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Stabilita mRNA a aktivita mikroRNA v myších oocytech

Messenger RNA stability and microRNA activity in mouse oocytes

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Souhrn

Přerod oocytu v zygotu představuje jediný fyziologický děj během životního cyklu savců, při kterém se z diferenciované buňky stává buňka pluripotentní. Toto buněčné přeprogramování je z velké většiny závislé na bezchybné post-transkripční regulaci maternálních mRNA. Porozumění mechanismům post-transkripční regulace v oocytech proto povede k významnému rozšíření našeho poznání v otázce buněčného přeprogramování. Mezi důležité post-transkripční regulátory v širokém spektru buněčných a vývojových procesů patří nedávno objevené krátké nekódující mikroRNA. Jejich funkce spočívá v represi cílených mRNA za pomoci proteinových komplexů, které spouštějí deadenylaci a odstranění ochranné čepičky z 5'-konce. Zmíněné komplexy se za normálních okolností sdružují v tzv. procesních tělískách (P-tělíska) v cytoplasmě. Tato práce přináší nečekané zjištění, že mikroRNA dráha je umlčená v plně dorostlých myších oocytech i během přerodu oocytu do zygoty. Toto zjištění je v souladu s pozorovaným rozpadem P-tělísek závislých na funkci mikroRNA během růstu oocytu a jejich absenci v plně dorostlých oocytech. Některé proteiny běžně obsažené v P-tělískách lokalizují v plně dorostlých oocytech do kortikální oblasti. Spolu s dalšími RNA-vazebnými faktory vytvářejí tyto proteiny ve finální fázi oocytárního růstu sub-kortikální domény, v nichž je uložena maternální mRNA. Dalším důležitým zjištěním je fakt, že součásti komplexu odpovědného za odstranění čepičky z 5'-konce mRNA jsou kódované umlčenými maternálními mRNA, které dávají vzniknout proteinům teprve během meiotického zrání oocytu. Aktivace tohoto komplexu během meiotického zrání přispívá k první vlně degradace maternálních mRNA. Uvedené výsledky významně přispívají k našemu chápání post-transkripční regulace maternálních mRNA, protože ozřejmují některé z mechanismů, jež mají podíl na udržování stabilního prostředí nutného pro akumulaci maternálních mRNA během oocytárního růstu a zároveň v průběhu meiotického zrání přepínají toto prostředí na degradační.

Abstract

The oocyte-to-zygote transition represents the only physiological event in mammalian life cycle, during which a differentiated cell is reprogrammed to become pluripotent. For its most part, the reprogramming relies on the accurate post-transcriptional control of maternally deposited mRNAs. Therefore, understanding the mechanisms of post-transcriptional regulation in the oocyte will help to improve our knowledge of cell reprogramming. Short non-coding microRNAs have recently emerged as an important class of post-transcriptional regulators in a wide range of cellular and developmental processes. MicroRNAs repress their mRNA targets via recruitment of deadenylation and decapping complexes, which typically accumulate in cytoplasmic Processing bodies (P-bodies). The presented work uncovers an unexpected feature of the microRNA pathway which is found to be suppressed in fully grown mouse oocytes and through the entire process of oocyte-to-zygote transition. This finding is consistent with the observation that microRNA-related P-bodies disassemble early during oocyte growth and are absent in fully grown oocytes. Some of the proteins normally associated with P-bodies localize to the oocyte cortex. At the final stage of oocyte growth, these proteins, together with other RNA-binding factors, form subcortical maternal mRNA storage domains. Furthermore, we find that components of the decapping complex are encoded by dormant maternal transcripts and their activation during meiotic maturation is a prerequisite for the initial wave of maternal mRNA clearance. Together, these data contribute to our understanding of maternal mRNA regulation by elucidating some of the mechanisms responsible for the maintenance of mRNA stabilizing environment during mouse oocyte growth and the switch to mRNA degradation during meiotic maturation.

