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Intracellular Localization of Porcine Single-Strand Binding Protein 2

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ABSTRACT

We have previously cloned cofactor CLIM2 (Ldb1/NL1) as a binding protein for LIM homeodomain transcription factor and now seek a protein interacting with CLIM2. Ultimately, SSBP2 was cloned as CLIM2 binding protein from the adult porcine pituitary cDNA library by the Yeast Two-Hybrid System. The amino acid sequence of porcine SSBP2 shows a high identity (99%) with those of other mammalian species, man, and mouse. Using fluorescence protein-fused SSBP2 and its deletion mutants, we observed that SSBP2 overexpressed in CHO cells predominantly localizes in mitochondria. Expression of mutant SSBP2s demonstrated that the first 241 amino acid residues are responsive for the mitochondrial localization. When CLIM2 vector was co-transfected, SSBP2 changed its location to nuclei. The similar translocation was also observed when CLIM2 vector was transfected 17 h after the transfection of SSBP2 vector. The first 120 residues of SSBP2 are responsible for the nuclear localization by guidance with CLIM2. RT-PCR demonstrated that SSBP2 was expressed in the porcine pituitary from fetal 40 days to postnatal 230 days in both genders and in the variety of pituitary and non-pituitary tumor cell lines, indicating that SSBP2 is present ubiquitously and plays a universal function during fetal and postnatal pituitary development. J. Cell. Biochem. 106: 912–919, 2009.

KEY WORDS: SSBP2; CLIM2; NUCLEAR LOCALIZATION; MITOCHONDRIA; PITUITARY

Lhx2 and Lhx3, members of the LIM homeodomain protein family [Thor et al., 1999], are reported to modulate the expression of the pituitary glycoprotein α subunit gene (αGSU) by binding to a proximal site identified as the pituitary glycoprotein hormone basal element (PGBE) [Roberson et al., 1994]. Recently, it was demonstrated that Lhx3 modulates the expression of porcine follicle stimulating hormone β subunit (FSHβ) gene [West et al., 2004]. In addition, the knockout mouse of Lhx3 gene demonstrated that Lhx3 plays a critical role in pituitary organogenesis [Sheng et al., 1997; Sheng and Westphal, 1999]. Thus, LIM-homeodomain transcription factors are closely associated with pituitary function as well as hormone production. We are currently investigating the regulatory mechanisms of these LIM-homeodomain transcription factors and are now discovering novel responsive regions of these factors on αGSU and FSHβ (in preparation). We recently have reported that porcine Lhx2 interacts with CLIM2 (Lin-11, Isl-1, and Mec-3 (LIM) domain-binding protein 2; same as Ldb1 (LIM domain binding protein) and nuclear LIM interactor1 (NL1)), resulting in alteration of its activity [Susa et al., 2006].

One of the important functions of LIM domain is its ability to interact with other proteins that participate in gene regulation [Jurata et al., 1996; Jurata and Gill, 1997; Wadman et al., 1997; Howard and Maurer, 2000, 2001; Mochizuki et al., 2000; Parker et al., 2000; Bridwell et al., 2001; Thaler et al., 2002; Deane et al., 2003; Matthews and Visvader, 2003; Xu et al., 2003, 2007a, 2007b; Lahlil et al., 2004; Hashimoto et al., 2005], making it possible to expand and/or modify the regulatory mechanism. CLIM2 was revealed to interact with RING finger LIM domain-binding protein (RLIM) which is encoded by the RNF12 (RING finger12) gene and acts as E3 ubiquitin ligase for CLIM2, LMO2, and HDAC2 (histone deacetylase), thereby inducing degradation through the 26S proteasome pathway [Ostendorff et al., 2002; Hiratani et al.,

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2003]. Hence, we attempted to clarify the protein interacting with the LIM protein in the porcine anterior pituitary gland and succeeded in cloning a member of LIM-domain interacting protein, CLIM2. Ultimately, this LIM-binding factor participates positively and/or negatively, depending on the interacting partner LIM-homeodomain transcription factors against the αGSU gene expression [Susa et al., 2006].

