Thioredoxin-Interacting Protein Regulates Glucose Metabolism and Affects Cytoplasmic Streaming in Mouse Oocytes

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Abstract

Thioredoxin-interacting protein (Txnip) regulates intracellular redox state and prompts oxidative stress by binding to and inhibiting Thioredoxin (Trx). In addition, via a Trx-independent mechanism, Txnip regulates glucose metabolism and thus maintains intracellular glucose levels. Previously, we found Txnip mRNA highly expressed in immature germinal vesicle (GV) oocytes, but currently there is no report describing the role of Txnip in oocytes. Therefore, we conducted the present study to determine the function of Txnip in mouse oocytes’ maturation and meiosis by using RNA interference (RNAi) method. Upon specific depletion of Txnip, 79.5% of oocytes were arrested at metaphase I (MI) stage. Time-lapse video microscopy analysis revealed that the formation of granules in the oocyte cytoplasm increased concurrent with retarded cytoplasmic streaming after Txnip RNAi treatment. Txnip RNAi-treated oocytes had upregulated glucose uptake and lactate production. To confirm the supposition that mechanism responsible for these observed phenomena involves increased lactate in oocytes, we cultured oocytes in high lactate medium and observed the same increased granule formation and retarded cytoplasmic streaming as found by Txnip RNAi. The MII-arrested oocytes exhibited scattered microtubules and aggregated chromosomes indicating that actin networking was disturbed by Txnip RNAi. Therefore, we conclude that Txnip is a critical regulator of glucose metabolism in oocytes and is involved in maintaining cytoplasmic streaming in mouse oocytes.

Introduction

In female mammals, oocytes undergo meiotic division and are arrested at meiotic prophase I, referred to as the germinal vesicle (GV) stage, in primordial follicles [1]. Under the influence of a luteinizing hormone surge, meiotic maturation restarts shortly before ovulation following the onset of puberty [2]. Meiotic maturation is comprised of meiosis I and meiosis II [3]. In meiosis I, oocytes undergo GV breakdown (GVB) and progress to metaphase I (MI) [4]. Without an intervening S phase, oocytes enter meiosis II and progress to metaphase II (MII) until the first polar body extrudes. Oocytes are arrested then again at the MII stage by cytosstatic factor until fertilization [5].

In a previous study, we identified differentially expressed mRNAs between the GV and MII stages of mouse oocytes using annealing control primer-PCR [6]. Among differentially expressed genes, we found that Thioredoxin interacting protein (Txnip) mRNA highly expressed in GV oocytes compared to MI oocytes. Txnip, also known as Vitamin D3 upregulated protein 1 (VDUP1) and Thioredoxin binding protein 2 (Tbp2), was primarily identified as an inhibitor of Thioredoxin (Trx) [7–9] and TXNIP forms a mixed disulfide bond with TRX by disulfide exchange at catalytic active sites and inhibits its activity [10]. TRX is a critical protein conveying electrons from NADPH to protein disulfide bonds [11]. Thus, the interaction between TRX and TXNIP maintains the intracellular reducing environment [10].

In addition to TXNIP binding TRX, there are substantial evidences suggesting that Txnip has a major role in regulating glucose metabolism independent of Trx [12–14]. The Txnip-knockout mice exhibit problems in glucose and lipid metabolism such as low blood glucose, hyperlipidemia, and dysregulated response to fasting [15–18]. However, there is no report regarding the function of Txnip in the mouse oocytes. Therefore, the aims of the present study were the characterization of the expression of Txnip in mouse oocytes and the elucidation of functions of Txnip in oocytes.

Materials and Methods

Animals

All imprinting control region (ICR) mice were obtained from Koatech (Pyeoungtack, Korea) and maintained in the breeding facility at the CHA Stem Cell Institute of CHA University. All procedures described within this study were reviewed and
approved by the Institutional Animal Care and Use Committee of CHA University and performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals.

