

Antibodies against the C-terminal peptide of rabbit oviductin inhibit mouse early embryo development to pass 2-cell stage

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ABSTRACT

A full-length rabbit oviductin cDNA(1909bp) was cloned. It consists of a 5'-UTR of 52bp, an open reading frame (ORF) of 1374bp and a 3' -UTR of 483bp and has more than 80% homology with that of other mammal oviductins. N-terminal peptide (NTP) (384 residues) and C-terminal peptide (CTP) (73 residues) of deduced protein precursor has about 80% and 50% identity with that of other mammals respectively. Fusion proteins GST-NTP 368(1R-368N)and GST-CTP73 (369F-441A) were expressed and purified. NH₂-terminal of CTP sequencing reveals that the purified protein is consistent with the deduced one. In order to study the function of NTP and CTP the mouse anti-NTP and rabbit anti-CTP antisera were prepared. Tissue-specific (skeleton muscle, oviduct, uterus, ovary, liver, heart and brain) analysis indicated that rabbit oviductin was only found in oviduct. The conditioned medium derived from the rabbit oviduct mucosa epithelial cells has a function of overcoming the early embryonic development block of Kunming mouse cultured in vitro. Anti-CTP antiserum could totally inhibit the early embryo development at 2-cell stage cultured in the conditioned culture medium, but anti-NTP antiserum couldn't. There was a positive relationship between the ratio of early embryos at development block and the dosage of anti-CTP antiserum added in the conditioned culture medium. These results suggest that oviductin has a function not only on fertilization, but also on the release of early embryonic development block, and the later function domain of rabbit oviductin may be situate in its C-terminal.

Key words: *Rabbit oviductin, C-terminal peptide, early embryo, development, loss of function.*

INTRODUCTION

Up to date, little is known about the functions of oviductins in promoting the development of mammalian early embryo. When the fertilized eggs of mammals are cultured in composition defined medium in vitro, they are often blocked at certain developmental stage, called "early embryonic development block". The time of development block of early embryo is not consistent in different species.

In mouse it happened at 2-cell stage, so called the 2-cell block. In essence, the cause of the development arrest is due to the failure of its transition from maternal to zygotic control. There are two viewpoints concerning the function of oviductins in the development of early embryos. One thinks that the oviductins is necessary for the normal development of early embryos, the other thinks that the early embryo can develop normally in absence of oviductins, provided that the media were done more meticulously and the cultural environment is controlled more stringently. Recently there are many reports about the function of mammalian oviductins [1-4], but there is no report yet about any oviductin which has a definite function in the development of

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early embryo. In our previous studies, we have gained “loss of function” evidence to suggest that the rabbit 64 kDa oviductin has a function in overcoming the development block of early embryos *in vitro*. Anti-rabbit oviductin antibodies could totally inhibit the early development of mouse fertilized eggs cultured in conditioned culture medium derived from the rabbit oviduct mucosa epithelial cells. *In vivo* antifertility experiment also showed the fertility of female adult mice immunized with this oviductin is significantly decreased as compared to those of controls[5]. Northern blotting analysis of total RNA prepared from rabbit oviduct mucosa epithelial cells revealed that the full-length of rabbit oviductin mRNA was about 1.9kb[6]. A cDNA library to mRNA isolated from rabbit oviduct epithelial cells was constructed. By screening the library with anti-serum against the oviductin, a 0.7kb 3' terminal of its full length cDNA was got[6]. The main aims of this study were to obtain the full-length of rabbit oviductin cDNA and its protein amino acid sequence, and by using antibodies against different part of the oviductin, to reveal its function on early embryonic development.

MATERIALS AND METHODS

Materials

λ gt11 cDNA library of rabbit oviduct epithelial cells and pBS/0.7kb rabbit oviductin cDNA were obtained from our laboratory; Restriction enzymes, T4 DNA ligase, pUC/M13 reverse primer 5' -d(CAGGAAACAGC TATG A C) -3' , Prime-a-Gene System, Erase-a-Base System, WizardTM Lambda Preps DNA Purification System were products of Promega company; T7sequencingTM kit, RediPack GST Purification Module were purchased from Pharmacia company; [α -³²P]dCTP and [α -³⁵S]dATP were bought from Amersham company; CNBr-activated SepharoseTM4B was products of Amersham Pharmacia Biotech company; Freund' s complete and incomplete adjuvants, the reagents for cell culture were products of Sigma company; New Zealand white rabbits and BALB/c mice were purchased from B.K company (Shanghai, China); Kunming mice were purchased from Medical College of Fudan University.

