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Differential Expression of *Spin* Transcripts: Oocyte and Somatic Types

Bermseok Oh*, Sue-Yun Hwang[1], Davor Solter[2], and Barbara Knowles[3]

National Institute of Health, Division of Genetic Disease, Seoul 122-701, Korea;
[1]Institute of Immunobiology, Catholic Institutes of Medical Science, The Catholic University of Korea, Seoul 137-701, Korea;
[2]Max-Planck Institute of Immunology, Freiburg D79108, Germany;
[3]The Jackson Laboratory, 600 Main Street, BarHarbor, Maine 04609, USA

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***Spin* is an abundant maternal transcript comprising up to 0.2% of the total mRNA stock in mouse oocyte, whose protein product is associated with the meiotic spindle. We have identified a new isoform of *Spin* transcript containing a distinct 5′-untranslated region and the N-terminus of encoded protein. Northern blot and RT-PCR analysis showed that the new isoform is expressed in embryos and most of adult tissues, while the previously identified transcript is expressed solely in mouse oocyte. We thus designated these two *Spin* isoforms as somatic type and oocyte type, respectively. To investigate the underlying mechanism for the differential expression, genomic structure of *Spin* was examined. *Spin* exists as multiple copies in the genome, some of which appears to be pseudogenes, and characterization of *Spin* genomic clones indicates that oocyte- and somatic-isoforms were generated by alternative splicing. The complex organization of *Spin* genomic locus and its multifaceted control of expression provide a good model to study the molecular mechanisms of elaborate genome usage in mammals.**

The most unexpected surprise from the recently completed Human Genome Project was the announcement that the total number of human genes (appx. 40,000) appears to be only twice larger than that of *Drosophila*. The big question then is how the complexity of human body structure and physiology can be made and maintained by such relatively small set of genes. One possibility is that each human gene may encode multiple isoforms to fulfill the intricate network of mammalian metabolism. Another explanation is that mammals have been evolved to practice a complex combination of differential gene expressions. It is most likely that both of these mechanisms are employed at a variety of combinations to orchestrate the elaborated process of mammalian development.

There are several revenues how a single genomic locus can produce more than one protein products and regulates their expression differentially as well. In the simplest scenario, an alternative form of mRNA containing part of the coding region can be produced by using the second transcription initiation site. In some genes alternative splicing generates multiple forms of transcript encoding distinct protein products. During mammalian development, timely controlled alternative splicing often generates additional gene product specifically required at that stage. In this case, differential usage of alternative first exon containing distinct upstream regulator sequences provides a mean to control the transcription of each product in a timely- and spatially-modulated manner.

We previously reported that *Spin* is expressed in mouse oocyte and early preimplantation embryos as an abundant maternal transcript, comprising up to 0.2% of the total cellular mRNA (Oh et al., 1997). Its protein product, SPIN, associates with spindles during oocyte meiosis, leading to a hypothesis that *Spin* is critical for the completion of meiosis cell cycle and early cleavage division of fertilized egg (Oh et al., 1998). In the oocyte, *Spin* is transcribed as mRNAs of three different sizes, e.g. 0.8, 1.7, and 4.1 kb. Sequence analysis of these transcripts revealed that they all contain an identical coding sequence but different length of 3′-untranslated region (UTR), generated by differential usage of three polyadenylation signals. Interestingly, the 3′-UTR region of each transcript contains a unique combination of several cytoplasmic polyadenylation elements (CPEs) (Richter, 1999), leading to the differential activation of

* To whom correspondence should be addressed.
Tel: 82-2-380-1534, Fax: 82-2-388-0924
E-mail: ohbs@nih.go.kr

translation in the ovulated egg and fertilized zygote (Oh et al., 2000).

To add more complexity to the regulation of *Spin* expression, we found in the present study that two types of *Spin* transcript is encoded by the *Spin* genomic locus. The transcript of one isoform is expressed ubiquitously in embryos and adult tissues, while the other type is detectable solely in the oocyte. The differential expression of the oocyte- and somatic-type was investigated by studying the genomic structure of *Spin* gene and we report an exemplary case of differential gene usage observed in the mouse *Spin* locus.

