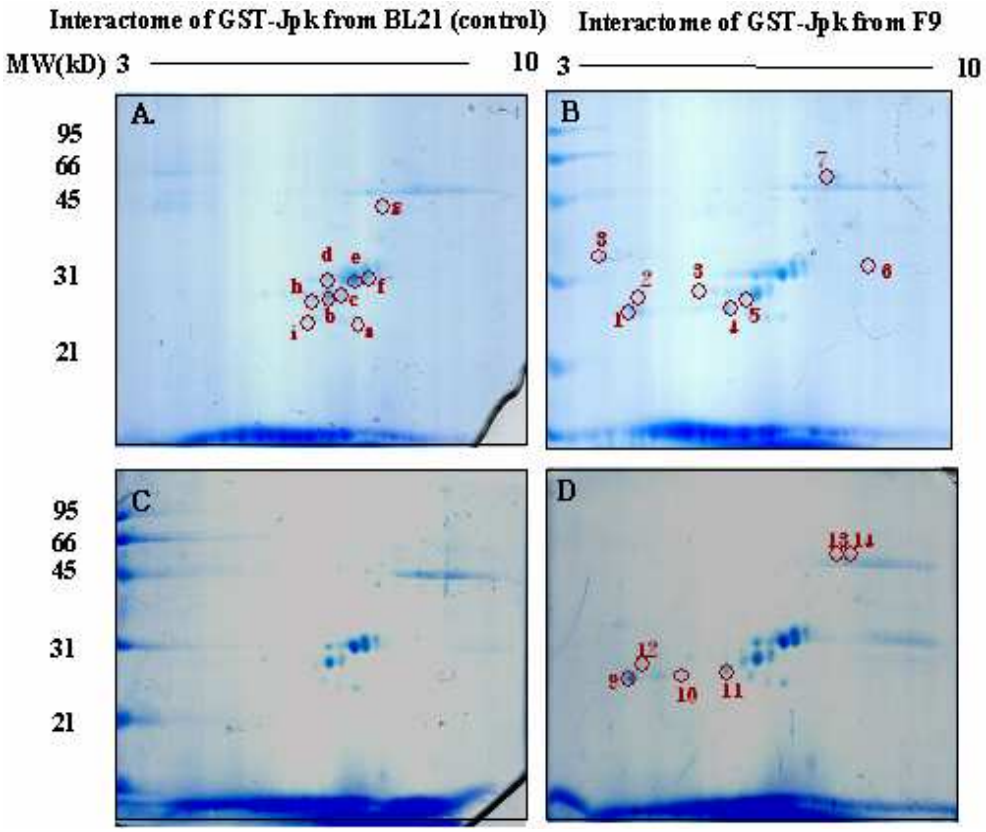

Figure 6. Two-dimensional gel electrophoresis. GST-Jpk (from BL21) and GST-Jpk (from F9) interacting proteins were isolated through GST-pull down method and analyzed by 2D gel electrophoresis. (A, C) The spots (a~i) are proteins interactng with GST-Jpk in *E.coli*, BL21 (B, D). The spots (1~14) appeared only in GST-Jpk interactome sample from F9 were selected after being compared with those of control A. The proteins obtained from (A) and (B, D) were analyzed by MALDI-TOF and then analyzed using MS-FIT and MOSCOT program through computer.

Table 1. The interacting proteins of Jpk in BL21(DE3)

	Protein	pI	MW (KDa)	Fuction	Assession No(s).
a	TraP	7.9	21.46	DNA transfer system	gi|1293085
b	endopeptidase	9.2	17.16		gi|15831437
c	OmpR	6	27.39	Signal receive dom ain	gi|15833501
d	putative amidase	9.6	45.62	Cellular processes and signaling	gi|38704120
e	OmpR	6	27.39	Signal receive dom ain	gi|15833501
f	hypothetical protein ECs4607	5.2	38.11		gi|15833861
g	Elonation Factor Tu (Dom ain1)	5.3	41.56	translation	gi|229900
h	endopeptidase	9.2	17.16		gi|15831437
i	Phenylpropionate dioxygenase	6	20.62		gi|15832659

Table 2. The interacting proteins of Jpk in F9 cells

	Protein	pI	MW (KDa)	Function	Assession No(s).
1	GST Yfyf	8.3	23.63	Class Pi Com plexes with Glutathione	gi|2781337
2	GST, MU 1	7.8	26.07		gi|28386202
3	cytokeratin KRT2-6HF	5.1	42.48	Interm ediate filam ent protein	gi|13272554
4	unnam ed protein product	8.6	36.97	unknown	gi|12854685
5	cytokeratin KRT2-6HF	5.1	42.48	Interm ediate filam ent protein	gi|13272554
6	PPIase	8	14.56	protein folding depend on catalytic /chaperon like activity	gi|38080301
8	**VCAM1**	5.3	38.92	cell- cell adhesion, lym phocyte migration	gi|423613
9	Expressedm sequence AA117069	8.8	36.32		gi|45768368
10	Renin 2	6	44.65	peptidase activity	gi|15029868
11	**Grb14 protein**	9.4	23.6	integral signaling cascade, signal transduction SH3/SH2 adapter activity	gi|18256070
12	unnam ed protein product	6.2	25.24		gi|26346194
13	1110001|14Rik protein	7.3	36.04		gi|23271651
14	40S ribosom al protein S2	10.4	25.19	Protein syntheses	gi|20896113

Table 3. Function-based classification of putative Jpk interactome.

Protein catagory	Spot no.	Gene Products	Origin
Folding, Modification and Degradation	a	TraP	BL21(DE3)
	b	endopeptidase	BL21(DE3)
	6	PPIase	F9
	8	VCAM1	F9
ROS regulation	i	Phenylproionate dioxygease	BL21(DE3)
	1	GST	F9
	8	VCAM1	F9
	10	Renin 2	F9
Signal Transduction and Metabolism	d	putative amidase	BL21(DE3)
	e	OmpR	BL21(DE3)
	5	Cytokeratin KRT2-6HF	F9
	11	Grb 14	F9
Protein Synthesis	g	EF-tu	BL21(DE3)
	14	40S ribosomal protein S2	F9

2. The location and function of Jpk in mammalian.

According to the resent reports, *Jpk* has a homology to the human *SelS* and is regulated by glucose deprivation and ER stress in mammalian. Moreover we inferred that Jpk is concerned with the capability of ER because Jpk interacting proteins were detected such as renin secreted through ER and VCAM1 and PPlase translocated through ER to outer membrane. Thus, we attempted to resolve the location of Jpk and observed the its transcripts under the ER stress in mammalian.