In the present study, we employed the Yeast Two-Hybrid System for the porcine anterior pituitary cDNA library using a porcine CLIM2 as a bait protein. Finally, we cloned and identified porcine SSBP2 (single-stranded DNA-binding protein 2) as a CLIM2 interactor and demonstrated that SSBP2 localizes in the mitochondria and that coexpression of CLIM2 forced its localization to the nuclei.

MATERIALS AND METHODS

CLONING OF CLIM2-BINDING PROTEIN

The coding region of porcine CLIM2 [Susa et al., 2006] was amplified by PCR using a set of primers listed in Table I from the porcine CLIM2 clone in pAD-GAL4 vector. The PCR product was cloned into *Eco*RI/*Xho*I sites of the yeast expression vector pBD-GAL4 (Stratagene, La Jolla, CA) as a bait protein vector, CLIM2/pBD-GAL4. The Two-Hybrid System was employed by co-transfection of yeast cells YRG-2 with each 10 μg of CLIM2/pBD-GAL4 and an adult porcine pituitary cDNA library constructed in pAD-GAL4 vector (Stratagene) by the LiAcetate method as described previously [Kato et al., 1997]. The yeast transformants were selected on a synthetic medium lacking tryptophane, leucine, and histidine, which are selection markers for pAD-GAL4, pBD-GAL4 and reporter gene (*his3*), and in the presence of 3-amino-1-,2-,4-triazole to inhibit the leaky HIS3 expression from pHISi-1 gene [Kato et al., 1997].

The colony-lift β-galactosidase filter assay was performed for yeast cells which were grown at 30°C for 2 days on a Whatman No. 50 filter paper (Whatman International Ltd., Maidstone, England), followed by visualization as described previously [Kato et al., 1997]. DNA samples were prepared from *E. coli* by the alkaline mini-preparation method and were employed in the fluorescence labeled dye-terminator reaction using the Big Dye terminator system (Applied Biosystems, Foster City, CA), followed by analysis on the ABI PRISM 310 (Applied Biosystems).

CONSTRUCTION OF EXPRESSION VECTORS

Amplified porcine CLIM2 and SSBP2 were ligated to the *Eco*RI/*Xho*I site of mammalian expression vector pcDNA3.1Zeo$^+$ (Invitrogen, Carlsbad, CA), producing CLIM2/pcDNA3.1 and SSBP2/pcDNA3.1, respectively. Using specific primer sets (Table I), the full-length porcine SSBP2 cDNA and five fragments encoding a part of SSBP2 were amplified and inserted in the frame to the *Eco*RI/*Sal*I sites of pEGFP-C1, pECFP-C1 or pEYFP-C1 plasmid vector (Clontech, Palo Alto, CA), producing SSBP2/YFP, SSa/GFP, SSb/CFP, SSc/CFP, SSab/CFP, and SSbc/YFP, respectively. The mitochondria localization vectors, Mit/CFP and Mit/YFP, were constructed by fusing with a mitochondria target signal (oligonucleotide encoding a peptide sequence MSVLTPLLLRGLTGSARRLPVPRAKIHSL [Rizzuto et al., 1995]) to CFP or YFP vector (Clontech).

SUBCELLULAR LOCALIZATION

CHO cells were obtained from the RIKEN Cell Bank (Tsukuba, Japan). Cells were maintained in monolayer cultures in Ham's F12 (GIBCO BR, Gaithersburg, MD) for CHO cells supplemented with 10% (v/v) fetal bovine serum (ICN Biomedical, Inc., Aurora, OH) and antibiotics (Sigma–Aldrich Corp., St. Louis, MO) in humidified 5% CO_2—95% air at 37°C. Cells were seeded in a 96-well plate at 1×10^4 cells/100 μl/well. Twenty-four hours after seeding, CHO cells were transfected with the complex of FuGENE6 (0.3 μl, Roche Diagnostics GmbH, Mannheim, Germany) and SSBP2 fluorescence vector/well with or without Mit/CFP and Mit/YFP. After incubation for 24 h, fluorescence was detected directly in the presence of media using fluorescent microscopy. The nucleus was visualized using propidium iodide (PI) staining.

To examine whether CLIM2 influences the prior localization of SSBP2 in the mitochondria, CHO cells were transfected with SSBP2/YFP and cultured for 17 h, followed by washing with medium twice. Thereafter, CLIM2/pcDNA3.1 or pcDNA3.1Zeo$^+$ together with CFP vector was supplementarily transfected and cultured further for 8 and 24 h, respectively.

