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—Original Article—

Molecular Cloning of Rat and Porcine Retina-derived POU Domain Factor 1 (POU6F2) from a Pituitary cDNA Library

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Abstract. Homeobox transcription factors are known to play crucial roles in the anterior lobe of the pituitary gland. During molecular cloning with the Yeast One-Hybrid System using a 5'-upstream region of the porcine *Fshβ* as a bait sequence, we have cloned a cDNA encoding a partial sequence of the retina-derived POU domain factor 1 (RPF1) from the porcine pituitary cDNA library and confirmed its specific binding to the bait sequence. *In situ* hybridization was performed to examine localization of *Rpf1* and showed that this gene is expressed in the stem/progenitor cells of the rat pituitary primordium as well as the diencephalon and retina. In addition, real-time PCR demonstrated that *Rpf1* transcripts are abundant in early embryonic periods but that this is followed by a decrease during pituitary development, indicating that this factor plays a role in differentiating cells of the pituitary. The transcriptional activity of RPF1 for genes of *Prop1*, *Prrx1* and *Prrx2*, which were characterized as genes participating in the pituitary stem/progenitor cells by our group, was then examined with full-length cDNA obtained from the rat pituitary. RPF1 showed regulatory activity for *Prop1* and *Prrx2*, but not for *Prrx1*. These results indicate the involvement of this retina-derived factor in pituitary development.

Key words: Differentiation , Gene regulation, Pituitary, POU6F2, Retina, RPF1

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The anterior pituitary (adenohypophysis) is composed of anterior and intermediate lobes, and is an indispensable endocrine organ responsible for the homeostasis of vital functions and the synthesis of many hormones. To develop this organ, various transcription factors are essential for organogenesis and maintenance of pituitary hormone production. Several approaches to clarify the molecular mechanisms have been used, and many transcription factors governing the basal and cell-specific expression have been discovered [1]. Transcription factors definitely recognize particular nucleotide sequences located near the target gene. We previously demonstrated that the 5'-upstream region of the porcine *Fshβ* (named Fd2, −852/−746 base (b) region) is bound with many nuclear proteins of the porcine anterior pituitary [2]. In addition, we have demonstrated that the 5'-upstream region of the porcine *Fshβ* (−852/+12 b region) is responsible for directing pituitary-specific expression in the gonadotropes, the cells that synthesize LH and FSH, by generation of transgenic rats [3]. Since then, we have continuously performed molecular cloning with the Yeast One-Hybrid System using Fd2 as a bait sequence. Consequently, we succeeded in cloning multiple pituitary transcription factors and published three reports describing the homeobox transcription factors PROP1, PRRX2 and LHX2, which specifically bind to an AT-rich region in Fd2 [4–6].

PROP1 is well known to play an essential role in the normal production of gonadotropins, as well as in the development of PIT1 lineage cells, GH-, PRL- and TSHβ-producing cells, in both humans and mice [7]. Immunohistochemistry of PROP1 demonstrated that this factor starts to be expressed in invaginating oral ectodermal cells at rat embryonic day 11.5 (E11.5), and at E13.5, it occupies all cells. It expressed the stem/progenitor cell marker *Sox2* in Rathke's pouch, a primordium of the anterior pituitary [8, 9], indicating that PROP1 has a crucial role in pituitary stem/progenitor cells. Subsequently, PRRX2 was first cloned from the mouse adult spleen [10], and is now known as a mesenchymal transcription factor that plays important roles in craniofacial and limb morphogenesis [11–13]. We demonstrated that PRRX2, as well as its close relative PRRX1, is present in the stem/progenitor cells of the anterior pituitary [14–16]. Finally, LHX2 is a member of the LIM-homeodomain protein family and has been previously identified as a transcription factor for *Cga* [17]. This factor shows a higher expression level during the embryonic period in the anterior pituitary than during the postnatal period [18]. Thus, past and present investigations of the three factors have revealed that they are important for pituitary organogenesis and maturation.

This paper further describes the molecular identification of

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transcription factors from uncharacterized clones screened by the Yeast One-Hybrid System using Fd2 as a bait sequence. The newly characterized gene, retina-derived POU domain factor 1 (RPF1; same as POU6F2), is a member of the POU-homeodomain transcription factors that was originally cloned from a human retina cDNA library. Specific binding of RPF1 to the AT-rich sequence and expression in the embryonic anterior pituitary were confirmed by DNase I footprinting and real-time PCR, respectively. We demonstrated using *in situ* hybridization that *Rpf1* without a doubt plays a role by expressing in rat's Rathke's pouch.

Materials and Methods

Animals

Wistar-Imamichi rats were housed individually in a temperature-controlled room under a 12 h light/12 h dark cycle. The present study was approved by the Institutional Animal Care and Use Committee, Meiji University, based on the NIH Guidelines for the Care and Use of Laboratory Animals.