A. Jpk protein was located at ER membrane through TM domain in COS7 cells.

To understand the structure of Jpk protein, we performed the computer analysis. As a result, it revealed that the N-terminus of Jpk contained one highly hydrophobic transmembrane domain (TM) and is predicted to be located in the outside of cells and ER lumen (Figure 7A and B). The C-terminus of Jpk, which was predicted to be located in the cytoplasm, contained 3 plausible glycosylation sites (AA-144,

-145 and -184) and several potential phosphorylation sites (AA -68, -121, -127, -132, -154, -184 and -185) (Figure 7C). We used the protein secondary structure prediction program through 3D-PSSM Server to analyze possible structural elements in Jpk. C-terminal 65 residues of the protein are predicted to form the coiled-coil structure. α-helix structure was predicted to be located along the TM domain and a putative nuclear localization site (NLS) (http://www.sbg.bio.ic.ac.uk/) (Figure 7C).

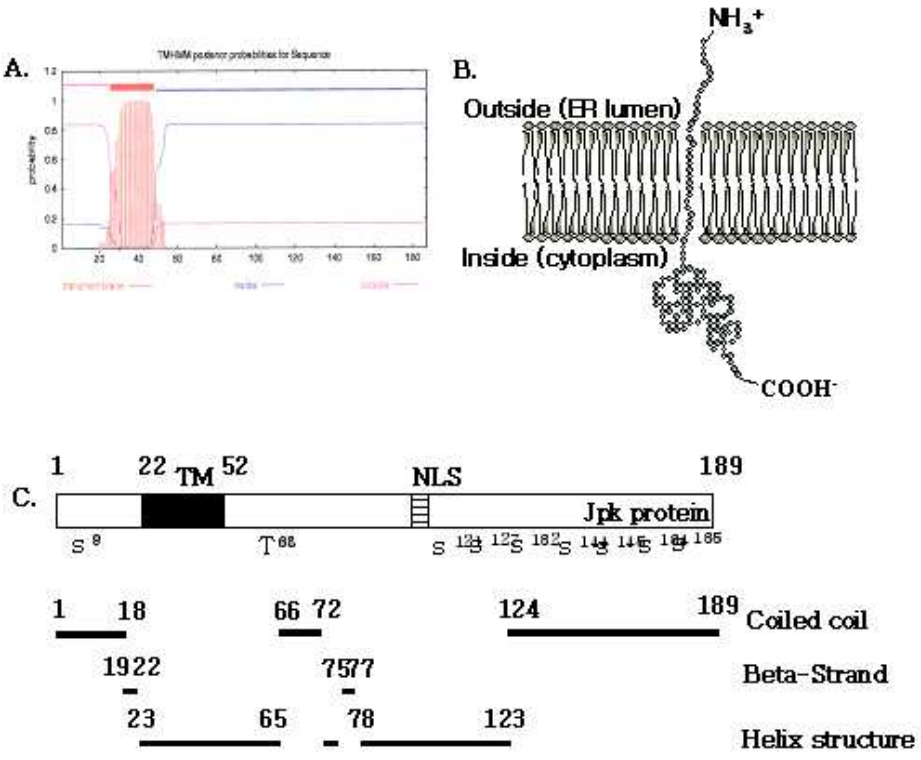

Figure 7. Computer analysis of Jpk protein structure. (A) The transmembrane domain of Jpk was predicted by researching program (http://www.cbs.dtu.dk/ services). (B) Diagram to show the Jpk in mammalian cells. (C) Analysis of Jpk secondary structure. Jpk has 189 amino acid including selenocystein. Jpk protein contained coiled-coil, extended (β-strand) and Helix structures. Computer analysis revealed that it contained a transmembrane domain (amino acid 22-52; black box), 3 plausible glycosylation sites (AA-144, -145 and -184) and several potential phosphorylation sites (AA -9, -68, -121, -127, -132, -154, -184 and -185); http://www.cbs.dtu.dk /services

To examine further where the Jpk protein is located among the cytoplasmic membraneous organelles, the COS7 cells, which are certainly distinguished between nucleus and cytoplasm, were transfected with each pEGFP-Jpk and pJpk-EGFP constructs and co-stained with the Mito-Tracker and ER-Tracker. They were visualized under the fluorescence microscope Image analyzer. As shown in Figure. 8, both EGFP-Jpk and Jpk-EGFP proteins were likely located in ER. However the expression aspects of EGFP-Jpk and Jpk-EGFP were different each other as shown in Figure 8B and C. EGFP-Jpk seemed to be located in the small vesicles and transported from ER to Golgi and plasmamembrane (Figure 8B), while Jpk-EGFP looked like to build up large vesicles and reside in ER (Figure 8C). Moreover EGFP-Jpk induced the cell death but not Jpk-EGFP (not shown data).

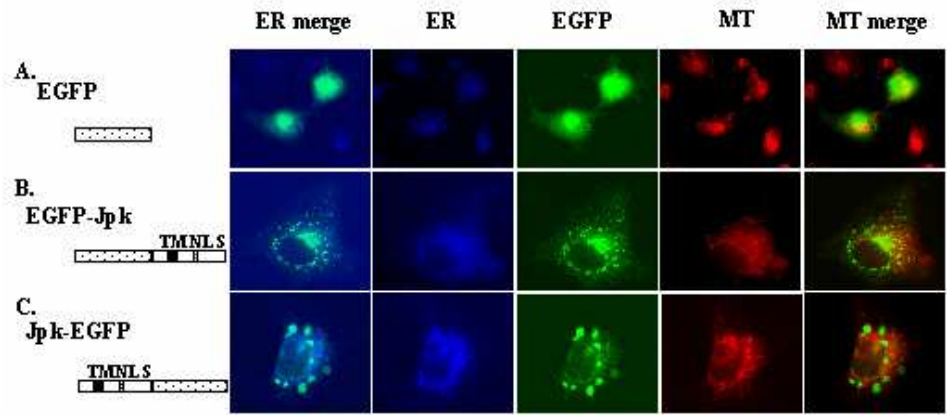

Figure 8. Subcellular localization of the EGFP tagged-Jpk. COS7 (monkey) cells were transiently transfected with each plasmid pEGFP (A), pEGFP-Jpk (B) and pJpk-EGFP and cultured for 20 hours. The cells were treated with 50 nM MitoTracker and 200 nM ER-Tracker to stain mitochondria and ER, respectively. The TM and a putative NLS were indicated along the plasmid construct, and the EGFP was indicated as a dotted box.

