Association of Maternal mRNA and Phosphorylated EIF4EBP1 Variants With the Spindle in Mouse Oocytes: Localized Translational Control Supporting Female Meiosis in Mammals

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ABSTRACT In contrast to other species, localized maternal mRNAs are not believed to be prominent features of mammalian oocytes. We find by cDNA microarray analysis enrichment for maternal mRNAs encoding spindle and other proteins on the mouse oocyte metaphase II (MII) spindle. We also find that the key translational regulator, EIF4EBP1, undergoes a dynamic and complex spatially regulated pattern of phosphorylation at sites that regulate its association with EIF4E and its ability to repress translation. These phosphorylation variants appear at different positions along the spindle at different stages of meiosis. These results indicate that dynamic spatially restricted patterns of EIF4EBP1 phosphorylation may promote localized mRNA translation to support spindle formation, maintenance, function, and other nearby processes. Regulated EIF4EBP1 phosphorylation at the spindle may help coordinate spindle formation with progression through the cell cycle. The discovery that EIF4EBP1 may be part of an overall mechanism that integrates and couples cell cycle progression to mRNA translation and subsequent spindle formation and function may be relevant to understanding mechanisms leading to diminished oocyte quality, and potential means of avoiding such defects. The localization of maternal mRNAs at the spindle is evolutionarily conserved between mammals and other vertebrates and is also seen in mitotic cells, indicating that EIF4EBP1 control of localized mRNA translation is likely key to correct segregation of genetic material across cell types.

THE oocytes of many species, both invertebrate and vertebrate, contain a large collection of localized determinants in the form of proteins and translationally inactive maternal mRNAs. Similar localized determinants in mammalian oocytes have been proposed (Ciemerych *et al.* 2000), but this aspect of mammalian reproduction remains controversial (Hürragi *et al.* 2006). Indeed, early mammalian embryogenesis is considered to be quite plastic and regulative in nature, so that localized determinants would not be expected to play essential functions. Embryo splitting can be used for twinning, and blastomere extirpation does not prevent elaboration of normal body plans and term develop-

ment. Additionally, much of the volume of the mammalian oocyte eventually becomes allocated to cells that do not contribute to embryonic development, being destined instead to generate the placenta. Accordingly, prepatterning of the mammalian oocyte through localization of maternal mRNAs or proteins, if it occurs, appears to be dispensable for mammalian embryogenesis.

One potential exception to this would relate to localization within the oocyte of maternal mRNAs that support a vital process that is evolutionarily conserved between mammals and other species, namely the formation and maintenance of the meiotic spindle. Recent studies in *Xenopus* revealed enriched localization to spindle microtubules of mRNAs encoding spindle proteins (Blower *et al.* 2007). The spindle is a complex structure; proteomic studies of isolated spindles have identified >1100 spindle-associated proteins, of which nearly 400 are specific to spindles and shared with proteomic studies that incorporated DNase digests to deplete DNA-associated proteins (Sauer *et al.* 2005; Bonner *et al.* 2011), indicating that a large array of

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proteins is needed to support spindle formation, maintenance, and function. Localized maternal mRNAs could be translated *in situ* to provide a local high concentration of proteins, while minimizing potential deficiencies related to limitations in the speed or extent of protein accumulation from elsewhere within the ooplasm.

Many maternal mRNAs that undergo translational recruitment and degradation in the mouse oocyte encode spindle-associated proteins (Chen *et al.* 2011). Some recruited mRNAs contain recognizable cytoplasmic polyadenylation elements (CPEs), which participate in translational regulation, and other mRNAs contain binding motifs for DAZL (deleted in azoospermia-like), a CPEB-regulated protein that is critical for translational control of maternal mRNAs encoding spindle proteins (Chen *et al.* 2011). Many other mRNAs that are recruited stage specifically lack recognizable CPEs, indicating that multiple translational regulatory mechanisms may operate at different stages (Potireddy *et al.* 2010).

Given the complex and dynamic pattern of maternal mRNA recruitment during oocyte maturation and early embryogenesis (Potireddy *et al.* 2006, 2010; Mtango *et al.* 2008; Chen *et al.* 2011) and the prevalence of spindle-encoding mRNAs among these, we wished to test oocytes of a mammalian species for conservation of localized maternal mRNAs at the spindle. We tested whether the key translational regulator, EIF4EBP1, might likewise be enriched at the spindle as part of the overall regulatory mechanism. We find by cDNA microarray analysis enrichment for maternal mRNAs encoding spindle proteins and other proteins on the mouse oocyte MII spindle. We also find that EIF4EBP1 undergoes a dynamic and complex spatially regulated pattern of phosphorylation at sites that regulate its association with EIF4E and its ability to repress translation. These phosphorylation variants appear at different positions along the spindle–chromosome complex (SCC) at different times throughout meiotic maturation. These results indicate that dynamic spatially restricted patterns of EIF4EBP1 may promote localized translation within the mammalian oocyte that contributes to spindle formation, maintenance, and function, and other nearby processes. Thus, localization of maternal mRNAs at the spindle is evolutionarily conserved between mammals and other vertebrates, and spatially regulated EIF4EBP1 phosphorylation may control the translation of these mRNAs, providing a means for coordinating spindle formation and maintenance with progression through the cell cycle.

Materials and Methods

Oocyte isolation and culture

Hybrid C57Bl/6 X DBA/2 (B6D2F1) females were obtained from the National Cancer Institute (NCI) at 5–6 weeks age and used from 6 to 10 weeks age. Mice were injected intraperitoneally with 5 IU of equine chorionic gonadotropin (eCG) and were sacrificed by cervical dislocation 44–48 hr

later. Ovaries were dissected in 37° HEPES-buffered M2 medium with 0.2 μ M isobutyl methyl xanthine (IBMX) (Sigma-Aldrich, St. Louis) to inhibit meiotic resumption of oocytes. Ovaries were held with forceps and punctured with a 27.5-gauge needle to release cumulus-enclosed oocytes (COCs) into the dish. All abnormal and dead COCs were excluded. COCs were cultured for 1 hr in 50 μ L mineral oil-covered microdrops of MEM α (Life Technologies/Invitrogen, Grand Island, NY) supplemented with 10% fetal bovine serum (Life Technologies/Gibco) that had been preequilibrated overnight. Attached cumulus cells were removed by mouth pipetting using a narrow bore pipette with a diameter slightly larger than that of an oocyte. The appearance of a perivitelline space (PVS) between the oocyte plasma membrane and the zona pellucida after the 1-hr culture provided a reliable indicator of oocyte meiotic and developmental competence; only oocytes with a centrally located germinal vesicle (GV) and present PVS after 1 hr recovery were used for experiments (Inoue *et al.* 2007). To release meiotic arrest, oocytes were washed six times in MEM α + 10% FBS lacking IBMX. The following times were used to isolate and fix oocytes at major meiotic cell cycle events: germinal vesicle breakdown (GVBD) at 2 hr after IBMX removal, metaphase I (MI) at 6 hr after IBMX removal, and MII at 16 hr after IBMX removal. To obtain *in vivo* matured MII oocytes, mice were injected with eCG, followed 48 hr later by 5 IU of human chorionic gonadotropin (hCG). Sixteen hours later, cumulus cells were removed by incubation in M2 medium containing hyaluronidase (120 IU/ml, Sigma).