Cloning of Rpf1 from porcine and rat pituitary cDNA libraries and construction of expression vectors

Cloning of porcine *Rpf1* was performed by the Yeast One-Hybrid System from porcine pituitary cDNA using Fd2 (–852/–746 b) of the porcine *Fshβ* promoter as a bait sequence, as described previously [6]. Rat *Rpf1* of the full-length clone was obtained directly by PCR amplification from a rat pituitary cDNA library using a specific primer set (forward, 5'-ATGATAGCTGGACAAGTCAGTAAGCCC-3', and reverse, 5'-TGCTTCCTTCTGATCTATGAACGGTGTG-3'). Both of the open reading frames (ORF) were ligated in frame into the expression vector pcDNA3.1 Zeo$^+$ (Invitrogen, Carlsbad, CA, USA) or pET32a (Novagen, Madison, WI, USA).

Production of recombinant protein and electrophoretic mobility shift assay (EMSA)

Bacterial recombinant proteins were expressed using the pET32a vector in E. coli BL21-CodonPlus(DE3)-RIPL (Stratagene, La Jolla, CA, USA) with an Overnight Express Autoinduction System 1 (Novagen), followed by purification using His-Tag Mag beads (Toyobo, Osaka, Japan).

The production of FAM-labeled DNA fragments from Fd2 was described previously [5]. EMSA was carried out as previously described [19]. FAM-labeling was then performed by PCR using a set of primers (forward, 5'-FAM-ATCGATAGGTACCGAGCTCTTACG-3', and reverse, 5'-TAATAAAAACCTGCCACGCGT-3'). The binding reaction was carried out in a mixture containing 10 fmol FAM-labeled double-stranded (ds) nucleotides, 100 ng rec-RPF1 and 250 ng ds-poly(dI-dC) in 10 µl of binding buffer (10 mM Hepes buffer, pH 7.9, containing 0.4 mM $MgCl_2$, 0.4 mM DTT, 50 mM NaCl and 4% (v/v) glycerol) by incubation at 37 C for 30 min. EMSA was performed by electrophoresis on a 4% polyacrylamide gel at 100 V for 60 min.

Ontogeny of expression of the rat pituitary genes by quantitative real-time PCR

Quantitative real-time PCR was performed for total RNAs prepared from the embryonic and postnatal pituitaries with the same conditions [16], and the nucleotide sequences of the primers used were as follows: rat *Rpf1*, 5'-AATCCGACGCCTGTCCCTTGGCC-3' and 5'-GGCCAGGGTTCAGTTTGGCTGTCAG-3'; and rat TATA box binding protein (*Tbp)*, 5'-GATCAAACCCAGAATTGTTCTCC-3' and 5'-ATGTGGTCTTCCTGAATCCC-3'. Each datum measured by duplicated experiments was calculated by the comparative C_T method (DDC_T method) to estimate the gene copy number relative to *Tbp* as an internal standard. The DNA sequence of each sample's PCR product was confirmed by nucleotide sequence determination (data not shown).

Transfection reporter assays

The expression vector of *Rpf1* in pcDNA3.1, and the serial truncated upstream regions of the mouse *Prop1* (NC_000077.6), *Prrx1* (NC_000067.6) and *Prrx2* (NC_000068.7) genes were amplified using primers (Table 1) and were ligated to the secreted alkaline phosphatase (SEAP) plasmid vector pSEAP2-Basic (BD Biosciences Clontech, Palo Alto, CA, USA). This resulted in the following reporter vectors: *Prop1* (–2293/+21), *Prrx1* (–2997/+103), *Prrx1* (–450/+103), *Prrx2* (–5060/+21) and *Prrx2* (–372/+21). The culture and transfection conditions for Chinese Hamster Ovary (CHO) cells (obtained from RIKEN Cell Bank, Ibaraki, Japan) and a reporter assay for secreted alkaline phosphatase activity were described previously [5, 20].

In situ hybridization and immunohistochemistry

In situ hybridization was performed according to a previous report (Fujiwara *et al*. 2007a). The full-length DNA of rat *Rpf1* was amplified by PCR and labeled with digoxigenin (DIG)-labeled cRNA probes using a Roche DIG RNA Labeling Kit (Roche Diagnostics, Penzberg, Germany). Cryosections (7 µm thickness) from the sagittal plane were hybridized with a DIG-labeled cRNA probe at 55 C for 16 h and were visualized with an alkaline phosphatase-conjugated anti-DIG antibody (Roche Diagnostics) using 4-nitroblue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP; Roche Diagnostics). Immunohistochemistry was performed according to our previous report [9], with a primary antibody for goat IgG against human SOX2 (1:500 dilution, Neuromics, Edina, MN, USA) and secondary antibody for Cy3-conjugated AffiniPure donkey anti-goat IgG (1:500 dilution; Jackson ImmunoResearch, West Grove, PA, USA).

