—Full Paper—

Identification of ZAG1, a Novel Protein Expressed in Mouse Preimplantation, and Its Putative Roles in Zygotic Genome Activation

Toshiki MATSUOKA¹⁾, Manabu SATO¹⁾, Mikiko TOKORO¹⁾, Seung-Wook SHIN¹⁾, Atsuto UENOYAMA¹⁾, Kazunari ITO¹⁾, Syuji HITOMI¹⁾, Tomoko AMANO¹⁾, Masayuki ANZAI²⁾, Hiromi KATO²⁾, Tasuku MITANI²⁾, Kazuhiro SAEKI^{1,2)}, Yoshihiko HOSOI^{1,2)}, Akira IRITANI^{1,2)} and Kazuya MATSUMOTO^{1,2)}

¹⁾Division of Biological Science, Graduate School of Biology-Oriented Science and Technology, Kinki University, Wakayama 649-6493, ²⁾Institute of Advanced technology, Kinki University, Wakayama 642-0017, Japan

Abstract. We isolated a mouse cDNA, *zag1* (zygotic gene activation-associated gene 1), that has an open reading frame of 1,728-bp encoding a protein of 66.2 kDa including both a bipartite nuclear targeting sequence and a P-loop motif containing nucleoside triphosphate hydrolase motifs. Northern blot analysis of mouse tissues showed that *zag1* was widely expressed but was especially prominent in the ovary and testis. RT-PCR analysis of *in vitro* fertilized embryos showed that the abundance of *zag1* transcripts in oocytes decreased after fertilization, and *zag1* mRNA was detected at 15 h post insemination (hpi) in fertilized embryos indicating that the gene was expressed at the start of zygotic gene activation at the mouse 1-cell stage. The nuclear-localization of ZAG1 protein in mouse preimplantation embryos at 15 hpi was confirmed by both subcellular analysis of enhanced green fluorescent protein (EGFP)-tagged ZAG1 and immunocytochemical analysis with anti-ZAG1 antibody. Subsequently, using yeast two-hybrid screening, we identified U2 small nuclear ribonucleoprotein B (U2B''), which is associated with pre-mRNA splicing, as a putative interacting partner of ZAG1 protein. Furthermore, knockdown of *zag1* expression by an antisense DNA plasmid induced arrest and/or delay of embryonic development in injected 1-cell embryos. These results suggest that ZAG1 may be closely associated with zygotic gene expression in mouse preimplantation embryos.

Key words: Embryonic gene activation (EGA), Gene expression, Maternal, Mouse, Preimplantation embryo, Zygotic gene activation (ZGA)

(J. Reprod. Dev. 54: 192–197, 2008)

The development of preimplantation embryos is dependent on stored maternal factors in oocytes [1–3]. During meiosis in both male and female germ cells, transcriptional genome activation does not occur. In the mouse, minor zygotic gene activation (ZGA) first occurs during the latter stage of mouse 1-cell embryo development followed by major ZGA during the 2-cell stage [4]. Thus, ZGA depends on transcripts and proteins derived from maternal-effect genes. Several maternal-effect genes have been identified, including *Mater* [5], *Dnmt1o* [6], *Npm2* [7], *Zar1* [8], *Oog1* [9, 10], *Zfp3612* [11], *Stella* [12, 13], *Hsf1* [14] and *Formin2* [15]. Moreover, transcriptome analyses have revealed that maternal-effect genes are involved in oocyte maturation, maintenance of meiotic arrest at the MII stage, fertilization and/or early embryonic development [16–19].

The precise regulation of ZGA is considered to be essential for normal development of the preimplantation embryo because appropriate ZGA results in establishment of the totipotency of the fertilized egg and each blastomere of the embryo at the subsequent cleavage stage. However, the molecular mechanisms by which the nuclear reprogramming event at ZGA is regulated remain unclear. Recently, *Brg1*, a new member of the maternal-effect gene class,

was shown to regulate ZGA in the mouse [20].