Collection of oocytes and follicular cells

For collection of GV oocytes from preovulatory follicles, 3-week-old female ICR mice were treated with 5 IU pregnant mare’s serum gonadotropin (PMSG; Sigma-Aldrich, St. Louis, MO, USA) and then sacrificed 46 hours later. Cumulus-enclosed GV oocytes (COGs) were recovered from ovaries by puncturing the preovulatory follicles with needles. Lactate-free M2 containing 0.2 mM 3-isobutylyl-1-methyl-xanthine (IBMX; Sigma-Aldrich) was used to inhibit GVBD. Cumulus cells (CCs) were removed from COCs mechanically by aspiration with a fine-bore pipette. Mural granulosa cells (GCS) were recovered from preovulatory follicles.

To obtain MI oocytes, female mice were treated with 5 IU PMSG; followed by 5 IU human chorionic gonadotropin (hCG) after 46 hours. Supernovulated MI oocytes were obtained from the oviduct 16 hours after hCG injection. CCs surrounding MI oocytes were removed by treating COCs with hyaluronidase (300 U/ml, Sigma-Aldrich).

Preparation of Txnip dsRNA and microinjection

Txnip-A primers were used to amplify a region of Txnip cdNA, which then was cloned into pGEM-T Easy vector (Promega, Madison, WI, USA) and linearized with SpeI. RNA was synthesized using the MEGAscript RNAi Kit (Ambion, Austin, TX, USA) and T7 RNA polymerase. Single-stranded sense and anti-sense transcripts were mixed and incubated at 75°C for 5 minutes then cooled to room temperature. To remove contaminating single-stranded cRNA and DNA in the dsRNA samples, the preparation was treated with RNase (Ambion) and Dnase (Ambion), respectively, for 1 hour at 37°C. Formation of dsRNA was confirmed by 1% agarose gel electrophoresis. For microinjection, RNAs were diluted to a final concentration of 3.5 μg/μl.

Approximately 10 pl of dsRNA was microinjected into each GV oocyte cytoplasm in lactate-free M2 medium containing 0.2 mM IBMX using a constant-flow system (Femtojet; Eppendorf, Hamburg, Germany). Buffer-injected oocytes were used as a sham control to assess injection damage.

In vitro maturation of oocytes

Microinjected GV oocytes were cultured in lactate-free M16 medium containing 0.2 mM IBMX for 8 hours for degradation of target transcripts followed by culture in M16 medium for 16 hours in 5% CO2 at 37°C to determine the rate of maturation in vitro. Oocytes without GV’s or polar bodies were scored as MI. Emission of the first polar body was used as an indicator of progression to MI. When high levels of lactate were used to treat control oocytes, five times of lactate was added to M16 medium (Sigma-Aldrich).

Droplet culture for lactate production assay

To evaluate subtle changes in lactate concentration, we used lactate-free medium for culture. For droplet culture, 250 Txnip RNAi-treated oocytes and 250 control oocytes were placed in 20 μl droplets of lactate-free M16 and incubated for 16 hours under mineral oil in 5% CO2 at 37°C. Droplets and oocytes were removed and oocytes were mixed with lactate assay buffer. The culture medium and oocyte lysates were stored at −80°C until analysis. Changes in lactate concentration after Txnip RNAi treatment was measured by using a lactate colorimetric assay according to the manufacturer’s instructions (Eton Bioscience, San Diego, CA, USA). Lactate concentrations were calculated from the standard curve.

Evaluation of glucose uptake

A fluorescent glucose analogue, 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG; Molecular Probes, Eugene, OR, USA), was used to monitor glucose uptake during oocyte maturation. Oocytes from each group were incubated in M16 medium containing 200 μM NBDG for 20 minutes in 5% CO2 and 37°C. After thorough washing with M16 medium, oocytes were imaged at 488 nm by time lapse video microscopy according to the methods described in method of time lapse microscopy.