Molecular cloning of complete length of rabbit oviductin cDNA

The recombinant pBS/0.7kb rabbit oviductin cDNAs were proliferated in E.coli XL1-blue and then were extracted and purified according SDS-alkali lysis method [7]. After EcoRI and NotI digestion and agarose electrophoresis analysis, the 0.7kb rabbit oviductin cDNAs were recovered from the low melting point agarose. Approximately 200,000 phage plaques of λ gt11 cDNA

library derived from rabbit oviduct epithelial cells were screened with the 0.7kb rabbit oviductin cDNA. Probes were labelled with [α -³²P]dCTP by using Prime-a-Gene labelling system (Promega) to a final specific activity of 1.82×10^9 cpm/mg DNA, and hybridization was performed at 68°C with hybridization solution (6 \times SSC, 0.05 \times BLOTTO). Positive clones were purified through secondary screening, and the positive clone with 1.9kb full-length rabbit oviductin cDNA was identified by proliferating plaques, extracting lDNA (using WizardTM Lambda Preps DNA Purification System) and digesting the lDNA with EcoRI and NotI. Then the 1.9kb full-length rabbit oviductin cDNA was subcloned into pBS.

Preparation of deletion subclones

The subclones with appropriate deletions were prepared using Erase-a-Base System (Promega) according to the method described in manufacturer' s protocol.

Sequencing and sequence analysis

The subclones with appropriate deletions were sequenced using T7sequencingTM kit, pUC/M13 reverse primer 5' -d(CAGGAAACAGCTATGAC)-3' , and [α -³⁵S]dATP. The sequences were put together and the sequence of 1.9kb rabbit oviductin cDNA was obtained. 1.9kb rabbit oviductin cDNA was also sequenced using ABI DNA sequencer. Then the homology, enzymatic digesting sites of 1.9kb rabbit oviductin cDNA and the homology, motif and enzymatic digesting sites of deduced protein were analyzed with Genbank, EMBL and SEQUEUCE LIST 1.0.

Expression and purification of GST-NTP rabbit oviductin, GST-CTP rabbit oviductin and GST

By removing the 5' -UTR and signal peptide coding sequence of 1.9kb rabbit oviductin cDNA, the 1.8kb rabbit oviductin cDNA was obtained. It was further divided into two fragments, NTP (N-terminal peptide of rabbit oviductin) rabbit oviductin cDNA (1.1kb 5' terminal of 1.9kb rabbit oviductin cDNA) and CTP (C-terminal peptide of rabbit oviductin) rabbit oviductin cDNA (0.7kb 3' terminal of 1.9kb rabbit oviductin cDNA). The recombinant pGEX-4T-3/ NTP rabbit oviductin cDNA and pGEX-4T-3/CTP rabbit oviductin cDNA were constructed and transformed into E.coli DE3 competent cells. GST, GST-NTP rabbit oviductin (1R-368N) and GST-CTP rabbit oviductin (369F-441A) were expressed and purified using RediPack GST Purification Module (Pharmacia).

Protein sequencing

After digesting GST-CTP rabbit oviductin with thrombin, CTP rabbit oviductin was separated using 2-dimensional electrophoresis, and then was transferred to PVDF membrane (Amersco). After staining with Coomassie Brilliant Blue dye liquor and destaining, the CTP rabbit oviductin band was got and then its N-terminal 11 amino acids sequence was determined using ABI Procise 491 and 477A protein sequencer.

Preparation of mouse anti-NTP rabbit oviductin and rabbit anti-CTP rabbit oviductin antisera

Mouse anti-GST-NTP rabbit oviductin and rabbit anti-GST-CTP rabbit oviductin polyclonal antisera were prepared by im-

munizing BALB/c mouse and New Zealand white male rabbits with GST-NTP rabbit oviductin and GST-CTP rabbit oviductin respectively.

GST-Sepharose 4B affinity chromatography column was constructed by conjugating GST to CNBr-Sepharose 4B beads (Amersham Pharmacia Biotech). After removing the anti-GST antibodies in mouse and rabbit antisera by using GST-Sepharose 4B affinity chromatography column, the mouse anti-NTP rabbit oviductin antisera and rabbit anti-CTP rabbit oviductin antisera with a final titre of 1:320,000 (ELISA method[8]) were chosen for "loss of function" analysis.

Western blotting analysis

The purified GST-NTP rabbit oviductin and GST-CTP rabbit oviductin fusion proteins were digested with thrombin and then separated by 16% SDS-PAGE respectively. Western blotting analysis was performed according to the method described in the reference[9].

Tissue specific analysis of rabbit oviductin

The proteins were extracted respectively from seven different tissues (skeleton muscle, oviduct, uterus, ovary, liver, heart and brain) of healthy New Zealand white rabbit. The concentration of proteins were determined by Bradford method. The protein samples (20mg of each) were separated by 12% SDS-PAGE, the Western blotting analysis was done with the same method indicated above.

Preparation and identification of conditioned medium derived from rabbit oviduct mucosa epithelial cells

Conditioned medium derived from rabbit oviduct mucosa epithelial cells was made according to the method described in reference[10]. To identify whether the conditioned medium had the function of overcoming development block of early embryo of Kunming mouse, 34 and 33 Kunming mouse fertilized eggs at 22 h after coitus (late one-cell stage) were cultured in 100 μ l M16 medium (as control) and the conditioned medium respectively, meanwhile 33 Kunming mouse fertilized eggs at 38 h after coitus (late two-cell stage) were cultured in 100 μ l M16 medium (as control also).