TABLE I. Primer List for Construction of SSBP2 Fragment

Fragment name	Forward primers	Reverse primers
Full length		
Porcine CLIM2	5'-AGAGAATTCACCATGCTAGATCGGGATGTG-3'[a]	5'-GCACAGTTGAAGTGAACTTGCGG-3'[b]
SSBP2	5′-GAGAATTCAGCATGTACGGCAAAGGCAAG-3′	5′-CTCTCGAGGAGACTCGGTAATGGATCACAC-3′
Subfragment of SSBP2		
SSa	5′ -GAGAATTCAGCATGTACGGCAAAGGCAAG-3′	5′-CTCTCGAGTGGTGGTACAGGACCTACTGG- 3′
SSb	5′ -GACAATTGCCAGTAGGTCCTGTACCACCA-3′	5′-CTCTCGAGTACATAGTTCCCAGGAGATGC-3′
SSc	5′-GACAATTGGCATCTCCTGGGAACTATGTA-3′	5′-CTCTCGAGGAGACTCGGTAATGGATCACAC-3′
SSab	5′-GAGAATTCAGCATGTACGGCAAAGGCAAG-3′	5′-CTCTCGAGTACATAGTTCCCAGGAGATGC-3′
SSbc	5′-GACAATTGCCAGTAGGTCCTGTACCACCA-3′	5′-CTCTCGAGGAGACTCGGTAATGGATCACAC-3′
RT-PCR		
SSBP2 pig rodent	5′-GGAACACCCATCATGCCTAG-3′	5′-CTCTCGAGGAGACTCGGTAATGGATCACAC-3′
Cyclophilin A	5′-TGGTGACTTYACACGCCATAATG-3′	5′-ATTCCTGGACCCRAAACGCTCC-3′

[a]CLIM2 sequence is underlined.
[b]Sequence of pAD-GAL4 cloning site.

RT-PCR OF PORCINE PITUITARIES AND CELL LINES

Specific PCR primer sets of the porcine, rat and mouse SSBP2 and cyclophilin A are listed in Table I. Each cDNA of porcine pituitaries [Aikawa et al., 2005] and pituitary tumor-derived cell lines [Aikawa et al., 2006] was amplified in a reaction mixture (5 μl) containing a set of primers (5 pmol each) and 0.125 U AmpliTaqGold polymerase (Applied Biosystems) with 32 cycles for SSBP2 and 36 cycles for cyclophilin A of denaturation (94°C, 30 s), annealing (55°C, 30 s) and extension reaction (72°C, 2 min) steps, followed by the agarose gel electrophoresis.

RESULTS

SCREENING OF CLIM2 INTERACTING PROTEIN FROM PORCINE PITUITARY CDNA LIBRARY

Co-transformation with CLIM2/pBD-GAL4 and porcine pituitary cDNAs/pAD-GAL4 was carried out with YRG-2 competent cells at an efficiency of 1×10^4 colony forming units per 1 μg DNA. By repeated screenings four times with each 2×10^5 transformants, 2 colonies were obtained on the $SD^{-Trp-Leu-His}$-agar plates. BLAST search revealed that the clones show a high homology to a human single-stranded DNA binding protein 2, SSBP2 (Accession number, NM_012446). Two clones showed the same entire sequences consisting of 2,020 bp length with 17 bp of poly(A). When the deduced amino acid sequence (361 residues) was compared to those of human [Liang et al., 2005] and mouse [Chen et al., 2002] SSBP2, the amino acid sequence has a 99% identity with conservation of certain characteristic domains, LUFS (LUG/LUH, Flo8, single-strand DNA-binding protein) domain [Conner and Liu, 2000], LisH (Lissencephaly type 1-like homology) domain [van Meyel et al., 2003; Liang et al., 2005], FORWARD domain (GFLxxWWxxFWD) [Bayarsaihan, 2002], PxxGMxPP-like motif [Bayarsaihan, 2002] and PxxP motif [Bayarsaihan, 2002] (Fig. 1). Characteristically, the amino acid content is rich in proline (16%), glycine (15%), serine (10%), methionine (7%), and asparagine (7%).