All studies were approved by the Temple University Institutional Animal Care and Use Committee, consistent with National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, and with Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accreditation.

Expression microarray analysis

Total RNA was isolated from cells using the PicoPure RNA isolation kit (Invitrogen). Up to 50 ng of total RNA from each array sample were subjected to two rounds of cDNA synthesis using the RiboAmp HS Plus kit (Life Technologies/Arcturus/Invitrogen). Labeled cRNA was produced using the Affymetrix GeneChip Expression 3' Amplification for IVT Labeling kit. The biotin-labeled cRNA samples were fragmented and 10 μ g hybridized to arrays. Posthybridization washing, staining, and scanning were performed as described in the Affymetrix GeneChip Expression Analysis Technical Manual. Microarray data were preprocessed and analyzed with scripts written in R (R Development Core Team 2009), utilizing routines from Bioconductor (Gentleman 2004). Probeset expression values were summarized and normalized using robust multiarray analysis (RMA) (Irizarry *et al.* 2003). The Bioconductor implementation of Microarray Analysis Suite 5.0 algorithm was used to obtain probeset calls (present, absent, and marginal). Probesets detected

Table 1 Prevalence of cellular compartments and processes among SCC-enriched mRNAs

Category	≥1000	% of ≥1000	500–999	% of 500–999	100–499	% of 100–499	Total	% of all genes	% of all assignments
Number of genes in group	50		33		74		157		
Chromatin/nuclear	17	34	8	24	25	34	50	32	17
Signaling	13	26	12	36	22	30	47	30	16
Plasma membrane	7	14	12	36	19	26	38	24	13
Spindle/cytoskeleton	8	16	5	15	10	14	23	15	8
Other or unknown	3	6	4	12	18	24	25	16	9
ER	5	10	4	12	11	15	20	13	7
Vesicle/endocytosis/transport	6	12	7	21	6	8	19	12	6
Protein degradation/ubiquitin	5	10	3	9	11	15	19	12	6
Translation	4	8	4	12	6	8	14	9	5
Golgi	6	12	4	12	3	4	13	8	4
RNA binding	5	10	4	12	2	3	11	7	4
Cytoplasmic sequestration	4	8	4	12	1	1	9	6	3
Mitochondrial	1	2	0	0	7	9	8	5	3

Some genes can be members of more than one category, hence values in columns 3, 5, 7, and 9 are not additive to 100%.

in all SCC and intact MII samples but with absent calls for enucleated oocyte samples, and satisfying both threshold criteria for inclusions (see below) were retained. Probesets detected in all SCC and intact MII samples and satisfying both threshold criteria for inclusions (see below) were retained, regardless of present/absent calls for enucleated oocyte samples. Average intensities for three sample groups were calculated from the normalized and filtered probesets, and compared as described in *Results*. Array data were deposited with the Gene Expression Omnibus database (accession no. GSE46875).

Oocyte fixation and immunofluorescence

Immunocytochemistry steps were performed in nine-well glass dishes (Pyrex) using 200 μ l drops of solution for incubations. Oocytes were fixed in 3.7% paraformaldehyde/PBS (Electron Microscopy) for 30 min at room temperature, washed twice in blocking buffer [PBS containing 0.1% BSA (Sigma), 0.01% Tween-20 (Bio-Rad), and 0.02% sodium azide (Sigma)], and either stored at 4° or processed immediately. Samples were permeabilized in PBS containing 0.1% Triton X-100 (Bio-Rad) for 30 min and incubated in blocking buffer for 1 hr at room temperature. Primary antibodies were used at 1:50 dilution in blocking buffer and were from Cell Signaling Technology (Danvers, MA) with the exception of phospho-Ser111-EIF4EBP1 from Abgent (San Diego, cat. no. AP3473a) and MIS18A (also known as FASP1) from Santa Cruz Biotechnology (Dallas, S-18, cat. no. sc-83615). Primary antibodies included: EIF4EBP1 mAb (53H11 Cell Signaling cat. no. 9644S), EIF4EBP1 pAb (Cell Signaling cat. no. 9452S), phospho-Thr69-EIF4EBP1 pAb (Cell Signaling cat. no. 9455S), phospho-Ser64-EIF4EBP1 (Cell Signaling cat. no. 9451S), phospho-Ser235/236-S6 (D57.2.2E Cell Signaling cat. no. 4858P), and phospho-Ser240/244-S6 (D68F8 Cell Signaling cat. no. 5364P). The polyclonal antibodies against EIF4EBP1, phospho-Thr69-EIF4EBP1, and phospho-Ser64-EIF4EBP1 have been used extensively, and their specificity established in earlier

studies (Gingras *et al.* 2001a; Wang *et al.* 2003; Ohne *et al.* 2008; Ma *et al.* 2009; Fonseca *et al.* 2011; Fuchs *et al.* 2011) including one report (Ellederova *et al.* 2006) in which SDS-PAGE/Western blotting of *in vitro* matured pig oocytes showed specific reactions with bands of the appropriate sizes. After overnight incubation with primary antibody at 4°, the oocytes were washed three times in blocking buffer for 10 min each wash. Secondary antibody incubation used goat anti-rabbit-Alexa 594 (Life Technologies/Molecular Probes) at 1:300 dilution for 1 hr at room temperature. Oocytes were washed three more times and mounted on slides in Vectashield mounting solution containing 1.5 μ g/ml DAPI (Vector Laboratories, Burlingame, CA), covered with coverslips, and sealed with nail polish. Slides were stored in cases at 4° and protected from light with aluminum foil until use for confocal microscopy. Confocal microscopic images were obtained using a Leica TCS SP5 confocal microscope with a $\times 40$ 1.25 NA oil objective. For DAPI excitation, the sample was excited with a UV laser; For Alexa 594 excitation, the sample was excited with a 561-nm laser. Sequential scanning was used to eliminate cross-talk between channels. All settings were kept constant within groups. For cytoplasmic signal quantification, mean intensity comparisons were performed using ImageJ from the National Institutes of Health (Schneider *et al.* 2012).