"Loss of function" analysis

Early embryos of Kunming mouse at 22 h after coitus were collected and randomly distributed into four groups. Two groups as experimental groups, were cultured in the conditioned culture medium (composed of half of conditioned medium and M16, 10% fetal bovine serum, 5ng/ml EGF, 5 μ g/ml transferrin and 5mg/ml Insulin) adding mouse anti-NTP rabbit oviductin and rabbit anti-CTP rabbit oviductin antisera respectively. Two other groups as control groups, were cultured in the conditioned medium adding normal mouse and normal rabbit sera respectively. The antisera and normal sera were diluted in M16 and sterilized by filtration, they were in a final dilution of 1:400.

The dose-response analysis was performed by adding a serial different quantity of 1:400 anti-CTP rabbit oviductin antiserum

to the conditioned culture medium containing the mouse early embryos (35-37 fertilized eggs each group).

RESULTS

Characterization of rabbit oviductin cDNA

Eighteen positive clones isolated from the primary screening of cDNA library to mRNA isolated from rabbit oviduct epithelial cells were selected for purification. By proliferating phage, extracting lDNA and digesting lDNA with EcoRI and NotI, the positive phage plaque with 1.9kb rabbit oviductin cDNA was got and subcloned into pBS. Eight subclones with appropriate deletions were obtained. The 1.9kb rabbit oviductin cDNA was sequenced manually and automatically. Results revealed that the full length of 1909bp rabbit oviductin cDNA (GenBank Accession number: AF347052) consists of a 5' - untranslated region (5' -UTR) of 52bp, an open reading frame (ORF) of 1374bp and a 3'-UTR of 483bp. The coding region encodes a signal peptide of 16 amino acids, which was found by analyzing the deduced amino acids sequence of rabbit oviductin with program Signal P[11], and a 441-amino acid protein (Fig 1). The homology analysis of its sequence and its deduced amino acid sequence showed that the 5' -terminal sequence of rabbit oviductin cDNA (about 1.3kb) has about 86% homology with the oviductins mRNA or cDNA of other mammals (such as human, papio hamadryas, rhesus, bovine, hamster, sheep ect). In 384 amino acids overlap of N-terminal peptide and 73 amino acids overlap of C-terminal peptide of deduced rabbit oviductin precursor, there are respectively 74-82% and 42-53% identity with oviduct-specific glycoprotein precursors of human (GI 2493676), house mouse (GI 2493678), cow (GI 2493675), pig (GI 2493679) and sheep (GI 2493680) (Fig 2). The phosphorylation sites analysis by using PhosphoBase v2.0 showed that there are potential phosphorylation sites of casein kinase I (62, 74, 82, 92, 152, 193, 232, 356, 381, 399), calmodulin-dependent protein kinase II (411), casein kinase II (26, 160, 197, 343), glycogen synthase kinase 3 (74, 78, 148, 189, 193, 398), protein kinase p34cdc2 (252, 432), protein kinase p70s6k (411), protein kinase A (74, 114, 121, 331, 411) and protein kinase C (70, 219, 304). Motif analysis of the deduced protein also showed there was a N-glyco-

protein site(368) and six potential O-glycoprotein sites (354, 356, 377, 396, 423, 440).

Purification of fusion proteins and protein se-

quencing

SDS-PAGE analysis of purified GST-NTP rabbit oviductin and GST-CTP rabbit oviductin fusion proteins showed that about 50% GST-NTP rabbit