SUBCELLULAR LOCALIZATION OF SSBP2

To test the subcellular localization of SSBP2, the expression vector of SSBP2 fused with fluorescence protein YFP (SSBP2/YFP) was constructed and transfected to CHO cells (Fig. 2). Transfection of SSBP2/YFP resulted in the fluorescence being predominantly observed with punctuated distribution in the cytoplasm (Fig. 2A), but not in the nuclei as shown in Figure 2B,C. The empty YFP vector used as a control, on the other hand, gave diffusible fluorescence in the transfected cells (Fig. 2D).

Subcellular localization was further examined by constructing vectors with several fragments of SSBP2 as indicated in Figure 3A. Transfection of SSa/GFP, SSb/CFP, SSc/CFP and SSbc/YFP failed to form punctuated distribution similar to SSBP2/YFP, and only SSab/CFP showed the same intracellular distribution as SSBP2/YFP (Fig. 3B), demonstrating the presence of localization signals in the N-terminal.

To further determine the localization of punctuated fluorescence, several subcellular localization vectors were constructed and their distributions were compared with those of SSBP2/YFP and SSab/CFP (Fig. 3E). When SSBP2/YFP and SSab/CFP were co-transfected with the mitochondria localization vector (Mit/CFP or Mit/YFP), the distribution of two fluorescent proteins was the same (Fig. 3C,D). Ultimately, the mitochondria localization vector (Mit/CFP or Mit/YFP) showed a fluorescence pattern similar to those of SSBP2/YFP and SSab/CFP (data of other localization vectors not shown).

Fig. 1. Comparison of the amino acid sequences of SSBP2. The amino acid sequence of porcine SSBP2 was compared with those of man [Liang et al., 2005] and mouse [Chen et al., 2002] SSBP2. Each amino acid identical to that of porcine SSBP2 is indicated by hyphen (-). Characteristic domains are indicated: LUFS domain [Conner and Liu, 2000], LisH domain [van Meyel et al., 2003; Liang et al., 2005], FORWARD domain [Bayarsaihan, 2002], PxxGMxPP-like motif [Bayarsaihan, 2002], and PxxP motif [Bayarsaihan, 2002].

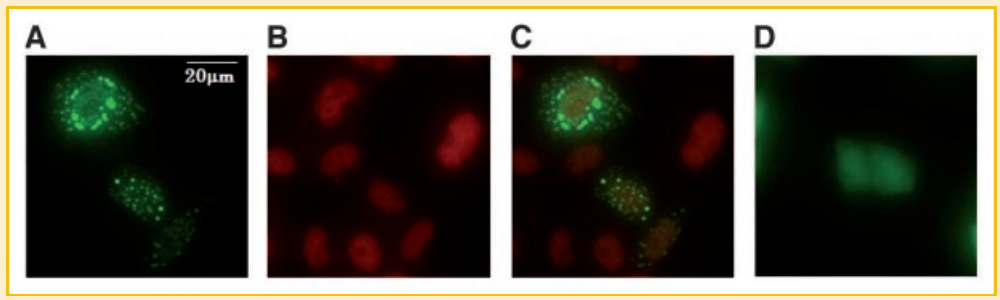

Fig. 2. Subcellular localization of SSBP2 in CHO cells. Cells were transfected with 10 ng of SSBP2/YFP (A) and nuclei were visualized with propidium iodide staining (B). Images of (A) and (B) were merged (C). YFP DNA only was transfected (D).

CO-EXPRESSION OF SSBP2 AND CLIM2

The present experiments hitherto show that SSBP2 localizes in mitochondria. However, SSBP2 is originally cloned as a protein interacting with CLIM2 and supposed to be present in nuclei. Hence, we examined the co-expression of SSBP2 and CLIM2 vectors. As shown in Figure 4A, the fluorescence of SSBP2 fusion protein with coexpression of CLIM2/pcDNA3.1 was observed in the PI-stained nuclei. To investigate the interaction region of SSBP2 with CLIM2, SSBP2 fragments used in the above experiments were similarly examined by coexpression of CLIM2/pcDNA3.1 as shown in Figure 4. Fluorescence of SSb/CFP (Fig. 4C), SSc/CFP (Fig. 4D), and SSbc/YFP (Fig. 4E,F) showed diffusible localization similar to that of CFP and YFP (data not shown). On the other hand, SSa/GFP (Fig. 4B) and SSab/CFP (Fig. 4E) showed fluorescence in the PI-stained nuclei, indicating that the N-terminal 120 residues of SSBP2 are responsive to nuclear localization together with CLIM2.