To understand the molecular basis for regulation of ZGA, we focused on identification and functional characterization of genes activated in the late 1-cell stage of the mouse embryo. In a previous study [21], we obtained cDNAs expressed specifically at minor ZGA by mRNA differential display between oocytes at the MII stage and embryos at the late 1-cell stage. In the present study, we characterized one of these cDNAs, *zag1* (zygotic gene activation-associated gene 1). The primary structure of the ZAG1 protein, deduced from the full-length cDNA (1728-bp), is composed of 575 amino acids (66.2 kDa) that include both a bipartite nuclear targeting sequence and P-loop motif containing nucleoside triphosphate hydrolase motifs. Here in, we demonstrate that *zag1* may be closely associated with ZGA in early preimplantation development of mouse embryos.

Materials and Methods

Animals

All mice (ICR strain) were purchased from Kiwa Experimental Animals (Wakayama, Japan) and maintained in light-controlled, air-conditioned rooms. All animal procedures conformed to the Guidelines of Kinki University for the Care and Use of Laboratory Animals.

Fig. 1. Mouse ZAG1 protein. The deduced amino acid sequence is numbered on the left and right. Abbreviations: Bipartite-NTS; Bipartite nuclear targeting sequence. The sequence data for the ZAG1 protein is available from GenBank™ under the accession number NP_958757.

A cDNA obtained by mRNA differential display in our previous study [21] was designated *zag1*. This gene was recently recorded as a mouse cDNA sequence (BC037393) and as a mouse homolog of human phosphonoformate immuno-associated protein 5 (NP_958757), neither of which have identified functions. The amino acid sequence of mouse ZAG1 was deduced (Fig. 1). The open reading frame of mouse *zag1* extends 1,728-bp and encodes a protein of 575 amino acids residues with a predicted molecular mass of 66.2 kDa. From database analyses (SMART and Bioinformatic Harvester III beta), the ZAG1 protein was suggested to be a soluble protein with a bipartite nuclear targeting sequence [amino acid (aa) 128–139] and a P-loop motif (aa 391–430) as a predicted functional domain. We isolated and cloned the full-length *zag1* cDNA by RT-PCR using mRNA extracted and purified from mouse ovaries.

Total RNA was extracted from nine different mouse tissues by the Trizol/phenol/ chloroform extraction method. Poly(A)+mRNA was prepared using Oligotex-dT30[super] (Takara, Shiga, Japan) according to the manufacturer's instructions. Two micrograms of mRNA was separated by electrophoresis on a 1% agarose gel containing formaldehyde and was transferred onto Hybond-N+ nylon membranes (GE Healthcare Bio-Sciences, Piscataway, NJ, USA). The blot was hybridized with a random-primed ^{32}P -radiolabelled *zag1* cDNA probe (full length) using Express-Hybrid TM buffer (GE Healthcare Bio-Sciences) according to the manufacturer's instructions and was detected using a Bio-Image Analyzer (BAS-2500; Fuji Film, Tokyo, Japan). Three independent experiments were performed to ensure reproducibility.

The procedures for *in vitro* fertilization were essentially those reported previously [22, 23]. In brief, spermatozoa were collected from the cauda epididymis of a male mouse. The sperm suspension was incubated for 1.5 h to allow for capacitation at 37 °C under 5% CO₂ in air. Oocytes were collected from the excised oviducts of female mice (2–3 months old) that had been superovulated with PMSG followed 48 h later by treatment with human chorionic gonadotropin (hCG; Puberogen; Sankyo, Tokyo, Japan). Cumulus masses were recovered in pre-equilibrated HTF medium. The sperm suspension was added to the oocyte-containing medium. Morphologically normal fertilized oocytes were collected from the sperm suspension 2 h post-insemination. The eggs were cultured in KSOM medium [24] at 37 °C under 5% CO₂ in air.

For immunization, a peptide (ETDTFFSSIDSKRR, residues 141–153), which was designed from the deduced amino acid sequence of the ZAG1 considering relative hydrophilicity, was synthesized and purified. Anti-ZAG1 antibody was obtained by injection of this peptide fused to keyhole limpet hemocyanin (KLH) followed by six booster injections at 1-week intervals into New Zealand White rabbits (Kitayama Labes, Kyoto, Japan). The titer of the antiserum was measured by ELISE. Finally, anti-ZAG1 antibody was fractionated with 40% ammonium sulfate and used in this study.