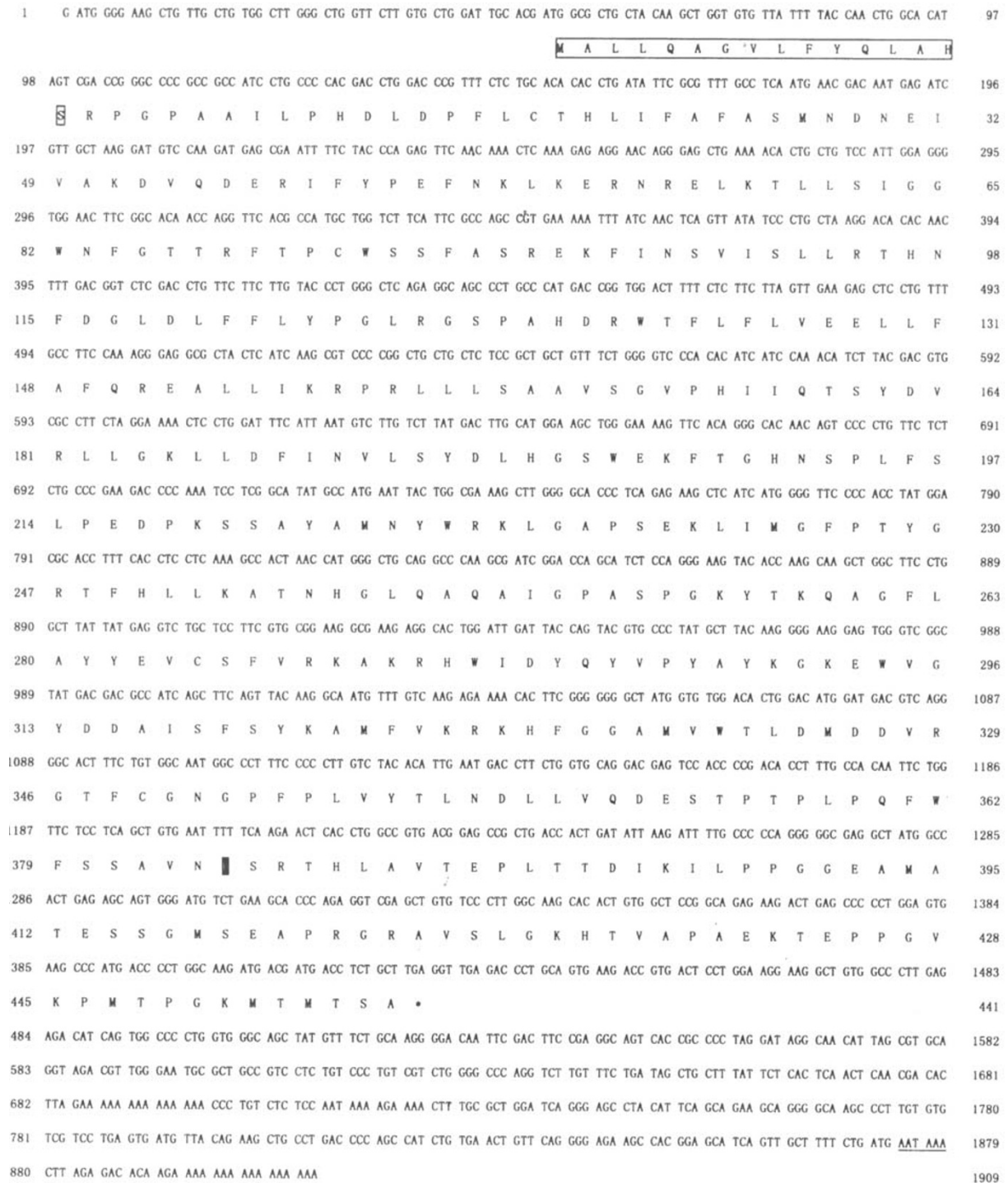


Fig 1. cDNA and deduced protein sequence of rabbit oviductin gene. The stop codon is replaced by asterisk; The letters in box is signal peptide; The polyadenylation signal (AAT AAA) is underlined; the first residue of the deduced CTP rabbit oviductin is in shadow.

oviductin (66kDa) was decomposed (Fig 3A, Lane 1) and presented as 30 kDa, but most of GST-CTP rabbit oviductin (34kDa) was intact (Fig 3B, Lane 1). GST-NTP rabbit oviductin and GST-CTP rabbit oviductin digested with thrombin showed that the decomposed GST-NTP rabbit oviductin (30kDa) and intact GST-CTP rabbit oviductin (34 kDa) could be digested completely, however the intact GST-NTP

rabbit oviductin was difficult to be digested (Fig 3A, Lane 2 and Fig 3B, Lane 2). CTP rabbit oviductin derived from GST-CTP rabbit oviductin was separated using 2-dimensional electrophoresis and then, eleven NH₂-terminal amino acids of CTP rabbit oviductin were sequenced. The sequencing results proved that they completely consistent with that of deduced from rabbit oviductin cDNA (Fig 4).

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rabbit -----MALLQAGVLFYQLAHSRPPGPAAILPHDLDPFLCGLHIFAFASMNNDNEIVAKDVQDERIFYPEFNKLKERNRELKTLLSIGGWNFGTTRFTPCWSSFASREKF
mouse MGRLLLLAGLVLLMKHSDGTAYKLVCFETNWAHSRPPGASIMPHDLDPFLCGLHIFAFASMSNNQIVAKNLGDENVLYPEFNKLKERNRELKTLLSIGGWNFGTSRFTAMLSTLANREKF
human MWKLLLLVWGLVLLKHHGAAHKLVCYFETNWAHSRPPGASILPHDLDPFLCGLHIFAFASMNNDQIVAKDLQDEKLYPEFNKLKERNRELKTLLSIGGWNFGTSRFTMLSTLANREKF
pig MGKLLLLVWGLVLLKHHNGAAHKLVCYFETNWAHSRPPGASILPRDLDPFLCGLHIFAFASMNDSQIVAKDARDESIFYPEFNKLKERNRELKTLLSIGGWNFGTSRFTMLSTLANREKF
cow ---LLLGVGLLLVLLKHHGAAHKLVCYFETNWAHSRPPGASILPRDLDPFLCGLHIFAFASMSNNQIVPKDPQDEKLYPEFNKLKERNRELKTLLSIGGWNFGTVRFTMLSTLANREKF
sheep MGKLLLLVWGLLMLKHHGAAHKLVCYFETNWAHSRPPGASILPRDLDPFLCGLHIFAFASMNNDQIVPKDPLDEKLYPEFNKLKERNRELKTLLSVGGWNFGTSRFTKMLSTLANREKF