1. Introduction

The oocyte-to-zygote transition (OZT), being the only naturally occurring cell reprogramming from a more differentiated state towards pluripotent state, represents a perfect model system for studying the molecular mechanisms that ensure proper and efficient establishment of pluripotency. Indeed, maternal factors are being identified that significantly improve reprogramming efficiency during the derivation of induced pluripotent stem cells from differentiated somatic cells using a defined set of transcription factors [1]. It is well documented that the driving force for reprogramming resides in the cytoplasm of a developmentally competent oocyte [2]. Therefore, dissecting the processes occurring in the ooplasm during OZT, including the post-transcriptional control of maternal mRNAs, will help complete the mosaic of cell reprogramming.

Overview of oocyte growth and oocyte-to-zygote transition

Oocytes are resting in the ovary arrested at the first meiotic prophase within primordial follicles. During every estrous cycle, a group of oocytes is recruited for growth and pass through primary, secondary, preantral and antral follicle stage to become fully-grown 'germinal vesicle' (GV; a designation of oocyte nucleus) oocytes capable of meiotic maturation. The final stage of oocyte growth before the resumption of meiosis is characterized by a large-scale chromatin rearrangement. For the most part of the growth phase chromosomes are dispersed in the nucleus with a large portion of chromatin being in the active euchromatin conformation. However, at the stage just prior to meiotic re-entry the entire chromatin content of the GV undergoes heterochromatinization, forming a densely packed heterochromatin ring around the oocyte nucleolus. Thereby, fully-grown GV oocytes are categorized based on their chromatin conformation as non-surrounded nucleolus (NSN) or surrounded nucleolus (SN) oocytes (reviewed in [3]). Changes occurring within the oocyte during this process are essential for the acquisition of developmental competence. Only eggs originating from SN oocytes can support further development, while the fertilized eggs of NSN origin arrest at the 2-cell stage [4]. The resumption of meiosis is triggered by a phosphodiesterase PDE3A-mediated decrease in cAMP levels, which can be blocked *in vitro* by PDE3A inhibitors, such as 3-isobutyl-1-methylxanthine (IBMX). During meiotic maturation, condensed chromosome segregation and asymmetric division of the cytoplasm occur, resulting in the first polar body extrusion. Meiosis arrests again at the metaphase II (MII) by the action of cytostatic factor complex containing the MOS kinase. Upon ovulation,

the MII egg is released from its follicle in order to be fertilized by sperm in the oviduct. Approximately 20 hours post-fertilization the first zygotic cleavage gives rise to two blastomeres of a 2-cell embryo. At this stage mouse zygotic genome is activated and the newly formed embryo discharges the maternal program and takes control of the further preimplantation development (reviewed in [5]).

The most part of mouse oocyte growth during folliculogenesis is characterized by significant transcriptional activity, resulting in a large maternal mRNA pool present in fully-grown GV oocytes. However, in the course of NSN to SN chromatin remodelling the oocytes become transcriptionally quiescent and the transcription remains shut down throughout OZT until the zygotic genome activation (ZGA) in the 2-cell embryo (reviewed in [6]). Therefore, an mRNA stabilizing environment needs to be created in the growing oocyte to enable maternal mRNA accumulation. In addition, a precise mechanism of post-transcriptional mRNA regulation ensuring timely translation and elimination of maternal transcripts is vital for correct orchestration of OZT.

Post-transcriptional regulation of maternal mRNAs

Cytoplasmic polyadenylation is the dominant mechanism of post-transcriptional maternal mRNA control during oocyte growth and meiotic maturation. It operates via two regulatory motifs in 3'UTRs of maternal mRNAs. A conserved polyadenylation hexanucleotide (Hex) AAUAAA is a common sequence motif in all mRNAs required also for nuclear polyadenylation. An additional cytoplasmic polyadenylation element (CPE) with consensus sequence UUUUUAU is present in the proximity of Hex motif in post-transcriptionally regulated transcripts. CPE is recognized by CPE binding protein (CPEB), which is currently considered a master regulator of cytoplasmic polyadenylation (reviewed in [7]). CPEB is found in oocytes within a large ribonucleoprotein (RNP) complex and plays a dual role in the regulation of maternal mRNAs during oogenesis. In growing oocytes, when maternal mRNAs are sythesized and stored in a translationally inactive state, CPEB associates with poly(A)-ribonuclease (PARN) and serves as a translation repressor. During meiotic maturation, CPEB is phosphorylated by Aurora A kinase, switching from a repressor to an activator function. Phosphorylation of CPEB impairs the interaction with PARN, which in turn leads to efficient polyadenylation of CPEB-bound mRNAs by a cytoplasmic poly(A) polymerase, and to translation activation (reviewed in [8]). In *Xenopus* oocytes, CPEB interacts with additional

