# Dynamics of Maternal Survivin mRNA in Mouse Oocytes and Pre-implantation Embryos

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**Abstract:** *Survivin is a bi-functional protein which has been shown to suppress apoptosis and regulate cell division in mammalian carcinoma cell lines and some somatic cells. In previous studies, we showed that survivin is an essential anti-apoptotic gene which is expressed in mouse pre-implantation embryos, and found that embryos in which survivin has been disrupted develop only to the blastocyst stage. Based on what is known about survivin in mammals and other organisms, we proposed that maternal survivin mRNA may be required for oocyte maturation and early embryonic development, including a role in regulation of chromosome segregation. To test this, we assessed changes in survivin mRNA levels over time in mouse oocytes and embryos, and asked if survivin mRNA levels change in a cell cycle-dependent manner in mouse oocytes and embryos. We found that maternal survivin mRNA levels were higher than zygotically transcribed survivin mRNA levels in mouse preimplantation embryos, that zygotic survivin was regulated in a cell-cycle dependent manner, and that survivin mRNA levels were high in young mouse oocytes but decreased as mice aged. Our data suggest that survivin may play a role as a maternal gene in the development of mouse oocytes and pre-implantation embryos.*

**Key words:** *Survivin, RT-PCR, Quantification, Single oocyte, Embryo*

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## Introduction

Survivin is a member of the inhibitor of apoptosis protein family [1]. Survivin inhibits apoptosis by directly binding to caspases [2] and has also been reported to be a cell cycle-regulated protein [3–6]. The expression of *survivin* mRNA is cell cycle-regulated at the transcriptional level, with 40-fold higher levels at the G2/M phase than at the G1 phase in HeLa cells [3]. Survivin is required for proper chromosome segregation in both budding and fission yeast [4–6]. Consistent with a role in chromosome segregation, the results of RNA interference assays in *Caenorhabditis elegans* have shown that in embryos lacking *survivin* display abnormal chromosome condensation, disrupted mitotic spindle formation and failure to complete cytokinesis, resulting in multinucleate embryos [7, 8]. Similarly, *survivin* null mouse embryos display polyploidy, abnormal mitotic spindles and failure of cytokinesis [9]. Taken together, the results of these studies suggest that *survivin* functions at the interface between programmed cell death and progression of the cell cycle.

Survivin is likely to have a similar function in embryos as has been found in cell lines and somatic cells. Indeed, survivin localizes to chromosomes and to the spindle midzone during meiosis and during embryonic mitosis [8, 10]. In previous studies, we showed that survivin has anti-apoptotic activity in pre-implantation embryos using an antisense approach [11]. Furthermore, *survivin* null mutant animals fail to complete embryogenesis [9].

In Xenopus, *survivin* mRNA accumulates during oogenesis, persists at constant levels up to the mid-

blastula stage of embryogenesis, and levels decrease by the time the embryos reach gastrulation [12]. In mouse embryos, zygotic activation of the genome occurs between the late 1- and early 2-cell stage [13, 14] and is followed by a precipitous decline in maternal mRNA levels [15–17]. Despite this, approximately 30% of maternal mRNA is still present at the blastocyst stage [15, 18, 19]. Until zygotic gene activation, maternal proteins and transcripts stored in oocytes control embryonic development, indicating the importance of maternal factors for development [20].

Based on the available evidence, we proposed that *survivin* is required maternally for oocyte maturation and for the early stages of embryonic development, with a specific role in the meiotic and mitotic cell cycles, and therefore, the disruption of *survivin* mRNA in oocytes or a lack of maternal *survivin* mRNA in early (i.e. pre-implantation) embryos would be lethal and result in cell cycle-related defects. To elucidate the role of *survivin* in oocytes and early embryos, we monitored changes in *survivin* mRNA expression levels over time in single mouse oocytes and embryos, and found evidence that expression of *survivin* mRNA levels increases at the G2/M phase in embryos and is reduced in aged oocytes.