CLIM2/pcDNA3.1 and empty pcDNA3.1, together with CFP, were transfected at 17 h after transfection with SSBP2/YFP and observation with fluorescence microscopy was carried out after culture for 8 and 24 h. We have previously confirmed that competent cells are able to receive plural vectors [Susa et al., 2008]. Transfection of SSBP2/YFP alone (Fig. 5A) and SSBP2/YFP together with CFP (Fig. 5B) showed a scattered fluorescence of SSBP2, while fluorescence of CFP showed diffuse distribution in the cytoplasm and nuclei. In contrast, transfection CLIM2/pcDNA3.1 and CFP showed that SSBP2-fluorescence localizes in the nuclei of CFP-fluorescence cells (Fig. 5C,D).

RT-PCR OF SSBP2 TRANSCRIPTS DURING PORCINE PITUITARY ONTOGENY AND IN CELL LINES

RT-PCR of SSBP2 using fetal (f) and postnatal (p) porcine pituitaries of both sexes was performed. The results showed that SSBP2 gene is already expressed at f40 and continues to express during the pituitary development and postnatal periods without pronounced change of expression (Fig. 6). There was no obvious difference between both sexes.

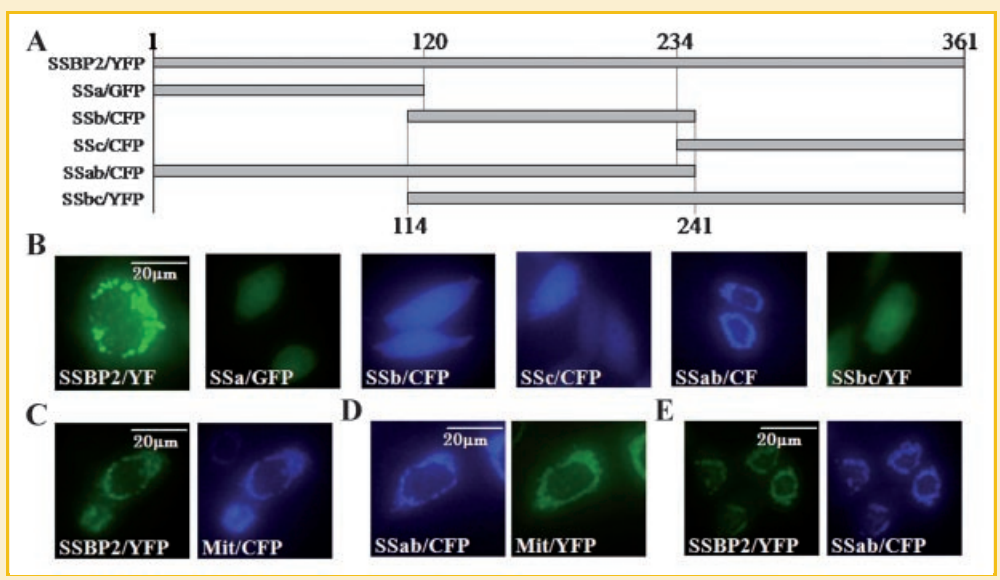

Fig. 3. Expression of SSBP2 fragments and mitochondria localization vector in CHO cells. A: Diagram of vectors fused with SSBP2 fragments with names indicated at left by fluorescence vectors (Y, yellow; G, green; C, cyan). B: Transfection of SSBP2 fragments (in each image). C: Co-transfection of SSBP2/YFP and mitochondria localization vector (Mit/CFP). D: Co-expression of SSab/CFP and mitochondria localization vector (Mit/YFP). E: Co-expression of SSBP2/YFP and SSab/CFP.

