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Identification and Predominant Expression of Annexin A2 in Epithelial-Type Cells of the Rice Field Eel

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Abstract *Annexin* is the largest family of genes encoding eukaryotic calcium-binding proteins that do not contain the EF hand motif. Annexin A2 has a common annexin core domain, consisting of four so-called annexin repeats, and each of these repeats has about 70 amino acids in length. Here we report identification of *annexin A2* from rice field eel by degenerate PCR and RACE techniques. Three-dimensional structure prediction shows that it has similar annexin repeat architecture. Phylogenetic analysis shows that this gene fits with the *annexin A2* clade of vertebrates. Subcellular co-localization and co-immunoprecipitation indicated annexin A2 interacted with its ligand S100A10, confirming characteristics of the rice field eel annexin A2. RT-PCR and Western blot results indicate *annexin A2* expressed ubiquitously in adult tissues. Immunofluorescence analysis shows obvious immunoreactivity in the nuclear membrane of developing oocytes and base membrane of mature oocytes in ovary and ovotestis. After the gonad differentiates into testis, annexin A2 protein expressed in the site of seminal vesicles epithelium in testis. The results provided a clue to the potential role of annexin A2 in the gonadal differentiation from ovary, via ovotestis to testis of the rice field eel. J. Cell. Biochem. 101: 600–608, 2007.

Key words: annexin A2; cloning; subcelluar localization; the rice field eel

Annexins are a family of calcium- and phospholipids-binding proteins that comprises more than 500 different members identified in both plants and animals. There are 12 annexin subfamilies (A1–A11 and A13) in vertebrates. They all have a common annexin core domain, which help them dock onto the cell membrane. This domain consists of four so-called annexin repeats, each repeat has about 70 amino acids in length [Crompton et al., 1988; Morgan and Fernandez, 1997; Gerke and Moss, 2002; Rescher and Gerke, 2004]. Annexin A2 (calpactin I heavy chain, p36) was first identified as a substrate of the transforming protein ($pp60^{src}$) of Rous sarcoma virus [Buss et al., 1986]. Later researchers found that it was present in cells with two forms, 36 kDa monomer and 90 kDa heterotetramer. In the heterotetramer, two annexin A2 molecules bind to the S100A10 dimer. Although the binding process does not depend on the Ca^{2+} existence, the complex itself has increased affinity toward Ca^{2+} and phospholipids as compared with the monomeric annexin A2 [Powell and Glenney, 1987].

Studies both in vitro and in vivo suggest that annexin A2 functions in a broad range of physiological processes, such as plasminogen activation [Ling et al., 2004], lipid microdomains formation [Chasserot-Golaz et al., 2005], insulin signal transduction [Biener et al., 1996], cholesterol transport [Smart et al., 2004], and cell migration [Tatenhorst et al., 2006], etc. Its expression in many kinds of cancers, for example, Lewis lung carcinoma tumor [Sharma et al., 2006b], breast cancer [Sharma et al., 2006a], acute myeloid leukemia [Olwill et al., 2005], and prostate cancer [Liu et al., 2003] suggest its important role in disrupted cell activities.

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Although a number of studies have examined the evolutionary history of annexin gene family [Morgan and Fernandez, 1997], phylogeny and origin of the gene family have not been understood well. Teleost is the most numerous group of vertebrates, which is composed of more than 24,000 species accounting for more than half of extant vertebrate species. It is also the most successful and diverse group of vertebrates. However, in this group of vertebrates, *annexin A2* gene has been characterized only in zebrafish [Farber et al., 2003], although similar sequences were found in *Fugu*, *Tetraodon*, *Medaka*, and *Stickleback* in Ensembl database.

The rice field eel, *Monopterus albus*, taxonomically belongs to teleosts, the family Synbranchidae of the order Synbranchiformes (Neoteleostei, Teleostei, Vertebrata). This fresh water fish is not only an economically important species of southeast Asia for food production, but also a good model for comparative genomic studies of distantly related vertebrate processes, and sexual differentiation, because of its primitive evolutionary status, relative small genome size, and naturally sex reversal from female via intersex into male during its life cycle [Zhou et al., 2001; Cheng et al., 2003]. Here we report molecular cloning of *annexin A2* from the rice field eel, show its predominant expression in epithelial type cells, especially in different gonads during sex reversal and interaction with S100A10, suggesting a potential role in sexual differentiation and cellular membrane association.

MATERIALS AND METHODS

Animals

The rice field eels (*M. albus*), freshwater teleost fish, were obtained from markets in the Wuhan area in China. Their sexes were confirmed by microscopic analysis of gonad sections. Zebrafish (*Danio rerio*) was obtained from Institute of Hydrobiology of the Chinese Academy of Science. The experiments were carried out in accordance with the International Guiding Principles for Biomedical Research Involving Animals as promulgated by the Society for the Study of Reproduction.