## Materials and Methods

*Animals*

IVCS mice (Institute for Animal Reproduction, Ibaraki, Japan) were used for all experiments. Young mice were 4 to 6 weeks old. Old mice were 40 weeks old. All procedures involving the care and use of animals were approved by the Animal Research Committee of Akita University School of Medicine.

*Collection of mouse oocytes and pre-implantation embryos*

*1) Collection of Oocytes*

Germinal vesicle (GV)-stage oocytes were obtained from the ovaries of mice injected with a single intraperitoneal injection of 10 IU of pregnant mare serum gonadotropin (PMSG; Sigma, St. Louis, MO, USA). Cumulus-free and GV-intact follicular oocytes were released from the large antral follicles by puncturing with a needle containing M2 medium (Sigma) supplemented with 60 $\mu$g/ml penicillin and 50 $\mu$g/ml streptomycin.

Cumulus cell-enclosed metaphase II-arrested eggs were obtained from oviducts of 4- to 6-week-old mice that were superovulated with a single intraperitoneal injection of 10 IU of pregnant mare serum gonadotropin followed 48 h later by injection of 10 IU of human chorionic gonadotropin (hCG; Sigma). Mice were sacrificed by cervical dislocation 15 h post-hCG injection. The cumulus cell masses surrounding the eggs were removed by brief exposure to 300 $\mu$g/ml hyaluronidase Type IV-S (Sigma) in M2 medium.

*2) Collection of Embryos*

Mice were superovulated with a single intraperitoneal injection of 5 IU of PMSG, followed 48 h later by an injection of 5 IU of hCG. The superovulated mice were placed with male mice for 2 h, 13 h post-hCG injection [21]. The oviducts or uterus of the mated mice were flushed at 30, 34, 48, 57, 67, 80 and 91 h post-hCG injection to obtain 1-cell, early 2-cell, late 2-cell, 4- and 8-cell, morula and blastocyst stage embryos, respectively. Fertilized 1-cell embryos were identified by the presence of second polar bodies or two pronuclei.

*Embryo culture with nocodazole and alpha-amanitin*

*1) Preparation of G2/M embryos*

Nocodazole (Sigma) was dissolved in dimethylsulfoxide at 3 mg/ml (10 mM) and stored at –20°C. Late 2-cell embryos and early compacting morulae were obtained by flushing the oviducts or uteri of the mated mice 46 and 75 h post-hCG injection, respectively. They were washed three times with M2 medium (Sigma). Subsequently, groups of 15 embryos were cultured separately in 30-$\mu$l drops of synthetic human tubal fluid medium [22] containing 0.05 $\mu$M or 0.5 $\mu$M nocodazole, and covered with mineral oil at 37°C in a humidified atmosphere containing 5% $CO_2$ to block them at the G2/M phase. For RT-PCR analysis, 4-cell, 8-cell, morula, blastocyst, and hatched blastocyst stage embryos were collected from the cultured embryos.

*2) $\alpha$-Amanitin treatment*

RNA synthesis from the zygotic genome was inhibited by 11 $\mu$g/ml $\alpha$-amanitin (WAKO, Osaka, Japan). $\alpha$-Amanitin was dissolved at 1 mg/ml in PBS and stored at –20°C. Early compacting morulae were transferred 75 h post-hCG injection into media containing 0.5 $\mu$M nocodazole and 11 $\mu$g/ml $\alpha$-amanitin [23] and were incubated for 12 h.