RNA-binding proteins, for example the well characterized translational repressor Xp54 (DDX6 in mammals, CGH-1 in *C. elegans*) [9]. In addition to translation repression, recent data from *C. elegans* implicate CGH-1 also in the maintenance of maternal mRNA stability [10, 11]. During oogenesis, CGH-1 is found in RNP granules that are independent of the decapping complex, and therefore distinct from somatic Processing bodies(see below). A subset of maternal mRNAs associated with CGH-1 is significantly destabilized upon CGH-1 depletion. This group of CGH-1-interacting mRNAs is rather small and contains mostly germ cell-specific transcripts [11]. In contrast, another RNA-binding protein MSY2 (YBX2) is considered to be a global mRNA stabilizing factor in mammalian germ cells [12] [13]. Reminiscent of CPEB regulation, MSY2 is also subject to phosphorylation during meiotic maturation. It has been suggested that phosphorylation of MSY2 might be a trigger for the global switch from mRNA stabilizing to a degradation-prone environment [13].

Deadenylation appears to be the dominant process resposible for inducing maternal mRNA degradation. In addition, recently discovered small non-coding microRNAs confer sequence specificity to maternal mRNA degradation in several organisms.

microRNA pathway

The majority of mammalian miRNAs are encoded in the genome and transcribed by RNA polymerase II into long primary miRNA (pri-miRNA) transcripts. Pri-miRNAs are processed in the nucleus by the Microprocessor complex consisting of an RNase III Drosha and its cofactor DGCR8, leading to precursor miRNA (pre-miRNA) stem-loop structures of approximately 70 nucleotides (nt) in length. Following the transport to cytoplasm, pre-miRNAs are cleaved by another RNase III Dicer to become 21-23 nt mature miRNAs that are loaded on one of the Argonaute (Ago) proteins. Such miRNA-loaded Ago constitutes the core of the effector miRNA-induced silencing complex (miRISC) [14].

In mammals, a single Dicer protein is responsible for both pre-miRNA processing and generation of small interfering RNAs (siRNAs) from long dsRNA substrates. Likewise, the mechanism of Dicer-processed short RNA loading onto Ago proteins is supposed to be similar for both miRNAs and siRNAs [15]. Therefore, sequence complementarity between the short RNA and its mRNA target is the only parameter distinguishing whether a short RNA will

act as a miRNA or a siRNA. Perfect complementarity, typically in case of a siRNA, results in a direct AGO2-mediated endonucleolytic cleavage of the target. In contrast, short RNA with only partial complementarity acts as a miRNA and requires additional protein components to form a fully functional miRISC complex capable of repressing translation and inducing indirect degradation of the target mRNA (reviewed in [15]). Nucleotides at positions 2-7 of the miRNA constitute the 'seed region', and their complementarity with the target is a critical determinant of the miRNA function [16]. The components of miRISC complex involved in the recruitment of deadenylation and decapping factors localize to cytoplasmic foci termed Processing bodies (P-bodies). These include the AGO2-interacting protein GW182 as well as the above mentioned DDX6 helicase. The physiological relevance of P-bodies is currently not clear. Nevertheless, it has been shown that the formation of P-bodies is a consequence, not the cause of miRNA-mediated repression [17]. Consequently, the presence or absence of P-bodies can be used as a robust readout for the evaluation of miRNA activity.

In *Drosophila*, *Xenopus* and zebrafish, zygotically expressed miRNAs have been shown to participate on maternal mRNA clearance. In mouse OZT the major wave of maternal mRNA degradation starts during meiotic maturation and the role of miRNAs in this process is unclear. Mouse oocytes contain significant amounts of mature miRNAs, to which the severe phenotype observed in Dicer knockout oocytes was originally attributed [18, 19]. However, the identification of a new class of Dicer-dependent endogenous siRNAs (endo-siRNAs) in mouse oocytes provoked the need to evaluate the role of miRNAs in more depth [20].