Degenerate PCR and RACE Cloning of the Rice Field Eel Annexin A2

SMART cDNAs were reversely transcribed from the RNAs of rice field eel testis using the SMART cDNA synthesis kit following commercial protocol (Clontech). Degenerate PCR was used to amplify the conserved region of the *annexin A2* using designed degenerate primers: AnA2-5Degenerate 5′-GACCGCC (GA)TCAA-(GA)AC(TC)AAAGG-3′ and AnA2-3Degenerate 5′-CTTGTA(TC)CT (GT)TCCAA(TC)ACTTTCTG-3′. PCR cycling conditions were: 35 cycles, each with 30 s, 94°C; 30 s, 54°C; and 40 s, 72°C in a 20 μl reaction mix. PCR products were sequenced. 3′-RACE was performed using common CDSIII primer 5′-ATTCTAGAGGCCGAGGCGGCCG-ACATG-d $(T)_{30}$N_1N-3′ (N = A, G, C, or T; N_1 = A, G, or C) and AnA2-5Degenerate primer at the conditions of 35 cycles, each with 30 s, 94°C; 30 s, 54°C; 60 s, 72°C. After 3′-RACE, nest PCRs were used to amplify specifically the 3′ part of the *annexin A2* gene. The nest primer pairs were the same CDSIII primer and 3RN primer: 5′-ATACTGGGTCTGATGAAGAGCAC-3′, 35 cycles, each with 30 s, 94°C; 30 s, 68°C; 60 s, 72°C. Based on the above sequences, 5′-RACE was performed using common SMARTIII primer, 5′-AAGCAGTGGTATCAACGCAGAG-TGGCCATTACGGCCGGG-3′ and 5RW primer: 5′-GTGACCTCTTTTGGTGCCTCTTG-3′ at the same conditions in 3′-RACE nest PCR. The nest PCR was done then using the same 5′-primer SMARTIII and nest primer 5RN: 5′-GAGA-CCTGGCATCGTCATCAATC-3′. All sequences were cloned and sequenced.

Phylogenetic Tree Construction

All known annexin A2 protein sequences were aligned using ClustalX 1.81 program. Phylogenetic tree was constructed using N–J (100 runs) method (Phylip).

RT-PCR

Reverse transcription PCR was used to amplify the *annexin A2* gene from different tissues and gonads. Amplification conditions were: 95°C, 15 s; 68°C (*annexin A2*) or 58°C (*beta-actin*), 30 s; and 72°C, 30 s for 30 cycles. Primers were as follows: *annexin A2* 5RN primer and 3RN primer, beta-actin 5′-GGG-AGTGATGGTTGGCATGG-3′ and 5′-AGGAA-GGAAGGCTGGAAGAG-3′.

Fluorescent Vectors Construction and Cell Transfection

The rice field eel *annexin A2* CDS was cloned into pDsRed-N1 vectors (Clontech) for fluorescence analysis. The YFP-S100A10 (human)

constructs were provided kindly by Dr Volker Gerke. COS-7 cells were maintained in DMEM medium with 10% fetal calf serum in 5% CO_2 incubator at 37°C. For transient transfections, cells were grown on cover slips and then transfected with Lipofectamine 2000 (Invitrogen). Twenty-four hours after transfection, living cells were observed using a DMLA fluorescence microscope (Leica, Germany).

Antibodies

The polyclonal antibodies anti human annexin A2 and S100A10 proteins were purchased from Santa Cruz Company. The second antibodies conjugated with FITC or AP were purchased from Pierce Company.

Immunofluorescence Analysis

Different tissues of adult rice field eel and zebrafish were cryosectioned. The sections (7 μm) were immobilized in acetone for 5 min in −20°C; washed with PBS three times; blocked by preimmune serum for 20 min; incubated with annexin A2-specific antibody at a final concentration of 1:50 in PBS at 4°C overnight; then incubated for 1 h at 37°C with the secondary antibody conjugated with FITC at a dilution of 1:50. Fluorescent images were taken under a DMLA fluorescence microscope (Leica).

Western Blot Assay

Proteins of freshly obtained eel tissues were extracted. The whole extract was analyzed by SDS–PAGE and transferred to Hybond-P membrane (Amersham Pharmacia, Sweden). The membrane was blocked with 5% low fat milk powder in TBST (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1% Tween 20) and incubated with annexin A2 or S100A10 antibody for 1 h at 37 or 4°C over night, and then with an AP labeled secondary antibody. The immunoreactive signal was revealed by NBT/BCIP regent.

Immunoprecipitation

For co-immunoprecipitation, the COS-7 cells were co-transfected with fluorescent vectors annexin A2-DsRed and YFP-S100A10. After 30 h, the cells were lysed with IP-lysis buffer (Shenergy Biocolor Bioscience & Technology, China) plus protease inhibitor. Fresh testis tissue of the rice field eel was also lysed. Two lysates were either boiled in SDS–PAGE sample buffer (input) or incubated with anti-S100A10 antibody together with protein G PLUS-Agarose (Santa Cruz), or with protein G PLUS-Agarose alone. The agarose beads were boiled in SDS–PAGE sample buffer, which was then subjected to SDS–PAGE and transferred to Hybond-P membrane. Western blot analysis was performed as described above.

RESULTS

Cloning and Structure Analysis of the Rice Field Eel Annexin A2

According to conserved sequences of all known *annexin A2* genes, we first designed a pair of degenerate PCR primers to amplify a partial fragment from rice field eel gonad SMART cDNAs. Based on this partial sequence, we synthesized primers to clone the full-length of the rice field eel *annexin A2* using RACE strategy. By combining the 5′-RACE and 3′-RACE products, *annexin A2* cDNA (GenBank access No. AY639382) was obtained, which encoded a putative protein of 338 amino acids with a poly(A) signal in the end. The predicted annexin A2 of the rice field eel showed the highest similarity with all known annexin A2 of vertebrates, which contained a common annexin core domain that comprised four repeats of about 70 amino acids. Phylogenetic analysis using N–J method further revealed that the rice field eel annexin A2 protein was clustered with all annexin A2 of vertebrates (Fig. 1).