*One-step real-time RT-PCR*

*1) RNA extraction*

Single oocytes or embryos were placed in sample tubes containing 350 $\mu$l denaturing solution (prepared according to the instructions for the RNeasy Micro Kit

**Table 1.** PCR primer and TaqMan probe sequences

| Gene | GenBank accession number | Species | | Sequence (5'→3') | Location |
|---|---|---|---|---|---|
| 18S rRNA | X00686 | Mouse | sense | AGTCCCTGCCCTTTGTACACA | 1679–1699 |
| | | | antisense | GATCCGAGGGCCTCACTAAAC | 1727–1747 |
| | | | probe | CGCCCGTCGCTACTACCGATTGG | 1701–1723 |
| survivin | AB013819 | Mouse | sense | ATTGCAAAGGAGACCAACAAC | 445–465 |
| | | | antisense | TGTCACTCAGGTCCAAGTTATCTC | 547–570 |
| | | | probe | TGCAAAGACTACCCGTCAGTCAATTG | 489–514 |

(QIAGEN, Valencia, CA, USA)) supplemented with guanidinium isothiocyanate and 3.5 $\mu$l of 14.3 M $\beta$-mercaptoethanol and were frozen immediately at –80°C. Total RNA was isolated using the RNeasy Micro kit following the manufacturer's instructions. Following thawing, total RNA was applied to a column, treated with DNase I for 15 min at room temperature, washed with RPE buffer and 80% ethanol, and resolved with 14 $\mu$l RNase-free water.

*2) Primers and probes*

Primers and TaqMan probes for quantifying 18S rRNA and survivin mRNA were designed using Primer Express V1.0 software (Applied Biosystems, Foster City, CA, USA). The sequences of primers and TaqMan probes are shown in Table 1. Survivin primers were designed to selectively amplify a 140-bp mouse survivin cDNA spanning the region of the alleles [23].

*3) One-step real-time quantitative RT-PCR*

One-step real-time quantitative RT-PCR was performed on a LightCycler System (Roche Diagnostics, Tokyo, Japan) in combination with the QuantiTect Probe RT-PCR Kit (QIAGEN) using a one-step method based on TaqMan as described by Konishi *et al.*. Briefly, each reaction contained 10 $\mu$l 2X QuantiTect Probe RT-PCR Master Mix, 4 mM $MgCl_2$, 0.2 $\mu$l Quantitect Probe RT Mix, 0.5 $\mu$l of 40 $\mu$M of each primer, 0.5 $\mu$l of 8 $\mu$M TaqMan probe, and 2 $\mu$l (for 18S) or 8 $\mu$l (for survivin) template RNA, adjusted to 20 $\mu$l with DNase/RNase-free water. The reaction mix was loaded into a glass capillary tube. Conditions of one-step real-time quantitative RT-PCR were as follows: 20 min at 50°C (reverse transcription), 15 min at 95°C (RT inactivation and initial activation), and then 50 cycles of amplification for 0 s at 95°C (denaturation) and 1 min at 60°C (annealing and extension). All heating and cooling steps were performed with a slope of 20°C/s. A single fluorescence reading at 530 nm was taken for each sample at the extension step.

*4) Plasmid constructions for the standard curve*

Control plasmids were generated as standards to quantify 18S rRNA and survivin mRNA as follows. RT-PCR was carried out using placental RNA from IVCS mice as the template and with primers specific for 18S rRNA and survivin mRNA (shown in Table 1). The PCR products were separated by 2% agarose gel electrophoresis (Agarose-LE, Nacalai Tesque, Inc., Kyoto, Japan) in the presence of ethidium bromide (Sigma) and visualized with a UV transilluminator (Funakoshi, Tokyo, Japan). To confirm the identity of the products, DNA bands were eluted from the agarose gel using the QIAquick gel extraction kit, ligated into the pDrive Cloning vector, and cloned in accordance with standard protocols (QIAGEN). Plasmid DNA was recovered using a Quantum Prep Plasmid Miniprep kit (Bio-Rad, Hercules, CA, USA), cycle sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and analyzed in an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) using T7 or SP6 site-specific primers. Purified plasmids were diluted to 10 ng/$\mu$l and stored at –20°C. The standard was serially diluted to generate a single standard curve for quantification of 18S rRNA and survivin mRNA. Standard curves were included in each run to verify the linearity and reliability of this procedure. Data analysis was performed using LightCycler Software version 3.5 (Roche Diagnostics). Single samples were assayed for both standards and unknowns. No signal was detected by the negative control assay in which RNase-free water was substituted for mRNA.