Results

Enrichment of maternal mRNAs at the meiotic spindle revealed by expression microarray analysis

If localized maternal mRNAs play a vital role in spindle formation, maintenance, and function in the oocyte, there should exist a significant number of maternal mRNAs that are highly partitioned to the spindle. To test for enriched localization of mRNAs at the MII spindle in mouse oocytes, we isolated three samples of >1000 SCCs each by microsurgery. We also collected four samples of 25 cytoplasts from which SCCs had been removed, and three samples of 25

Figure 1 Enrichment of MIS18A protein on the mouse oocyte MII spindle observed by confocal microscopy and image quantification. (A–C) MII oocytes were matured *in vivo*, fixed, and immunostained as described in *Materials and Methods*. The spindle region of a MII oocyte is shown with MIS18A immunoreactive signal in white. (B) DNA observed by fluorescent DAPI staining. (C) Merged image in which MIS18A is shown in red and DNA is shown in blue. (D) Quantitative analysis of increased intensity of MIS18A localization to the spindle as compared to cytoplasm for MIS18A. Box plot distribution is shown where the mean is represented by a black square ($n = 6$, $P < 0.0047$ using one-tailed *t*-test with unequal variance). MIS18A, MIS18 kinetochore protein homolog A; GV, germinal vesicle; GVBD, germinal vesicle breakdown; MI, metaphase I; MII, metaphase II.

intact MII oocytes. The samples were processed for RNA extraction; the mRNA was reverse transcribed, amplified, and labeled; and the cRNA was hybridized to Affymetrix arrays. After normalization, average raw intensity values were compared to identify probesets that were different between the three sample types based on fold changes as follows: Assuming that the SCC would comprise no more than 10% volume of the oocyte, we calculated that a twofold enrichment for mRNAs at the spindle would yield an expression ratio of 2.25 for SCC:cytoplasm and 1.125 for intact MII:cytoplasm. Threefold enrichment would yield corresponding ratios of 3.85 and 1.285.

We identified 50 mRNAs that satisfied both criteria for twofold enrichment, and an additional 3 that satisfied the SCC:cytoplasm ratio but not the MII:cytoplasm ratio with maximum raw intensity values of ≥ 1000 (Supporting Information, Table S1). The mRNAs with greatest levels of enrichment included two that encode known spindle or cytoskeleton-associated proteins, anillin and MIS18A. To evaluate the relationship of these localized mRNAs to the spindle and other cellular compartments, as well as the functions likely to be directed by their encoded proteins, we assigned the mRNAs to categories representing cellular compartments or processes (Table 1). The four most prominently affected categories included proteins associated with plasma membrane, chromatin/nuclear, signaling, and, as expected, spindle/cytoskeletal functions, followed by vesicle/endocytosis/protein transport, Golgi and endoplasmic reticulum, ubiquitination and protein degradation, and RNA binding.

For mRNAs with maximum raw intensity values between 500 and 999, 33 satisfied both criteria for enrichment and

one satisfied just the SCC:cytoplasm criterion. The most prominent cell compartment and functional categories for these mRNAs were again plasma membrane, chromatin/nuclear, signaling, and spindle/cytoskeleton. This group had a higher representation of mRNAs related to signaling and plasma membrane functions.

An additional 74 mRNAs satisfied both criteria and had maximum raw intensity values of 100–499, and an additional 20 fulfilled just the SCC:cytoplasm criterion for inclusion. The same top four categories were repeated for this group as the two higher signal intensity groups (with the exception of those listed as other or unknown functions), indicating that these categories are consistently seen across the range of signal intensity values. Protein degradation/ubiquitination and mitochondrial associations were more prominent for this group of mRNAs.

None of the SCC-enriched mRNAs fulfilled the criteria for threefold or greater enrichment on the SCC. We note that the list of SCC-enriched mRNAs includes those encoding proteins found previously enriched on the SCC, such as calmodulin, as well as proteins involved in endocytosis, also previously found to be related to spindle formation and function (Miyara *et al.* 2006; Han *et al.* 2010). Several of the genes associated with the plasma membrane are involved in interaction of the cell surface with the cytoskeleton or spindle.

We compared our list of mRNAs enriched at the SCC with a list of polysomal mRNAs enriched more than threefold in MII oocytes vs. one-cell embryos (Potireddy *et al.* 2006). Twelve of 50 (24%) SCC-enriched mRNAs were also selectively translated at the MII stage (*Atrx*, *Glce*, *Etnk1*, *Lbr*, *Rcn2*, *Sypl*, *Cdh1*, *Tex12*, *Hectd2*, *Dnajc3*, *Slc35a1*, and

Bmpr2). Conversely, comparing our SCC mRNA list to the list of mRNAs enriched on one-cell stage polysomes revealed only two mRNAs in common (*Atp6v1* and *Calm1*). This confirms that the SCC-associated mRNAs are selectively translated in MII oocytes, as needed to contribute to spindle formation, maintenance, and function.

Enriched localization of MIS18A at the spindle

Fully grown immature mouse oocytes from large antral follicles resume meiosis after the luteinizing hormone ovulatory surge or spontaneously when removed from the ovary. Nuclear envelope dissolution and chromatin condensation are followed by spindle formation and migration, extrusion of the first polar body containing homologous chromosomes, and arrest at metaphase II until fertilization or spontaneous activation. In rodents, maternal stores of maturation-promoting factor are adequate to initiate the process, but SCC formation requires protein synthesis (Hashimoto and Kishimoto 1988), indicating a possible role for translational control of localized mRNAs in SCC formation.

If enrichment of maternal mRNA at the spindle supports its formation and function, we would expect to observe enriched localization of that protein to the SCC. We tested for enriched localization to the SCC of MIS18A (MIS18 kinetochore protein homolog A), a protein that is enriched in the mitotic spindle of HeLa cells, binds and recruits centromere protein A (CENPA) to centromeres, and is essential for metaphase alignment and proper chromosome segregation (Fujita *et al.* 2007). This mRNA had an expression ratio of 3.55 for SCC:cytoplasm and 1.54 for intact MII:cytoplasm and therefore satisfied our criteria of being an mRNA with enriched localization to the SCC. Immunofluorescence detection of MIS18A in MII oocytes (Figure 1) revealed enriched localization of the protein at the SCC, with an average immunoreactive signal intensity that was 1.5-fold higher in the SCC compared to the surrounding cytoplasm.