Annexin A2 is Expressed Ubiquitously in Both mRNA and Protein Level

The transcription analysis of *annexin A2* gene of the rice field eel was determined by RT-PCR. RT-PCR result showed that *annexin A2* mRNA expressed in a ubiquitous pattern, except for a faint band observed in the heart (Fig. 2a). Because the rice field eel is a natural sex-reversal fish with a gonadal transformation from female via intersex to male during its life, we analyzed the *annexin A2* expression during the transformation. However, RT-PCR showed that *annexin A2* mRNA expression has no significant sex difference among ovary, ovotestis, and testis.

Protein extracts from adult tissues were further analyzed for annexin A2 expression by Western blotting (Fig. 2b). The antibody reacted

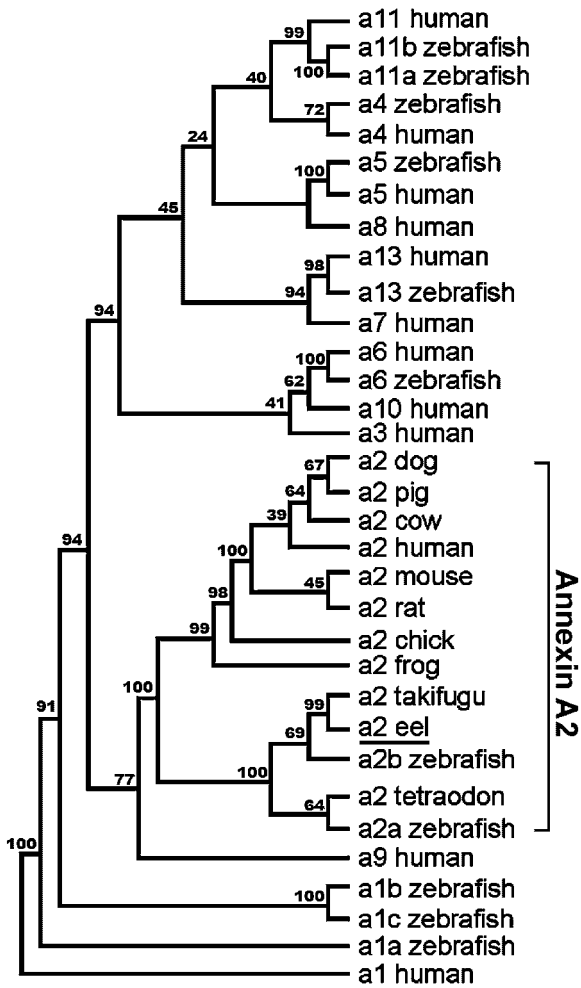

Fig. 1. Phylogenetic tree of the annexin A proteins. The rice field eel annexin A2 was clustered into a group of all known annexin A2. The annexin A protein names of different organisms are shown on the right. Numbers in the branches represent the bootstrap values (%) from 100 replicates obtained using N–J method. The accession numbers: a11a zebrafish AAO20275, a11b zebrafish AAO20276, a11 human P50995, a1b zebrafish AAO20268, a1c zebrafish AAO20269, a1-a zebrafish AAO20267, a1 human P04083, a9 human O76027, a2a zebrafish AAO20270, a2 eel AAV31758, a2b zebrafish AAO20271, a2 frog AAH42238, a2 chick P17785, a2 mouse P07356, a2 rat Q07936, a2 human P07355, a2 dog AAR00321, a2 pig AAU85387, a2 cow P04272, a3 human P12429, a10 human Q9UJ72, a4 human P09525, a4 zebrafish AAO20272, a6 human P08133, a6 zebrafish AAO20274, a7 human P20073, a13 human P27216, a13 zebrafish AAO20277, a8 human P13928, a5 human P08758, a5 zebrafish AAO20273, a2 takifugu NEWSINFRUP00000153511, and a2 tetraodon GSTENT-00015969001.

strongly with a major protein band that migrated with the expected molecular weight for the rice field eel annexin A2 (38 kDa), confirming ubiquitous expression of the rice field eel annexin A2 in protein level. Strong immunoreactivity was observed in gonads,

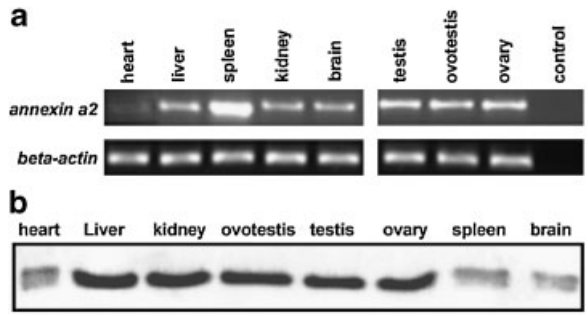

Fig. 2. Expression analysis of *annexin A2* mRNA and protein of the rice field eel in different adult tissues by RT-PCR and Western blot. **a**: Amplified fragment of *annexin A2* was 298 bp. The *beta-actin* (680 bp) was used as an inner control. **b**: Tissue extracts were subjected to SDS–PAGE and analyzed by Western blotting using annexin A2 specific antibody. A band about 38 kDa was observed.

kidney, and liver, whereas heart, spleen, and brain expressed lower.