*Statistical analysis*

Results are presented as means ± SE. The association between the numbers of oocytes and embryos and the expression of the 18S rRNA and survivin mRNA was assessed with Pearson's correlation coefficient. *p* values were calculated using Fisher's *r* to *z* test. Statistical differences between non-

treated embryos and treated embryos were determined using the Mann-Whitney *U* test. $p<0.05$ was considered significant.

## Results

*Quantification of 18S rRNA in single oocytes and pre-implantation embryos*

To verify our single-oocyte and single-embryo approach, we monitored quantitative changes in 18S rRNA expression levels and found that they change dramatically in the early stages of embryogenesis (Fig. 1). A reduction of 18S rRNA levels by approximately two-thirds was observed during oocyte maturation. There was an additional decrease by almost 50% at the late 2-cell embryo stage. This decrease can be interpreted as degradation of maternal 18S rRNA stores, as rRNA synthesis has been shown to initiate at the 2-cell stage in mouse embryos and is generally accompanied by degradation of maternal supplies [16]. By the 8- to 16-cell stages, zygotic rRNA synthesis occurs at a rate of 1.25–2.5 pg/cell/h [16]. Thus, the rate of rRNA synthesis may be similar to the rate of its decay at these stages and as a result, appears as a steady-state level of rRNA content [26]. At the start of the 8-cell stage, 18S rRNA levels began to increase and by the blastocyst stage, the level was five-fold higher than those observed at the 2- to 4-cell stages.

*Quantification of survivin mRNA in single oocytes and pre-implantation embryos*

Oocyte RNA synthesis is essentially absent after germinal vesicle breakdown [18] and there is a net loss of about 30% of polyadenylated mRNA between the fully grown and the ovulated oocyte stage [18, 27–29]. We were interested to learn how the levels of *survivin* mRNA compare to overall mRNA levels and thus, measured quantitative changes in *survivin* levels in early mouse embryos (Fig. 1). We found that there are approximately 8,000 copies of *survivin* mRNA per GV-stage oocyte, which is extremely high in comparison to mRNA levels for housekeeping genes such as actin, which has been estimated at 36,000 to 40,000 copies per mouse oocyte [30, 31], and for hypoxanthine guanine phosphoribosyltransferase, which has been estimated at 6,000 copies per mouse oocyte [32]. Overall, we found that maternal stores of *survivin* mRNA were slightly reduced in MII oocytes and levels abruptly decreased by the early 2-cell stage, consistent with previously reported results [18, 27–29].

At the late 2-cell stage, *survivin* mRNA levels declined precipitously to 10% of levels observed in early 2-cell stage embryos. At the start of the morula stage, *survivin* mRNA levels began to increase and by the blastocyst stage, *survivin* mRNA levels were three-fold higher than those observed at the 2- to 8-cell stages.