Ribosomal subunits indicative of active translation are present at the spindle

We reasoned that for localized mRNAs to direct the localized synthesis of their proteins in support of SCC formation and function, the translational machinery would need to be present at the SCC. A commonly used marker of active translation is phosphorylated ribosome protein S6 (RPS6). Phosphorylated RPS6 is associated with efficient formation of translation initiation complexes and entrance into polysomes (Duncan and McConkey 1982; Thomas *et al.* 1982). RPS6 is present at *Xenopus laevis* meiotic spindles (Blower *et al.* 2007). To test for RPS6 at mouse oocyte SCCs, we performed immunofluorescence detection of two phosphorylated RPS6 variants (Ser235/236-P-RPS6 and Ser240/244-P-RPS6) in MII oocytes (Figure 2). As expected, controls in which primary antibody was omitted from immunofluorescence showed no detectable signal. Both antibodies directed to phosphorylated RPS6 variants labeled the

Figure 2 Presence of phosphorylated RPS6 variants at the mouse oocyte MII spindle by immunofluorescence and confocal microscopy. (A–C) Immunoreactive signal produced by each antibody is shown in red and DNA is shown in blue. (A–C) Localization of Ser240/244-P-RPS6. (D–F) Localization of Ser235/236-P-RPS6. (G–I) Negative control in which all conditions are identical except omission of primary antibody. At least five oocytes were imaged for each condition. Bar, 20 μ m. RPS6, ribosomal protein S6.

cytoplasm of GV and GVBD oocytes. Ser235/236-P-RPS6 coalesced in a ring around the condensing chromosomes in GVBD oocytes (Figure 2E). Though not enriched substantially above the surrounding cytoplasm, both phosphorylated variants were present at the SCC (*i.e.*, there was no prominent void indicating absence of RPS6), consistent with translational capacity being present.

EIF4EBP1 expression and phosphorylation in oocytes

We next examined expression of EIF4EBP1. EIF4EBP1 is a key factor that regulates mRNA translation. This protein binds to EIF4E and inhibits its interaction with EIF4G, thereby interfering with translation initiation. EIF4EBP1 is an intrinsically disordered protein that undergoes dynamic folding and stabilization of tertiary structure when it binds to EIF4E (Fletcher and Wagner 1998). The effect of EIF4EBP1 phosphorylation on EIF4E binding affinity is likely due to an intrastructural modulation that prevents folding into a binding-compatible conformation, thereby leaving EIF4EBP1 disordered and unfolded (Tait *et al.* 2010). Of the seven serine/threonine phosphorylation sites reported in EIF4EBP1 (Thr36, Thr45, Ser64, Thr69, Ser82, Ser100, and Ser111 for mouse; human sequence numbers are greater by one), the first five are phylogenetically conserved among all species of organisms. The residues Ser100 and Ser111 are unique to EIF4EBP1 and not present in EIF4EBP2 or EIF4EBP3 orthologs. The other phosphorylation sites are present.

In general, hyperphosphorylated EIF4EBP1 is associated with EIF4E release leading to translation initiation, but controversy exists over the importance of particular sites in controlling the release of EIF4E binding (Gingras *et al.* 2001b; Harris and Lawrence 2003; Hay and Sonenberg

Figure 3 Model illustrating site-specific phosphorylation and regulation of EIF4EBP1. Motifs within mouse EIF4EBP1 are shown below the primary structure along with their amino acid sequences. Upstream signals (insulin, cell cycle M phase, and DNA damage) and downstream kinases (mTOR, CDK1, and ATM) impact the phosphorylation state of EIF4EBP1 at several residues including the sites (P)Ser-64, (P)Thr-69, and (P)Ser-111. Phosphorylation sites are shown as solid sites within the structure and motifs are shown as open boxes. DOG 2.0 protein domain structure illustrator software (Tsukiyama-Kohara *et al.* 2001) was used to generate the EIF4EBP1 protein structure model. mTOR, mammalian target of rapamycin; CDK1, cell division kinase 1; ATM, ataxia telangiectasia mutated.

2004). In somatic cells, site-specific phosphorylation may follow an ordered, sequential pattern of acquisition (Gingras *et al.* 2001a; Ayuso *et al.* 2010), but this has not been examined in detail for oocytes. Evidence for the role of specific phosphorylation sites in contributing to translation initiation is illustrated in Figure 3. We hypothesized that the translational control of mRNAs localized at the SCC could be facilitated by stage-dependent, spatially-restricted EIF4EBP1 phosphorylation. Specifically, we focused on three phosphorylated residues for detailed examination as potential regulatory candidates: two sites close to the EIF4E-binding region (Ser64 and Thr69) and one site in the C-terminal regulatory region (Ser111).

We examined EIF4EBP1 expression, phosphorylation, and localization in GV-intact oocytes before maturation, after meiotic resumption at GVBD, at the MI stage during *in vitro* maturation, and at the MII stage after *in vitro* maturation. These periods represent major nuclear and cytoplasmic maturation events in which the oocyte has stage-specific requirements for protein synthesis; GVBD and chromatin condensation do not require protein synthesis, but progression to MI and maintenance of MII arrest are dependent on protein synthesis (Schultz and Wassarman 1977; Siracusa *et al.* 1978). We examined expression of EIF4EBP1 with phosphorylation at Ser64, Ser111, and Thr69 (Figures 4 and 5). In addition, we examined the expression of EIF4EBP1 (independent of phosphorylation) using both a monoclonal and a polyclonal antibody against total EIF4EBP1 (Figures 4 and 5). For a summary of immunofluorescent staining results see Figure 6.

Staining GV-intact oocytes with the antibody for total EIF4EBP1 revealed diffuse staining throughout the entire cytoplasm and nucleus, except for an absence in the nucleolus. (Figure 4, row 1). Antibodies against Ser64-phosphorylated EIF4EBP1 (Ser64-P-BP1) showed localization to the GV and cytoplasm, with intense spots also visible within the nucleus (Figure 4, row 2). After GVBD, Ser64-P-BP1 showed an increase in cytoplasmic staining and a strong signal associated with the condensing chromosomes. The antibody specific for Ser111-phosphorylated EIF4EBP1 (Ser111-P-BP1) (Figure 4,

row 3) produced a very similar pattern to Ser64-P-BP1, with an increase in cytoplasmic staining and chromosome-associated signals concomitant with germinal vesicle breakdown. The antibody specific for Thr69-phosphorylated EIF4EBP1 (Thr69-P-BP1) (Figure 4, row 4) showed a low-level homogeneous distribution within the nuclear and cytoplasmic compartments at the germinal vesicle stage, in addition to signals on cytoplasmic foci. After GVBD, overall cytoplasmic levels for Thr69-P-BP1 did not change, but staining was acquired in specific association with the condensing chromosomes.