To map the region of Jpk which locates it into ER, various *Jpk* deletion mutants were constructed. Since Jpk contained a TM domain and a putative NLS (Figure 7), several combinational deletion constructs of *Jpk* were made for the presence or absence of TM and NLS (Figure 9B). Jpk-EGFP protein, which contains a TM domain and NLS, was located in ER membrane with ER tracker (Figure 9B, and 10). And pJpk(1-92)-EGFP, which include a TM domain, and pJpk(1-132)-EGFP containing a TM domain and a NLS were detected in the ER in COS7 cells (Figure 9C, D, and 10). However the pJpk(89-182)-EGFP and pJpk(122-182)-EGFP having no TM domain, were located in the whole cell without localizing at specific organelles (Figure 9E, F, and 10).

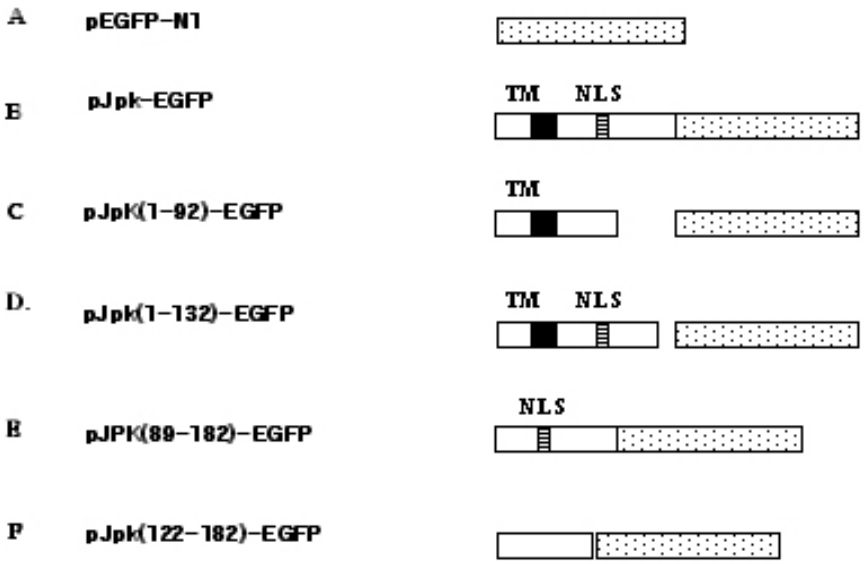

Figure 9. EGFP-tagged Jpk constructs. Jpk-EGFP harbors one TM and NLS, and Jpk(1-92)-EGFP and Jpk(1-132)-EGFP constructs contains a TM domain. But the constructs of Jpk(89-182)-EGFP and pJpk(122-182)-EGFP have a TM deleted. The TM and a putative NLS were indicated along the plasmid construct, and the EGFP was indicated as a dotted box.

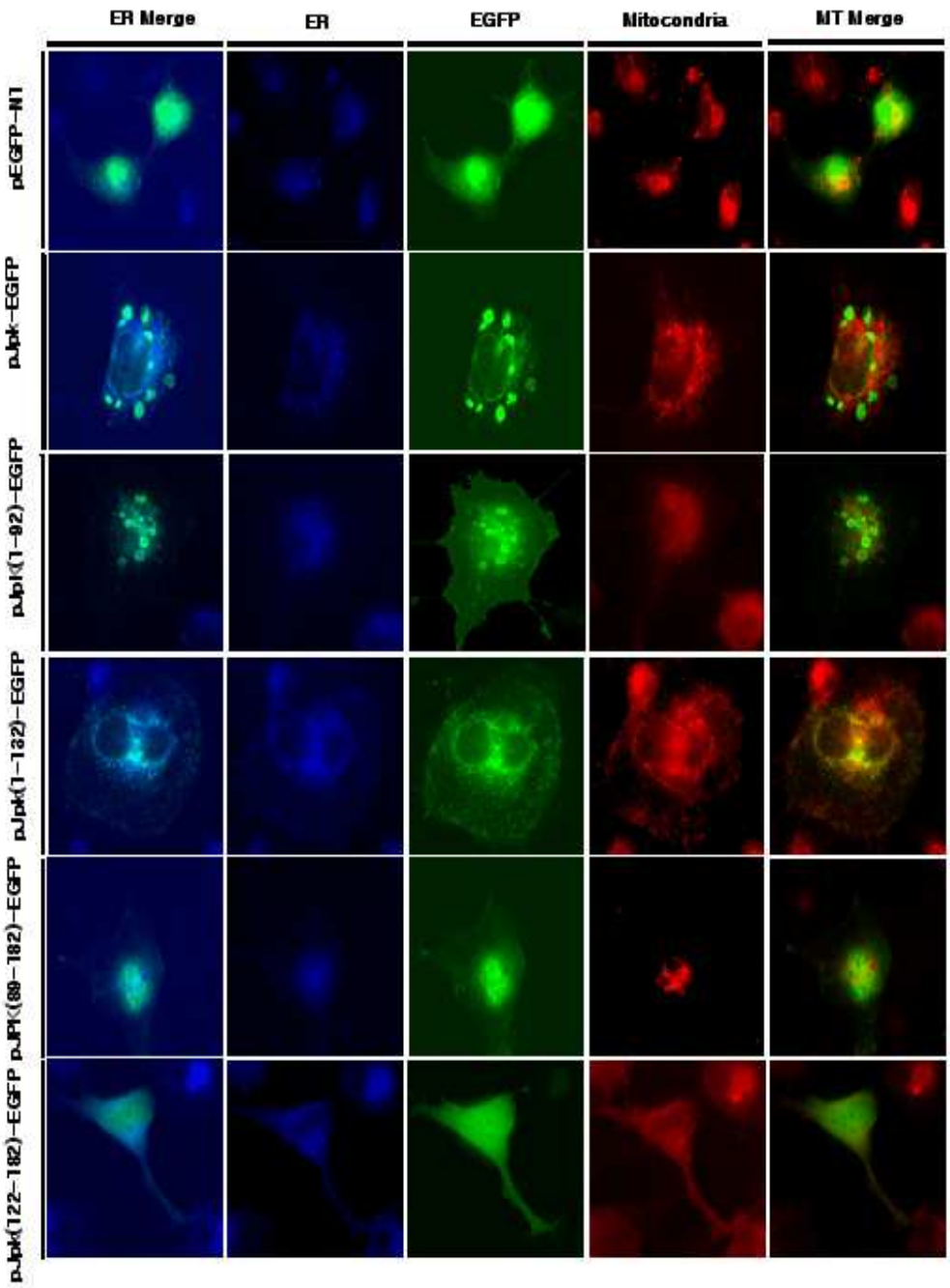
ER Merge
ER
EGFP
Mitocondria
MT Merge
pEGFP-N1
pJpk-EGFP
pJpk(1-92)-EGFP
pJpk(1-132)-EGFP
pJPK(89-182)-EGFP
pJpk(122-182)-EGFP