The procedures for RT-PCR and Western blot analysis were essentially the same as those reported previously [25]. In brief, in RT-PCR analysis, mRNA were isolated from ten oocytes or embryos. Then, the mRNA were supplemented with Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA) to synthesize first-strand cDNA. A semi-quantitative PCR was carried out using the following primer set for *zag1*: 5'-GTGGCCACCAT-TAACTTCAG-3' and 5'-GGGCCTATGAATGCTTTACT-3'. The reaction parameters were one cycle of 94 C for 5 min and 40 cycles of 94 C for 1 min, 55 C for 1 min and 72 C for 1 min. For the positive control, *g3pdh*, was amplified using sense (5'-ACCA-CAGTCCATGCCATCAC-3') and antisense (5'-TCCACC-ACCCTGTTGCTGTA-3') primers. Three independent experiments were performed for each. The bands were quantified by densitometry using a Molecular Imager FX (BioRad Laboratories, Hercules, CA, USA). In Western blot analysis, mouse preimplantation embryos were harvested from culture by rinsing with 0.1% PVA-Phosphate-buffered saline (PBS) dissolved in SDS buffer. The lysates were resolved on 10 or 15% SDS-PAGE gels and electrophoretically transferred to PVDF membranes (GE Healthcare Bio-Sciences). The membranes were blocked with Block Ace (Dainippon-Pharm, Osaka, Japan) and then incubated with anti-ZAG1 polyclonal or anti-actin polyclonal (sc-1616; Santa Cruz Biotechnology, Santa Cruz, CA, USA) antibodies in PBS containing 0.2% Tween 20, followed by incubation with donkey anti-rabbit IgG-HRP conjugate (GE Healthcare Bio-Sciences) for anti-ZAG1 polyclonal antibody or donkey anti-goat IgG-HRP conjugate (GE Healthcare Bio-Sciences). The signal was detected by

enhanced chemiluminescence (ECL; GE Healthcare Bio-Sciences). Three independent experiments were performed for each antibody.

His-tagged ZAG1 was constructed by subcloning the full-length sequence of *zag1* into pQE32. The His-tagged fusion protein was purified on a Ni²⁺-NTA resin column (Qiagen, Valencia, CA, USA), dialyzed in buffer (20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM EDTA, 10% glycerol and 5 mM MgCl₂) and used directly for experiments. The His-tagged *zag1* expression vector was used to transform *Escherichia coli* M15. The fusion protein was purified using a glutathione-Sepharose column (GE Healthcare Bio-Sciences) from the extract of the IPTG-induced transformant. Three independent experiments were performed.

Immunofluorescence staining

Embryos were fixed in 4% PFA in PBS (Invitrogen) for 10 min at room temperature, and then the fixed samples were incubated in PBS containing 0.1–0.2% Triton X-100 (Nacalai Tesque, Kyoto, Japan) overnight at 4 °C. The samples were incubated with anti-ZAG1 polyclonal antibody (1:200) in PBS containing 30 mg/ml BSA (3% BSA-PBS) overnight at 4 °C. After incubation, the samples were reacted with Alexa Fluor 594-labeled donkey anti-rabbit IgG secondary antibody (1:1,000; Invitrogen) for 1 h at room temperature and then mounted on glass slides in Vectashield mounting medium (Vector Laboratories, Burlingame, CA, USA) containing 2–5 µg/ml DAPI (Invitrogen). Embryos were observed using a fluorescence microscope for evaluation of each signal. At least three independent experiments were performed.

Microinjection of plasmid DNA vectors

An expression vector (*pβ-act/zag1-EGFP/SV40*) consisting of a fusion gene of *zag1* cDNA and enhanced green fluorescence protein (EGFP) under the control of the chicken β-actin promoter was injected into the pronucleus of 1-cell embryos. After culture, the subcellular localization of the EGFP signal in 2- to 8-cell embryos was detected using a single photon imaging system with a GFP filter.

In order to investigate the effect of knockdown of *zag1* on the development of preimplantation embryos, a *zag1* antisense vector (*pβ-act/antisense-zag1/IRES/luc+/SV40*) with bicistronic expression of *zag1* antisense RNA and humanized firefly luciferase was injected into the pronucleus of 1-cell embryos at 8 to 10 hpi. Injected embryos showing luciferase activity at 14 h after injection were selected and cultured to confirm the effect of antisense DNA expression by observation of subsequent development to the blastocyst stage. In these experiments, *pβ-act/luc+/SV40* was used as a control expression vector. The results from three experiments were pooled.