rabbit INSVISLLRTHNFDGLDFFLYPGLRGSPAHRWTFVFLVEELLFAFQREALLIKRPRLLLSAAVSGVPHI IQTSYDVRLLGKLLDFINVLSDYDLHGSWEKFTGHNSPLFSLPEDPKSSA
mouse IDSVISFLRIGHFDGLDFFLYPGLRGSPHWRWTFVFLVEELLFAFQREALLIQHPRLLLSAAVSGVPHISIIHTSYDALLGRRLLDFINVLSDYDLHGSWEKFTGHNSPLFSLPEDSKSSA
human IASVISLLRTHDFDGLDFFLYPGLRGSPHWRWTFVFLVEELLFAFQREALLTMRPRLLLSAAVSGVPHIVQTSYDVRFLGRLLDFINVLSDYDLHGSWEKFTGHNSPLFSLPEDPKSSA
pig IRSAIGLLRTHGFDGLDFFLYPGLRGSPRRDRWTFVFLVEELLFAFQREALLTMRPRLLLSAAVSGADPHVQKAYDVRLLGRLLDFINVLSDYDLHGSWEKFTGHNSPLFSLSDDPKSSA
cow VSSVIALLRTHGFDGLDFFLYPGLRGSPARDRWFVFLVEELLQAFKNEAQLTMRPRLLLSAAVSGDPHVQKAYEARLLGRLLDFISVLSYDLHGSWEKFTGHNSPLFSLPGDPKSSA
sheep VKSVIALLRTHGFDGLDFFLYPGLRGSPARDRWFVFLVEELLQAFKNEAQLTMRPRLLLSAAVSGDPHVQKAYDARLLGRLLDFISVLSYDLHGSWEKFTGHNSPLFSLPGDPKSSA

rabbit YAMNYWRKLGAPSEKLMGFPTYGRTFHLLKATNHGLQAQAI GPASPGKYTKQAGFLAYEVCFSVRKAKRHWIDYQYVPYAYKGEWVGYDDAISFSYKAMFVKRKHFGGAMVWTLDM
mouse YAMNYWRKLGTPADKLMGFPTYGRNFYLLKESKNGLQASMGSPASPGKYTKQAGFLAYEVCFSVQRAKKNIDYQYVPYAFKGEWLYDDTISFSYKAMYVREHFGGAMVWTLDM
human YAMNYWRKLGAPSEKLMGFPTYGRFRLKASKNGLQARAI GPASPGKYTKQAGFLAYEIGSFVWGAKKHWIDYQYVPYANKGEWVGYDDAISFSYKAWFIRREHFGGAMVWTLDM
pig YTMNYWRKLGAPPEKLMGFPTYGRFRLKASKNEIGAEAVGPASPGKYTKQAGFLAYEVCFSVQRAKKNIDYQYVPYAYKGEWVGYDDTISFSYKAFKREHFGGAMVWTLDD
cow YAMNYWRKLGVPPEKLMGLPTYGRTFHLLKASQNEIQAQAVGPASPGKYTKQAGFLAYEIGCFVRRAKRHWINDYQYVPYAFKGEWVGYDDAISFGYKAFKREHFGGAMVWTLDD
sheep YAMSRYRGLGVPPEKLMGLPTYGRTFHLLRASQNEIGAGAVGPASPGKYTKQAGFLAYEVCFSVQRAKKNINDYQYVPYAFKGEWVGYDDAISFGYKAFKREHFGGAMVWTLDD

rabbit DVRGTCGNGPFPVYTLNDLLVQDESTPTPLPQFWFSSAVNFSRT---HLAVTEPLTTDI-KILPPGGEAMATESG---MSEAPRGA-----VSLGKHTVAPAEKTEPPGVKPM
mouse DVRGTCGNGPFPVHILNELLVQTESNSTPLPQFWFSSVNASGGPSENTALTEVLTDTIKILPPGGEAMTEVHRRYENITVPSDGSVTPG-GTASPRKHAVTEENNTMAAEAKTM
human DVRGTCGNGPFPVYVYVNDILVRAEFSSLSLPQFWLSSAVNSSSDPERLAVITAWITDS-KILPPGGEAGVTEIHQKCENTITITPRGTTVTPKTEVSLGKHTVALGEKTEITGAMTM
pig DVRGTCGNGPFPVYVYVNDLLKAEVSSLSLPGGLSTTVNSRTPCESLAVITKDLTDL-GILPLGGEAVATEHARSDNITVTPGGLVAPTRPTLSFGKLTVAPEGKTESPGEKAM
cow DFRGTCGNGPFPVYVYVNDLLVNDVDFSSLSPKFWFSTAVNSSRIGPEMPTMTRDITLGL-GILPLGGEAVATETHRKSATITITPKGEIATPTRTPLSFGRTAAPEGKTESPGEKPL
sheep DFRGTCGNGPFPVLAHTLNDLLVNDVDFSSLSPKFWFSTAVNSSRIGPEMPTMTRDITLGL-GILPLGGEAVATETHRKSATITITPRGETATPTRTPLSFGRTAAPEGKTESPGEKPL

rabbit TP-GKMTMTSA* -----
mouse STLDFFSKTTTGVSKITITGSKTTT---GVSKTTTGVS-K-ATAGISKTIPKISKATAGV
human ISVGHQSMTPGEKALTPVGHQSVTTGQKTLTSVGYQSVTPGEKTLTPVGHQSVTP-----
pig ITPVGHPSVTPGDMSVPPVPIQT---GDR-ITPPRRQAVAP-EKMTLPSGKRSD-----
cow ITPVGHAVSPGGI AVGPVRLQT---GQK-VTPPGRKAGVP-EKVTPPSGKMTVTP-----
sheep ISVGRILAVSPGGI AVGPVHLQI---GQK-VTPPGRKAGVP-EKVTPSGKMTVTP-----