Expression Pattern of Annexin A2 Protein in Gonads

We analyzed expression site of the annexin A2 in three types of gonads of the rice field eel by immunofluorescence. In testis, annexin A2 immunoreactivity was detectable in seminal vesicles epithelium (Fig. 3A). These epithelial cells are somatic cells. While in ovary, annexin A2 was immunolocalized to the nuclear membrane of developing oocytes and base membrane of mature oocytes (Fig. 3G). In the ovotestis of intersex rice field eel (Fig. 3D), positive signals were similar to those in ovary. We also detected expression pattern of annexin A2 in zebrafish gonads. Annexin A2 protein was localized to the seminal vesicles epithelium of testis (Fig. 4D). Annexin A2 in zebrafish ovary was detected only within early developing oocytes (Fig. 4A), which was a little different compared with that in the rice field eel ovary.

Expression of Annexin A2 Protein in Epithelial Type Cells of Kidney and Liver

We further investigated annexin A2 protein distribution in kidney and liver. In kidney, immunofluorescence results showed that annexin A2 mainly localized in the lining epithelium of collecting tubule, while negative in the outer epithelial cell of tubule and glomerula. Peripheral staining at the plasma membrane was very strong (Fig. 5A). In liver,

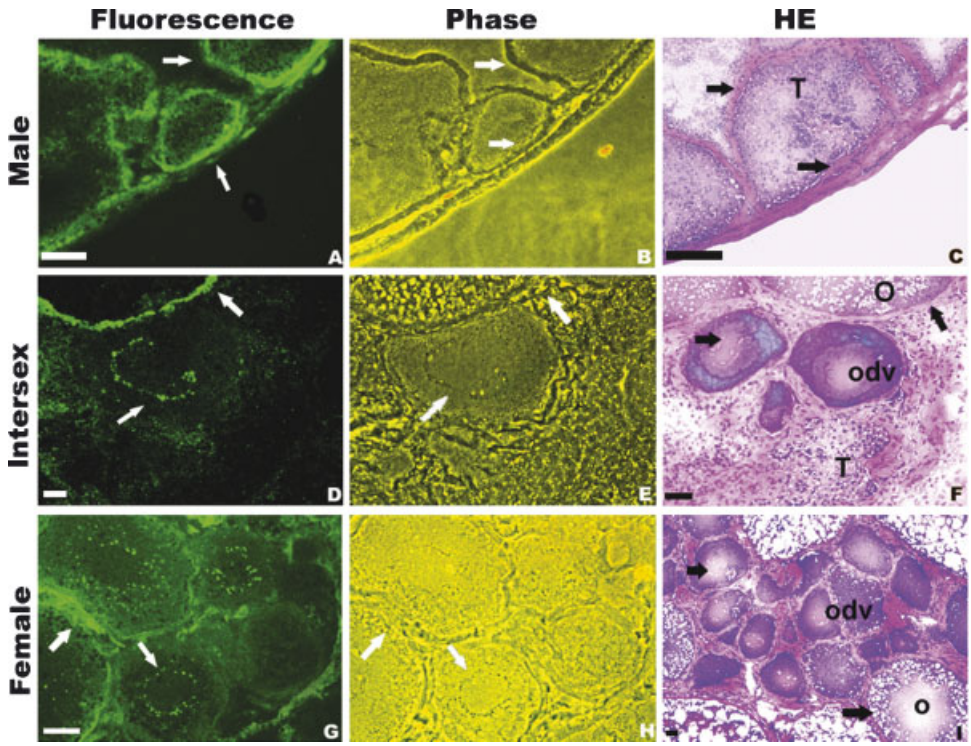

Fig. 3. Expression analysis of the rice field eel annexin A2 protein in different gonads by immunofluorescence. The positive signals of testis (**A**), ovotestis (**D**), and ovary (**G**) are indicated by arrows. The corresponding phase contrast images are shown in (**B**), (**E**), and (**H**). HE staining of testis (**C**), ovotestis (**F**), and ovary (**I**) is shown on the **right panel**. Arrows in HE show seminal vesicles epithelium (testis), the nuclear membrane of developing oocytes, and base membrane of mature oocytes (ovotestis, ovary). T, seminiferous tubules; O, mature ova; odv, developing oocytes, Scale bar: 100 μm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