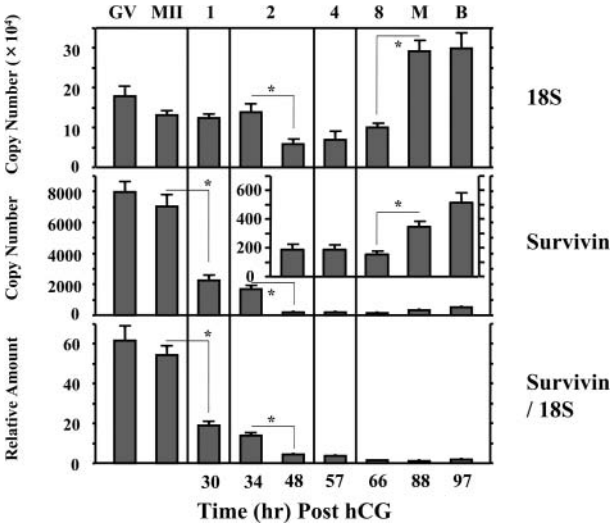


**Fig. 1.** Expression of 18S rRNA and *survivin* mRNA in mouse oocytes and preimplantation embryos.
The levels of 18S rRNA and *survivin* mRNA in single GV-stage oocytes, MII stage oocytes, and pre-implantation embryos were quantified by RT-PCR using specific primers. The estimated number of copies per oocyte or embryo is shown. The inset in the center panel shows a magnification of the graph for *survivin* mRNA from the late 2-cell through to the blastocyst stage. Bars indicate the mean value obtained for each stage (n = 14–30) and error bars represent SEM. Morphological stages are indicated above the graphs (GV-stage oocyte, MII oocyte, 1-cell through 8-cell stages, morula, and blastocyst).Significant differences are indicated by $p<0.05$.

*Expression of survivin mRNA during the embryonic cell cycle*

We next determined if *survivin* mRNA expression was regulated in a cell cycle-dependant manner in mouse embryos as has been observed in somatic cells. To arrest the cells, we chose nocodazole instead of colcemid because it is considered less toxic to the cells [33–35]. Yu *et al.* [36] reported that the optimum concentration of nocodazole for cell cycle arrest in metaphase of mouse late 2-cell stage embryos is 0.05 $\mu$M. Otaegui et al. [34] reported that the minimum effective concentration of nocodazole (>95% of arrested nuclei) for mouse 16-cell embryos was 5 $\mu$M but that

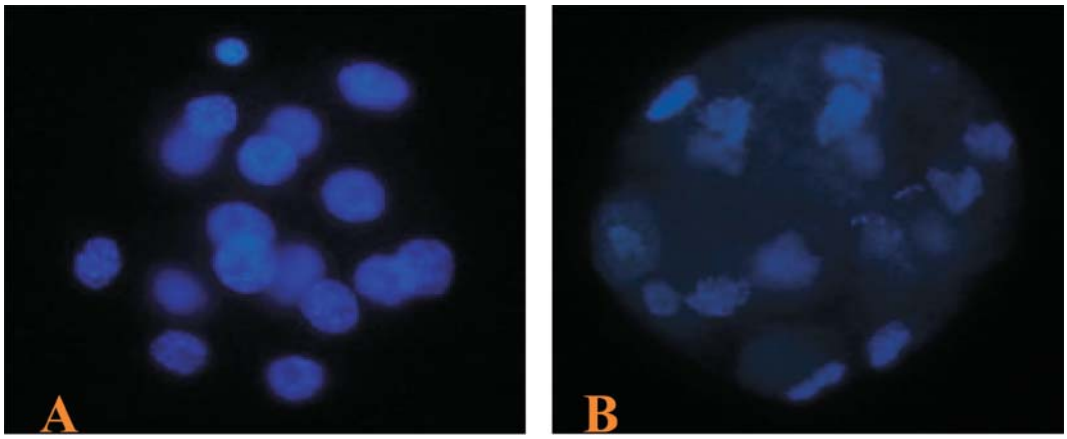


**Fig. 2.** Morphology of nuclei in nocodazole-treated embryos.
Representative epifluorescence images of optical sections of (A) an early compacting morula and (B) an embryo arrested by nocodazole treatment for 12 h are shown. Each embryo was stained with 1.0 $\mu$g/ml Hoechst 33342. Magnification, × 400.

their rate of development to the blastocyst stage was less than half of that for 2-, 4- or 8-cell stage embryos. We sought a concentration of nocodazole that would have little effect on embryonic viability but would still be effective at blocking cell cycle progression. We found that treatment of morulae for 12 h with 0.5 $\mu$M nocodazole resulted in a moderate block of the cell cycle (75% of cells arrested, data not shown). Thus, a 12-h exposure appeared to be the minimum period required to block the cell cycle of 16-cell embryos, as G1, S and G2/M phases are estimated to be at least 2, 8–9 and 2 h, respectively, at these stages [37]. Blockage of the cell cycle in morulae was determined by fluorescence microscopy after staining of mitotic nuclei with the DNA dye, Hoechst 33342 (Fig. 2). Compared with untreated embryos at the 2-cell (Fig. 3) or morula stage (Fig. 4), nocodazole-treated embryos had a slight but not significant increase in 18S rRNA levels, suggesting that nocodazole did not alter RNA stability in the embryos.