Materials And Methods

cDNA isolation and sequencing

The 1.0 kb somatic type *Spin* clone was isolated from the mouse 2-cell stage cDNA library (Rothestein et al., 1992), using 0.7 kb *Bam*HI-*Hin*dIII fragment of the 4.1 kb transcript containing the coding sequence (Oh et al., 1997). This somatic type clone was inserted into the pBS vector (Stratagene) and sequenced with T3 and T7 primers and *Spin*-specific primers using Sequenase kit (US Biochemical) according to the manufacturers instruction.

In vitro translation

In vitro translation was performed with TNT system (Promega) as described in the instruction manual. Briefly, 1 μg of 0.8 kb oocyte type *Spin* plasmid or 1.0 kb somatic type plasmid was mixed with T7 polymerase and rabbit reticulocyte lysate in the presence of L-[^{35}S]-methionine (specific activity >1,000 Ci/mmol; Amersham), and incubated for 2 h at 37℃. The polypeptide produced was analyzed on a SDS-PAGE, which was dried and exposed on the X-ray film.

RT-PCR and northern hybridization

Mouse oocyte RNA was prepared according to the method of Huarte et al. (1987). Embryos were recovered from the pregnant female at the appropriate days considering the day of the plug as day 0.5 of development. Embryos and tissues were dissected and rinsed once in phosphate-buffered salin (PBS), and were homogenized in TRI REAGENT (MRC) solution. The RNAs were extracted according to the manufacturers instruction. All RNAs were treated with DNase before RT-PCR. Reverse transcription was performed with total cellular RNA extracted from 100 mouse oocytes or 1 μg of tissue RNAs in a 20 μl reaction using Superscript™ reverse transcriptase (Gibco), according to the manufacturers protocol. For each PCR, 1 μl of RT reaction was amplified using a common downstream primer and upstream primers specific for the oocyte type (Type A) and the somatic type (Type B) PCR amplification was performed through 35 cycles of 95℃ for 30 sec, 60℃ for 45 sec, and 72℃ for 30 sec. For northern hybridization, 10 μg of total RNAs from embryos and tissues were hybridized with the radiolabeled 0.7 kb *Bam*HI-*Hin*dIII *Spin* fragment containing part of the coding region.

Southern hybridization

For zoo blot analysis, genomic DNAs were extracted from mouse C57BL/6, rat, human, monkey, cow, pig, dog and rabbit by the method of Sambrook et al. (1989). For each lane, 10 μg of DNA was digested with HindIII and run on 0.7% agarose gel, and transferred to a nylon membrane (Micro Seperation). Genomic DNAs from mouse was further analyzed on additional Sourthern analysis after digestion with *Bam*HI, *Eco*RI and *Hin*dIII. Hybridization probes are prepared by radiolabeling fragments of *Spin* cDNA with [^{32}P]-dCTP as shown in Fig. 5.

Characterization of Spin genomic clones

To obtain *Spin* genomic clones, bacteriophage λ genomic library made from the B6Cl.4 T-cell line was screened with the 0.7 kb *Bam*HI-*Hin*dIII *Spin* fragment. Isolated clones were sequenced using *Spin* specific primers by the Sequenase™ kit (US Biochemical).

Results

A new type of Spin transcript expressed in embryos and adult tisssues

In addition to the original maternally transcribed *Spin* clones, we identified another type of *Spin* cDNA from the mouse 2-cell library. Sequence analysis of the new clone showed that it has a different 5′-UTR and N-terminus of the coding region, but shares the 228 amino acid residues and the 3′-UTR with previously identified 0.8kb maternal transcripts (Fig. 1A). For the sake of brevity, we named the original maternal cDNA as Type A, and the newly found form as Type B. To confirm the difference in the N-terminus of encoded proteins, *in vitro* translation was performed on Type A and Type B cDNA clones. As expected, the Type B clone produced a polypeptide with larger molecular weight than one of the Type A clone, reflecting the size difference of N-terminal peptide unique to each protein (Fig. 1B).