At first metaphase, staining with the antibody for total EIF4EBP1 showed continued diffuse staining throughout the cytoplasm. Antibodies for Ser64-P-BP1 and Ser111-P-BP1 showed strikingly similar immunostaining patterns, which included signals at both poles of the spindle and on small foci throughout the cytoplasm (Figure 4, rows 2 and 3). Localization was also seen on kinetochores of MI chromosomes for both of these phosphorylated forms. Interestingly, Thr69-P-BP1 was enriched along the polar spindle microtubules.

After separation of homologous chromosomes and extrusion of the first polar body, oocytes bypass interphase without DNA replication and arrest at the second metaphase of meiosis. Protein synthesis is required for the maintenance of this arrest (Siracusa *et al.* 1978), and synthesis of cyclin B is believed to play a major role in this process (Hashimoto and Kishimoto 1988). Staining with the antibody for total EIF4EBP1 revealed diffuse cytoplasmic staining, similar to earlier stages of maturation. An ~40% decrease in EIF4EBP1 cytoplasmic levels was seen in MII oocytes compared to GV-intact stage oocytes (Figure 5). The monoclonal and polyclonal antibodies produced similar results for all stages examined, with the exception that at MII, the monoclonal antibody showed an enrichment on the polar spindle microtubules that matches the enrichment pattern of Thr69-P-BP1. Thr69-P-BP1 displayed a low level of staining throughout the cytoplasm that did not change significantly during maturation (Figure 5), but showed a striking signal along the polar microtubules in all MII oocytes examined (Figure 4, row 4). Ser64-P-BP1 and Ser111-P-BP1 displayed

Figure 4 Localization and phosphorylation of EIF4EBP1 during major stages of meiotic maturation. Row 1 (A–D) shows confocal immunofluorescence results of oocytes stained with polyclonal antibody against total EIF4EBP1 ($n = 9, 11, 5,$ and 12 for GV, GVBD, MI, and MII, respectively); row 2 (E–H) shows Ser64-P-BP1 ($n = 7, 12, 6,$ and 8); row 3 (I–L) shows Ser111-P-BP1 ($n = 11, 10, 8,$ and 14); row 4 (M–P) shows Thr69-P-BP1 ($n = 5, 7, 7,$ and 8). All antibodies were used in two to four separate experiments, and separate lots were tested when available. The signal produced by each antibody is shown in red and DNA is shown in blue. Bar, $20\ \mu\text{m}$.

intense signals on the spindle poles and in spots throughout the cytoplasm (Figure 4, rows 2 and 3). Ser64-P-BP1 staining was also enhanced in the cortical granules of all oocytes examined. Ser111-P-BP1 did not show cortical granule staining (Figure 4). Ser64-P-BP1 and Ser111-P-BP1 were again enriched on the kinetochores of chromosomes, but this was less intense than seen in MI oocytes. Whereas total levels of EIF4EBP1 decreased throughout maturation, cytoplasmic signals for both Ser64-P-BP1 and Ser111-P-BP1 showed an ~ 4.5 -fold relative increase as compared with GV-intact stage oocytes (Figures 4 and 5).

Discussion

Maternal mRNA localization has been broadly observed in invertebrate and anamniote vertebrates, but has not been associated with mammalian oocytes. Our results demonstrate that mRNAs encoding proteins associated with the spindle chromosome complex are spatially enriched at the SCC. Moreover, our data demonstrate the presence at the SCC of the protein translation apparatus and the developmentally regulated phosphorylation of a key translational control protein, EIF4EBP1, as well as enriched expression of one protein, MIS18A, encoded by one of the localized mRNAs. Localization of mRNA to the spindle has also been reported for *Xenopus* oocytes (Blower *et al.* 2007). Collectively, our results demonstrate that mammals and amphibians share this aspect of maternal mRNA localization in oocytes. Such localization is also seen in somatic cells (Mili and Macara 2009), indicating

that it likely plays a key role in the proper formation and function of both meiotic and mitotic spindles and chromosome segregation during meiosis and during mitosis in diverse cell types. However, differences between cell types suggest that distinct modes of regulation exist.

The translational control of these SCC-associated mRNAs is likely to be complex, as maternal mRNAs, including some encoding spindle proteins, are regulated by a combination of binding proteins (Chen *et al.* 2011). Our results demonstrate that the regulated phosphorylation of EIF4EBP1 may contribute to this regulation. An increase in EIF4EBP1 phosphorylation was previously seen in porcine and bovine oocytes by Western blotting (Tomek *et al.* 2002; Ellederova *et al.* 2006), and is confirmed here for mouse oocytes. Because EIF4EBP1 phosphorylation releases EIF4E binding to permit initiation, the overall increase in phospho-EIF4EBP1 in the ooplasm may facilitate maternal mRNA translational recruitment in the ooplasm.

The dynamic spatial and temporal pattern of localization of phosphorylated EIF4EBP1 at the spindle is indicative of a novel mechanism promoting localized protein production. We propose that, as the spindle forms, it captures mRNAs that remain translationally repressed by the presence of EIF4EBP1. Phosphorylation of EIF4EBP1 may allow localized mRNA translation to sustain spindle formation and provide an ongoing supply of proteins for spindle maintenance. This may allow diverse cellular signals to be integrated to control the timing of localized mRNA translation in support of spindle formation and meiotic progression.

Figure 5 Quantification of cytoplasmic expression of EIF4EBP1 and phosphorylated variants in GV- and MII-stage oocytes. GV and MII oocytes were isolated, matured (for MII oocytes), fixed, and immunostained as described in *Materials and Methods*. Cytoplasmic fluorescence intensities were calculated using ImageJ software (NIH) and compared using a two-tailed *t*-test. Error bars represent SEM. Solid represents GV oocytes and shading represents MII oocytes. **P* < 0.05; NS, not significantly different.