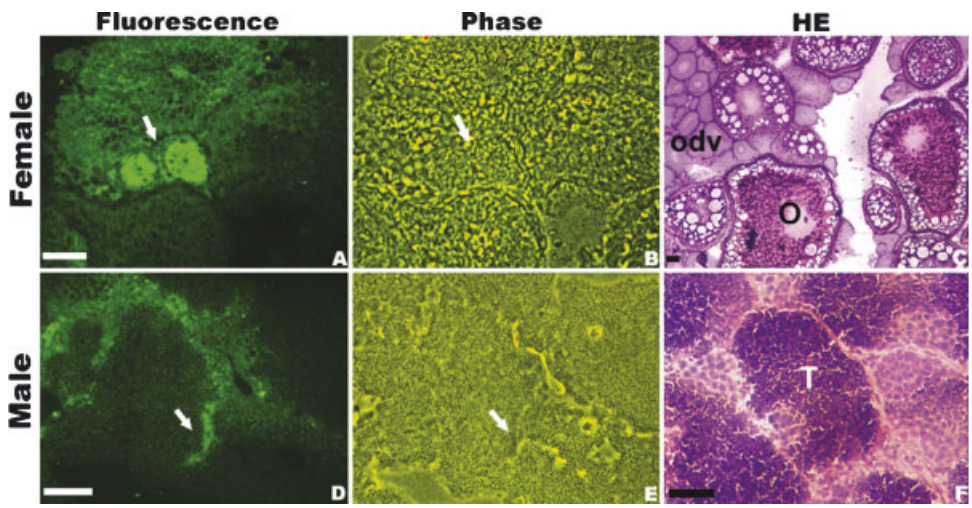

Fig. 4. Expression analysis of zebrafish annexin A2 protein in gonads by immunofluorescence. The arrows show the positive signals in ovary (**A**) and testis (**D**). The corresponding phase contrast images (**B**) and (**E**) are in the **middle panel**. HE staining of ovary (**C**) and testis (**F**) is shown on the **right panel**. Arrows in HE show early developing oocytes (ovary) and seminal vesicles epithelium (testis). T, seminiferous tubules; O, mature ova; odv, developing oocytes, Scale bar: 100 μm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

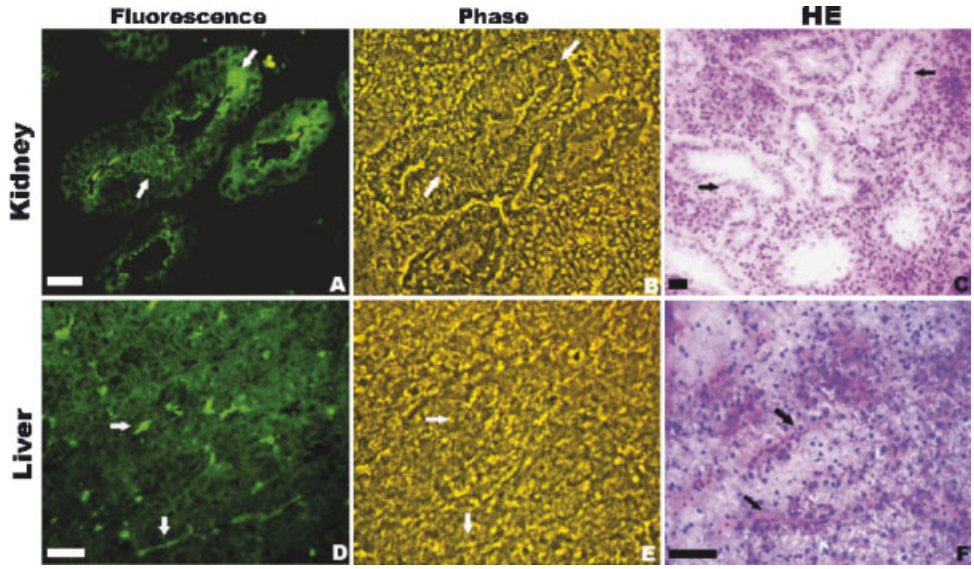

Fig. 5. Expression of Annexin A2 protein in liver and kidney of the rice field eel. Strong immunoreactivity was detected in lining epithelium membrane of collecting tubules of kidney (**A**) and bile ducts of liver (**D**) (arrow). **B**, **E**: Show their phase images, respectively. **C**, **F**: Show HE staining images. Arrows in HE show the lining epithelium of collecting tubule (kidney) and bile duct (liver). Scale bar: 100 μm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

annexin A2 immunoreactivity was localized exclusively in bile duct (Fig. 5D). No staining was observed in the liver hepatocytes, annexin A2 was concentrated at the bile ducts which build up by simple columnar epithelium. These results showed that annexin A2 is highly expressed in epithelial type cells.

Annexin A2 Protein Located in the Cytoplasm and Interacted With S100A10 in Transfected COS-7 Cells

We constructed an annexin A2-DsRed fluorescent protein chimera to investigate its distribution in COS-7 cells. In this case, red fluorescence protein was fused to the C-terminal. The red fluorescence mainly appeared in cytoplasm in the transfected cells, absent in the nucleus (Fig. 6a). In addition, peripheral staining at the plasma membrane was observed. The subcellular localization of the rice field eel annexin A2 is in line with that observed in human annexin A2, and is reminiscent of what has been observed for endogenous annexin 2 in a number of cultured albeit fixed cells [Zobiack et al., 2001], indicating that the DsRed tag does not interfere with the binding of annexin 2 to its target membrane. It appears likely that the cytosolic plus membrane distribution reflects the dynamic nature of the annexin 2-membrane interaction and the protein most likely shuttles between membrane and cytosol as discussed before [Gerke and Moss, 1997]. In contrast to annexin A2-DsRed, the YFP-S100A10 chimera, empty vectors pDsRed-N1 and pEYFP-C1 showed both cytosolic and nuclear distribution.