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Fig 2. Alignment of the deduced rabbit oviductin amino acid sequence with deduced sequence from human, both sequences include signal peptide. Identical residues with that of rabbit oviductin are in shadow, the first residue of NTP rabbit oviductin is in pane, the first residue of CTP rabbit oviductin is underlined, gaps are indicated by a dash and the termination of rabbit oviductin sequence is indicated by asterisk.

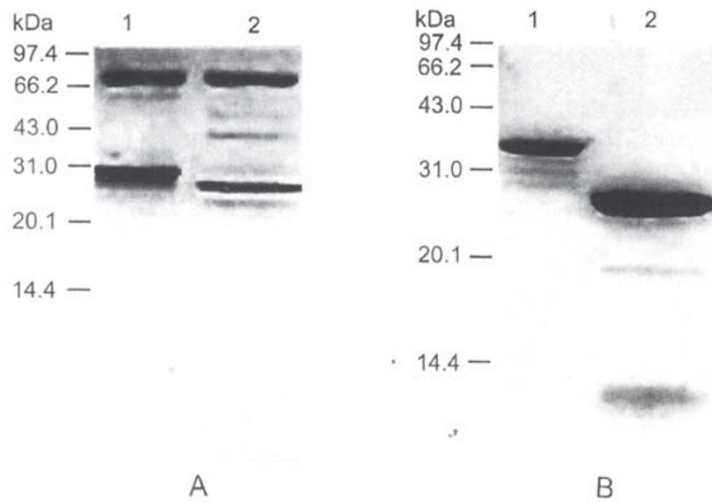


Fig 3. (A) 16%SDS-PAGE analysis of GST-NTP rabbit oviductin fusion protein purified by affinity chromatography and that digested by thrombin. Lane 1. Purified GST-NTP rabbit oviductin fusion proteins; Lane 2. Purified GST-NTP rabbit oviductin fusion proteins digested by thrombin. (B) 16% SDS-PAGE analysis of GST-CTP rabbit oviductin fusion protein purified by affinity chromatography and that digested by thrombin. Lane 1. Purified GST-CTP rabbit oviductin fusion protein; Lane 2. Purified GST-CTP rabbit oviductin fusion protein digested by thrombin.

GCT GTG AAT TTT TCA AGA ACT CAC CTG GCC GTG ACG GAG CCG CTG ACC ACT
A V N F S R T H L A V T E P L T T
F S R T H L A V T E P

Fig 4. Eleven amino acids sequence of CTP rabbit oviductin determined by N-terminal amino acid micro-sequence analysis. *Italic letters* indicated amino acids deduced from rabbit oviductin cDNA and **bold letters** indicated amino acids sequenced.

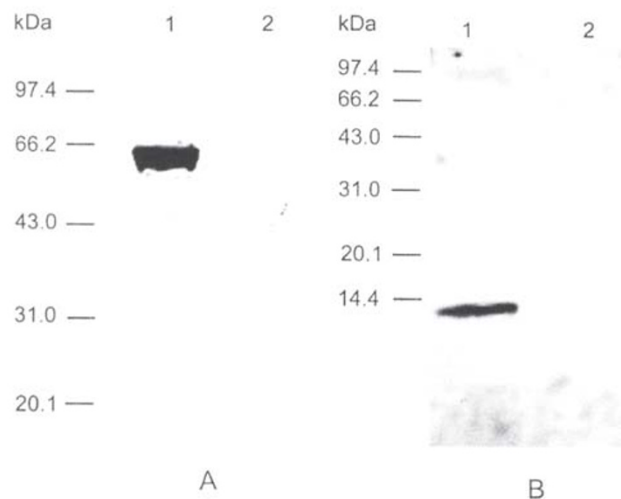


Fig 5. (A) The specificity analysis(western blotting method) of mouse anti-NTP rabbit oviductin antiserum after GST-absorbtion. Lane 1. GST-NTP rabbit oviductin digested by thrombin; Lane 2. Purified GST. (B) The specificity analysis(western blotting method) of rabbit anti-CTP rabbit oviductin antiserum after GST-absorbtion. Lane 1. GST-CTP rabbit oviductin digested by thrombin; Lane 2. Purified GST.

