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Identification of a Spindlin homolog in gibel carp (*Carassius auratus gibelio*)

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Abstract

Spindlin has been suggested to play an important role during the transition from oocyte maturation to embryo development in mouse, but its homolog similar to the mouse *Spindlin* in molecular and expression characterization has not been identified up to now in other vertebrates. In this study, a full length of cDNA sequence is cloned and sequenced from the gibel carp (*Carassius auratus gibelio*). It contains 1240 nucleotides with an open reading frame of 771 nt encoding 257 amino acids. Based on its amino acid sequence alignment and comparison analysis with the known Spin family proteins, the newly cloned Spin is named *Carassius auratus gibelio Spindlin* (*CagSpin*). Its product could be detected from mature eggs to blastula embryos, but its content decreased from the two-cell stage, and could not be detected after the gastrula stage. It suggests that the CagSpin should be a maternal protein that is expressed during oocyte maturation, and plays a crucial role in early cleavage of embryogenesis. *CagSpin* is the first homolog similar to mouse *spindlin* identified in fish, and also in other vertebrates. GST pull-down assay reveals the first biochemical evidence for the association of CagSpin and β-tubulin, the microtubule component. Therefore, CagSpin may play important functions by interacting with β-tubulin and other spindle proteins during oocyte maturation and egg fertilization.

Keywords: Early embryogenesis; Embryo; *Carassius auratus gibelio*; Oocyte maturation; Fertilization; β-tubulin; Microtubule; Spindlin (Spin)

1. Introduction

Oocyte maturation is one of the most critical events in animal life cycling. The maturation period can be further divided into two distinct aspects: (1) cytoplasmic maturation, which refers to the expression and organization of factors essential for egg activation, pronuclear development, axis specification, and maternal inheritance (Eppig et al., 1994; Antczak and van Blerkom, 1997; Gardner, 1999; Cheung et al., 2000), and (2) nuclear maturation, including chromosome recombination, crossing-over and segregation in meiosis (Albertini, 1992a,b; Albertini et al., 1993).

Spindlin (*Spin*), first reported in mouse, has been suggested to play a role in cell cycle regulation during the transition from oocyte to embryo (Oh et al., 1997). Spin, as a member of Spin/Ssty (*Ssty*, Y-linked spermiogenesis specific transcript) protein family, contains a conserved motif of about 50 amino acids (Spin/Ssty repeat), which is predicted to form a four-stranded β-structure in its secondary structure (Staub et al., 2001). Three separate Spin/Ssty repeat modules, generally thought to be independent functional units, are considered to indispensably compose the structural and functional integrity of all known spin family proteins. In mice, *Spin* transcripts first appear in unfertilized eggs and two-cell stage embryos, but disappear after the eight-cell stage (Oh et al., 1997); while *Ssty* transcripts are specifically expressed in sperm cells (Bishop and Hatat, 1987). It has been discovered in mice that phosphorylation of *Spin* at the serine or threonine residue is essential for its proper function

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interacting with the spindle (Oh et al., 1997, 1998). However, different results were reported in chicken, where *chSpin*-Z representing Spin's homologous gene localizing on the long arm of the Z chromosome was transcribed in various tissues of adult chickens and *chSpin*-W representing the counterpart gene was transcribed most prominently in ovarian granulosa and thecal cells. Additionally, both genes are transcribed in early embryos. The function of chSpin was suggested to be associated with chromosomes during mitosis (Itoh et al., 2001).

Gibel carp (*Carassius auratus gibelio*) is a unique gynogenetic fish distributed in China (Gui, 1996). Because of its specific polyploidy background and two different reproduction modes (Gui et al., 1993; Zhou et al., 2000), it has been used as an intriguing study model for understanding regulatory mechanisms of oocyte maturation, egg fertilization and early embryogenesis (Xie et al., 2001, 2003; Dong et al., 2004). Some important genes, such as ZP3 (Fan et al., 2001), cyclin A1 (Fan et al., 2000), cyclin B, cyclin A2 (Xie et al., 2001, 2003), SNX (Wen et al., 2003), C-type lectin (Dong et al., 2004), and C1q-like factor (Chen and Gui, 2004), have been identified in the gibel carp. In this study, we are focusing on the *Spin* gene in the gynogenetic fish, because Spin has been suggested to play an important role during oocyte maturation and early embryogenesis in mouse (Oh et al., 1997, 1998). The aims for this study are to clone the *Spin* gene from the gibel carp, to analyze its association with tubulin, and to reveal its expression profile during oocyte maturation and early embryogenesis.