Yeast strains and screening of the two-hybrid ovary cDNA library

The yeast two-hybrid MATCHMAKER LexA system (Takara) was employed to detect specific protein-protein interactions [26]. The mouse *zag1* sequence was amplified by PCR and subcloned into the pGilda vector (Takara). A mouse ovary cDNA library in the vector pB42AD was screened. The EGY48 yeast strain used for the screening assay contained both *Leu2* and *lacZ* reporter

genes under the control of a LexA-responsive upstream activation site. For the assay, bait and library plasmids were used to simultaneously transform yeast using the lithium acetate procedure. Double transformant cells grown on Ura⁻, His⁻, Trp⁻, Leu⁻ plates were incubated for 5 days at 30 °C. Positive colonies were picked up and assayed for the lacZ phenotype. Putative positives were picked up and then further tested by assaying the colonies for β-galactosidase activity. Following confirmation of the specificity of the interaction, ZAG1 binding partners were identified by sequence analysis. The transformation of pGilda-p53 and pB42AD-T antigen were used as negative and positive controls, respectively.

Results

Mouse zag1 was expressed ubiquitously but was especially abundant in the testis and ovary

The tissue distribution of *zag1* gene expression was examined by northern blot analysis of poly(A)⁺ RNAs from various tissues of adult mice using full-length *zag1* cDNA as a probe. Transcripts of approximately 1.8 kbp were detected in all tissues examined, but were detected particularly strongly in the gonad (Fig. 2A). The blots were stripped and reprobed with β-actin cDNA to ensure equivalent mRNA loading.

Embryonic zag1 transcripts were expressed at minor ZGA in 1-cell embryos, whereas ZAG1 protein was constantly detected from the oocyte to at least the 2-cell embryo stage

RT-PCR and Western blot analysis were performed to investigate the developmental profiles of *zag1* gene expression in fertilized mouse eggs. Stored *zag1* transcripts in unfertilized eggs decreased after fertilization. *Zag1* transcript levels increased by approximately four-fold at 15 hpi compared with those at 6 hpi (Fig. 2B and C). These results indicated that embryonic *zag1* was expressed as part of the minor ZGA. In Western blot analysis, the anti-ZAG1 antibody recognized a protein of approximately 66.2 kDa, which is the molecular weight predicted for the mouse ZAG1 protein. Western blot analysis of mouse preimplantation embryos at 0, 6, 15 and 24 hpi showed ZAG1 protein was detected constantly, but increased gradually at 15 and 24 hpi (Fig. 2D), indicating embryonic *zag1* transcripts expressed in late 1-cell embryos were translated.

Mouse ZAG1 was localized to the nuclei of mouse preimplantation embryos

We observed the subcellular localization of ZAG1 protein by immunofluorescence in the 1- to 2-cell stage embryos. The nuclei of preimplantation embryos were labeled with DAPI (Fig. 3Ak–o). At the pronuclear stage, ZAG1 protein was localized to the cytoplasm (Fig. 3Af). At the syngamy stage, localization of the ZAG1 protein was observed in around the syngamy (Fig. 3Ag). At anaphase of the first division, ZAG1 protein was localized to between sister chromosomes (Fig. 3Ah). At the early 2-cell stage, we observed the localization of ZAG1 protein around the nuclear membrane (Fig. 3Ai), whereas the localization of ZAG1 protein was observed in the inner nucleus at the late 2-cell stage (Fig. 3Aj).

Fig. 2. Expression profile of the *zag1* gene during preimplantation development of *in vitro* fertilized eggs. (A) Northern blot analysis of the *zag1* gene in mouse tissues. (B) RT-PCR analysis of *zag1* gene expression. (C) Expression profile of *zag1* gene in preimplantation embryos. The *zag1* gene shows the expression profile of a maternal zygotic gene [15]. (D) Western blot analysis of mouse ZAG1 during early development of *in vitro* fertilized eggs.

In addition, after microinjection of an expression vector encoding ZAG1-EGFP fusion protein into the male pronucleus of 1-cell embryos, EGFP signals were detected in the nuclei of 2- to 8-cell embryos (Fig. 3B). These results revealed that the previously predicted bipartite nuclear targeting sequence spanning aa 128 to 139 in the ZAG1 protein was confirmed to be functional in the mouse preimplantation embryo.