To realize whether annexin A2 protein of the rice field eel has similar characteristics to interact with S100A10 as those observed in mammals, cell co-localization and co-immunoprecipitation assays were used. In co-transfected cells, red annexin A2 signals mixed with S100A10 green signals, which showed a uniform yellow staining, especially they congregated to form large dots in the cytoplasm (Fig. 6b). These results suggested that two proteins interacted with each other and were capable of forming a complex. Co-immunoprecipitation experiment further confirmed this interaction (Fig. 6c). Lysates of co-transfected cells with annexin A2-DsRed and YFP-S100A10 were subjected to immunoprecipitation using anti-S100A10 antibody, following Western blotting of annexin a2 and S100A10 antibodies, respectively. This analysis revealed that annexin A2 co-immunoprecipited S100A10. A control analysis (first antibody absent in immunoprecipitation step) showed no corresponding bands, indicating the specificity of the interaction.

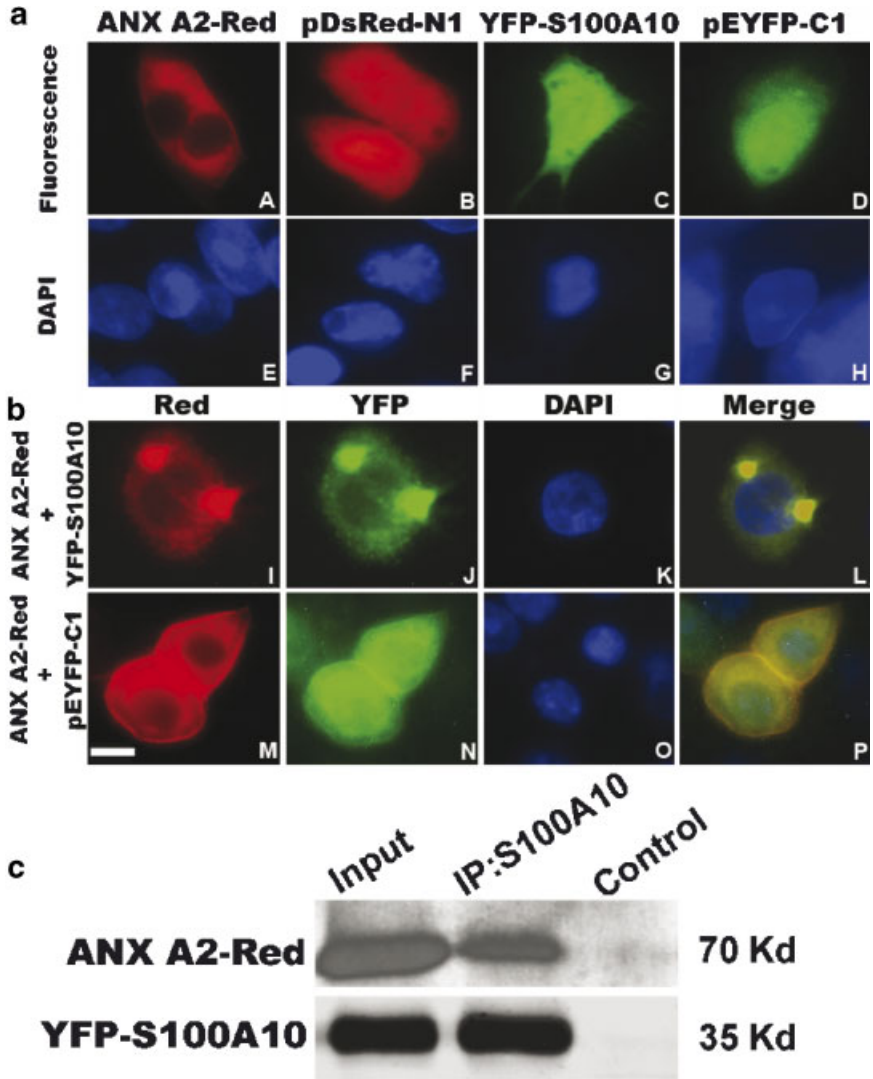

Fig. 6. Intracellular localization of the rice field eel annexin A2 (**a**), co-localization of annexin A2 and S100A10 (**b**), and co-immunoprecipitation experiment (**c**) in transfected COS-7 cells. a: Annexin A2 expression resulted in cytosolic staining, while nucleus was devoid of signals (**A**). The YFP-S100A10 fusion (**C**), empty vector pDsRed-N1 (**B**), and pEYFP-C1 (**D**) all resulted in diffuse cytosolic and nuclear fluorescence. The corresponding nuclei were stained with DAPI (**E–H**). b: Cells co-transfected with annexin A2-DsRed and YFP-S100A10 showed co-localization and congregation of annexin A2-DsRed (**I**) and YFP-S100A10 (**J**). Cells co-transfected with annexin A2-DsRed and pEYFP-C1 were shown as control. The red fluorescence appeared in cytoplasm and membrane (**M**), while the YFP fluorescence appeared in both cytoplasm and nuclei (**N**). The nuclei were shown on (**K**, **O**). The merge pictures were shown on **right panel** (**L**, **P**). Scale bar: 10 μm. c: The cell extracts were incubated with protein G PLUS-Agarose alone (control) or with anti-S100A10 antibody. Co-immunoprecipitated proteins were analyzed by Western blotting with anti-Annexin A2 and anti-S100A10 antibodies. A 70 kDa band (ANX A2-DsRed) and a 35 kDa band (YFP-S100A10) were shown on the membrane. The input lane represents 1/10 of the lysate volume used for the immunoprecipitation. IP, immunoprecipitation. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Annexin A2 Protein Interacted With S100A10 in the Testis of the Rice Field Eel