Statistical analysis

All results are presented as the mean ± SEM of quadruplicate transfections in two independent experiments. The statistical analysis was assessed by Student's *t*-test for independent groups and Dunnett's test for the remaining groups. Differences between groups were considered to be statistically significant at a P value of less than 0.05.

Results

Cloning and characterization of porcine Rpf1

One-hybrid cloning performed for 4.1 x 10^6 transformants efficiently produced a 1 x 10^5 colony by forming units for each 1 µg of DNA, and ultimately yielded 11 clones by confirming a specific interaction with the bait sequence, Fd2. Confirmation of both amino

Table 1. List of primers used for construction of the 5'-upstream region of *Prop1*, *Prrx1* and *Prrx2*

Prop1		
Forward primer	–2993	5'-aataacgcgtCTAAGATTCAGAGCCAAGCTAG-3'
Reverse primer	+21	5'-aatactcgagGCTAGATACCTGTTTTCTCACAG-3'
Prrx1		
Forward primer	–2297	5'-aatacgcgtTCTAGAACAATGGGGGAG-3'
	–450	5'-aatacgcgtTCTCCGCCAAAACAAAGCTG-3'
Reverse primer	+103	5'-acacactcgcgaTCCACTTAATAGGAGCCTGTA-3'
Prrx2		
Forward primer	–5060	5'-aatacgcgtAGGAGGATTTGTGTGGCTTG-3'
	–372	5'-aatacgcgtCAAATTCGAGGCTAATCTGC-3'
Reverse primer	+21	5'-acacactcgcgaGTGCCGGATCTCAAGTCAGT-3'

Upper and lowercase letters indicate the sequence of the gene to be amplified and adaptor containing the recognition sequence for restriction enzymes (*Mul* I for the forward primer and *Xho* I or *Nru* I for the reverse primer), respectively.

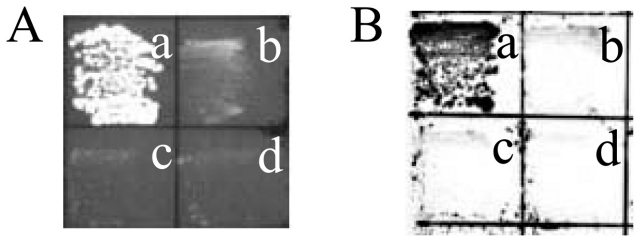

Fig. 1. Amino acid requirements and β-galactosidase activity of yeast transformants. Amino acid requirements (A) and β-galactosidase activity (B) of transformants were examined. Transformants contained cDNA/pADGAL4 with the bait clone (a), pADGAL4 with the bait clone (b), cDNA /pADGAL4 without the bait clone (c) and the bait clone alone (d).

acid requirements (Fig. 1A) and expression of the reporter gene β-galactosidase (Fig. 1B) of yeast transformants was performed.

Two of the selected clones showed a very similar identity to the nucleotide sequence of mammalian *Rpf1* (accession No. **AB907853**) but were missing a part of the N-terminal region. Subsequent re-screening and direct PCR using several newly developed cDNA libraries resulted in failure to obtain the full-length clone. Instead, a full-length *Rpf1* cDNA was obtained by direct PCR for the rat pituitary cDNA library (accession No. **AB907852**), and the amino acid sequence was compared with those of pigs and humans (Fig. 2). Rat and human RPF1s had greatly similar sequences and were identical in the POU-specific domain and POU homeodomains (Fig. 2). Meanwhile, porcine RPF1 lacked half of the POU-specific domain in the carboxyl region, including a 36-amino acid insertion present in the human RPF1 [21], but had a conserved POU homeodomain for DNA binding (Fig. 2). Other clones selected during this procedure and described elsewhere were *Prop1* [22], *Prrx2* [5] and *Lhx2* [6].

EMSA was carried out for FAM-labeled Fd2 with RPF1 in the presence and absence of unlabeled Fd2. Binding between a labeled probe and RPF1 gave remarkably large complexes at the top of the well (Fig. 3A). Those complexes were mostly dissociated by the addition of unlabeled probes in excess molar amounts. DNase I footprinting was then performed to examine the binding region of porcine RPF1. As shown in Fig. 3B, signals between –848/–800 were apparently protected by DNase I action, confirming the specific binding of RPF1 to Fd2. Notably, the region protected was different from those of PROP1, PRRX2 and LHX2 [5, 6, 22], which were cloned as Fd2-binding proteins showing different binding characteristics for each homeobox factor.

Rpf1-expressions during rat pituitary development

Real-time PCR during rat pituitary development demonstrated that *Rpf1* expression was observed at a level of 0.024 against the TATA box binding protein (*Tbp*) early on E12.5, and gradually decreased by about 0.01-fold at P0 and 0.002-fold at P60, respectively, in the anterior lobe (Fig. 4). In the postnatal intermediate/posterior lobes, there was also a low level of *Rpf1* expression.