Specificity of the antisera used in analysis

Results obtained from Western blotting analysis showed that mouse anti-NTP rabbit oviductin and rabbit anti-CTP rabbit oviductin antisera were specific to GST-NTP rabbit oviductin (66kDa) and CTP rabbit oviductin (8kDa) respectively (Fig 5A, Lane 1 and Fig 5B, Lane 1), whereas no immunoreactivity was shown with GST (26kDa) (Fig 5A, Lane 2 and Fig 5B, Lane 2). These results indicated that the antisera had high specificity to rabbit oviductin.

Tissue-specific analysis of rabbit oviductin



Western blotting results (Fig 6A and 6B) of proteins extracted from seven different tissues of healthy New Zealand white rabbit by using the polyclonal antibodies against NTP rabbit oviductin or against CTP rabbit oviductin showed that the oviductin was found only in oviduct. Interestingly, a about 38kDa protein in proteins extracted from rabbit brain was revealed when anti-CTP rabbit oviductin antibodies were used as probes.

“Loss of function ” analysis

34 fertilized eggs of Kunming mouse at 22 h after



Fig 6. (A) Western blotting analysis of proteins extracted from seven different rabbit tissues using anti-NTP rabbit oviductin polyclonal antibodies. Lane 1-7. Proteins extracted from oviduct, skeleton muscle, uterus, ovary, liver, heart and brain respectively. (B) Western blotting analysis of proteins extracted from seven different rabbit tissues using anti-CTP rabbit oviductin polyclonal antibodies. Lane 1-7. Proteins extracted from skeleton muscle, oviduct, uterus, ovary, liver, heart and brain respectively.

coitus (late one-cell stage) cultured in M16 medium were totally arrested at 2-cell stage, however 33 fertilized eggs of Kunming mouse at 38 h after coitus (late two-cell stage) could further develop and 60.6%(20/33) of early embryos developed to blastocysts. As experimental group, among 33 fertilized eggs of Kunming mouse at 22 h after coitus cultured in conditioned medium, 29 embryos (88%) overcame 2-cell block. In control group only 14%(5/36) of embryos passed the 2-cell block (Fig 7).

After adding mouse anti-NTP rabbit oviductin and rabbit anti-CTP rabbit oviductin polyclonal antibodies in the conditioned medium the 2-cell block ratio of experimental groups were 11%(4/35) and 100%(37/37) respectively. In the control groups, they were 20%(7/35) in that of adding normal mouse serum and 15%(5/33) in that of adding normal rabbit serum (Fig 8). As seen in the dose-response curve to

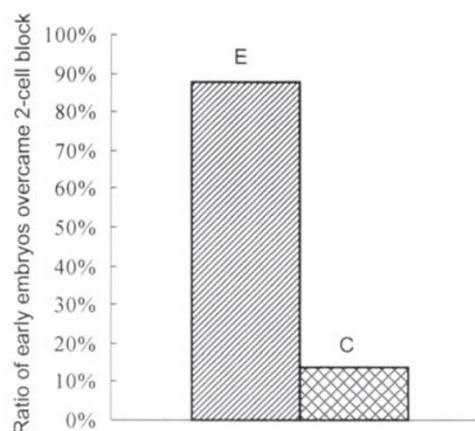


Fig 7. Analysis of the percentage of early embryos passed 2-cell stage in the conditioned medium. E: Experimental group (in the conditioned medium); C: Control group (in M16 medium).

were 17%, 29%, 57%, 81.8% and 100% respectively. These indicated that there was a positive correlation between the ratio of early embryos of Kunming mouse blocked at 2-cell stage and the quantity of anti-CTP rabbit oviductin antiserum added into the conditioned medium.

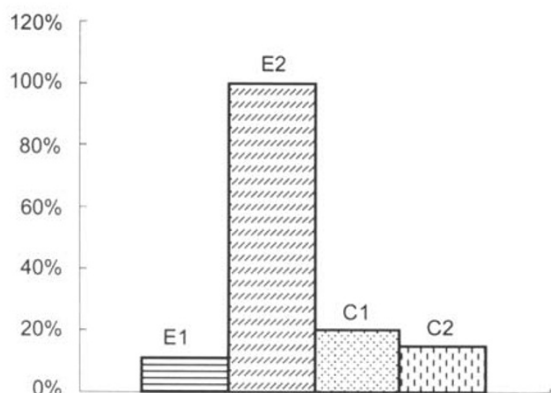


Fig 8. Results of loss of function analysis. E1: Experimental group added anti-NTP rabbit oviductin antiserum; E2: Experimental group added anti-CTP rabbit oviductin antiserum; C1: Control group added normal mouse serum; C2: Control group added normal rabbit serum.

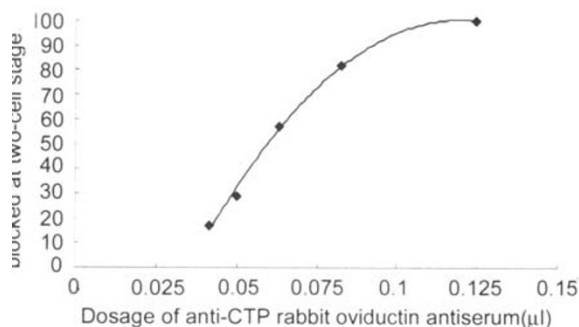


Fig 9. Dose-response curve of correlation between development of mouse fertilized eggs and anti-CTP rabbit oviductin antiserum added to the conditioned medium. Dosage of anti-rabbit oviductin antiserum indicated the volume (ml) added to the culture medium.