Messenger RNA isolation, RT-PCR analysis and quantitative real-time RT-PCR

Messenger RNA was isolated from oocytes using the Dynabeads mRNA DIRECT kit (Dynal Asa, Oslo, Norway), according to the manufacturer’s instructions. Briefly, oocytes were resuspended in 300 μl lysis/binding buffer and mixed with 20 μl prewashed Dynabeads oligo dT25 for annealing for 5 minutes at room temperature. After the beads were separated with a Dynal MPC-S magnetic particle concentrator and washed with buffer A twice followed by buffer B, poly(A)+ RNAs were eluted by incubation in 13 μl 10 mM Tris-HCl (pH 7.5) at 73°C for 2 minutes.

Purified mRNA and a 0.5 μg oligo (dT) primer were mixed and incubated at 70°C for 10 minutes, and cDNA was synthesized with Reverse transcriptase and RNase inhibitor (Promega). Single oocyte-equivalent cDNA was used as the template for PCR analysis. Primer sequences for the genes used in this study and its PCR conditions are listed in Table 1. The mouse Txnip gene has two variants, so we selected conserved sequences of these variants for primer design. PCR products were electrophoresed on a 1.5% agarose gel followed by analysis using an image analyzer (Gel Logic 112; Kodak, Rochester, NY, USA). For the comparison of the level of Txnip mRNA expression with that of the other genes, quantitative real-time RT-PCR was performed as previously described [19].

Western blot analysis

Protein extract (50 oocytes per lane) was separated using 12% SDS-PAGE and transferred onto a poly(vinylidene difluoride) membrane (Bio-Rad Laboratories, Hercules, CA, USA). The membrane was blocked for 1 hour in Tris-buffered saline-Tween (TBS-T) containing 5% skim milk and then incubated with mouse monoclonal anti-TXNIP antibody (1:400; MBL, Nagoya, Japan) or rabbit monoclonal anti-α-Tubulin antibody (1:1000; Cell Signaling Technology, Danvers, MA, USA) in TBS-T. After incubation, membranes were incubated with horseradish peroxidase-conjugated anti-mouse IgG or anti-rabbit IgG (1:1000; Sigma-Aldrich) in TBS-T for 1 hour at room temperature. After each step, the membranes were washed three times with TBS-T,
and bound antibody was detected using an enhanced chemiluminescence (ECL) detection system (Amersham Biosciences, Piscataway, NJ, USA) according to the manufacturer’s instructions. Protein levels were quantified by measuring the density of area for each band using Image J software (NIH). These values were then normalized to that of α-Tubulin and were expressed as a percentage of control oocytes.

**Immunofluorescence staining**

Oocytes were fixed in PFA solution (4% paraformaldehyde and 0.2% Triton X-100) for 40 minutes at room temperature, washed three times in PVA-PBS for 10 minutes, and then stored overnight in 1% BSA-supplemented PVA-PBS (BSA-PVA-PBS) at 4°C. The oocytes were blocked with 3% BSA-PVA-PBS for 1 hour and incubated in 1% BSA-PVA-PBS containing mouse monoclonal α-Tubulin antibody (1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4°C overnight. After washing three times in PVA-PBS, oocytes were incubated for 1 hour at room temperature with a second antibody diluted 1:100 (fluorescein isothiocyanate-conjugated counterstained anti-mouse IgG; Sigma-Aldrich) in 1% BSA-PVA-PBS. After washing three times, they were incubated in a propidium iodide (PI) solution (1 mg/ml; Sigma-Aldrich) for 30 minutes to counterstain DNA, and then mounted between a microscope slide and a clean coverslip.

**Statistical analysis**

Each experiment was repeated at least three times. Data were presented as mean ± SEM derived from at least three separate and independent experiments and were evaluated using one-way analysis of variance (ANOVA) and a log linear model. A p-value <0.05 was considered as statistically significant.