In situ hybridization

In situ hybridization of *Rpf1* was performed for the rat embryo's cephalic portion at E16.5 and E13.5. While a sense probe did not show any positive signals, an antisense probe gave apparent positive signals in the retina at E16.5 (Fig. 5A), and this was where the presence of cells immunopositive for anti-human RPF1 antiserum in the mouse at E13-16 was reported previously [21], verifying the specificity of this probe. Under the same conditions as for the sections at E13.5, an anti-sense probe gave specific signals in most of the cells that composed Rathke's pouch, i.e., a primordium of the anterior pituitary with low-level signals in those of the rostral tip, a prospective area of medial eminence at the caudal area, in addition to the surrounding cells of the diencephalon, the prospective posterior lobe (Fig. 5B). In addition, the immunohistochemistry of SOX2, a pituitary stem/progenitor marker, showed that SOX2-positive cells occupy all of the primordium cells except for those of the rostral tip (Fig. 5C).

Promoter assay of Prop1, Prrx1 and Prrx2 with RPF1

Rat *Rpf1* full-length cDNA was cloned from a rat pituitary cDNA library by PCR using a specific primer set designed to accomplish

Fig. 2. Diagram of RPF1 and amino sequence comparison. In diagram (A), Gln-rich, proline, serine and POU-specific domains and POU homeodomains are indicated. The sequence identity (%) of the domain between the rat and human is shown for each domain (B). Amino acid sequences were compared. The POU-specific domain and homeodomain are boxed.

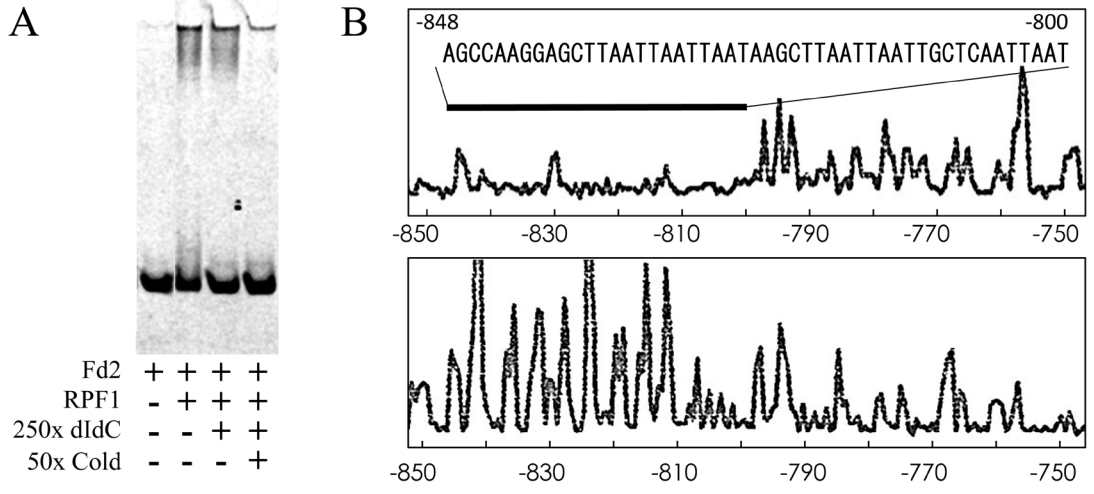

Fig. 3. Electrophoretic gel mobility shift assay and DNase I footprinting analysis of complexes between recombinant RPF1 and FAM-labeled Fd2. (A) Electrophoretic gel mobility shift was analyzed on 4% polyacrylamide gel followed by visualization with a fluorescence viewer. The composition of each binding mixture is indicated under the electrogram. The number indicates the molar excess amount. (B) DNase I footprinting analysis for a complex the same as the above. In the comparison between DNase I digests with (upper panel) and without (lower panel) RPF1, the protected region of Fd2 is indicated with a thick horizontal line together with the nucleotide sequence.

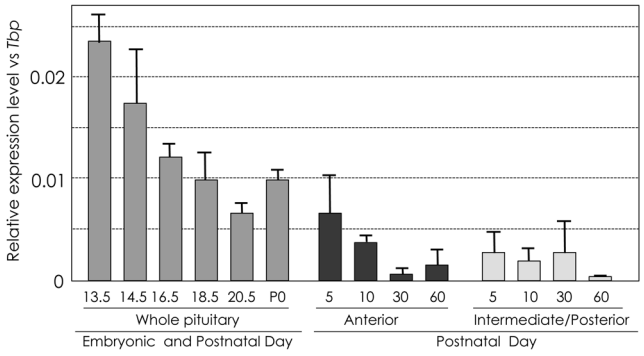

Fig. 4. Expression profile of *Rpf1* during rat pituitary development. Quantitative real-time PCR was performed to estimate the mRNA level of *Rpf1* using total RNAs extracted from whole pituitaries between embryonic day (E) 13.5 and postnatal day (P) 0 and from anterior lobes and intermediate and posterior lobes between P5 and P60. Each sample was measured in duplicate in two independent experiments, and data were calculated by the comparative CT method to estimate the copy number relative to the TATA box binding protein (*Tbp*) as an internal standard.