Figure 10. Subcellular localization of the EGFP tagged-Jpk. Each *Jpk* construct was transfected into the COS7 (monkey) cells, and then treated with Mito- and ER-Tracker to stain mitochondria and endoplasmic reticulum, respectively. We visualized them under the fluorescence microscope. Jpk-EGFP harboring a TM seemed to be localized in the endoplasmic reticulum.

B. *Jpk* was strongly induced during ER stress mediated apoptosis.

Since Jpk localizes in ER and is reported to be increased by ER stress, the expression pattern of *Jpk* was analyzed using RT-PCR under ER-stress condition.

To understand the capability of *Jpk* in ER, we analyzed its expression pattern under ER stress condition using F9 cells. But F9 cells were dead in serum free media because they rapidly proliferated, so that the experiment was performed in the complete media supplemented with serum. As shown in Figure 11, F9 cells expressed *GRP78* in the absence of DTT, which is ER stress inducer. The level of *GRP78* expression did not change by up to 3 mM DTT but was slightly increased at 4 and 5 mM DTT. The expression of *Jpk* and *CHOP-10* were increased about 7 to 16 times at 4 mM DTT compared to those of non-treated control (Figure 11), and the cell viability was reduced to about 50% (Figure 12). At 5 mM DTT, the expression pattern of *Jpk*, *GRP78* and *CHOP-10* were similar to those of cells treated with 4 mM DTT and the

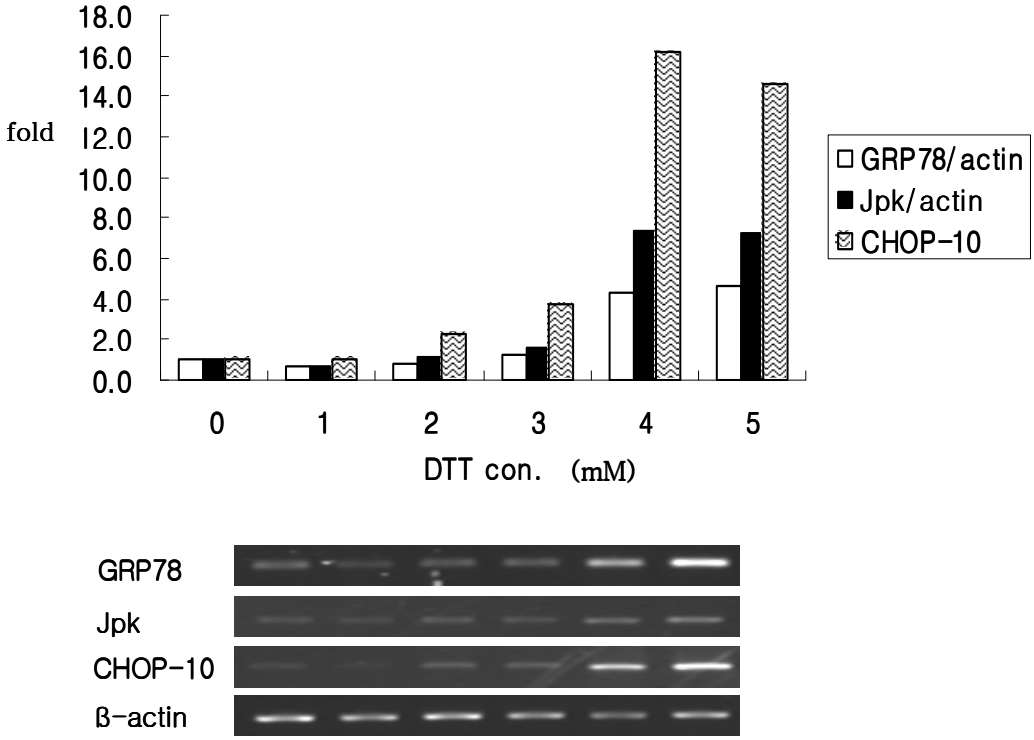

Figure 11. Effect of DTT on Jpk expression. F9 cells were treated with complete DMEM medium containing different concentrations (0, 1. 2, 3, 4, and 5 mM) of DTT for 24h. RT-PCR was performed with total RNA isolated from each sample. The relative expression level of GRP78 (white), *Jpk* (black) and *CHOP-10* (wave strip) were shown after normalizing the intensity of the PCR band with that of *β-actin*.