The regulation of EIF4EBP1 phosphorylation at the spindle is likely to be temporally and mechanistically distinct from its regulation in the rest of the oocyte. We observe some level of EIF4EBP1 phosphorylation before and after maturation, but a spatially dynamic pattern with enriched localization at the spindle on a background of overall diminishment of total EIF4EBP1 content (Figure 6). Thr69-P-BP1 is present at a low, diffuse level at both GV and MII stages, but undergoes dramatic enrichment at the spindle. Kinases associated with the spindle and cell cycle progression are obvious likely controllers. Kinases implicated in EIF4EBP1 phosphorylation include mTOR, polo-like kinases, cyclin-dependent cell division kinases, and several others (Lawrence *et al.* 1997; Yang and Kastan 2000; Heesom *et al.* 2001; Shang *et al.* 2012). For example, PLK1-mediated phosphorylation in the region of human EIF4EBP1 residues 77–118, which includes Ser112, is accompanied by localization to spindles in mitotic cells. The roles of mitotic kinases in EIF4EBP1 phosphorylation suggests that EIF4EBP1 may help regulate spindle formation and function and cell cycle progression, including proper oocyte maturation.

We observe significant differences between meiotic and mitotic spindles in the regulation of EIF4EBP1 phosphorylation and localization. We observe two distinct localization patterns for phosphorylated variants of EIF4EBP1 on meiotic spindles of mouse oocytes: Thr69-P-BP1 along the polar microtubules, and Ser64-P-BP1 (with Ser111-P-BP1) at the spindle poles and kinetochores. In HeLa cells, cell-cycle-dependent phosphorylation of EIF4EBP1 occurs, and CDC2 was identified as the critical kinase during mitosis at Ser65 and Thr70 (Heesom *et al.* 2001). Identical phosphorylation patterns at Thr70-P-BP1 and Ser65-P-BP1 in mitotic HeLa cells (Heesom *et al.* 2001) contrasts with the distinct locations during oocyte meiosis. We also observe differences

between metaphases I and II in the phosphorylation of EIF4EBP1. These differences between MI, MII, and mitotic spindles point to possible functional heterogeneity for EIF4EBP1 in supporting the formation and function of spindles that participate in different types of cellular division.

The functional categories represented by the mRNAs enriched at the SCC bears mention. Aside from the expected prevalence of spindle- and cytoskeleton-related proteins and signaling proteins, many of the SCC-enriched mRNAs encoded nuclear/chromatin-associated proteins, proteins associated with the Golgi and endoplasmic reticulum, and proteins related to protein degradation and ubiquitination. The presence of mRNAs encoding chromatin-associated proteins may provide a ready supply of proteins needed for chromatin compaction or nuclear functions after meiosis. The mRNAs encoding Golgi proteins may be important for spindle positioning, as inhibition of Golgi-based membrane fusion by brefeldin A treatment disrupts asymmetric spindle positioning in both MI and MII mouse oocytes (Wang *et al.* 2008). The mRNAs encoding ER proteins may be related to a dynamic association of the ER with the spindle just prior to the first metaphase, when the SCC is translocated to the cell surface (FitzHarris *et al.* 2007). The presence of mRNAs encoding proteins associated with the plasma membrane and the cytoskeleton may also contribute to asymmetric spindle localization in the oocyte. The presence of mRNAs encoding proteins related to ubiquitination would also be consistent with a local role for these proteins in controlling spindle formation and function (Mtango *et al.* 2012).

The decline in total EIF4EBP1 expression during maturation with a coincident increase in phosphorylation of Ser64 and Ser111 raises the possibility that phosphorylation regulates EIF4EBP1 stability as well as its binding to EIF4E. A dual effect of phosphorylation of EIF4EBP1 was reported elsewhere, either reducing affinity of EIF4EBP1 for EIF4E, or promoting polyubiquitination and decreased EIF4EBP1 stability (Elia *et al.* 2008). Our data are consistent with EIF4EBP1 degradation subsequent to phosphorylation. Overall, the data suggest that phosphorylation and possibly ubiquitination regulate the availability and function of EIF4EBP1 during meiosis. Regulating EIF4EBP1 expression at the spindle may thus comprise one aspect of the critical role for the ubiquitin pathway previously seen in oocytes (Mtango *et al.* 2012).

The 7-methylguanosine cap (m^7G) and the activities of its direct and indirect binding proteins, including EIF4EBP1, contribute to the regulation of maternal mRNA translation in the mouse oocyte. The m^7G cap is present on the majority ($\geq 80\%$) of mRNA molecules from both unfertilized and fertilized mouse eggs, and nearly all mRNA extracted from unfertilized mouse eggs are translated *in vitro* and sensitive to inhibition by m^7GTP (Schultz *et al.* 1980). In addition, mRNA decapping via maternally recruited DCP1A and DCP2 is involved in the degradation of maternal transcripts during maturation and proper genome activation in mouse (Ma *et al.* 2013). Thus, the cap-binding protein EIF4E and its

Figure 6 Summary of immunofluorescence results. Illustration of phosphorylated EIF4EBP1 localization in GV-intact oocytes and MII oocytes. Shaded circles in GV and solid circles in MII represent cortical granules.

binding partner EIF4EBP1 are important for the recruitment and translation of maternal mRNAs during maturation and early development.

We note that EIF4EBP1 null mice are viable and fertile, but display selective effects in tissues where the ratio of EIF4EBP1 to other EIF4EBP orthologs is highest, as well as hypoglycemia, reduced fat deposition, and increased metabolic rates (Tsukiyama-Kohara *et al.* 2001). The potential roles for other EIF4EBP orthologs in compensating for an absence of EIF4EBP1 in oocytes remains to be evaluated.

Defective regulation of EIF4EBP1 may contribute to diminished oocyte quality. Age-related increases in oocyte aneuploidy are accompanied by defects in the meiotic spindle (Chiang *et al.* 2012; Nagaoka *et al.* 2012) and aberrant regulation of maternal mRNA (Pan *et al.* 2008). Oocytes from diabetic mice also display meiotic defects, including chromosome misalignment and spindle abnormalities, which can be reversed by islet transplantation (Cheng *et al.* 2011). Insulin signaling may promote the production of high-quality oocytes (Wang and Moley 2010) via mTOR-mediated phosphorylation of EIF4EBP1. Future studies to determine the mechanisms by which insulin regulates meiosis should add to our mechanistic understanding of how dysregulated insulin signaling might affect oocyte quality and developmental competence. Our discovery of localized maternal mRNAs and phosphorylated EIF4EBP1 at the spindle also provide renewed incentive for dissecting the mechanisms that link maternal age, genotype, and environmental exposures to diminished oocyte quality arising out of defective spindle formation and function.