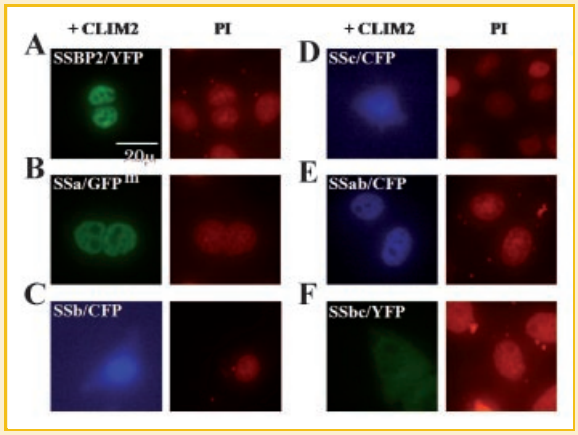

Fig. 4. Co-expression of SSBP2 or SSBP2 fragments together with CLIM2/pcDNA3.1 in CHO cells. Cells were transfected with 10 ng of SSBP2/YFP (A), SSa/GFP (B), SSb/CFP (C), SSc/CFP (D), SSab/CFP (E) and SSbc/YFP (F) together with same amount of CLIM2/pcDNA3.1. Nucleus was visualized using propidium iodide staining (PI: right panel of each fluorescence image).

To examine whether SSBP2 gene expresses in unique cells, RT-PCR was performed for pituitary tumor-derived and other cell lines. All cell lines including non-pituitary cell line L929 showed PCR product (Fig. 7) exhibiting ubiquitous expression of this gene in the cells examined.

DISCUSSION

In the present study, we cloned SSBP2 as CLIM2-binding protein and demonstrated that SSBP2 alone locates in the mitochondria but changes its location in the nuclei by co-expression of CLIM2 vector. SSBP2 is a member of the gene family with SSBP1, SSBP3, and SSBP4, and is known to exhibit a binding ability to LIM domain interacting proteins, such as CLIM1 and CLIM2, that modify or convert the function of LIM homeodomain transcription factors [Bach et al., 1997]. Though the DNA binding specificity of SSBP2 itself is yet unclear, the chicken orthologue of SSBP3 showed exquisite specificity for pyrimidine-rich single-stranded mirror repeat element of the α2(I) collagen promoter with electrophoretic mobility shift assay (EMSA) [Bayarsaihan et al., 1998]. In this study, although we attempted to examine the DNA binding ability of porcine SSBP2 with EMSA for single-stranded DNA probes including pyrimidine-rich oligonucleotide, and failed to observe any binding properties (data not shown), the possibility remains that SSBP2 binds to some unexamined sequences.

Transfection of SSBP2 expression vector fused with fluorescent protein showed an intriguing distribution and scattered fluorescence in the cytoplasm (Fig. 2A). Using the mitochondrial localization vector, we concluded that SSBP2 localizes in the mitochondria. Hitherto, the subcellular localization of *Drosophila* Ssdp, which