transcriptional activity. The clone showed conservation in the POU-specific and homeobox domains very similar to those of humans. A transfection assay in CHO cells was performed to examine whether the rat RPF1 regulates genes; *Prop1*, *Prrx1* and *Prrx2* were chosen, and we sought to determine if they might play important roles in the pituitary stem/progenitor cells [9, 15, 23, 24]. Rat RPF1 showed significant stimulation of *Prop1* (–2997/+21), by 3.6-fold, and significant repression of *Prrx2* (–372/+21), by 0.61-fold (Fig. 6), respectively, indicating that RPF1 has an ability to modulate opposing effects depending on the target gene. On the other hand, rat RPF1 did not show a significant effect for *Prrx1*.

Discussion

In the present study, we first cloned the *Rpf1* cDNA, which is known to express specifically in retina cells, from the porcine anterior lobe of the pituitary and confirmed specific binding to the bait DNA fragment. Ontogenic *Rpf1* expression in the rat pituitary was high in an early embryonic period. *In situ* hybridization revealed that *Rpf1* was expressed in most progenitor cells of the rat embryonic pituitary at E13.5. Using a full-length rat *Rpf1* clone, we demonstrated that RPF1 has the potential ability to modulate expression of *Prop1* and *Prrx2*, which are important transcription factors in pituitary stem/progenitor cells.

Rpf1 (POU6F2) was first cloned as retina-derived POU-domain factor-1 (aliases: POU6F2, RPF-1, WT5, WTL, Wilms tumor suppressor locus) [21]. Localization of RPF1 was first observed in the developing mouse retina at E11, at which time it localized to neuroblasts that migrated from the mitotic zone to the future ganglion cell layer. Hence, RPF1 is considered to be involved in the early differentiation of amacrine and ganglion cells. Since then, very few reports have been published regarding this factor. Cloning of this retina-specific transcription factor from the pituitary cDNA library was unexpected. However, several investigators postulated that the anterior pituitary and eyes have a close embryological relation.

More than 25 years ago, a high-level of transient expression of δ-crystallin gene in the chicken Rathke's pouch was reported [25], implying a close relationship of the cell's state of development between the early lens and pituitary primordium. At the end of the last century, Kondoh *et al.* reported that mutations in gli-mediated hedgehog signaling in Zebrafish led to lens transdifferentiation from the adenohypophysis primordium [26]. Thereafter, it was reported that proper expansion of retinal and pituitary precursor cell populations is regulated with Six6/CKI regulatory network [27] and that *Pitx3* defines an equivalence domain for the lens and anterior pituitary placodes [28]. Taken together, the lens and pituitary develop from the same cell lineage via the action of many signaling and transcription factors. RPF1 may play a role as one of the common factors, both

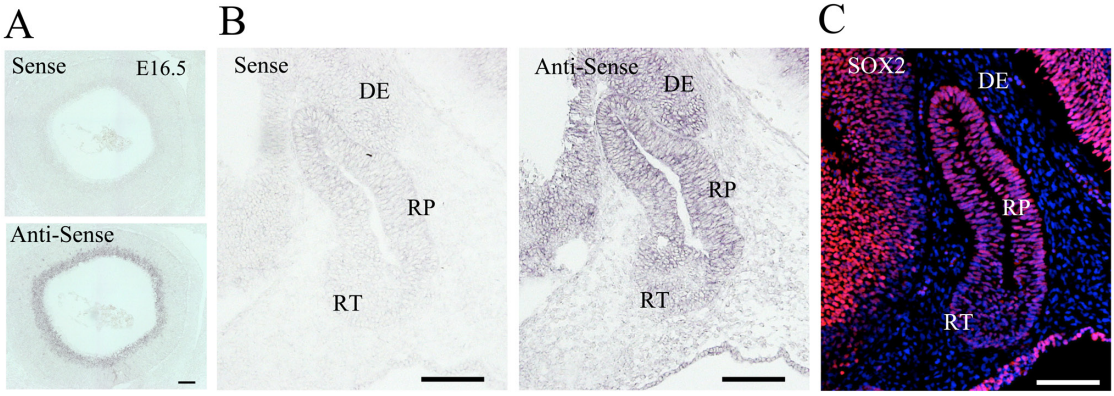

Fig. 5. *In situ* hybridization and immunohistochemistry. *In situ* hybridization with DIG-labeled sense and anti-sense probes was performed for sections of retina at E16.5 (A) and the cephalic portion at E13.5 (B). (C), Immunohistochemistry of SOX2 was performed for sections at E13.5 and merged with nuclear staining with DAPI (blue). RP, Rathke's pouch; DE, diencephalon; RT, rostral tip. The bar indicates 100 μm.