ZAG1 protein was confirmed to be associating with U2B'' by a yeast two-hybrid system

In order to identify a functional context for ZAG1 in mouse embryos, we performed yeast two-hybrid screens using full-length ZAG1 as the bait and a mouse ovary cDNA library as the prey to identify possible interacting protein partners. Since the *zag1* gene was expressed in the mouse oocyte, we used an ovary cDNA library in this study. As a result, U2 small nuclear ribonucleoprotein B (U2B'') was identified as a putative interacting partner for full-length *zag1*. To identify the specificity of the protein interac-

Fig. 3. Subcellular localization of mouse ZAG1 in preimplantation embryos. (A) Differential interference contrast (DIC) (a-e). Mouse preimplantation embryos were immunostained with anti-ZAG1 polyclonal antibody (f-o) and DAPI (k-o). (B) Subcellular localization of mouse ZAG1-EGFP fusion protein in preimplantation embryos by microinjection of an expression vector encoding ZAG1-EGFP. EGFP signals were detected in the nuclei of 2- to 8-cell embryos. The scale bars are 100 μ m.

tion, pGilda-ZAG1 was co-transformed into yeast with pB42AD-U2B'' or pB42AD-T antigen. The results showed that ZAG1 gave strong positive signals for interaction with U2B'' but not with T antigen as the negative control (Fig. 4).

Injection of zag1 antisense DNA plasmid induced arrest and/or delay of development of preimplantation embryos

We investigated the effect of knockdown of *zag1* expression using an antisense vector (*pβ-act/antisense-zag1/IRES/luc+/SV40*) on the development of preimplantation embryos. In this system, which used an antisense vector with internal ribosomal entry sites (IRES), both *zag1* antisense RNA and luciferase protein were produced from a message transcribed from the *β-actin* promoter (Fig. 5A). After injection of the *zag1* antisense vector, 79 (47/60), 3 (2/60), 3 (2/60) and 15% (9/60) of luciferase positive embryos developed to the 1-cell, 2-cell, morula and blastocyst stages, respectively. The developmental rate of luciferase positive 1-cell embryos injected with the *zag1* antisense vector to the 2-cell stage was significantly lower compared with that of the control (20 vs. 93%, $P < 0.05$). In addition to this cleavage-stage arrest, we observed a developmental delay in luciferase positive 1-cell embryos injected with the *zag1* antisense vector compared with that of the control, as shown in Fig. 5. Sixty seven percent (32/48) of luciferase positive 1-cell embryos at 24 hpi developed into 2-cell

Fig. 4. Identification of a protein that interacts with ZAG1 protein by a yeast two-hybrid system. For the U2B''-binding assays, the full-length ZAG1 was tested for binding using a yeast two-hybrid system.

embryos at 48 hpi, and subsequently 81% (26/32) of injected 2-cell embryos at 48 hpi developed to the 4-cell, 8-cell or morula stages at 72 hpi. By contrast, the luciferase positive 2-cell embryos developed from 1-cell embryos injected with *zag1* antisense vector developed to the blastocyst stage at the same rate as the control [69 (9/13) vs 60% (18/28), respectively]. These results showed that *zag1* may have some involvement in the development of preimplantation embryos. However, the current findings did not provide definitive evidence of a key role of *zag1*, and it remains to be determined if a substantial reduction of endogenous *zag1* at transcriptional and/or translational levels is induced by expression of antisense RNA.

Discussion

In this study, we analyzed a functionally unknown gene [renamed as zygotic gene activation-associated gene 1 (*zag1*) for its speculated function]. The mouse ZAG1 amino acid sequence has a bipartite nuclear targeting sequence and P-loop motif (Fig. 1). The ZAG1 orthologs of rodents and humans have clearly diverged, although conserved sequence stretches are found in the bipartite nuclear targeting sequence and P-loop motif of *zag1* proteins. For example, the mouse full-length ZAG1 amino acid sequence shows 87% identity to that of the rat, whereas the mouse and human proteins share only 65% identity (data not shown). This species variability of the ZAG1 protein at the structural level could be indicative of relatively rapid evolution in this protein. As shown in Fig. 3A, subcellular localization of ZAG1 protein was observed in the inner nuclei of embryos except for at the mitosis stage. In addition, in mouse 1-cell embryos injected with the ZAG1-EGFP expression vector, subcellular localization of fused ZAG1-EGFP was detected in the nuclei of 2- to 8-cell embryos (Fig. 3B). Taken together, the present study demonstrates that the bipartite nuclear targeting sequence of ZAG1 protein is a functional domain in mouse preimplantation embryos. Furthermore, during our search