DISCUSSION

It was in 1989 that Bleau and St. Jacques first raised the definition of “oviductin” which referred to a class of oviduct-specific glycoproteins and may

be conducted to development of egg and early embryo[12]. Over the last several years, considerable progress on oviductin research has been made[13]. Some reports demonstrated that they associated with the zona pellucida of the ovulated oocyte and with the early embryo, some confirmed that the synthesis of oviductin is dependent on estrogen, some revealed that the nonciliated epithelial cells of the oviduct are solely responsible for the synthesis of oviductins, some analyzed the chemical characteristics of oviductins, such as the size of their mRNA, the molecular weight of the polypeptides and their isoelectric point, some compared the alignment of the available deduced amino acid sequences of oviductins from various species, and inferred the role (s) of oviductin in reproductive events. However the research on oviductins was restricted because of their difficulties to be purified, so there was few report about the oviductin on its definite function in the development of early embryo.

In our previous studies, we have gained “loss of function” evidence (in vitro and in vivo), and suggested that 64kDa rabbit oviductin exhibit some positive functions in overcoming early embryo developmental block. We also have revealed its molecular weight, isoelectric point and demonstrated the synthesis and secretion of this rabbit oviductin were not dependent exclusively on 17 β -estradiol or progesterone, the rabbit oviductins could pass through zona pellucida easily and aggregated tightly around the early embryonic cell membrane[5]. The rabbit oviductin cDNA sequence revealed significant identities and similarities (about 80%) to that of oviductal glycoproteins from a number of species. The NTP rabbit oviductin (384 amino acids) has greater (74-82%) identity with oviduct-specific glycoprotein precursors of other mammals, as compared to that of CTP rabbit oviductin (73 amino acids, 42-53% (Fig 2), suggesting that the differences of oviductal glycoproteins between species may be found in the later region. So it is necessary to study the function of CTP rabbit oviductin in the development of early embryo. The homology comparisons also showed that there were 40-50% similarities between rabbit oviductin and some enzymes of chitinase gene family, but the oviductins showed no chitinase activity[14]. In recent years, few researches concerning the roles of oviductins in the development of early embryo

were reported[15],[16].

The full-length of rabbit oviductin cDNA (1909bp), which may play a pivotal role in overcoming the development block, was identified and sequenced. The experiment of culturing one-cell fertilized eggs of Kunming mouse in conditioned medium derived from rabbit epithelial mucosa cells showed that the proteins secreted by oviduct epithelial cells have the function of overcoming 2-cell development block of mouse early embryo (Fig 7). We have gained “loss of function” evidence to suggest that the rabbit 64 kDa oviductin has a function in overcoming the development block of early embryos in vitro[5]. For the sake of clarifying that which part of the rabbit oviductin, NTP rabbit oviductin (N-terminal peptide of the rabbit oviductin, 1R-368N) or CTP rabbit oviductin (C-terminal peptide of the rabbit oviductin, 369F-441A), involves in early embryonic development, mouse anti-NTP rabbit oviductin and rabbit anti-CTP rabbit oviductin antisera were prepared and purified by using immunoaffinity chromatography method. Western blotting analysis (Fig 5A, 5B) showed these purified antisera had high specificity to NTP rabbit oviductin and CTP rabbit oviductin respectively.

The “loss of function” experiment made with anti-CTP rabbit oviductin antiserum (Fig 8 and Fig 9) indicated that anti-CTP rabbit oviductin antibodies have the function of inhibiting mouse early embryos development to pass 2-cell block, indicated that the rabbit oviductin may have a function of improving the development of early embryo. Due to anti-NTP rabbit oviductin antiserum couldn't inhibit the early embryo development of mouse cultured in the conditioned culture medium, the function domain of the rabbit oviductin may be at its C-terminal.

Since the late one-cell stage embryos cultured in M16 medium were totally blocked at two-cell stage, and the two-cell stage embryos obtained from oviduct then cultured in M16 medium could further develop, indicated the rabbit oviductin play a key role on two-cell stage embryos. However, the late two-cell stage embryos never derived from in vitro fertilized eggs cultured in M16 medium, so it cannot rule out the possibility that the rabbit oviductin also play roles in the further development of early embryo after two-cell stage. With regard to whether the rabbit oviductin has that functions, we will fur-

ther research. All these function will be further confirmed after obtaining its “gain of function” evidences. Nevertheless, its “loss of function” evidence is somewhat sufficient to reveal the rabbit oviductin is probably a key inducer in realizing the transition from maternal to zygotic control of early embryo. In the future, “gain-of-function” experiments may help to confirm the importance of the C-terminal peptide of the rabbit oviductin in the development of early embryo. It was reported that oviductin has a function on fertilization[3],[4],[16], while our research suggested that oviductin also has a function on the release of early embryonic development block.

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