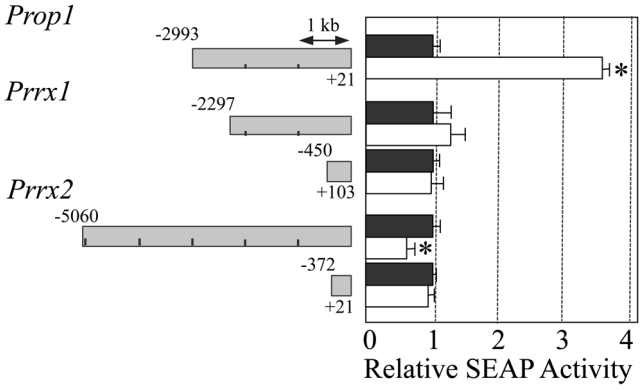

Fig. 6. Transient transfection assay of promoter activity in CHO cells. The pSEAP2-Basic vector fused each mouse's *Prop1*, *Prrrx1* and *Prrx2* promoters with the SEAP gene transfected with pcDNA3.1 harboring *Rpf1* (dark-gray bar) or not harboring *Rpf1* (open bar). The expression level of the reporter gene is indicated relative to that of pcDNA3.1. Data (mean ± SD) are means of quadruplicate transfections from two independent experiments. * P<0.01.

in the pituitary and lens.

Real-time PCR of the ontogenic expression and *in situ* hybridization of the rat embryo strongly suggests that RPF1 is involved in early development of the anterior pituitary, and modulates genes acting in pituitary stem/progenitor cells. We recently demonstrated that the transcription factors PROP1, PRRX1 and PRRX2 are present in pituitary stem/progenitor cells and are involved in the differentiation and development of pituitary hormone-producing cells [9, 15, 23, 24]. Moreover, transfection assays showed that RPF1 is able to modulate gene expression of *Prop1* by stimulating it and that of *Prrx2* by repressing it. Considering the results in the CHO cell line directly, RPF1, which is expressed in the early embryonic pituitary, may stimulate expression of *Prop1* in pituitary primordium cells [29] and inversely repress that of *Prrx2* appearing in a small number of postnatal pituitary cells [15]. Cloning of pituitary RPF1 thus provides a novel clue for clarifying the molecular mechanism of pituitary organogenesis. We observed that PRRX1 appears in embryonic pituitary stem/progenitor cells around day E15.5 in the rat by changing *Prop1* expression [15], while RPF1 did not show any effect on the *Prrx1* promoter. Hence, the mechanism of *Prrx1* expression in the PROP1-positive cells remains to be clarified. Moreover, in the near future, it is an urgent issue to clarify the role of RPF1 during early pituitary organogenesis especially in the period of ectodermal invagination (E9-10) and PROP1 appearance (E11.5), and to examine whether SOX2 and RPF1 participate in the regulation of *Prop1 in vivo*. Additionally, expression of *Rpf1* in the diencephalon, a presumptive posterior lobe first found in the present study, has not yet been investigated, and the role of RPF1 in this tissue remains to be clarified.

Alternative splicing variants of *Rpf1* have been reported in retina transcripts [21]. In the present study, we obtained clones of the same length and sequence but a partial one from the pig pituitary cDNAs and a single PCR product from rat cDNAs. In addition, we confirmed that rat and human RPF1s are mostly of the same length and have highly homologous nucleotide and amino acid sequences. These results suggest the presence of tissue-dependent splicing in the *Rpf1* gene. On the other hand, RPF1 has a bipartite DNA binding domain consisting of a POU-specific domain and a POU homeodomain, which are known to have overlapping recognition sequences but different sequence specificities [30]. The POU-specific domain of RPF1 has a 36-amino acid insertion in comparison with that of the typical POU-homeodomain factor OCT1, and thereby RPF1 may have a slightly different binding specificity. The left and right halves of the consensus sequence [a(a/t)TATGC(A/T)AAT(t/a)t] are recognized by the POU-specific domain and POU homeodomain, respectively [30]. Although porcine RPF1 has a deletion of the carboxyl half of the POU-specific domain, the binding region identified by DNase I footprinting contains a very similar sequence to the OCT1 binding consensus in three segments, AAGGAGCTTAATT, AATAAGCTTAATT and TAATTGCTCAATT (Fig. 3B), respectively, indicating multiple bindings of RPF1 to the bait DNA fragment and an alternative explanation for the large complex formation in the EMSA (Fig. 2A). Moreover, since SOX2 is known to interact with POU domain factors [31], colocalization of RPF1 and SOX2 might indicate a possibility to cooperatively modulate expression of genes during pituitary organogenesis.