**Results**

**Expression of Tnip during oocyte maturation**

During oocyte maturation process, Tnip mRNA expression was higher in GV, GVBD, and MI than in MII oocytes (Fig. 1A). Likewise, a higher level of endogenous TXNIP protein expression was detected at the GV stage compared to the MII stage (Fig. 1B). Notably, cycle threshold (CT) value from quantitative real-time RT-PCR ranged from 21 to 23 indicating that Tnip transcripts are extremely abundant in GV oocytes compared to other genes previously studied in our laboratory such as Sebox [19], Bcl2l10 [20], and Obox4 [21], which had CT values of 24, 26, and 29, respectively (Fig. 1B). Interestingly, TXNIP protein expression was barely present at the GVBD and MI stages although Tnip mRNA was still highly expressed in both stages, at a level similar to that of α-Tubulin and were expressed as a percentage of control oocytes.

| Genes   | Accession no. | Primer sequences a | Annealing temperature | Product size |
|---------|---------------|-------------------|-----------------------|--------------|
| Tnip-a  | NM_001009935.2 | F: CAGCCAACTCAAGGCGCAAA R: ATTTGCCAGGTCTCTGGTCT | 60°C | 506 bp |
| Tnip-b  | NM_001009935.2 | F: TGCTCCAAAGAAGTCATCC R: TTAGAGTGGTGGCCTACAT | 60°C | 229 bp |
| Sebox   | NM_008759     | F: AAAGCCAGGAGCCCTAAACT R: TTAGAGTGGTGGCCTACAT | 60°C | 334 bp |
| Bcl2l10 | AF067660      | F: CTCTGTGACTAGGCAGATCG R: GAAGTGGCAGTCATGCTC | 60°C | 551 bp |
| Obox4   | AF619109      | F: CCCTCTTCATGTGGCCTAGCT R: GCCAGGTCCAATCTCAAAGA | 60°C | 240 bp |
| Glut1   | NM_011400.3   | F: AAGAAGAGGGCTGCGCATG R: ACAGCGACCACAGCTCAGA | 60°C | 244 bp |
| Glut2   | NM_031197.2   | F: GCCGTGTGACTAGGCACATTG R: GAAGTGGCAGTCATGCTC | 60°C | 205 bp |
| Glut3   | NM_011401.4   | F: GAACCGACTATGCGGACAT R: GCCAGGTCCAATCTCAAAGA | 60°C | 240 bp |
| Glut4   | NM_009204.2   | F: CAACAGCTCTAGGCATCAA R: GCTAAGAGGACCGCAAAAGC | 60°C | 297 bp |
| Glut5   | NM_019741.3   | F: TCTCCGTGAGAAGCTCCTCTC R: CCCAAAGGCTCTACACACAA | 60°C | 201 bp |
| Glut6   | NM_00177627.1 | F: GAGGTCCTAGGAGGAGTGGTGA R: AATACACTGCTGACCACTGGT | 60°C | 228 bp |
| Glut7   | NM_00108529.1 | F: GATTTCTTCGCTCGTCGGCTAT R: GATGGATGGAAAGGCTGCTC | 60°C | 274 bp |
| Glut8   | NM_0019488.4  | F: TTTCTCTCATGTTCTCCAGAR R: AGGCTGCTGTCATGCTC | 60°C | 256 bp |
| Glut9   | NM_001012363 | F: GAATGCCACATTGCTGGTCA R: TGCAAGTAAAGGATGGCAGT | 60°C | 221 bp |
| Glut10  | NM_130451.2   | F: GGTGCGCTTGGAGGACATG R: TAGATGAGGACCGAGGCAA | 60°C | 210 bp |
| Glut11  | NM_178934.4   | F: AGACGACGGCTCTGTATG R: TGACACCCAGTTCATGCTA | 60°C | 294 bp |
| Glut12  | NM_00103633.3 | F: GTCATAAGGTCGATGTCGA R: CTGACACCCAGTTCATGCTA | 60°C | 278 bp |
| Fshr    | NM_031523     | F: TCTCTATGAGGACCTAGGTT R: AGAGGCTTCCGACCAACAT | 60°C | 165 bp |
| Gdf9    | NM_008610     | F: GTGTCCTAATGATGGCGGAG R: GGTTGAAGGGAGGAGGAGG | 60°C | 446 bp |
| Gapdh   | BC092294      | F: ACCACATCCATGCACTAC R: TCAACACCCAGTTCATGCTA | 60°C | 451 bp |
| Hif1oo  | NM_008872     | F: GCCAACCAGAAAGGAGTCCAGAA R: TGGAGGAAGTTCTGGAGAAGTAA | 60°C | 378 bp |

*aF, forward; R, reverse.