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GENETICS

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Association of Maternal mRNA and Phosphorylated EIF4EBP1 Variants With the Spindle in Mouse Oocytes: Localized Translational Control Supporting Female Meiosis in Mammals

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Table S1. Probesets for mRNAs showing increased abundance in the spindle-chromosome complex

Probe Set ID	Gene Symbol	Gene name	SCC/EN	MI/EN	EN Avg. Intensity	MI Avg. Intensity	SCC Avg. Intensity	Gene Function	Cytoplasmic sequestration	Vesicle/endocytosis/transport	Plasma membrane	Spindle/cyto-skeleton	Chromatin/nuclear	RNA binding	Golgi	Signaling	Other	Mitochondrial	Translation	Protein degradation/biotinylation
Probesets with maximum raw intensity values 1000 or greater																				
1423256_a_at	Atp6v1g1	ATPase, H ⁺ transporting, lysosomal V1 subunit G1	3.65	1.30	536.65	697.46	1961.11	hydrogen-exporting ATPase activity				1								
1439648_at	Anln	Anillin	3.57	1.30	677.13	878.95	2419.65	actin binding, cytokinesis					1							
1453314_x_at	Mis18a	MIS18 kinetochore protein homolog A	3.55	1.54	807.05	1239.05	2864.96	mitosis					1							
1460856_a_at	SFT2d1	SFT2 domain containing 1	3.47	1.37	771.32	1058.16	2675.60	vesicle transport		1										
1433537_at	Atrx	Alpha thalassemia/mental retardation syndrome X-linked homolog	3.30	1.18	793.15	936.99	2619.49	chromatin binding, DNA repair					1							
1451154_a_at	Celf2 (Cuxbp2)	CUGBP, Elav-like family member 2	3.26	1.22	276.54	338.23	902.71	mRNA splice site selection						1						
1455402_at	Socs7	Suppressor of cytokine signaling 7	3.13	1.27	643.04	816.07	2012.06	cytokine signaling					1				1			
1428374_at	Glice	Glucuronyl C5-epimerase	3.12	1.30	818.18	1064.18	2549.11	glycosaminoglycan synthesis, Golgi								1				
1433514_at	Enk1	Ethanolamine kinase 1	3.10	1.36	313.79	426.38	972.68	lipid metabolism									1			
1436900_x_at	Leprpt	Leptin receptor overlapping transcript	3.03	1.27	635.13	808.05	1924.36	receptor		1										
1422241_a_at	Ndufa1	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 1	2.97	1.33	539.85	716.28	1605.93	Mitochondrial function										1		
1415829_at	Lbr	Lamin B receptor	2.94	1.30	1155.51	1500.91	3399.25	DNA binding					1							
1439257_x_at	Rpn1	Ribophornin 1	2.92	1.32	455.37	599.81	1329.02	protein glycosylation, ER component		1										
1456438_x_at			2.85	1.29	382.77	494.32	1091.87													
1438943_x_at			2.77	1.27	309.50	391.77	858.46													
1422449_s_at	Rcn2	Reticulocalbin 2	2.89	1.23	398.79	491.30	1154.39	calcium ion binding, ER		1										
1424799_at	Eif1	Eukaryotic translation initiation factor 1	2.86	1.30	1122.96	1464.08	3213.92	translation initiation												
1455286_at	Kif5c	Kinesin family member 5C	2.80	1.17	390.84	457.84	1095.94	motor activity					1							
1449731_s_at	Nkfbia	Nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, alpha	2.76	1.20	370.54	446.16	1024.26	cytoplasmic sequestration of transcription factor; interacts with Chuk in NF-kappa-B signaling pathway	1								1			1
1449039_a_at	Hnrndll	Heterogeneous nuclear ribonucleoprotein D-like	2.73	1.29	308.75	397.95	841.55	mRNA metabolism, DNA binding												
1428103_at	Adam10	A disintegrin and metalloprotease domain 10	2.71	1.30	623.14	809.22	1698.66	endopeptidase												
1454612_at	Mex3c	Mex3 homolog C	2.65	1.38	406.78	562.76	1076.61	RNA binding, binds ARE												
1452176_at	Nup153	Nucleoporin 153	2.55	1.35	870.39	1171.70	2217.51	negative regulation of RNA export						1	1	1				
1436451_a_at	Tmed2	Transmembrane emp24 domain trafficking protein 2	2.53	1.49	2195.06	3278.57	5554.91	Golgi organization												
1436452_x_at			2.42	1.44	1535.81	2209.19	3722.76													
1455968_x_at			2.37	1.43	2555.28	3644.35	8065.00													
1422880_at	Sypl	Synaptophysin-like protein	2.52	1.32	1359.61	1797.84	3425.04	Transport, cytoplasmic vesicle												
1423390_at	Siah1a	Seven in absentia 1A	2.50	1.35	390.18	525.69	974.01	ubiquitin-protein ligase activity												
1424807_a_at	Calm2	Calmodulin 2	2.49	1.33	418.69	555.71	1043.84	calcium signaling activation of adenylate cyclase		1										
1428272_at	Eif1b	Eukaryotic translation initiation factor 1B	2.47	1.18	1008.29	1189.08	2495.81	translation initiation												
1448261_at	Cdh1	Cadherin 1	2.46	1.32	1214.31	1598.34	2984.59	calcium ion binding, cell adhesion, actin cytoskeleton				1	1							
1445684_s_at	Hdac2	Histone deacetylase 2	2.45	1.40	631.12	883.27	1544.70	chromatin modification, transcription repression												
1435122_x_at	Dnmt1	DNA methyltransferase (cytosine-5) 1	2.43	1.24	1066.93	1319.78	2592.76	DNA methylation												
1421183_at	Tex12	Testis expressed gene 12	2.43	1.34	482.07	647.67	1169.62	synaptonemal complex												
1422669_at	Ebag9	Estrogen receptor-binding fragment-associated gene 9	2.42	1.42	380.20	541.24	921.66	apoptotic process, Golgi												
1429043_at	Smnnc1	Survival motor neuron domain containing 1	2.42	1.30	318.83	413.63	772.82	mRNA processing												
1416509_at	Tm9sf3	Transmembrane 9 superfamily member 3	2.39	1.23	661.95	811.08	1579.76	membrane binding												
1433887_at	Onsac3	Onsac (nsp60) homolog, subfamily C, member 3	2.38	1.38	746.77	1032.60	1776.20	Protein kinase inhibitor, ER unfolded protein response	1	1										