In summary, the present study identified the retina-derived POU domain factor RPF1 for the first time in the stem/progenitor cells of the anterior pituitary, indicating a close developmental relation between the pituitary and eye. Since POU domain factors including pituitary-specific transcription factor 1 (PIT1) are known to play a role in cell-type-specific differentiation, the finding of RPF1 in the pituitary might provide a clue to clarify the molecular mechanism of pituitary organogenesis.

Acknowledgments

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References

1. **Jorgensen JS, Quirk CC, Nilson JH.** Multiple and overlapping combinatorial codes orchestrate hormonal responsiveness and dictate cell-specific expression of the genes encoding luteinizing hormone. *Endocr Rev* 2004; **25**: 521–542. [Medline] [CrossRef]
2. **Kato Y, Tomizawa K, Kato T.** Multiple binding sites for nuclear proteins of the anterior pituitary are located in the 5′-flanking region of the porcine follicle-stimulating hormone (FSH) β-subunit gene. *Mol Cell Endocrinol* 1999; **158**: 69–78. [Medline] [CrossRef]
3. **Cai LY, Kato T, Ito K, Nakayama M, Susa T, Aikawa S, Maeda K, Tsukamura H, Ohta A, Izumi S, Kato Y.** Expression of porcine FSHbeta subunit promoter-driven herpes simplex virus thymidine kinase gene in transgenic rats. *J Reprod Dev* 2007; **53**: 201–209. [Medline] [CrossRef]
4. **Aikawa S, Susa T, Sato T, Kitahara K, Ono T, Suzuki K, Nakayama M, Kato T, Kato Y.** Prop-1 activation of porcine FSHβ subunit gene requires AT-rich recognition sites. *Jpn J Reprod Endocrinol* 2005; **10**: 43–48.
5. **Susa T, Ishikawa A, Kato T, Nakayama M, Kato Y.** Molecular cloning of paired related homeobox 2 (prx2) as a novel pituitary transcription factor. *J Reprod Dev* 2009; **55**:

502–511. [Medline] [CrossRef]