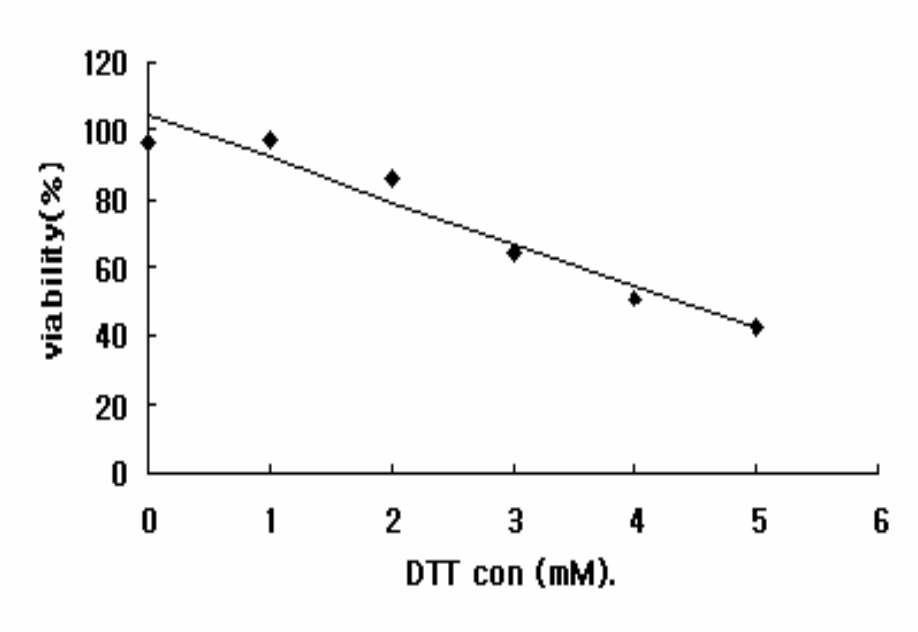

Figure 12. Effect of DTT on F9 cell viability (•). F9 cells were growing in complete DMEM media containing different concentrations (0, 1, 2, 3, 4 and 5mM) of DTT for 24h, and then the cell viability was measured with trypan blue exclusion analysis

cell viability was reduced to about 40% (Figure 11 and 12).

In order to examine if the expression pattern of *Jpk* in F9 cells is similar to that in other cells under the ER stress, we performed the same experiment using MCF7 cell, human breast cancer cell line.

The MCF7 cells are treated with the various concentrations (0, 0.01, 0.1, 0.5, 1 and 10 mM) of DTT and EGTA, ER stress inducers, for 24 hours and then cell viability were measured using Trypan blue assay and *Jpk* transcripts were analyzed by comparing with those of *GRP78* through RT-PCR. *Jpk* was expressed three-fold higher (Figure 13A and B) and viability was lost about 10% when cells were treated with 0.01 mM DTT and EGTA (Figure 13). At 10 mM DTT, cell viability was reduced to about 30% and the expression of *Jpk* and *GRP78* were upregulated about 12 and 8 fold compared to those of non-treated cells (Figure 13A and 14). At 10 mM EGTA, most of the cells died, and the expression of *Jpk* and *GRP78* were increased 14 and 10 fold higher than those of non-treated cells (Figure 13). In MCF7 cells treated with low concentration (from 0.01 to 1 mM) of DTT and EGTA, the

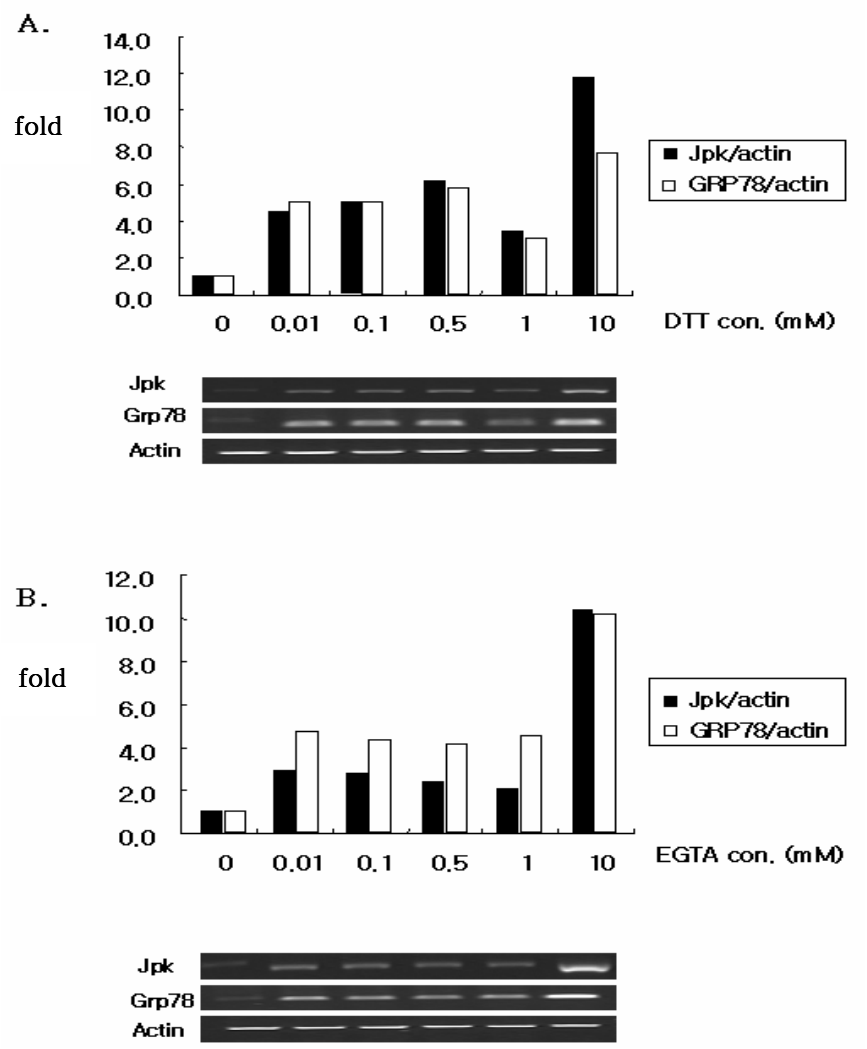

Figure 13. Effect of DTT and EGTA on Jpk expression. MCF7 cells were treated with serum-frees DMEM containing different concentrations (0, 0.01, 0.1, 0.5, 1.0 and 10.0 mM) of DTT or EGTA for 24h. RT-PCR was performed with total RNA isolated from each sample. The relative expression level of *Jpk* (black) and *GRP78* (white) (A and B) were shown after normalizing the