To address whether S100A10 presents in gonads of the rice field eel, and if so, whether it interacts with Annexin A2, the polyclonal antibody anti-human S100A10 was used to detect S100A10 expression in gonads of the rice field eel. Western blot analysis showed that it expressed highly in testis, and weak signal was also observed in ovary; however, it was almost undetected in ovotestis (Fig. 7a). Testis lysate was further subjected to co-immunoprecipitation assay. We revealed that annexin A2 protein co-immunoprecipited a S100A10 homolog protein, which indicated that annexin A2 interacted with S100A10 in male gonad (Fig. 7b).

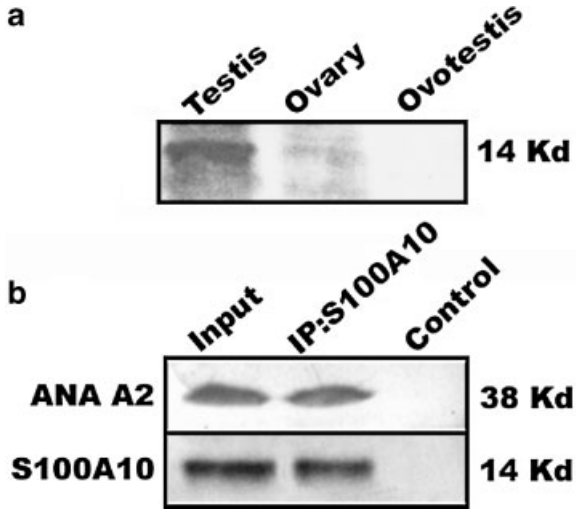

Fig. 7. Expression of S100A10 homolog in gonads of the rice field eel (**a**) and co-immunoprecipitation performed in testis tissue (**b**). Gonad extracts were subjected to SDS–PAGE and analyzed by Western blotting using S100A10 specific antibody. A band about 14 kDa was observed in testis and ovary; however, it was undetected in ovotestis in pre-intersex stage (a). Co-immunoprecipitation of annexin A2 and S100A10 protein from testis lysate (b): the extracts were incubated with protein G PLUS-Agarose alone (control) or with anti-S100A10 antibody. Co-immunoprecipitated proteins were analyzed by Western blotting with anti-Annexin A2 and anti-S100A10 antibodies. A 38 kDa band (ANX A2) and a 14 kDa band (S100A10) were shown on the membrane.

DISCUSSION

We have identified an evolutionarily conserved member of the annexin family, annexin A2 from the rice field eel, a freshwater fish that undergoes natural sex reversal from female to male during its life. The eel annexin A2 gene had a unique annexin core domain with four α-helical rich annexin repeats. Its three-dimensional structure was very similar to those of other annexin members [Liemann and Lewit-Bentley, 1995; Rosengarth and Luecke, 2003]. Phylogenetic analysis showed that it clustered with other vertebrate annexin A2 genes. Like human annexin A2, expression of annexin A2 of the rice field eel was broad-spectrum. Further immunofluorescent analysis showed that as observed in human annexin A2 [Dreier et al., 1998], it is highly expressed in epithelial type cells of kidney and liver of the rice field eel (Table I). Interestingly, we observed special expression pattern of the annexin A2 in different gonads during sex reversal of the rice field eel. Strong immunoreactivity in nuclear membrane of developing oocytes, besides in base membrane of mature oocytes, was observed during oogenesis, and this pattern persisted in ovotestis. As the sex transformation, the oocytes will be apoptotic and seminal vesicles epithelium start to develop. Accordingly, annexin A2 protein was expressed in the site of seminal vesicles epithelium in testis. The results suggested a potential role of annexin A2 in the gonadal differentiation from ovary, via ovotestis and testis of the rice field eel, probably by a mechanism via intracellular membrane organization and epithelium differentiation.

COS7 cells expressing annexin A2-DsRed fusion protein showed a cytosolic fluorescence, which indicated that annexin A2 did not enter the nuclei. Human annexin A2-CFP fusion protein showed almost the same localization in transfected HepG2 cells [Zobiack et al., 2001]. A notable feature of annexin A2 was that it could form a stable complex with ligand S100A10. We validated this ability of the rice field eel annexin A2 by co-transfecting it with YFP-S100A10. Both proteins not only co-expressed in cytoplasm, but also, especially congregated to form large complex in the cytoplasm, which suggested that two proteins interacted with each other. Co-immunoprecipitation analysis further confirmed the interaction of annexin A2 with S100A10. The annexin A2 of the rice field eel may function by forming annexin A2/S100A10 complex, in intracellular membrane organization and epithelium development. Further function analysis is needed to show the involvement of annexin A2 in the gonadal differentiation. Other models must be used to carry out

TABLE I. Comparison of Annexin A2 Expression Between the Rice Field Eel and Human

Tissue	Positive structure in the rice field eel	Positive structure in human[a]
Ovary	Nuclear membrane of developing oocytes, base membrane of matured oocytes	Germinal epithelium, tunica albuginea, ovarian stroma
Testis	Seminal vesicles epithelium	Sertoli cells
Kidney	Lining epithelium of collecting tubules	Bowman's capsule, loop of Henle, distal convoluted, collecting tubules
Liver	Bile ducts	Bile ducts

[a]Data from reference Dreier et al. [1998].

these studies, as the rice field eel also has its disadvantages in functional analysis such as long life cycle and poor genetic background. However, this species is a good model in findings of genes involved in sexual transformation, and these genes should be analyzed functionally in the other systems.