Fig. 5. Injection of a *zag1* antisense DNA plasmid induced arrest and/or delay of development of preimplantation embryos (hpi; hour post insemination). (A) Development of preimplantation embryo after microinjection of pβ-act/luc+/SV40 (positive control). (B) Development of preimplantation embryo after microinjection of pβ-act/anti-*zag1*/IRES/luc+/SV40. Degenerated (D; -), 1-cell (1C; □), 2-cell (2C; ▤), 4-cell (4C; ▥), 8-cell (8C; ▨), Morula (M; ▩), Blastocyst (B; ■).

through databases, we found a P-loop motif in the ZAG1 protein, which is a highly conserved feature of ATP- and GTP-binding proteins that it has been implicated in cleavage of the β-γ-phosphate bond of the NTP [27]. Thus, we speculated that *zag1* is involved in DNA replication, chromatin-remodeling, transcriptional regulation, RNA synthesis, RNA processing and RNA transport.

ZAG1 is assumed to play a role in the nucleus of the preimplantation embryo. Using a yeast two-hybrid screen, we showed that U2B'' was a binding partner of ZAG1 (Fig. 4), although it is necessary to determine whether U2B'' interacts with ZAG1 *in vivo* by immunoprecipitation analysis using preimplantation embryos. U2B'' is a component of 12S U2 snRNP complexes in the nucleus [28], which binds hairpin IV of U2 snRNP only when it is complexed with U2A' [29]. U2 snRNPs are involved in pre-spliceosome assembly and the pre-mRNA splicing mechanisms in the nucleus [30], which is localized from the germinal vesicle to the oocyte cytoplasm and to the pronuclei during ovulation and fertilization [31]. Interestingly, although the central segment of U2B'' has an nuclear localization signal (NLS), nuclear import of U2B'' does not depend on this NLS [32]. Nuclear import of U2B'' has been shown to be important for pre-spliceosome formation [33], which is also a requirement for hydrolysable ATP [34]. Therefore, it is assumed that ZAG1, with its P-loop motif, may be involved in nuclear import of U2B'', although it remains to be determined whether the ATP-binding motif of ZAG1 has ATPase activity.

The peptide α-amanitin induces suppression of transcription during the minor ZGA phase of the mouse 1-cell embryo [35, 36], but development to the 2-cell stage can still occur by a zygotic clock [37]. Cycloheximide also induces suppression of the minor ZGA during the mouse 1-cell embryo stage [17, 38]. These reports imply that translation of maternal RNA transcripts is necessary for subsequent development of embryos, while embryonic transcripts are not required in late 1-cell embryos. In general, the process of gene expression comprises transcription of RNA, RNA synthesis, RNA processing, mRNA capping, translation of protein, protein synthesis, protein processing and protein modification. However, little is known about the necessity of pre-mRNA splicing in late 1-cell embryos. In this study, knockdown of ZAG1, which was

assumed to be involved in pre-mRNA splicing mechanisms, resulted in arrest and/or delay of development to the 2-cell stage (Fig. 5). Pre-mRNAs, mature mRNAs and proteins originating from maternal and/or embryonic genes likely exist in late 1-cell embryos. Thus, pre-mRNA splicing mechanisms may be important in development of 1-cell to 2-cell embryos. Further investigation is needed to confirm the mechanism of pre-mRNA splicing by the spliceosome in late 1-cell embryos.

In conclusion, our results suggest that *zag1* may be a new member of the ZGA regulating genes in the mouse embryo at the late 1-cell stage.