2. Aims of the project

The primary focus of the presented work was to determine the contribution of mouse oocyte miRNAs to meiotic maturation and maternal mRNA degradation, and to monitor the distribution of P-bodies during the oocyte-to-zygote transition.

After the initial surprising finding that fully grown GV oocytes are devoid of microscopically visible P-bodies, we addressed the following objectives:

- monitor the presence of P-bodies and the dynamics of P-body disassembly during oocyte growth

- ascertain the distribution of P-body components in fully grown oocytes and their potential role in dormant maternal mRNA regulation
- evaluate the contribution of the decapping complex, which was found to be translated only during meiotic maturation, to the initial wave of maternal mRNA degradation
- test the miRNA pathway activity in fully grown oocytes

3. Materials and Methods

Mice

We used 6-10 week old females of C57Bl6 strain or mixed background of C57Bl6 and BALBc to collect fully grown GV oocytes. Two day old and 12 day old mice were used to obtain primordial and growing oocytes, respectively.

Oocyte collection and culture

Fully grown oocytes were isolated in M2 medium (Sigma) supplemented with 0.2mM IBMX and cultured in CZB medium (Chemicon) containing 0.006% Glutamine with or without 0.2mM IBMX. Primordial and growing oocytes were liberated from the follicles by a short incubation in 1 mg/ml collagenase in PBS.

Immunofluorescence

Oocytes were washed in PBS and fixed for 1 hour in 3.7% paraformaldehyde (PFA) in PBS at RT. Following permeabilization in 0.1% Triton X-100 in PBS, oocytes were blocked in blocking solution (0.1% BSA in PBS with 0.05% Tween 20) and incubated with primary antibody at 4°C O/N. Alexa 488- or Alexa 594-conjugated secondary antibodies were applied to visualize the proteins of interest. Oocytes were mounted in Vectashield medium with DAPI. Confocal images were acquired using Leica SP5 laser scanning confocal microscope.

Whole-mount fluorescence in situ hybridization

Oocytes were fixed in 3.7% PFA in PBS and dehydrated in 70% ethanol. After rehydration, oocytes were treated with 1 μg/ml proteinase K and subsequently post-fixed in 3.7% PFA/0.25% glutaraldehyde. Oligodeoxyribonucleotide probe of 50 bases containing 5 fluorescein-conjugated dTs was used for hybridization.

Luciferase reporters and microinjection

Luciferase reporter RNA for oocyte injection was prepared by *in vitro* transcription using mMESSAGE mMACHINE T7 kit. Oocytes were injected using FemtoJet microinjector (Eppendorf). Luciferase activity was measured with Dual-Luciferase Reporter Assay System (Promega).

Microarray analysis

RNA from oocytes was amplified and converted into biotinylated complementary RNA which was then hybridized to Affymetrix MOE430.2 GeneChip arrays. Bioinformatic analyses were performed using R and Bioconductor software.

Detailed experimental procedures are described in the publications originating from this PhD project (see List of publications below).

4. Results and discussion

To study the RNP complexes in mouse oocytes we first optimized the protocol for whole-mount indirect immunofluorescence and verified that the critical steps in the protocol, such as fixation and mounting, do not introduce artifacts to RNP distribution. In addition, we developed a protocol for whole-mount fluorescence in situ hybridization (FISH) in oocytes with subcellular resolution, which had not been described before. We optimized this technique, using fluorescently labelled 50-mer oligodeoxynucleotide as a probe, to detect the abundant *c-Mos* mRNA. We also developed a staining procedure for the colocalization of poly(A) mRNA with proteins (publication 4. in the list of publications (LOP)).

P-body dynamics during oocyte growth and mRNA storage domains in fully-grown oocytes (publication 1. in LOP)