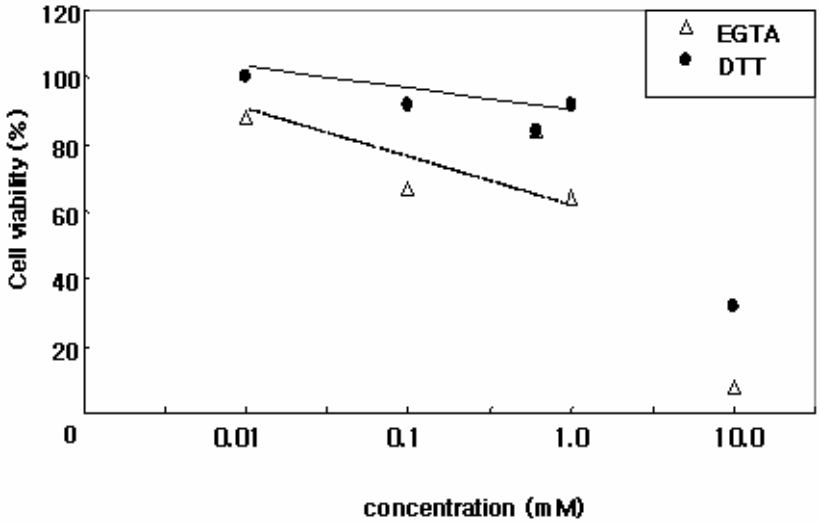

Figure 14. Effect of DTT(•) and EGTA(△) on MCF7 cells viability. MCF7 cells were treated with serum-free DMEM containing different concentrations (0, 0.01, 0.1, 0.4, 1.0 and 10.0 mM) of DTT or EGTA for 24h, and then the cell viability was measured with trypan blue exclusion analysis.

expression of *Jpk* was similarly increased to the expression of *GRP78*. However, many MCF7 cells were observed to be in the process of apoptotic cell death when treated with high concentration of DTT and EGTA (10 mM) and the *Jpk* transcripts were dramatically increased compared to *GRP78* mRNA.

In order to test whether *Jpk* affect the expression of *GRP78* and *CHOP-10*, the *GRP78* and *CHOP-10* mRNA levels were analyzed after transiently transfecting the *Jpk* constructs; pEGFP-C1, pEGFP-Jpk, pEGFP-N1 and pJpk-EGFP. As shown in Figure 9, the expression of *GRP78* and *CHOP-10* were not changed by overexpression of *Jpk* in F9 cells (Figure 15).

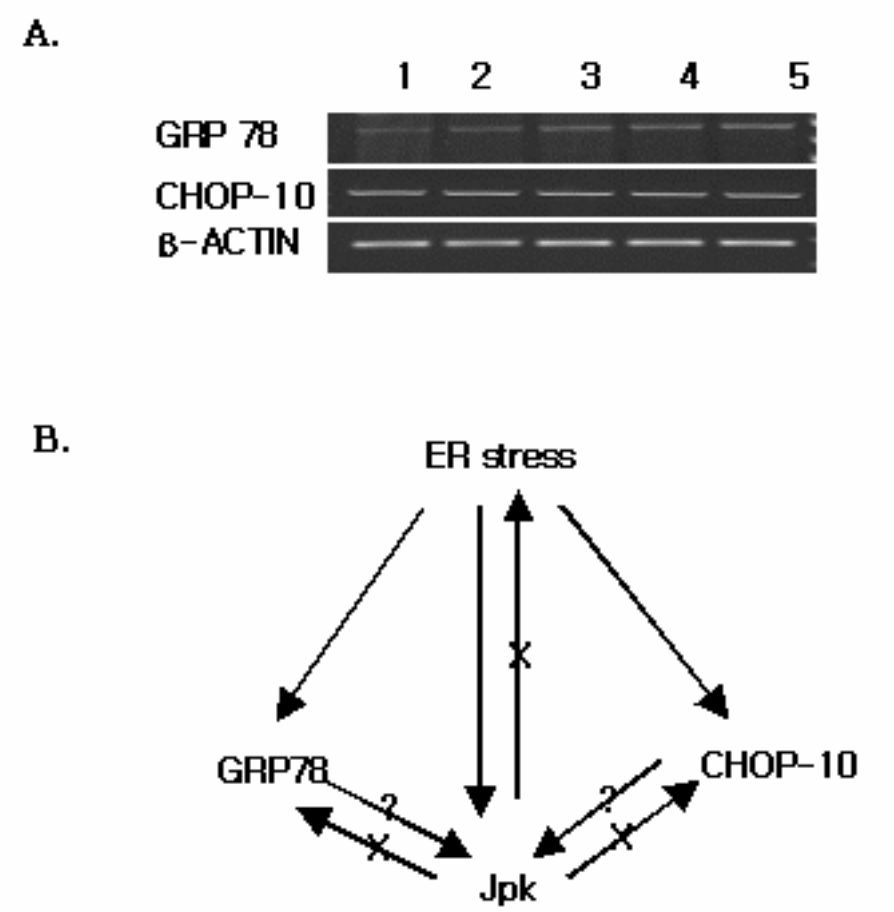

Figure 15. The effect of Jpk on the expression of GRP 78 and CHOP-10. The constructs of EGFP-tagged Jpk (lane 1, no construct; lane 2, pEGFP-C1; lane 3, pEGFP-Jpk; lane 4, pEGFP-N1; lane 5 pJpk-EGFP) were transfected into F9 cells using lipofectamin plus reagents. And then the total RNAs were purified from each cell and the expression levels of *GRP78* and *CHOP-10* were analyzed through RT-PCR method (A). (B) A diagram showing the effect of *Jpk* on expression of *GRP78* and *CHOP-10*, as well as ER-stress.

Ⅳ. DISCUSSION

In this report, we supposed that Jpk interacts with various proteins in F9 cells and in *E.coli* strain BL21(DE3) (Table 1 and 2) and plays an important role in ER because renin2, VCAM1 and PPIase of these proteins is known to pass through ER. In addition, Jpk is analyzed to be located in ER membrane and has various domains such as TM domain, NLS and several putative modification sites. We demonstrated that *Jpk* mRNA level increased robustly during ER stress-mediated apoptosis and this phenomenon is similarly appeared in F9 and MCF7 cells. However, F9 cells affected the ER stress on being treated DTT of higher concentration compared with MCF7 cells.