Tnip-a primers were used for preparation of dsRNA.

Tnip-b primers were used for confirmation target mRNA knockdown after RNAi treatment.

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**Table 1. Sequences of primers used in this study and RT-PCR conditions.**
complete *Txnip* mRNA degradation was verified by 8 hours after microinjection of *Txnip* dsRNA (Fig. 2A). To confirm depletion of TXNIP protein expression, Western blot analysis was performed, and results showed that TXNIP protein level decreased markedly with *Txnip* RNAi treatment (Fig. 2B). The residual TXNIP protein found in Fig. 2B is thought as an endogenous protein translated before *Txnip* RNAi was performed. These results suggest that *Txnip* RNAi caused sequence-specific suppression of *Txnip* expression. The maturation rate of *Txnip* RNAi-treated GV oocytes to MII stage (5.3%) significantly decreased compared with that of oocytes in control (81.25%) or buffer-injected (76.3%) groups. Most of the oocytes were arrested at the MI stage (79.5%) after *Txnip* RNAi (Table 2).

**Granule formation with retarded cytoplasmic streaming during in vitro maturation.** While control oocytes released the first polar body to complete meiosis within 16 hours (Fig. 3A), *Txnip* RNAi-treated oocytes were arrested at the MI stage (Fig. 3B). During in vitro maturation after *Txnip* RNAi treatment, enormous

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**Figure 1. Expression of *Txnip* during oocyte maturation.** (A) Expression of *Txnip* mRNA during oocyte maturation. For quantitative real-time RT-PCR, cDNA equivalent to a single oocyte was used as a template for amplification. GV, GVBD, MI, and MII oocytes were obtained after in vitro culture for 0, 2, 8, and 16 hours, respectively. Expression levels were calculated from C_T values after normalization with H1foo mRNA. Experiments were repeated at least three times, and data are expressed as mean ± SEM. Asterisk represents statistically significant differences compared with GV oocytes (*p*<0.05). (B) Comparison of C_T value of *Txnip* with that of other genes studied in our laboratory (*Sebox*, *Bcl2l10*, and *Obox4*). (C) Expression of TXNIP protein during oocyte maturation. Proteins were extracted from 50 mouse oocytes at each stage. α-Tubulin was used as a loading control. doi:10.1371/journal.pone.0070708.g001
amount of intracellular granules were formed in the cytoplasm of oocytes (Fig. 3B). In addition, we observed that the cytoplasmic streaming was retarded in Txnip RNAi-treated oocytes compared to the fast cytoplasmic streaming found in the control oocytes (Video S1 and Video S2; Control oocytes and Txnip RNAi-treated oocyte, respectively).

**Affected spindle and chromosomal organization.** While control MI oocytes showed normal, barrel-shaped spindles and well-aligned chromosomes at the metaphase plate during oocyte maturation, the Txnip RNAi-treated oocytes exhibited condensed chromosomes in one mass with scattered tubulin dots throughout the cytoplasm (Fig. 4).

**Increased lactate production with glucose uptake.** Because Txnip was known as a negative regulator of lactate production [14], we measured the lactate production during *in vitro* maturation. As we expected, the concentration of lactate was significantly increased by 39.5% in Txnip RNAi-treated oocytes (Fig. 5). Because of increased lactate production by Txnip RNAi treatment, we presumed that glucose uptake into oocytes were also upregulated by Txnip RNAi. Following sufficient time (8 hours) for Txnip knockdown in IBMX-supplemented M16 medium, we directly visualized glucose uptake using a non-metabolizable fluorescent glucose analogue, 2-NBDG [22,23]. Within the first 5 minutes rapid NBDG uptake occurred and accumulated maximally after 15–20 minutes. As soon as oocytes were transferred to the M16 medium, Txnip RNAI-treated oocytes showed twice as strong fluorescence intensity compared with control oocytes as depicted in Figure 6. These results suggested that glucose uptake into oocytes increased during degradation of Txnip mRNA in IBMX-supplemented medium.