Acknowledgments

We are grateful to Mr. M Hayakumo for technical support. This study was supported by a Grant-in-Aid for the 21st Century COE Program of the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

- Schultz RM. Regulation of zygotic gene activation in the mouse. *Bioessays* 1993; 15: 531–538.
- Latham KM. Mechanisms and control of embryonic genome activation in mammalian embryos. *Int Rev Cytol* 1999; 193: 71–124.
- Minami N, Suzuki T, Tsukamoto S. Zygotic gene activation and maternal factors in mammals. *J Reprod Dev* 2007; 53: 707–715.
- Matsumoto K, Anzai M, Nakagata N, Takahashi A, Takahashi Y, Miyata K. Onset of paternal gene activation in early mouse embryos fertilized with transgenic mouse sperm. *Mol Reprod Dev* 1994; 39: 136–140.
- Tong ZB, Gold L, Pfeifer KE, Dorward H, Lee E, Bondy CA, Dean J, Nelson LM. Mater, a maternal effect gene required for early embryonic development in mice. *Nat Genet* 2000; 26: 267–268.
- Howell CY, Bestor TH, Ding F, Latham KE, Mertineit C, Trasler JM, Chaillet JR. Genomic imprinting disrupted by a maternal effect mutation in the *Dnmt1* gene. *Cell* 2001; 104: 829–838.
- Burns KH, Viveiros MM, Ren Y, Wang P, DeMayo FJ, Frail DE, Eppig JJ, Matzuk MM. Roles of NPM2 in chromatin and nucleolar organization in oocytes and embryos. *Science* 2003; 300: 633–636.
- Wu X, Viveiros MM, Eppig JJ, Bai Y, Fitzpatrick SL, Matzuk MM. Zygote arrest 1 (*Zar1*) is a novel maternal-effect gene critical for the oocyte-to-embryo transition. *Nat Genet* 2003; 33: 187–191.
- Minami N, Aizawa A, Ihara R, Miyamoto M, Ohashi A, Imai H. Oogenesis is a novel mouse protein expressed in oocytes and early cleavage-stage embryos. *Bio Reprod* 2003; 69: 1736–1742.
- Tsukamoto S, Ihara R, Aizawa A, Kishida S, Kikuchi A, Imai H, Minami N. Oog1, an oocyte-specific protein, interacts with Ras and Ras-signaling proteins during early embryogenesis. *Biochem Biophys Res Commun* 2006; 343: 1105–1112.
- Ramos SB, Stumpo DJ, Kennington EA, Phillips RS, Bock CB, Ribeiro-Neto F, Blackshear PJ. The CCCH tandem zinc-finger protein Zfp3612 is crucial for female fertility and early embryonic development. *Development* 2004; 131: 4883–4893.
- Saitou M, Barton SC, Surani MA. A molecular programme for the specification of germ cell fate in mice. *Nature* 2002; 418: 293–300.
- Payer B, Saitou M, Barton SC, Thresher R, Dixon JP, Zahn D, Colledge WH, Carlton MB, Nakano T, Surani MA. Stella is a maternal effect gene required for normal early development in mice. *Curr Biol* 2003; 13: 2110–2117.
- Christians E, Davis AA, Thomas SD, Benjamin IJ. Maternal effect of Hsf1 on reproductive success. *Nature* 2000; 407: 693–694.
- Leder P. The formins: connecting embryonic pattern formation and hypofertility in the mouse. *Ann NY Acad Sci* 2004; 1038: 75–79.
- Zeng F, Baldwin DA, Schultz RM. Transcript profiling during preimplantation mouse development. *Dev Biol* 2004; 272: 483–496.
- Hamatani T, Carter MG, Sharov AA, Ko MS. Dynamics of global gene expression changes during mouse preimplantation development. *Dev Cell* 2004; 6: 117–131.
- Potireddy S, Vassena R, Patel BG, Latham KE. Analysis of polysomal mRNA populations of mouse oocytes and zygotes: dynamic changes in maternal mRNA utilization and function. *Dev Biol* 2006; 298: 155–166.
- Su YQ, Sugiura K, Woo Y, Wigglesworth K, Kamdar S, Affourtit J, Eppig JJ. Selective degradation of transcripts during meiotic maturation of mouse oocytes. *Dev Biol* 2007; 302: 104–117.
- Bultman SJ, Gebuhr TC, Pan H, Svoboda P, Schultz RM, Magnuson T. Maternal BRG1 regulates zygotic genome activation in the mouse. *Genes Dev* 2006; 20: 1744–1754.