The identification of Jpk interactome is important to understand *Jpk* function. Thus, we employed a proteomics-based approach to identify novel interactome of Jpk. The 20 spots on 2D gel were analyzed and classified according to the function; 1) protein modification and degradation, 2) ROS regulation, 3) protein synthesis and 4) signal transduction and metabolism.

TraP, endopeptidase, PPIase and VCAM1 are components

related to protein folding, modification and degradation. Next, phenylproionate dioxygenase, GST, VCAM1 and renin2 have been catagolized as a group associating with ROS regulation. Proteins, which are involved in signaling transduction and metabolism, are putative amidase, OmpR, cytoskeratin KRT-6HF and Grb14. The last proteins categorized as protein synthesis in the present study was EF-tu and 40S ribosomal protein S2. Considering the relation of Jpk with the response to glucose stimulus, redox signal, unfolded protein response (UPR), and ER-associated protein degradation (ERAD),[7-9] Jpk seems likely to perform these functions with putative interactome analyzed in this study. Especially, VCAM1, Grb14 and renin2 among them seemed to be interesting because they do the similar function to Jpk protein.

Vascular cell adhesion molecule 1, VCAM1, is expressed in the cell surface and is important in inflammation, immune responses and intracellular signaling events.[16] VCAM-1 is reported to scavenge reactive oxygen species (ROS) by activating endothelial cell nicotinamide adenine dinucleotide phosphate (NADPH) oxidase[16] and is involved in eNOS attenuation in post-ischemic inflammatory injury[16]. Moreover VACM1 is revealed to

increase in type 2 (non-insulin dependent) diabetes. Since Jpk was strongly expressed in liver and blood of type 1 (insulin dependent) diabetic rats,[7] it inferred that Jpk may function in diabetes as interacting with VCAM1.

Growth factor receptor bound protein 14 (Grb14) is a member of the Grb7 family and expressed in testis, ovary, liver and kidney. [17,18] Overexpression of Jpk in hepatoma cells reduced insulin action which uptake glucose and synthesis glycogen. Recently, it has been reported that Jpk regulates the glucose homeostasis[5] and overexpression of Jpk interrupt insulin action on glucose uptake and glucose synthesis. This function of Jpk to inhibit insulin action is similar to the function of Grb14 to inactivate insulin receptor by recruiting PDK and transduce insulin signal. Therefore, we suggest that Jpk could be possibly involved in reducing insulin action by prohibiting Grb14 from activating insulin receptor signaling and ultimately lead to diabetes.[17,18]

renin2 is a secretory protein which is processed by cleavage and glycosylation of prorenin during passing from ER to golgi apparatus.[19,20] *renin* gene was specially expressed in kidney in murine embryo and suggested to play a role in vascular

development of the kidney.[20] Similarly, *Jpk* was also expressed in kidney at day 11.5 p.c. (data not shown) and looked like to form the secreting vesicles around ER and golgi at the cellular level. These results altogether imply that Jpk might be incorporated in maturation and secretion of renin through regulation of post-translational modification such as cleavage, glycosylation occurring in ER and golgi.

In the case of PPIase and endopeptidase, they were categorized as functional proteins involved in protein modification and degradation. PPIase is revealed to interact selectively with calcium ions (Ca^{2+}) in ER and accelerates protein folding like chaperon. Endopeptiase has known to eliminate other proteins by hydrolyzing peptide bonds. Lately, Jpk is revealed to act in unfolded protein response and ER-associated protein degradation (ERAD). It might be possible that PPIase interacting with Jpk in ER detached from Jpk under ER stress stimulus to reprocess the misfolded proteins and detached Jpk might be relevant to this ERAD pathway, which the unfolded proteins transport from ER to cytoplasm and then degrade them by various peptidase. It is inferred that Jpk may function in diabetes as interacting with

VCAM1 and reducing insulin action with Grb14 by prohibiting from activity insulin receptor signaling. In addition, Jpk might be incorporated in maturation and secretion of renin 2 through regulation of post-translational modification such as cleavage and glycosylation occurring in ER and golgi.

To study the capacity of Jpk, its protein structure analyzed using the computer analysis. In these results, N-term is analyzed to be located at ER lumen and C-term is exposed to cytoplasm. The C-term, which has various putative glycosylation and phosphorylation sites, seemed to play an important role in signal pathway. When pEGFP-Jpk and pJpk-EGFP were trnasfected into COS7, both constructs were located in ER. However the expression aspects of them were different to each other. While the EGFP-Jpk containing selenocystein was likely to be located in small vesicles, probably secreting vesicle from ER, the pJpk-EGFP having deletion of selenocystein at its most C-term end looked like to be located in large vesicles and accumulated in ER. Since EGFP-Jpk induced apoptosis[7] but not Jpk-EGFP (not shown data), the translocation from ER to golgi and plasmamembrane as well as

the toxicity of Jpk seemed to be associated with selenocystein.

Endoplasmic reticulum (ER), where Jpk is located, is the site for the synthesis and folding of secretory proteins.[21] This organelle can build up stresses, so called ER stress, by perturbations of Ca^{2+} homeostasis and accumulation of unfolded protein.[12,13] When ER is under stress, several ER stress response such as translational attenuation, upregulation of the genes for ER chaperones and degradation of unfolded proteins are turned on as a quality-control system.[12,13] However, when ER stress response is not sufficient to relieve cells from the stress, ER elicits apoptotic signals.[12, 13, 21] Furthermore, apoptosis is important to form the pattern of embryo during embryogenesis and this is induced by ER stress according to cases.