1433944_at	Hectd2	HECT domain containing 2	2.38	1.45	410.49	595.04	976.23	E3 ubiquitin protein ligase												
1433857_at	Fat1	FAT tumor suppressor homolog 1	2.38	1.21	869.94	1052.98	2067.54	actin filament organization, cell adhesion				1	1							
1424002_at	Pdcd3	Phosducin-like 3	2.36	1.29	501.23	646.18	1184.99	apoptosis inhibition		1										
1455111_at	Yip6f	Yip1 domain family, member 6	2.35	1.28	1169.49	1495.97	2753.37	ER to Golgi transport vesicle		1	1									
1426776_at	Wsl	Wiskott-Aldrich syndrome-like	2.34	1.33	741.88	985.47	1733.93	actin cytoskeleton organization												
1417538_at	Slc35a1	Solute carrier family 35 (CMP-sialic acid transporter), member 1	2.32	1.28	447.29	571.42	1039.62	nucleotide-sugar transport, Golgi												
1422462_at	Ube2t	Ubiquitin-conjugating enzyme E2T (putative)	2.32	1.26	673.19	846.30	1561.25	Ubiquitin ligation, DNA repair												
1455724_at	Pm1	Proline rich G1a (C-carboxyglutamic acid) 1	2.30	1.1	828.30	928.59	1910.04	calcium ion binding, possible p53 target												
1437313_x_at	Hmgb2	High mobility group box 2	2.30	1.36	307.43	416.88	705.60	DNA binding, chromatin structure, base excision repair					1	1						
1427221_at	Slc6a20a	Solute carrier family 6 (neurotransmitter transporter), member 20A	2.29	1.32	370.14	490.18	847.33	amino acid transmembrane transport												
1434394_at	N4bp2	NEDD4 binding protein 2	2.28	1.35	1514.79	2049.51	3457.59	5'-polynucleotide kinase and nicking endonuclease activity, DNA repair	1											
1434901_at	Zbtb2	Zinc finger and BTB domain containing 2	2.27	1.23	374.44	460.11	849.54	nucleic acid binding												
1434310_at	Bmpr2	Bone morphogenetic protein receptor, type II (serine/threonine kinase)	2.26	1.23	555.29	683.42	1253.29	ATP and growth factor binding												
1459833_a_at	Rnaseh1	Ribonuclease H1	2.26	1.33	476.64	633.69	1075.63	DNA replication												
1434856_at	Ankrd44	Ankyrin repeat domain 44	2.25	1.18	668.21	787.77	1506.51	Possible binding to protein phosphatase 6												
1434030_at	Gtpbp10	GTP-binding protein 10 (putative)	2.25	1.26	441.24	557.11	993.95	Ribosome biogenesis and nuclear function												
							SUM		4	5	6	7	8	17	9	6	13	3	1	4
Probesets satisfying one of two criteria for inclusion at the level of two-fold enrichment																				
1423648_at	Pdia6	Protein disulfide isomerase associated 6	2.61	1.07	349.32	373.30	911.19	cell redox homeostasis, ER-Golgi intermediate compartment protein disulfide isomerase activity		1										
1433834_at	March6	Membrane-associated ring finger (C3HC4) 6	2.52	1.11	2264.01	2512.22	5698.12	ligase activity, zinc ion binding												
1427275_at	Smc4	Structural maintenance of chromosomes 4	2.34	0.97	1346.88	1310.31	3152.97	chromosome, condensin complex												
Probesets with maximum raw intensity values of 500-999																				
1448937_at	Slc35b3	Solute carrier family 35, member B3	4.16	1.41	122.83	172.62	511.56	Translocate PAPS from cytosol/nucleus to Golgi lumen		1										
1434161_at	Lin52	Lin-52 homolog (C. elegans)	3.26	1.38	219.20	302.16	713.71	Component of DREAM complex; repress cell cycle-dependent genes												
1424438_a_at	Leprpt	Leptin receptor overlapping transcript	3.15	1.30	149.40	194.26	469.90	Regulates cell surface expression of leptin and growth hormone receptors												
1448148_at	Gm	Granulin	3.04	1.24	141.20	174.66	429.00	blastocyst hatching, embryo implantation, signaling												
1423247_at	Erp44	Endoplasmic reticulum protein 44	2.93	1.26	175.89	221.82	515.41	Oxidative folding in ER		1										
1428603_at	Glo1	Glucocorticoid induced transcript 1	2.89	1.40	284.80	397.59	822.93	Early marker for glucocorticoid-induced apoptosis												
1436499_at	Sgms1	Sphingomyelin synthase 1	2.84	1.47	171.73	251.69	487.76	Synthesize sphingomyelin, cell growth and prevention of cell death												
1418072_at	Hist1h2bc	Histone cluster 1, H2bc	2.71	1.35	209.58	281.93	568.47	Nucleosome component												
1438324_at	R330182L06Rik	RIKEN cDNA R330182L06 gene	2.70	1.30	142.03	184.86	383.48	BMP signaling pathway component; differentiation												
1418327_at	R110058L19Rik	RIKEN cDNA R110058L19 gene	2.66	1.28	204.82	261.65	545.59	Unknown function												
1420106_at	Siah1a	Seven in absentia 1A	2.66	1.30	111.72	144.81	296.81	E3 ubiquitin protein ligase												
1452053_a_at	Tmem33	Transmembrane protein 33	2.62	1.44	199.12	287.68	522.07	Membrane protein, possible DNA damage signaling												
1455372_at	Cpeb3	Cytoplasmic polyadenylation element binding protein 3	2.61	1.38	169.67	234.62	442.83	Binds CPEs in mRNA 3'UTR to regulate translation												
1438839_a_at	Ywhae	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide	2.57	1.35	232.24	313.48	597.45	Phosphoserine/threonine-binding signaling protein of 14-3-3 pathway; regulates translation during mitosis		1										
1423215_at	Spcs2	Signal peptidase complex subunit 2 homolog (S. cerevisiae)	2.52	1.27	228.89	289.99	577.88	Remove signal peptides from nascent proteins in ER as part of microsomal complex			1									
1415943_at	Sdc1	Syndecan 1	2.51	1.28	209.63	269.14	525.99	Cell surface proteoglycan that connects surface to cytoskeleton												

[illegible]

Abbreviations: SCC, spindle chromosome complex; EN "enucleated" or SCC-depleted cytoplast; MII intact MII oocyte.