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REFERENCES

Biener Y, Feinstein R, Mayak M, Kaburagi Y, Kadowaki T, Zick Y. 1996. Annexin II is a novel player in insulin signal transduction. Possible association between annexin II phosphorylation and insulin receptor internalization. J Biol Chem 271:29489–29496.

Buss JE, Kamps MP, Gould K, Sefton BM. 1986. The absence of myristic acid decreases membrane binding of p60src but does not affect tyrosine protein kinase activity. J Virol 58:468–474.

Chasserot-Golaz S, Vitale N, Umbrecht-Jenck E, Knight D, Gerke V, Bader MF. 2005. Annexin 2 promotes the formation of lipid microdomains required for calcium-regulated exocytosis of dense-core vesicles. Mol Biol Cell 16:1108–1119.

Cheng H, Guo Y, Yu Q, Zhou R. 2003. The rice field eel as a model system for vertebrate sexual development. Cytogenet Genome Res 101:274–277.

Crompton MR, Moss SE, Crumpton MJ. 1988. Diversity in the lipocortin/calpactin family. Cell 55:1–3.

Dreier R, Schmid KW, Gerke V, Riehemann K. 1998. Differential expression of annexins I, II and IV in human tissues: An immunohistochemical study. Histochem Cell Biol 110:137–148.

Farber SA, De Rose RA, Olson ES, Halpern ME. 2003. The zebrafish annexin gene family. Genome Res 13:1082–1096.

Gerke V, Moss SE. 1997. Annexins and membrane dynamics. Biochim Biophys Acta 1357:129–154.

Gerke V, Moss SE. 2002. Annexins: From structure to function. Physiol Rev 82:331–371.

Liemann S, Lewit-Bentley A. 1995. Annexins: A novel family of calcium- and membrane-binding proteins in search of a function. Structure 3:233–237.

Ling Q, Jacovina AT, Deora A, Febbraio M, Simantov R, Silverstein RL, Hempstead B, Mark WH, Hajjar KA. 2004. Annexin II regulates fibrin homeostasis and neoangiogenesis in vivo. J Clin Invest 113:38–48.

Liu JW, Shen JJ, Tanzillo-Swarts A, Bhatia B, Maldonado CM, Person MD, Lau SS, Tang DG. 2003. Annexin II expression is reduced or lost in prostate cancer cells and its re-expression inhibits prostate cancer cell migration. Oncogene 22:1475–1485.

Morgan RO, Fernandez MP. 1997. Annexin gene structures and molecular evolutionary genetics. Cell Mol Life Sci 53: 508–515.

Olwill SA, McGlynn H, Gilmore WS, Alexander HD. 2005. Annexin II cell surface and mRNA expression in human acute myeloid leukaemia cell lines. Thromb Res 115:109–114.

Powell MA, Glenney JR. 1987. Regulation of calpactin I phospholipid binding by calpactin I light-chain binding and phosphorylation by p60v-src. Biochem J 247:321–328.

Rescher U, Gerke V. 2004. Annexins—Unique membrane binding proteins with diverse functions. J Cell Sci 117: 2631–2639.

Rosengarth A, Luecke H. 2003. A calcium-driven conformational switch of the N-terminal and core domains of annexin A1. J Mol Biol 326:1317–1325.

Sharma MR, Koltowski L, Ownbey RT, Tuszynski GP, Sharma MC. 2006a. Angiogenesis-associated protein annexin II in breast cancer: Selective expression in invasive breast cancer and contribution to tumor invasion and progression. Exp Mol Pathol 81:146–156.

Sharma MR, Rothman V, Tuszynski GP, Sharma MC. 2006b. Antibody-directed targeting of angiostatin's receptor annexin II inhibits Lewis Lung Carcinoma tumor growth via blocking of plasminogen activation: Possible biochemical mechanism of angiostatin's action. Exp Mol Pathol 81:136–145.

Smart EJ, De Rose RA, Farber SA. 2004. Annexin 2-caveolin 1 complex is a target of ezetimibe and regulates intestinal cholesterol transport. Proc Natl Acad Sci USA 101:3450–3455.

Tatenhorst L, Rescher U, Gerke V, Paulus W. 2006. Knockdown of annexin 2 decreases migration of human glioma cells in vitro. Neuropathol Appl Neurobiol 32: 271–277.

Zhou RJ, Cheng HH, Tiersch TR. 2001. Differential genome duplication and fish diversity. Rev Fish Biol Fisheries 11:331–337.

Zobiack N, Gerke V, Rescher U. 2001. Complex formation and submembranous localization of annexin 2 and S100A10 in live HepG2 cells. FEBS Lett 500:137–140.