We first focused on the distribution and dynamics of P-bodies at different stages of mouse oocyte growth. Using an autoimmune human serum 18033 which stains GW182 and EDC4 proteins and is commonly used as a marker of P-bodies, together with antibodies against DDX6, AGO2 and a decapping activator DCP1A, we detected large P-bodies in oocytes at primordial and primary follicle stages. Surprisingly, the detected P-bodies gradually decreased

in size as the oocytes entered the growth phase, and eventually disassembled completely in secondary oocytes. No P-body-like structures were observed later in oocyte development. Closer inspection of P-body components in fully-grown oocytes revealed two interesting features. The 18033 signal and DDX6 were enriched in the cortex of fully-grown oocytes, while DCP1A levels dramatically increased during meiotic maturation. The distribution of cortically enriched DDX6 and 18033 signal underwent dynamic changes in fully-grown oocytes. In surrounded nucleolus (SN) oocytes the proteins detected by these antibodies accumulated in transiently forming irregularly shaped foci, which we termed subcortical aggregates (SCAs). We analyzed the localization of additional RNA-binding proteins and found MSY2 (YBX2) and CPEB localizing into SCAs in SN oocytes. The protein composition of SCAs therefore suggested that these might be cytoplasmic storage domains for maternal mRNAs. In line with this, we also detected poly(A) mRNA signal in SCAs. The mRNAs stored in SCAs are likely untranslated, as an exon junction complex (EJC)-component eIF4AIII is also enriched in SCAs. EJC remains associated with processed mRNAs until the first round of translation and therefore the EJC staining can be used to localize stored untranslated transcripts in the cytoplasm [21]. Finally, we also performed RNA-FISH with a specific probe targeting a classical dormant maternal transcript *c-Mos* and we found that *c-Mos* signal is enriched in subcortical patches resembling SCAs in SN oocytes. Taken together, we established the transiently forming SCAs in SN oocytes as storage domains for untranslated maternal mRNAs. While the generation of SCAs remains a mystery, we were able to follow SCA fate during meiotic maturation. After germinal vesicle breakdown SCAs moved towards the oocyte centre and simultaneously started to disperse, likely reflecting the release of stored mRNAs. Interestingly, DCP1A signal started to accumulate in the oocyte centre concomitant with SCA disappearance.

Role of decapping in maternal mRNA degradation (publication 5. in LOP)

Although DCP1A signal, and similarly decapping enzyme DCP2 signal, increased during meiotic maturation, we noticed that *Dcp1a* and *Dcp2* mRNAs are abundant in growing oocytes. Thereby, the nature of *Dcp1a* and *Dcp2* transcript regulation resembled the classical dormant maternal transcripts controlled by CPEB. We cloned the *Dcp1a* and *Dcp2* 3'UTRs, both containing multiple candidate CPE elements, and fused them with the firefly luciferase coding sequence. As expected, injection of these fusion constructs in the form of

non-polyadenylated RNA resulted in an increased luciferase activity only when the injected oocytes were matured to MII eggs. Moreover, mutations introduced into the CPE elements of *Dcp1a* and *Dcp2* 3'UTRs significantly reduced the luciferase activity in MII eggs. CPEB-dependent dormancy of decapping presents an elegant way for the switch between stabilizing and degradation-prone environments at the point when maternal mRNAs need to be degraded. We thereby focused on the potential role of decapping activation in the initiation of maternal mRNA clearance.

We used an siRNA approach to downregulate both DCP1A and DCP2 during meiotic maturation and compared the transcriptomes of DCP-siRNA- and control-siRNA-injected MII eggs by microarrays. Depletion of DCP1A and DCP2 had a generally stabilizing effect, as evidenced by upregulation of hundreds of transcripts compared to only 35 transcripts significantly downregulated. Interestingly, the majority of transcripts upregulated in *Dcp1a* and *Dcp2* knockdown eggs fell into the group of significantly downregulated under normal conditions during meiotic maturation (previously published data [22]). Although only a fraction of the MII egg degradome was stabilized upon decapping suppression, we may conclude that decapping contributes to the initiation of maternal mRNA degradation.

Given the growing evidence for the requirement of maternal mRNA degradation to allow proper preimplantation development [23], we also tested the effect of morpholino-induced maternal DCP1A and DCP2 depletion on ZGA. We observed decreased BrUTP incorporation and lower levels of active promoter marker histone H3K4me3 in decapping deficient 2-cell parthenogenotes, indicating compromised ZGA in these embryos. It will be interesting to reproduce these experiments in physiologically fertilized knockout embryos lacking maternal decapping activity. The complete removal of decapping activity in knockout oocytes will have a much stronger impact on ZGA and therefore this system will be more suitable for mechanistical studies.