It has long been reported and accepted that the oocytes are deficient in their ability to utilize the glucose thus require the surrounding CCs and GCs to utilize the glucose [22,24]. According to the contradiction between this concept and our results in Figure 6, we decided to evaluate the existence of glucose transporters (*Gluts*) in the follicular cells including oocyte itself. As depicted in Figure 7, CCs and GCs express most of Glut family members. Expression of Glut 2 and 7 was not or weakly detected in CCs and GCs, respectively. In addition, we observed that oocytes also express substantial level of Glut 1, 10, and 12. Therefore, we concluded that glucose uptake can occur in oocytes that may be not actively used in nature, and the increased glucose and lactate in the oocyte cytoplasm after Txnip RNAi may induce the gluconeogenic pathway in oocytes and those intracellular granules may indicative of that.

**Effect of high lactate treatment was comparable to Txnip RNAi treatment**

We investigated how the high concentration of lactate affects oocyte maturation. The addition of five folds higher lactate to the culture medium resulted in a remarkable reduction of cytoplasmic streaming compared to the control oocytes with increased formation of small granules in the oocyte cytoplasm (Video S3, Fig. 6, column of High lactate). As we presumed, the glucose uptake increased and that was similar to the phenomena found in Txnip RNAi-treated oocytes.

**Discussion**

In the present study, we firstly report the expression and function of Txnip in mouse oocytes. Txnip RNAi treatment increased oocytes’ glucose uptake and lactate production and resulted in retarded cytoplasmic streaming with intracellular granule formation, abnormal spindle assembly, and chromosome arrangement.

Both abundant mRNAs and proteins are accumulated in the oocytes during oocyte development. Wang and colleagues reported a number of differentially expressed proteins among

### Functional Analysis of Txnip in Mouse Oocytes

**Table 2.** Txnip RNAi-treated oocytes arrested at the MI stage during *in vitro* maturation.

| Treatment         | Number of oocytes (%) |
|-------------------|-----------------------|
|                   | Total | Germinal vesicle (GV) | Metaphase I (MI) | Metaphase II (MII) |
| Control           | 96    | 0 (0)                | 18 (18.75)       | 78 (81.25)          |
| Buffer-injected   | 97    | 0 (0)                | 23 (23.7)        | 74 (76.3)           |
| Txnip RNAi        | 132   | 20 (15.2)            | 105 (79.5)*      | 7 (5.3)*            |

*Values with statistical significance (*p*<0.05) compared to control or buffer-injected groups.

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the different stages of mouse oocytes [25]. They demonstrated that the abundant proteins in GV oocytes are mainly related to actin binding, the microfilament motor, primary transporters, and amino acid transporters. In addition, many metabolism-related proteins were expressed in GV oocytes for supporting oocyte maturation [25]. We found that \textit{Txnip} was highly expressed in GV oocytes than in MII oocytes in this study. Thus, we focused the investigation to the role of \textit{Txnip} in regulation of glucose metabolism during oocyte maturation.


txnip has been reported to inhibit glucose uptake and lactate production [14]. Based on these previous reports, we assumed that increased glucose uptake and lactate production by \textit{Txnip} RNAi would lead to the formation of glycogen granules in oocytes. To prove this hypothesis, we measured glucose uptake and lactate production after \textit{Txnip} RNAi, and as we anticipated, \textit{Txnip} RNAi-treated oocytes showed increased glucose uptake and lactate production compared with control oocytes. Thus, we concluded that the \textit{Txnip} RNAi treatment resulted in enhanced lactate production by increased glucose uptake and lactate production.