The expression pattern of Type A and Type B was investigated by RT-PCR of RNAs from oocytes, embryos and tissues (Fig. 2A). It was found that Type A is solely expressed in the ovary and oocyte, indicating an oocyte-specific expression. In contrast, Type B was detectable in most tissues. To further confirm the oocyte-specific expression of Type A, another set of RT-PCR was performed using RNAs from the F9 embryonic carcinoma cell line, the 13.5 d

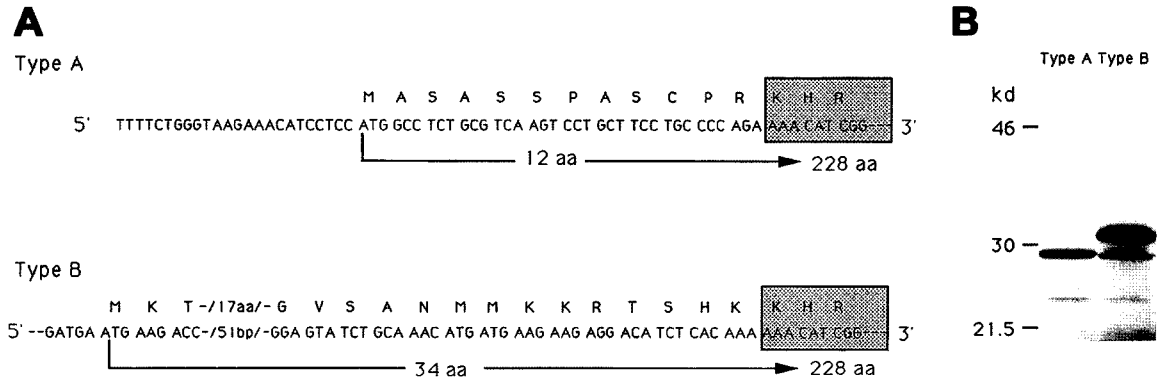

Fig. 1. A new type of *Spin* cDNA. cDNA sequence between type A and type B isoforms were compared (A) and *in vitro* translation of type A and type B cDNA clones was performed (B).

post coitum (dpc) embryo and oocytes (Fig. 2B). Again the Type A transcript was detected only in oocyte but neither in F9 cell line nor 13.5 dpc embryo. Thus we named the Type A isoform of *Spin* transcript as oocyte type, and the Type B isoform as somatic type.

To analyze the expression of somatic type *Spin* during embryonic development and in adult tissues, we performed northern hybridizations (Fig. 3A). The amount of Type B transcript gradually increased during post implantation stages, peaking at 13.5 dpc. Among tissues, higher expression was observed in the kidney and brain (Fig. 3B).

Structure of the Spin genomic locus

There are two possible explanations for the generation of oocyte- and somatic-type *Spin* transcripts. One possibility is the existence of separate *Spin* genes each producing different types of *Spin* transcript; another possibility is that both oocyte- and somatic-type transcripts are produced from a single locus by alternative splicing. To understand the organization of *Spin* genomic structure, we set on to do a series of analyses described below.

First, to examine the evolutionary conservation of *Spin* gene, a zoo blot containing the genomic DNA of various mammals was analyzed (Fig. 4). Judged from the intensity of hybridized bands, *Spin* appears to have homologs in most of tested mammals, though the sequence similarity outside rodent may vary from species to species.

Next, the mouse genomic DNA was analyzed by Southern hybridization to infer how many copies of *Spin* gene exist in the genome. Three different fragments of *Spin* cDNA, one encompassing the coding region and two from the 3-untranslated region, were used to probe mouse genomic DNA digested

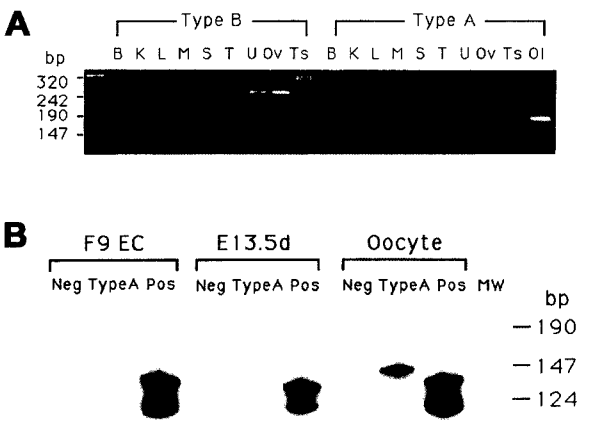

Fig. 2. Expression pattern of two *Spin* isoforms. RT-PCR of oocytes and tissues with type A- and B-specfic primers was performed (A) and RT-PCR of F9 embryonic carcinoma cell line, 13.5 dpc embryo and oocyte was carried out, showing specific expression of type A in the oocyte. B: brain, K: kidney, L: liver, M: muscle, S: spleen, T: thymus, U: uterus, Ov: ovary, Ts: testis, Ol: primary oocyte, Neg: RT reaction without template, Pos: RT reaction with type A cDNA.