MicroRNA pathway suppression in mouse oocytes (publication 2. in LOP)

We noticed that together with P-body disassembly the colocalization of AGO2 with GW182, an interaction essential for miRNA-mediated translational repression [24], was also lost during oocyte growth. Therefore, we created a luciferase reporter system to study miRNA activity in mouse oocytes. It was previously documented that fully-grown oocytes express mature forms of

miRNAs, with members of Let-7 and miR-30 families being among the most abundant [18, 19]. We introduced one site perfectly complementary to Let-7a or miR-30c to the 3'UTR region following the renilla luciferase coding sequence to generate a siRNA-mimicking 'perfect' reporter. Next, we inserted three or four binding sites for Let-7a or miR-30c respectively, with mismatches at positions 10 and 11 to generate a miRNA-mimicking 'bulged' reporter. Finally, we mutated two nucleotides in the seed region of each miRNA binding site in the bulged reporter to generate a control 'mutated' reporter. Testing the reporters in cultured cells revealed more than 80% downregulation of the perfect reporter compared to controls and the bulged reporter was a target to an even stronger repression. In small oocytes from 12 day old mice both perfect and bulged reporters were repressed significantly compared to mutated control. However, the repression of bulged reporters was largely relieved in fully-grown oocytes and MII eggs, while perfect reporters remained repressed. These data indicate that the miRNA pathway is perturbed in fully-grown mouse oocytes, likely at the level of translational repression and recruitment of mRNA degradation factors to the target mRNA.

To confirm these results suggesting the miRNA pathway suppression on a large scale, we compared transcriptomes of the wild type and Dicer deficient oocytes using microarrays. The numbers of misregulated transcripts were low compared to changes in transcriptomes of other cell types upon loss of Dicer. Furthermore, no miRNA seed footprint was identified in the upregulated transcripts.

Together, our data showed that miRNA activity is compromised in fully-grown mouse oocytes. Our conclusions were supported by a parallel study analyzing DGCR8 knockout oocytes [25]. Despite a complete loss of canonical maternal miRNAs caused by DGCR8 depletion, the knockout oocytes showed no abnormal phenotypes and could give rise to viable and fertile offspring.

Regulation of Dicer-dependent pathways during mouse oocyte-to-zygote transition (publication 3. in LOP)

Given that canonical miRNAs are dispensable during mouse OZT, it remains to be tested whether miRNA pathway suppression is required in this largest reprogramming event of mammalian development. It is possible that transient suppression of the miRNA pathway during reprogramming creates a window in which differentiation-supporting miRNAs are disengaged from regulating their target mRNAs, and are replaced by pluripotency-promoting miRNAs.

Uncovering the mechanism of miRNA pathway suppression will allow to restore miRNA function and test this hypothesis.

In addition, our finding that miRNAs are inactive in mouse oocytes sheds new light on the interpretation of Dicer knockout phenotype during meiotic maturation and brings endo-siRNAs in the centre of research focus. Whether disrupting endo-siRNA-dependent regulation of target mRNA levels could explain spindle defects observed in Dicer null mouse oocytes is a subject to ongoing research. Encouragingly, comparison of genes upregulated in Dicer and AGO2 knockout oocytes [26] with the genes matching endo-siRNAs [20] revealed numerous microtubule-associated genes as potential endo-siRNA targets.

5. Conclusions

Studies in various model organisms strengthen our knowledge concerning the general principles of post-transcriptional regulation during the oocyte-to-zygote transition. While most of the currently known mechanisms controlling maternal mRNA stability and degradation have been uncovered using *Xenopus*, zebrafish and invertebrate systems, the data constituting this thesis represent progress in the understanding of post-transcriptional processes in a mammalian system. The identification of maternal mRNA storage domains in fully grown oocytes sets the basis for future functional studies of individual components. Similarities in the protein composition of these domains between invertebrates, vertebrates and mouse indicate that the function of at least some of these proteins might be conserved in metazoans. Following up on the results presented in this work, the function of DDX6 helicase in the regulation of maternal mRNA stability is currently being addressed. It has become evident that an mRNA stabilizing environment needs to be created in growing oocytes to permit maternal mRNA accumulation. Our data on the dormancy of decapping and its activation during meiotic maturation offer one of the first insights into the mechanisms underlying the global switch from mRNA stability to degradation. In addition, suppression of the miRNA pathway might also contribute to stabilizing conditions during oocyte growth. Future work will clarify whether the reduction of miRNA activity is a prerequisite for a successful oocyte-to-zygote transition, and eventually for cell reprogramming in general. In such a case, deciphering the mechanisms of miRNA pathway suppression would provide an invaluable

penetration into the processes by which differentiated cells acquire the self-renewing potential, with possible application in the iPSC technology and cancer research.