Figure 3. \textit{Txnip} RNAi treatment resulted in MI arrest and granule formation. Microphotographs of (A) control oocytes and (B) \textit{Txnip} RNAi-treated oocytes after \textit{in vitro} culture for 16 hours in M16 medium following 8 hours incubation in IBMX-supplemented M16 medium. Bars = 100 \textmu m. doi:10.1371/journal.pone.0070708.g003

Figure 4. Immunofluorescence staining of spindles and chromosomes. Spindles were stained with \textalpha-Tubulin antibody (green) and chromosomes were counterstained with propidium iodide (red). Control MI oocytes were cultured for 8 hours \textit{in vitro}. MI-arrested oocytes of \textit{Txnip} RNAi group showed aggregated chromosomes at the center of the oocytes and scattered dots of spindle. Bars = 10 \textmu m. doi:10.1371/journal.pone.0070708.g004

Figure 5. Lactate production was increased in \textit{Txnip} RNAi-treatment oocytes. Lactate production was measured by a lactate colorimetric assay kit after droplet culture in 20 \textmu l of M16 medium. The y-axis indicates the concentration of lactate from a total of 250 oocytes used for each group. Asterisk indicates statistically significant difference compared to the control (p<0.05). doi:10.1371/journal.pone.0070708.g005
glucose uptake and this state promoted the formation of intracellular granules.

We used denuded oocytes when we measure glucose uptake and lactate production, since we used them for microinjection of dsRNA. By using 2-NBDG, we identified glucose uptake in denuded oocytes suggesting that glucose can enter oocytes in the absence of CCs. In fact, we verified the expression of Glut family members in denuded oocytes as well as in follicular cells suggesting the oocytes’ ability of glucose uptake. It has long been known that oocytes are deficient in their ability to utilize glucose and require CCs to take up glucose via Gluts system and metabolize it into pyruvate which then is provided to oocytes [22,24]. According to our results, we verified that the oocytes has the machinery for glycolytic metabolism and can uptake glucose. The carrier-mediated uptake of pyruvate and glucose in human and mouse oocytes has been reported [30].

Our second question was why cytoplasmic streaming was retarded after Txinp RNAi treatment. Cytoplasmic streaming, also known as cyclosis, is the movement of cytoplasm which transports cytoplasmic nutrients, microscopic particles, and organelles toward different parts of the cell where they are needed [31]. Cytoplasmic streaming is driven by actin filaments in plant cells [32]. In C. elegans oogenesis, cytoplasmic streaming driven by the actomyosin cytoskeleton has been shown to play a role in oocyte enlargement and both microfilaments and microtubules migrate in the direction of actin movement [33,34]. Moreover, recent reports have suggested that actin movements induce cytoplasmic streaming in mouse oocytes [35,36].

Interactions between microtubules and actin filaments are notably important in oogenesis [37]. Cytoplasmic actins play a role in nuclear anchoring [38], meiotic spindle positioning that is required for asymmetric cell division [39], and chromosome assembly [40]. In the present study, although we did not directly observe actin movement, scattered dots of tubulin and aggregated chromosomes support the idea that actin flow was disturbed by Txinp RNAi treatment. Therefore, we concluded that the Txinp RNAi contributes to abnormal actin flow and retarded cytoplasmic streaming. We supposed that granules formed by Txinp RNAi treatment physically obstructed normal microtubule-actin filament movement. The results are summarized in Figure 8.

In conclusion, our findings demonstrated that Txinp is a critical regulator of glucose metabolism and regulates cytoplasmic streaming and meiotic maturation of the mouse oocytes. It is worth to note that our results provide the first evidence of Txinp expression and its role in mouse oocytes. Further study of molecular mechanisms involved in the function of Txinp would...
contribute towards elucidating the association between metabolic abnormalities and female fertility.

Supporting Information

Video S1 Time-lapse microscopy of control oocytes during in vitro maturation. (AVI)

Video S2 Time-lapse microscopy of Txnip RNAi-treated oocytes during in vitro maturation. The cytoplasmic streaming was retarded in Txnip RNAi-treated oocytes compared to the relatively fast cytoplasmic streaming found in the control oocytes (Video S1). (AVI)

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