To examine whether Jpk, which is located in ER, is concerned with ER stress response, the expression pattern of it was observed under the ER stress and compared to those of *GRP78* and *CHOP-10* which are known to be related to ER stress response. As a result, both *Jpk* and *GRP78* transcripts tended to be increased in mild ER stress condition. In severe stress condition, many cells were dead and *Jpk* mRNA level was strongly

increased more than that of *GRP78*. Interestingly, this induction pattern was similar to that of *CHOP-10*, although *CHOP-10* was more strongly induced than *Jpk*. Recently, it has been reported that the expression of *CHOP-10* is weakly expressed in non or mild stress condition, but induced strongly in severe ER stress.[16,17] Consequently, this induction of *CHOP-10* triggers cell cycle arrest and apoptosis.[23-26] In the case of *Jpk*, it seemed to play a similar role to *GRP78*, protecting cells, in mild ER stress, but seemed to be involved with apoptotic pathway under intense ER stress. In this study, *Jpk* was localized in ER and play an essential role in ER stress mediated apoptosis but not regulate *GRP78* and *CHOP-10*.[26]

Ⅴ. CONCLUSION

The aim of this study was to understand the function of Jpk by observing the *Jpk* expression pattern under the ER stress and identifying the interactome of *Jpk* during embryogenesis. Firstly, GST-pull down assay and proteomics method was applied to find the interacting proteins of *Jpk* in developing embryos as well as in F9 cells. Secondly, we observed the subcellular location of Jpk and analyzed their expression pattern under the ER stress using RT-PCR.

We identified the interactome of Jpk; 1) protein modification and degradtion (TraP, endopeptidase, PPlase and VCAM1); 2) ROS regulation (pheneylproionate dioxygease, GST, VCAM1 and renin2); 3) signal transduction and metabolism (putative amidase, OmpR, cytoskeratin KRT2-6HF, and Grb14); 4) protein synthesis (EF-tu and 40S ribosomal protein S2). Especially, VCAM1 and Grb14 of these proteins were likely to have a relationship with Jpk in the function of glucose metabolism in diabetes. Jpk and renin2 might act in mesonephric tubule during kidney development.

Although the Jpk was located at the ER, it seemed to have different aspects developing on the presence or absence of selenocystein at its most C-term end: the selenocystein seemed to be necessary for the localization in the small secretory vesicle. DTT and EGTA caused ER stress and apoptosis by impairing the formation of disulfide bonds and perturbing the steady state Ca^{2+} level in the ER, respectively. *Jpk* was induced strongly like *GRP78* in MCF7 and F9 cells when DTT or EGTA was treated. Especially, *Jpk* dramatically increased during ER-stress mediated apoptosis. In these events, Jpk proteins seem to play an important role. Since overexpression of Jpk did not change the expression level of CHOP and GRP78, Jpk dose not seem to regulate the expression of GRP78 and CHOP-10 even if they were altogether induced by ER stress and apoptosis.

Jpk, which is increased by ER stress induced apoptosis, plays a critical role to form the embryo pattern as interacting with the interactome during embryogenesis.

In conclusion, these results altogether will provide new insight into the function of *Jpk* during embryogenesis.

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ABSTRACT (IN KOREA)

배자 발생시 Jpk 단백질의 기능과 Interactome 분석에 관한 연구

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Jpk (Jopock) 유전자는 생쥐 (murine)의 형태 형성에 관여하는 *Hoxa-7* 유전자의 발현은 조절하는 인자로 분리되었으며, 세포 내에서 과 발현시킨 경우 prokaryotic cell과 eukaryotic cell에서 세포사멸을 유도하였다. 특히 F9 embryonic teratocarcinoma cell에서는 mitochondrial transmembrane potential을 낮추고 ROS 생성을 증대시키며 Bax와 Bad의 발현을 증가시켜 세포사멸을 유도하는 것으로 보고되었다.

본 실험에서는 배자 발생과정 (embryogenesis) 중에서 Jpk의 기능을 이해하기 위하여 Jpk에 상호 작용하는 단백질을 F9 세포에서 찾아 MALDI-TOF로 분석하였다. 그 결과 Jpk의 interactome으로 여러 단백질들이 분석되었으며, 그 기능에 따라 1) protein modification and degradation; TraP, endopeptidase, PPlase 그리고 VACM1, 2) ROS regulation; phenylproionate dioxygease, GST, VACM1 그리고

Renin2 3) signal transduction 그리고 metabolism; putative amidase, cytoskeratin KRT2-6HF 4) protein synthesis; EF-tu, 40S ribosomal protein S2로 나누었다. 이들 단백질 중에서 VCAM1, Grb14 그리고 renin2이 특히 glucose과 ROS 조절에 관여하는 Jpk의 기능과 일치하는 것으로 보아 직접 또는 간접적으로 상호작용하고 있을 것으로 보여졌다. 또한 ER을 거쳐 분비되거나 세포막으로 이동하는 단백질인 renin2, VCAM1 그리고 PPIase을 통해서 Jpk가 ER에서 어떠한 중요한 기능을 할 것으로 생각된다.

세포 내에서의 Jpk의 발현위치를 보기 위하여, EGFP와 융합시키고 ER과 mitochondria를 염색함으로써 ER membrane에 위치하는 것을 확인하였다. 그리고 여기서의 Jpk의 역할을 이해하기 위하여 ER stress 유도물질인 DTT와 EGTA를 농도 별로 처리한 후 RT-PCR로 *Jpk, GRP78* and *CHOP-10*의 발현양상을 분석하였다. 그 결과 *Jpk*는 ER stress로 세포사멸이 유도된 세포에서 강하게 발현되는 것을 확인하였다. 즉 발생 과정 중에 형태형성을 위하여 때에 따라서 ER stress에 의한 세포사멸이 유도되는데 이때 과 발현되는 Jpk가 interactome과 함께 중요한 역할을 할 것으로 생각된다.

결과적으로 이 보고서에서는 *Jpk*는 ER에 존재하면서 ER stress에 의해 조절되며, *Jpk*가 glucose에 의해 발현이 조절되는 것

으로 보아 glucose regulation pathway에 관여하는 VCAM1과 Grb14 과 함께 상호작용하였을 것으로 생각이 되었다. 또한, 발생과정 중 신장 (kidney)발생에 관여하는 것으로 알려진 renin은 ER을 거쳐 golgi을 통해 분비되는 단백질로서 Jpk가 ER로 부터 golgi로 이동시키는 것을 도와줄 것으로 추정되었다.

결론적으로 이번 연구는 배자발생 과정 중 *Jpk*의 기능에 대하여 새로운 관점에서 논하고 있다.

핵심 되는 말: 세포사멸, ER stress, F9 세포, GST pull down system, Hox, interactome, Jpk, Proteomics, RT-PCR, subcellular localization