2. Materials and methods

2.1. Broodfish and embryos

Matured gibel carps used in this paper were selected from Guanqiao Experimental Station, Institute of Hydrobiology, Chinese Academy of Sciences (CAS). During reproduction season, spawning was artificially induced by two intraperitoneal injections with a mixture of acetone-dried carp pituitary, HCG and LRH-A (Gui, 1999). Ovulated eggs were inseminated with sperms from male red common carp (*Cyprinus carpio*) to stimulate gynogenesis (Jiang et al., 1983). The inseminated eggs were incubated at about 20 °C.

2.2. RNA isolation, mRNA purification and SMART cDNA library construction

Full mature eggs were sampled and used for RNA isolation according to the RNA Extraction Kit (Pharmaica) Manual. mRNA was purified with PolyATract mRNA Isolation System (Promega) Manual and used further for SMART cDNA library construction. Purified mRNA was transcribed into single strand SMART cDNA with SMART™ PCR cDNA Synthesis Kit (Clontech) according to the instruction of library construction. Then, single strand SMART cDNA was synthesized into double-strand SMART cDNA. Total RNA from various tissues were isolated with RNA Quick Extraction Kit (Pharmacia) and used for Reverse Transcription Polymerase Chain Reaction (RT-PCR) detection.

2.3. Degenerate-PCR and rapid amplification of cDNA ends PCR (RACE-PCR)

Degenerate primers were designed corresponding to the conserved amino acid sequences of Spin/Ssty: skr1, skr2, skf1 and skf2 (Table 1). SMART cDNA library was used as Degenerate PCR template. To obtain full-length cDNA for Spin, specific primers smr and smf (Table 1) were designed and used with smart 5′ or 3′ primers (Table 1) by RACE PCR. RACE PCR was performed with a touch-down PCR protocol: 94 °C for 20 s, 60 °C for 20 s, 72 °C for 2 min, followed by 9 cycles in which annealing temperature was lowered 1 °C per cycle from 60 °C until it reached 50 °C, then 30 cycles of 50 °C for 20 s and 72 °C for 2 min.

PCR products were separated on 1.0% agarose gel, recovered by DNA Extraction Kit (Fermentas) and cloned into pGEM-T easy plasmid (Promega, US) for sequencing.

Table 1
Primers used for PCR in this paper

Primer codes	Sequences
smr	5′-GAGAGCACCAGACCTCTCCA-3′
smf	5′-GGTCAGTGACACTCGTCTGG-3′
smart 5′race	5′-AACGCAGAGTACGCGGG-3′
smart 3′race	5′-CAGAGTACT$_{16}$-3′
skr1	5′-TA(A/G)TC(A/G)TGCAT(C/G)A(A/G)(C/T)TG(A/G)TAC-3′
skr2	5′-TACAT(A/G)TA(C/G)A(A/G)(C/G)A(T/C)(A/T/C/G)GG(A/G)TC-3′
skf1	5′-T(T/A)(T/C)(T/C)T(C/G)(A/G)T(T/C/G)AA(A/G)TA(T/C)GA(T/C)GG-3′
skf2	5′-GG(A/T/C/G)(T/A/G)T(T/C/G)GA(T/C)TG(T/C)GT(C/G)GG-3′
β-actinF	5′-GTGCACTGGTCTTCAGGGGTT-3′
β-actinR	5′-GGGAAGTGGATGCGTGGGTAT-3′
rtF	5′-ATGGAGTGTGTGTACAGAAGC-3′
rtR	5′-ACGATCCAACCATGTCATAGACG-3′