At the 2-cell stage, *survivin* mRNA levels were significantly lower in nocodazole-treated embryos as compared with untreated embryos (Fig. 3). In contrast, *survivin* mRNA levels were two-fold higher in treated morula stage embryos than in the untreated control ($p$<0.0001, Fig. 4). To confirm that this increase in *survivin* mRNA levels was due to zygotic transcription, we examined the effect of $\alpha$-amanitin, an inhibitor of mRNA synthesis [23]. Treatment with $\alpha$-amanitin caused a slight but not significant reduction in the ability of nocodazole to upregulate embryonic *survivin* mRNA levels in the morula (Fig. 4). These results indicate that *survivin* mRNA expression is regulated by the cell cycle and that zygotic transcript levels are much lower than the levels of residual maternal transcript. Therefore, at early developmental stages, maternal transcripts contribute much more than zygotic transcripts to overall *survivin* mRNA levels.

*Quantification of survivin mRNA in GV and MII phase oocytes from young and old mice*

We found the *survivin* mRNA level was extremely high at the GV and MII stages compared to the other stages. Therefore we hypothesized that the decrease in the maternal *survivin* mRNA level found in oocytes obtained from aged mice leads to failure of complete embryogenesis. To assess this, we quantified survivin mRNA levels in oocytes obtained from young and old mice. At both the GV and MII stages, *survivin* mRNA levels were significantly lower in oocytes from old mice than in those from young mice (Table 2). In order to exclude the possibility of degeneration of RNA due to aging, *survivin* mRNA levels were normalized to 18S rRNA levels.

## Discussion

Previously, we showed that *survivin* mRNA and protein are present in unfertilized oocytes and in early embryos through the blastocyst stage [11]. Moreover, the results of RT-PCR-based analysis showed no significant differences in *survivin* mRNA levels in

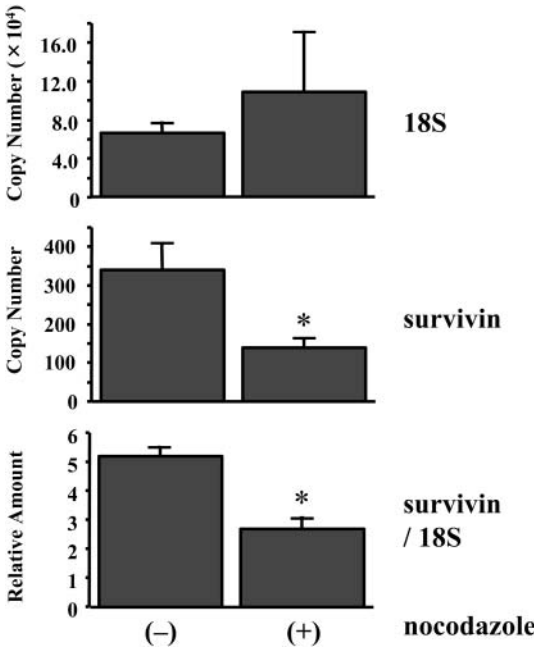


**Fig. 3.** Effect of nocodazole on *survivin* mRNA levels in late 2-cell stage embryos.
Late 2-cell embryos were obtained 46 h post-hCG injection. Left, embryos cultured in 0.05 $\mu$M nocodazole for 12 h. After the treatment, all embryos we observed were arrested. Bars, mean values; error bars, SEM. *, $p<0.05$. An untreated control group was also included in the assay.