- Matsumoto K, Nakagami K, Otake S, Tanaka H, Hashimoto Y, Yamada S, Saeki K, Hosoi Y, Iritani A. Identification of genes expressed in mouse unfertilized and fertilized eggs using differential display of RNA. *J Fertil Implant* 2001; 18: 5–7.
- Mizuno S, Sono Y, Matsuoka T, Matsumoto K, Saeki K, Hosoi Y, Fukuda A, Morimoto Y, Iritani A. Expression and subcellular localization of GSE protein in germ cells and preimplantation embryos. *J Reprod Dev* 2006; 52: 429–438.
- Anzai M, Nishiwaki M, Yanagi M, Nakashima T, Kaneko T, Taguchi Y, Tokoro M, Shin SW, Mitani T, Kato H, Matsumoto K, Nakagata N, Iritani A. Application of laser-assisted zona drilling to *in vitro* fertilization of cryopreserved mouse oocytes with spermatozoa from a subfertile transgenic mouse. *J Reprod Dev* 2006; 52: 601–606.
- Ho Y, Wigglesworth K, Eppig JJ, Schultz RM. Preimplantation development of mouse embryos in KSOM: augmentation by amino acids and analysis of gene expression. *Mol Reprod Dev* 1995; 41: 232–238.
- Matsumoto K, Nakayama T, Sakai H, Tanemura K, Osuga H, Sato E, Ikeda JE. Neuronal apoptosis inhibitory protein (NAIP) may enhance the survival of granulosa cells thus indirectly affecting oocyte survival. *Mol Reprod Dev* 1999; 54: 103–111.
- Estojak J, Brent R, Golemis EA. Correlation of two-hybrid affinity data with *in vitro* measurements. *Mol Cell Biol* 1995; 15: 5820–5829.
- Smith CA, Rayment I. Active site comparisons highlight structural similarities between myosin and other P-loop proteins. *Biophys J* 1996; 70: 1590–1602.
- Behrens SE, Tye K, Kastner B, Reichelt J, Luhrmann R. Small nuclear ribonucleoprotein (RNP) U2 contains numerous additional proteins and has a bipartite RNP structure under splicing conditions. *Mol Cell Biol* 1993; 13: 307–319.
- Scherly D, Boelens W, Dathan NA, Van Venrooij WJ, Mattaj IW. Major determinants of the specificity of interaction between small nuclear ribonucleoproteins U1A and U2B⁺ and their cognate RNAs. *Nature* 1990; 345: 502–506.
- Caspary F, Shevchenko A, Wilm M, Seraphin B. Partial purification of the yeast U2 snRNP reveals a novel yeast pre-mRNA splicing factor required for pre-spliceosome assembly. *EMBO J* 1999; 18: 3463–3474.
- Dean WL, Seufert AC, Schultz GA, Prather RS, Simerly C, Schatten G, Pilch DR, Marzluff WF. The small nuclear RNAs for pre-mRNA splicing are coordinately regulated during oocyte maturation and early embryogenesis in the mouse. *Development* 1989; 106: 325–334.
- Kambach C, Mattaj IW. Nuclear transport of the U2 snRNP-specific U2B⁺ protein is mediated by both direct and indirect signaling mechanisms. *J Cell Sci* 1994; 107: 1807–1816.
- Caspary F, Seraphin B. The yeast U1A/U2B⁺ complex is required for pre-spliceosome formation. *EMBO J* 1998; 17: 6348–6358.
- Hetzler M, Mattaj IW. An ATP-dependent, Ran-independent mechanism for nuclear import of the U1A and U2B⁺ spliceosome proteins. *J Cell Biol* 2000; 148: 293–303.
- Kidder GM, Green AF, McLachlin JR. On the use of α -amanitin as a transcriptional blocking agent in mouse embryos: a cautionary note. *J Exp Zool* 1985; 233: 155–159.
- Warner CM, Hearn TF. The effect of α -amanitin on nucleic acid synthesis in preimplantation mouse embryos. *Differentiation* 1977; 7: 89–97.
- Wiekowski M, Miranda M, DePamphilis ML. Regulation of gene expression in preimplantation mouse embryos: effects of the zygotic clock and the first mitosis on promoter and enhancer activities. *Dev Biol* 1991; 147: 403–414.
- Wang Q, Latham KE. Requirement for protein synthesis during embryonic genome activation in mice. *Mol Reprod Dev* 1997; 47: 265–270.