6. **Kato T, Ishikawa A, Yoshida S, Sano Y, Kitahara K, Nakayama M, Susa T, Kato Y.** Molecular cloning of LIM homeodomain transcription factor *Lhx2* as a transcription factor of porcine follicle-stimulating hormone beta subunit (FSHβ) gene. *J Reprod Dev* 2012; **58**: 147–155. [Medline] [CrossRef]
7. **Kioussi C, Carrière C, Rosenfeld MG.** A model for the development of the hypothalamic-pituitary axis: transcribing the hypophysis. *Mech Dev* 1999; **81**: 23–35. [Medline] [CrossRef]
8. **Yako H, Kato T, Yoshida S, Inoue K, Kato Y.** Three-dimensional studies of Prop1-expressing cells in the rat pituitary primordium of Rathke's pouch. *Cell Tissue Res* 2011; **346**: 339–346. [Medline] [CrossRef]
9. **Yoshida S, Kato T, Susa T, Cai L-Y, Nakayama M, Kato Y.** PROP1 coexists with SOX2 and induces PIT1-commitment cells. *Biochem Biophys Res Commun* 2009; **385**: 11–15. [Medline] [CrossRef]
10. **Kongsuwan K, Webb E, Housiaux P, Adams JM.** Expression of multiple homeobox genes within diverse mammalian haemopoietic lineages. *EMBO J* 1988; **7**: 2131–2138. [Medline]
11. **Meijlink F, Beverdam A, Brouwer A, Oosterveen TC, Berge DT.** Vertebrate aristaless-related genes. *Int J Dev Biol* 1999; **43**: 651–663. [Medline]
12. **Mitchell JM, Hicklin DM, Doughty PM, Hicklin JH, Dickert JWJ Jr, Tolbert SM, Peterkova R, Kern MJ.** The Prx1 homeobox gene is critical for molar tooth morphogenesis. *J Dent Res* 2006; **85**: 888–893. [Medline] [CrossRef]
13. **Balic A, Adams D, Mina M.** Prx1 and Prx2 cooperatively regulate the morphogenesis of the medial region of the mandibular process. *Dev Dyn* 2009; **238**: 2599–2613. [Medline] [CrossRef]
14. **Higuchi M, Yoshida S, Ueharu H, Chen M, Kato T, Kato Y.** PRRX1 and PRRX2 distinctively participate in pituitary organogenesis and a cell-supply system. *Cell Tissue Res* 2014; **357**: 323–335. [CrossRef] [Medline]
15. **Higuchi M, Kato T, Chen M, Yako H, Yoshida S, Kanno N, Kato Y.** Temporospatial gene expression of Prx1 and Prx2 is involved in morphogenesis of cranial placode-derived tissues through epithelio-mesenchymal interaction during rat embryogenesis. *Cell Tissue Res* 2013; **353**: 27–40. [Medline] [CrossRef]
16. **Susa T, Kato T, Yoshida S, Yako H, Higuchi M, Kato Y.** Paired-related homeodomain proteins Prx1 and Prx2 are expressed in embryonic pituitary stem/progenitor cells and may be involved in the early stage of pituitary differentiation. *J Neuroendocrinol* 2012; **24**: 1201–1212. [Medline] [CrossRef]
17. **Roberson MS, Schoderbek WE, Tremml G, Maurer RA.** Activation of the glycoprotein hormone α-subunit promoter by a LIM-homeodomain transcription factor. *Mol Cell Biol* 1994; **14**: 2985–2993. [Medline]
18. **Zhao X, Monson C, Gao C, Gouon-Evans V, Matsumoto N, Sadler KC, Friedman SL.** Klf6/copeb is required for hepatic outgrowth in zebrafish and for hepatocyte specification in mouse ES cells. *Dev Biol* 2010; **344**: 79–93. [Medline] [CrossRef]
19. **Kato Y, Koike Y, Tomizawa K, Ogawa S, Hosaka K, Tanaka S, Kato T.** Presence of activating transcription factor 4 (ATF4) in the porcine anterior pituitary. *Mol Cell Endocrinol* 1999; **154**: 151–159. [Medline] [CrossRef]
20. **Susa T, Kato T, Kato Y.** Reproducible transfection in the presence of carrier DNA using FuGENE6 and Lipofectamine2000. *Mol Biol Rep* 2008; **35**: 313–319. [Medline] [CrossRef]
21. **Zhou H, Yoshioka T, Nathans J.** Retina-derived POU-domain factor-1: a complex POU-domain gene implicated in the development of retinal ganglion and amacrine cells. *J Neurosci* 1996; **16**: 2261–2274. [Medline]
22. **Aikawa S, Kato T, Susa T, Tomizawa K, Ogawa S, Kato Y.** Pituitary transcription factor Prop-1 stimulates porcine follicle-stimulating hormone β subunit gene expression. *Biochem Biophys Res Commun* 2004; **324**: 946–952. [Medline] [CrossRef]
23. **Chen M, Kato T, Higuchi M, Yoshida S, Yako H, Kanno N, Kato Y.** Coxsackievirus and adenovirus receptor-positive cells compose the putative stem/progenitor cell niches in the marginal cell layer and parenchyma of the rat anterior pituitary. *Cell Tissue Res* 2013; **354**: 823–836. [Medline] [CrossRef]
24. **Yoshida S, Kato T, Yako H, Susa T, Cai L-Y, Osuna M, Inoue K, Kato Y.** Significant quantitative and qualitative transition in pituitary stem/progenitor cells occurs during the postnatal development of the rat anterior pituitary. *J Neuroendocrinol* 2011; **23**: 933–943. [Medline] [CrossRef]
25. **Ueda Y, Okada TS.** Transient expression of a 'lens-specific' gene, delta-crystallin, in the embryonic chicken adenohypophysis. *Cell Differ* 1986; **19**: 179–185. [Medline] [CrossRef]
26. **Kondoh H, Uchikawa M, Yoda H, Takeda H, Furutani-Seiki M, Karlstrom RO.** Zebrafish mutations in Gli-mediated hedgehog signaling lead to lens transdifferentiation from the adenohypophysis anlage. *Mech Dev* 2000; **96**: 165–174. [Medline] [CrossRef]
27. **Li X, Perissi V, Liu F, Rose DW, Rosenfeld MG.** Tissue-specific regulation of retinal and pituitary precursor cell proliferation. *Science* 2002; **297**: 1180–1183. [Medline]
28. **Dutta S, Dietrich JE, Aspöck G, Burdine RD, Schier A, Westerfield M, Varga ZM.** pitx3 defines an equivalence domain for lens and anterior pituitary placode. *Development* 2005; **132**: 1579–1590. [Medline] [CrossRef]
29. **Sornson MW, Wu W, Dasen JS, Flynn SE, Norman DJ, O'Connell SM, Gukovsky I, Carrière C, Ryan AK, Miller AP, Zuo L, Gleiberman AS, Andersen B, Beamer WG, Rosenfeld MG.** Pituitary lineage determination by the Prophet of Pit-1 homeodomain factor defective in Ames dwarfism. *Nature* 1996; **384**: 327–333. [Medline] [CrossRef]
30. **Verrijzer CP, Alkema MJ, van Weperen WW, Van Leeuwen HC, Strating MJ, van der Vliet PC.** The DNA binding specificity of the bipartite POU domain and its subdomains. *EMBO J* 1992; **11**: 4993–5003. [Medline]
31. **Dailey L, Basilico C.** Coevolution of HMG domains and homeodomains and the generation of transcriptional regulation by Sox/POU complexes. *J Cell Physiol* 2001; **186**: 315–328. [Medline] [CrossRef]