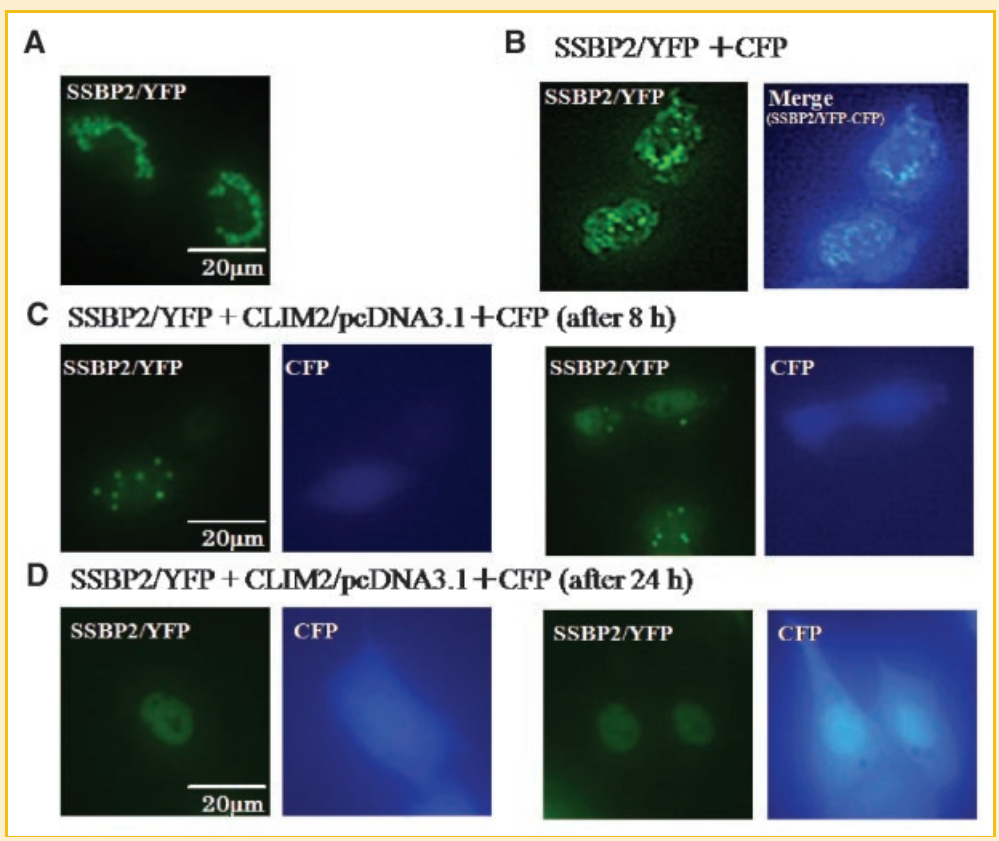

Fig. 5. Expression of CLIM2/pcDNA3.1 in the SSBP2/pcDNA3.1-transfected CHO cells. CHO cells were transfected with CLIM2/pcDNA3.1 or pcDNA3.1 together with CFP vector 17 h after transfection and fluorescence were observed. A: SSBP2/YFP. B: SSBP2/YFP, pcDNA3.1, and CFP. C: SSBP2/YFP, CLIM2/pcDNA3.1, and CFP after 8 h. D: SSBP2/GFP, CLIM2/pcDNA3.1, and CFP after 24 h. Two typical figures are indicated (C,D).

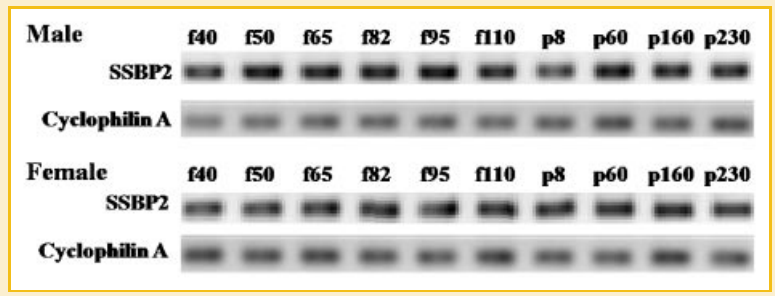

Fig. 6. Ontogeny of porcine SSBP2 gene expression. Total RNAs prepared from fetal (f) and postnatal (p) porcine pituitaries of male (upper) and female (lower) were analyzed by RT-PCR. Amount of cDNA sample, which produces the same amounts of PCR product for cyclophilin A, was subjected to PCR reaction for SSBP2.

shows a high similarity to the amino-terminal 75 residues of SSBP2 except for other regions, is reported to be diffusible in cytoplasm [van Meyel et al., 2003]. Therefore, the mitochondrial localization of SSBP2 is a new observation. Furthermore, our attempts using several SSBP2 fragments revealed that the amino-terminal 241 residues are required for mitochondrial localization (Fig. 3D). By reference to the homology between SSdp and SSBP2, residues beyond position 75 may be responsive to mitochondrial localization. Meanwhile, the bacterial single-stranded mitochondrial DNA-binding protein (mtSSB) is previously cloned [Tiranti et al., 1993] and shows similarity to vertebrate SSBP1. However, the primary structure of mtSSB (148 residues) as well as SSBP1 has no obvious homology (18%) within the amino-terminal region of porcine SSBP2. The molecular basis to conduct the mitochondrial localization of SSBP2 remains an interesting issue.