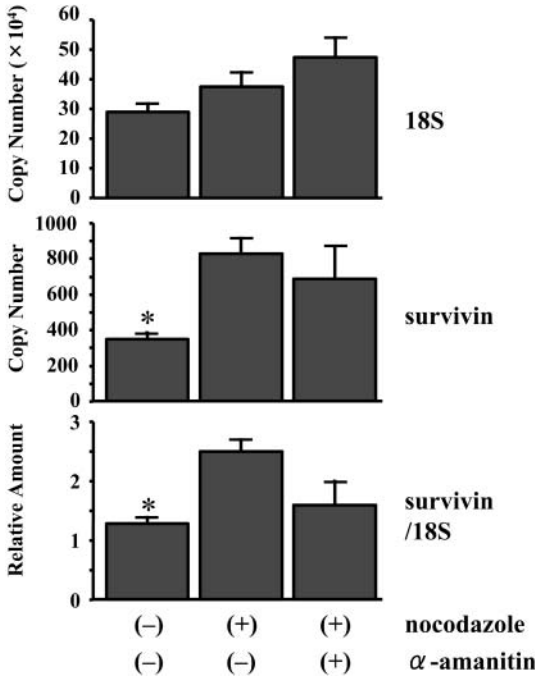


**Fig. 4.** Effect of nocodazole on *survivin* mRNA level in morula stage embryos. Early compacting morulae were obtained 75 h post-hCG injection. Left, embryos cultured in 0.5 $\mu$M nocodazole for 12 h, or in 0.5 $\mu$M nocodazole and 11 $\mu$g/ml α-amanitin for 12 h. After the treatment, 75% of the embryos we observed embryos were arrested. Only arrested embryos were examined. Bars, mean values; error bars, SEM. *, $p<0.05$. An untreated control group was also included in the assay.

**Table 2.** *Survivin* mRNA levels in GV and MII phase oocytes from young and old mice

| Stage | relative amount (survivin/18S) | | |
|---|---|---|---|
| | young | old | |
| GV | 9.8 + 3.8 | 5.6 + 2.5 | $p<0.05$ |
| MII | 9.1 + 2.9 | 5.2 + 4.1 | $p<0.05$ |

GV = germinal vesicle; MII = metaphase II.

oocytes as compared with early embryos. In the present study using real-time RT-PCR, we contrastingly detected significant differences in *survivin* mRNA levels in single oocytes and early embryos. We attribute the difference in the results to the use of different primers in the reverse transcription reactions (i.e., previously, we used an oligo dT primer and here we used a gene-specific antisense primer). In mice, an additional round of polyadenylation of maternal mRNA occurs after fertilization [28, 29]. Most inherited maternal mRNAs remain in a deadenylated state through to the end of the 2-cell stage [30]. Consistent with this, our observations suggest that most maternal *survivin* mRNA remains in a deadenylated state in oocytes and is polyadenylated some time after fertilization.

Growth of primary mouse oocytes occurs over a period of approximately two weeks and results in a 100-fold increase in volume and a 20-fold increase in total RNA content [18]. More specifically, total RNA, including poly (A)-containing mRNA, accumulates in a quasi-linear fashion until oocytes become 65 $\mu$m in diameter, after which there is little increase in RNA levels [38]. Similar to our mouse oocyte findings, *survivin* mRNA levels also increase during *Xenopus* oogenesis [12]. Taken together, this suggests that the extremely high level of *survivin* mRNA detected in mouse GV stage oocytes in the present work results from accumulation of *survivin* mRNA during oogenesis.

Although here we did not address the survivin protein

levels, one of our previous studies revealed that survivin is present in the cytoplasm of the mouse oocyte [11]. *Survivin* mRNA contains a nuclear polyadenylation element (NPE) in the 3' untranslated region but lacks a cytoplasmic polyadenylation element (CPE) [24]. NPE-containing transcripts lacking CPE are immediately translated during oocyte growth as they are exported to the oocyte cytoplasm [39]. Therefore, survivin protein in mouse oocytes seems to be translated from accumulated *survivin* mRNA during oogenesis.