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List of publications

Publications related to this thesis:

1. Flemr, M., Ma, J., Schultz, R.M., and Svoboda, P. (2010). P-body loss is concomitant with formation of a messenger RNA storage domain in mouse oocytes. Biol Reprod *82*, 1008-17. (IF2010 = 3.87)

2. Ma, J., Flemr, M., Stein, P., Berninger, P., Malik, R., Zavolan, M., Svoboda, P., and Schultz, R.M. (2010). MicroRNA activity is suppressed in mouse oocytes. Curr Biol *20*, 265-70.
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3. Svoboda, P., and Flemr, M. (2010). The role of miRNAs and endogenous siRNAs in maternal-to-zygotic reprogramming and the establishment of pluripotency. EMBO Rep *11*, 590-7.
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4. Flemr, M., and Svoboda, P. (2011). Ribonucleoprotein localization in mouse oocytes. Methods *53*, 136-41.
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5. Ma, J., Flemr, M., Strnad, H., Chen, J., Svoboda, P., and Schultz, R.M. (2012) Maternally-recruited DCP1A and DCP2 regulate mRNA degradation during oocyte maturation and genome activation in mouse. Development - under revision for submission

Other publications:

Nejepinska, J., Malik, R., Filkowski, J., Flemr, M., Filipowicz, W., and Svoboda, P. (2012). dsRNA expression in the mouse elicits RNAi in oocytes and low adenosine deamination in somatic cells. Nucleic Acids Res *40*, 399-413.

Nejepinska, J., Flemr, M., and Svoboda, P. (2012). Control of the interferon response in RNAi experiments. Methods Mol Biol *820*, 133-61.

Vinopal, S., Cernohorska, M., Sulimenko, V., Sulimenko, T., Vosecka, V., Flemr, M., Draberova, E., and Draber, P. (2012) γ-Tubulin 2 nucleates microtubules and is downregulated in mouse early embryogenesis. PLoS One *7*, e29919.

Curriculum vitae

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EDUCATION

2007 - 2012 Charles University Prague, 1st Faculty of Medicine, Ph.D. program in cell and developmental biology

2002 - 2007 Institute of Chemical Technology Prague, Faculty of Food and Biochemical Technology, M.Sc. program in general and applied biochemistry, graduated with honours

RESEARCH EXPERIENCE

Sep 2012 - Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland (Postdoc position in the laboratory of Dr. Marc Bühler)

2007 - 2012 Laboratory of Epigenetic Regulations, Institute of Molecular Genetics, Prague (Head: Petr Svoboda Ph.D.) Ph.D. project focused on mechanisms of maternal mRNA degradation during oocyte-to-zygote transition in the mouse

2004 - 2007 Laboratory of Plant Biochemistry, Institute of Chemical Technology, Prague (Head: Prof. Olga Valentova Ph.D.) research on phospholipid signalling pathways involved in plant systemic acquired resistance
diploma thesis: Phospholipase D regulated transcriptome of systemic acquired resistance in Arabidopsis thaliana.

02-06/2006 Laboratory of Plant Cellular and Molecular Physiology, University Pierre et Marie Curie, Paris (Head: Prof. Alain Zachowski Ph.D.) Erasmus study stay - research on phospholipid-modifying enzymes involved in plant defence responses
final report: Role of Phospholipase D in Response to Salicylic Acid in A. thaliana.

ADDITIONAL INFORMATION

2007 Josef Hlavka Award dedicated to the best students and talented young investigators from Czech universities and Academy of Sciences

2007 Dean’s Award for excellence in undergraduate studies, ICT Prague

2011 Scientia foundation Award for the best 2010 publication record of PhD students of the 1st Faculty of Medicine, Charles University