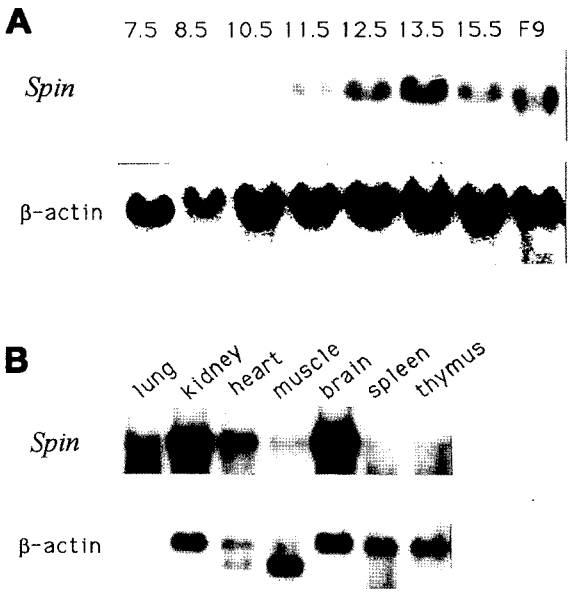

Fig. 3. Expression of type B transcript. Northern analysis of post implantation embryos (A) and adult tissues (B) was performed with *Spin* cDNA probe. Numbers in (A) represent days post coitum.

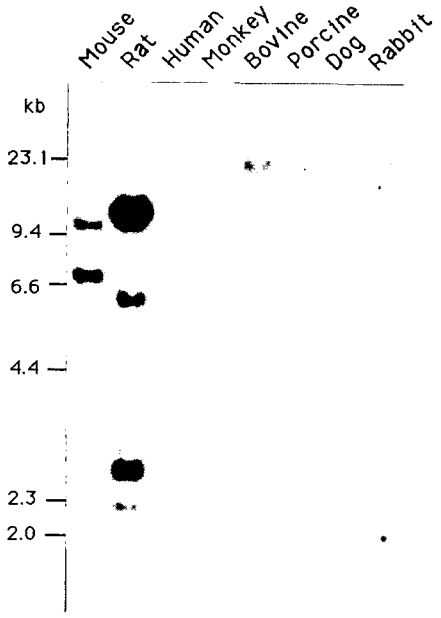

Fig. 4. Zoo blot analysis of mammals. The *Spin* coding region probe hybridized to *Hin*dIII-digested genomic DNAs from various mammals.

with *Bam*HI, *Eco*RI, or *Hin*dIII (Fig. 5). All three probes recognized at least three bands, suggesting that more than one copy of *Spin* gene exist.

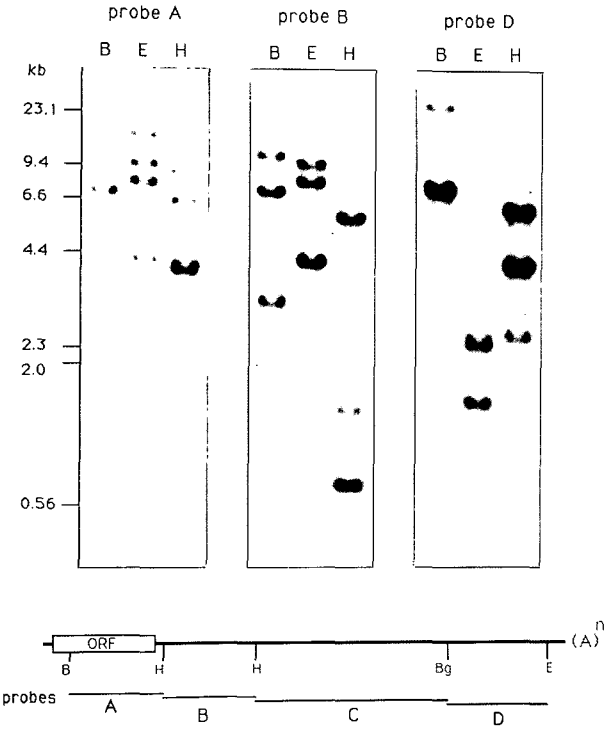

Fig. 5. Southern hybridization of mouse genomic DNA. *Spin* probes containing different regions of cDNA (shown on the bottom) hybridized to multiple bands of mouse genomic DNA digested with three different restriction enzymes. B: *Bam*HI, E: *Eco*RI,; H: *Hin*dIII. ORF: open reading frame.