The timing of expression of *survivin* mRNA during development suggests that as for most mouse genes, zygotic transcription of *survivin* occurs in early to late 2-cell stage embryos. Despite this, the level of *survivin* transcript is very low at these stages and we observed a decrease in the level of *survivin* mRNA level per blastomere later in embryogenesis. Conversely, we observed an increase in *survivin* mRNA levels during the transition from the 8-cell stage to the blastocyst stage. This trend is apparent as a gradual decrease in relative *survivin* mRNA levels (as found by determining the ratio of *survivin* RNA levels to those of 18S rRNA) during the course of development (Fig. 1). Murphy *et al.* [12] reported the same pattern during *Xenopus* embryogenesis. In contrast to all other zygotic transcripts reported, which are expressed at much higher levels in blastocysts than in mature oocytes, we found that *survivin* mRNA levels at the blastocyst stage are only 55% of the levels present in mature oocytes. Thus, even after zygotic transcription, the level of maternal *survivin* mRNA is still much higher than zygotic transcript levels, despite the gradual decrease in maternal levels over time. This finding agrees with the results of a previous study, in which 30% of labeled maternal RNA was shown to remain at the blastocyst stage (day 3.5) [15, 19].

Expression of *survivin* mRNA is regulated in a cell cycle-dependent manner in most mammalian carcinoma cell lines as well as in some somatic cells. However, the regulation process of *survivin* mRNA expression in oocytes and embryos remains unknown. In this work, we assessed the expression of *survivin* mRNA at the G2/M phase in embryos after imposing a cell cycle block with nocodazole. The *survivin*-like *C. elegans* protein BIR-1 is localized to chromosomes and to the spindle midzone during mitosis and meiosis in *C. elegans* oocytes and one-cell embryos [8]. Based on sub-cellular localization of BIR-1, which is presumably related to function, it is suspected that mouse survivin protein plays a role in chromosome segregation. Consistent with this, we previously found that antisense targeted embryos lack proper mitotic spindle structures [11]. Thus, a decrease of normal maternal *survivin* mRNA levels in oocytes may lead to errors in chromosome segregation in early embryos.

In nocodazole-treated morulae, most of which are believed to be arrested at G2/M, *survivin* mRNA expression was approximately 2-fold higher than that observed for untreated morulae, the majority of which are thought to be in the S phase [37]. The effect of nocodazole in our study was much less dramatic than was reported in several previous studies using cell lines [2, 3, 24, 40, 41] or somatic cells [42]. This lack of effect may be due to masking of increases in zygotic *survivin* mRNA levels by the presence of maternal *survivin* mRNA. Nocodazole treatment significantly decreased *survivin* mRNA levels in 2-cell embryos, indicating that degradation of maternal mRNA occurred in the presence of nocodazole. Therefore, the results include the possibility that maternal *survivin* mRNA may be involved in the cleavage of cells in pre-implantation stage embryos even after zygotic activation.

Although the role of the remaining maternal mRNA plays in embryos between the pre-implantation and blastocyst stages remains unclear, it is known that this maternal mRNA does affect blastocyst formation via interaction with zygotic genetic information [43]. We found that (1) maternal *survivin* mRNA levels exceed zygotic levels in mouse oocytes and preimplantation embryos; (2) *survivin* mRNA is regulated in a cell cycle-dependent manner in mouse embryos, as has previously been shown for some somatic cells and cell lines; and (3) *survivin* mRNA levels are significantly lower in oocytes from older mice as compared with those from younger mice. In conclusion, we suggest that maternal *survivin* regulates meiosis in oocytes and mitosis in pre-implantation embryos up to the point that zygotic transcription begins, and that a decrease in maternal *survivin* levels can lead to abnormal chromosomal segregation, presumably as cells lack proper mitotic spindles and the tubulin network is disrupted. Our results are consistent with the observation that although *survivin* antisense and *survivin* null mouse embryos do not survive to term, they can develop as far as the blastocyst stage, presumably due to the presence of maternal supplies of *survivin* mRNA. Moreover, our results suggest that oocyte competence may be assessed via the quantification of specific transcripts in their mRNA pools. Ultimately, we hope that the quantification of *survivin* mRNA can be applied to the selection of oocytes for clinical *in vitro* fertilization.

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