Since SSBP2 is postulated to play its role in the nuclei by interacting with the CLIM2 LUFS domain [van Meyel et al., 2000], the subcellular localization of SSBP2 was unexpected, and then we attempted to co-express CLIM2 vector and SSBP2 vector. The result showed a dramatic change in that the fluorescence of SSBP2 in the mitochondria disappeared, and the nuclei have emerged (Fig. 4). SSBP2 has no nuclear location signal, so this nuclear localization must due to CLIM2. Indeed, between the dimerization domain and LIM interaction domain, CLIM2 has a nuclear location signal [Cassata et al., 2000], by which the SSBP2-CLIM2 complex migrated into the nuclei. We found that the responsive region in the SSBP2 to locate in nuclei is present within the first 120 amino acid residues (Fig. 4B). Since it was previously demonstrated that the residues 1–100 of mouse SSBP2 are responsive to interaction with the residues 214–223 of mouse CLIM2 [van Meyel et al., 2003], nuclear migration of SSBP2 by CLIM2 may arise with interaction between both amino-terminal regions. A similar translocation was reported according to which *Drosophila* Ssdp altered its cytoplasm localization to nuclei by interaction with Chip, a *Drosophila* orthologue of CLIM2 [van Meyel et al., 2003]. Whether CLIM2 guides the mitochondrial SSBP2 to nuclei has remained a question. When CLIM2 vector was transfected 17 h after transfection of SSBP2/YFP, a few cells showed fluorescence of both SSBP2/YFP and CFP after 8-h culture with scattered fluorescence in cytoplasm and concentrating fluorescence in nuclei (Fig. 5C). With prolonged cultivation of 24 h, the scattered fluorescence of SSBP2 was mostly lost and fluorescence was observed in the nuclei (Fig. 5D). Taken together, SSBP2 located in the mitochondria may be guided to nuclei with CLIM2 synthesized later. This result also indicates the importance of stoichiometry between SSBP2 and CLIM2. However, we could not exclude the possibility that turnover of SSBP2 is very rapid, it thus disappeared, and that newly synthesized SSBP2 and CLIM2 interact in cytoplasm and translocate to nuclei. When SSBP2 plays its role in nuclei, the stoichiometry between SSBP2 and CLIM2 is important, as pointed out previously [van Meyel et al., 2000]. More recently, a novel interaction of SSBP2 was reported, namely, the adenoviral oncoprotein E1B55K binds to SSBP2 and sequesters it into juxtanuclear bodies [Fleisig et al., 2007], suggesting that SSBP2 can alter its location in accordance with interactors.

Since the discovery of SSBP2 [Bayarsaihan et al., 1998; Raval-Fernandes et al., 1999], the precise function and molecular mechanism of this protein have been obscure. SSBP2 was discovered as a target of an unbalanced translocation in myeloid leukemia cells [Liang et al., 2005], while other members of the SSBP family have been widely studied as interaction partners CLIM2 and shown to have a function in axis formation in *Xenopus*, wing development in *Drosophila*, and head morphogenesis in mice [Chen et al., 2002; van

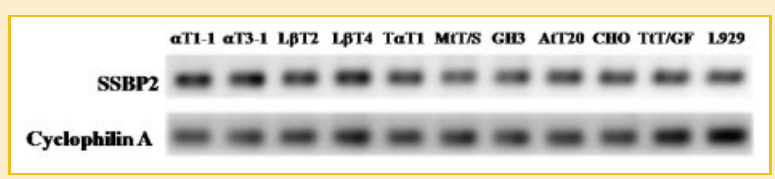

Fig. 7. RT-PCR analysis of SSBP2 gene expression in pituitary tumor and non-pituitary cell lines. Amounts of cDNA sample from pituitary tumor-derived cell lines (αT1-1, αT3-1, LβT2, LβT4, TαT1, MtT/S, GH3, AtT20, and TtT/GF) and non-pituitary cell lines (CHO and L929), which produce the same amounts of PCR product for cyclophilin A, were subjected to PCR reaction for SSBP2.

Meyel et al., 2003; Nishioka et al., 2005; Enkhmandakh et al., 2006]. Thus, SSBP2 plays an important biological function that accounts for CLIM2-dependent actions to protect against ubiquitination. The present study did not clarify the role of the translocation of SSPB2 from mitochondria to nucleus, but may be involved in nuclear-mitochondria interactions at the transcriptional and post-transcriptional level as reviewed [Cannino et al., 2007]. Further study is warranted on the mitochondrial location and role of SSBP2.

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