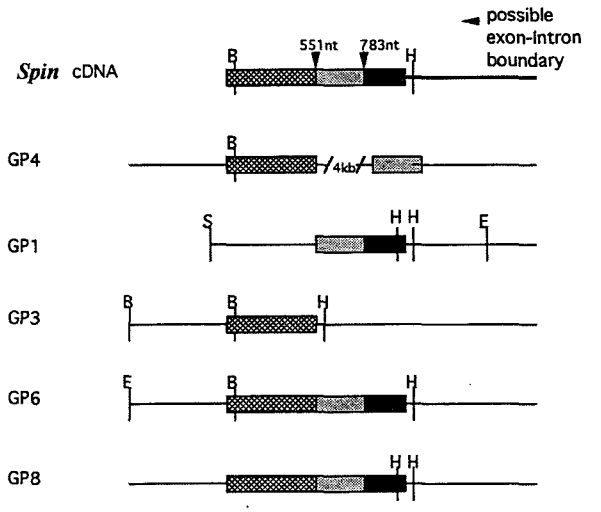

Fig. 6. Characterization of Spin genomic clones. Structures of 5 genomic clones that hybridized to the *Spin* coding region probe are shown. Boxed area represents the coding region, where each exon is designated by different colors. Arrowheads indicate the potential exon-intron boundary on the cDNA. Capital letters represent restriction sites. B: BamHI, H: HindIII, E: EcoRI.

To isolate genomic clones of *Spin*, bacteriophage λ genomic library was screened with the *Spin* coding region probe. Among the 5 genomic clones obtained (Fig. 6), GP3 and GP4 contained exon(s) with 100% nucleotide sequence homology to *Spin* cDNA. However, GP3 and GP4 clone differ in their restriction enzyme maps, indicating that these clones are derived from independent copies of *Spin* gene. Both GP3 and GP4 clones revealed exon-intron boundaries within the coding region, and the most upstream boundary falls right on to the site where the difference between oocyte- and somatic-type ends, thus subscribing to the possibility of alternative splicing. The other three genomic clones (GP1, GP6 and GP8) have regions with more than 90% sequence homology to that of the cDNA, however it is unlikely that these clones encode functional SPIN due to premature stop codons and no intron (Fig. 6). Possibly these clones represent pseudogenes of *Spin*.

Discussions

Spin encodes two types of isoform, one expressed solely in the oocyte and the other expressed in somatic cells. Previously, differential expression of oocyte- versus somatic-type isoforms has been reported from *Xenopus* genes encoding 5S ribosomal RNA (rRNA) and c-*myc*, however in these cases each type of transcripts are synthesized from separate copy of genes. The 5S rRNA is synthesized from about 400 copies of somatic-type genes and 20,000 copies of oocyte-type genes. Transcription from both types of genes in the oocyte contributes to the rapid accumulation of 5S rRNA during oocyte maturation, but after fertilization oocyte-type copies are transcriptionally

repressed (Wolffe and Brown, 1988). About *Xenopus* c-*myc*, the oocyte- and somatic-type transcripts share 91.3% nucleotide similarity in the coding region and 81% similarity in the untranslated region (Vriz et al., 1989). In these cases the sequence of oocyte and somatic type mRNA are quite different in the coding region as well as the untranslated region. In *Spin*, the sequence difference between two types is confined to the N-terminus. Unique regions consist of the first 12 amino acids in the oocyte type and 34 amino acids in the somatic type. Therefore it is most likely that the two *Spin* isoforms are produced by differential usage of the first exon. In many cases, choosing alternative first exons containing distinct 5′-UTRs has been reported to produce protein isoforms in timely- and spatially-controlled manner.

Characterization of *Spin* genomic clones reveals that the first exon-intron boundary in the coding region falls right into the site where the common sequence between the two types starts, indicating that oocyte- and somatic-isoforms were generated by alternative splicing. However, the possibility that both types are produced from two separate loci cannot be excluded, since *Spin* gene appears to exist as multiple copies. Equally unclear is the role of distinct peptides in the N-terminus of each isoform protein, since neither of them features known functional motifs. In any case, *Spin* displays a unusual example of multifaceted gene expression control, exercising alternative splicing as well as differential transcription and translation. Further characterization of detailed mechanisms in the production of two SPIN isoforms may provide useful information that will guide us to better understand the complex panoply of mammalian genome usage.

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