Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 347 (2006) 36–43
BBRC
www.elsevier.com/locate/ybbrc

Abundant transcripts from retrotransposons are unstable in fully grown mouse oocytes

Mareike Puschendorf [a], Paula Stein [b], Edward J. Oakeley [a], Richard M. Schultz [b,*], Antoine H.F.M. Peters [a,*], Petr Svoboda [a]

[a] *Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland*
[b] *Department of Biology, School of Arts and Sciences, University of Pennsylvania, PA, USA*

Received 17 May 2006
Available online 27 June 2006

Abstract

One physiological function proposed for RNA interference (RNAi) is to constrain expression of repetitive elements and thereby reduce the incidence of retrotransposition. Consistent with this model is that inhibiting the RNAi pathway results in an increase in expression of repetitive elements in preimplantation mouse embryos. Mouse oocytes are essentially transcriptionally quiescent providing a unique opportunity to assess the stability of repetitive element-derived transcripts in these cells. We compared the transcriptome of freshly isolated fully grown germinal vesicle (GV)-intact oocytes to that of oocytes in which meiotic maturation *in vitro* was inhibited for 48 h by milrinone. Consistent with the aforementioned function for RNAi is that the abundance of only a relatively small number of transcripts decreased in the cultured oocytes, when compared to changes that occur during maturation or following fertilization, and of those, several belonged to mobile elements.

Keywords: GV-oocyte; Meiosis; mRNA stability; mRNA degradation; Milrinone; L1; IAP; VL30; ETn

Approximately 40% of the human and mouse genomes are composed of a diverse group of transposable elements (TEs) [1–3], which differ in many aspects such as structure, copy-number, expression pattern, and rate and mechanism of mobility. Most of TEs are retroelements that transpose through a ''copy and paste'' mechanism utilizing an RNA intermediate. Although transcribed, these sequences are typically not mobile because the vast majority bear mutations, truncations, and deletions. For example, of the ~400,000 human L1 insertions (which occupy 17% of the genome [2]), only 90 are intact and full-length, of which 40 are active in a cell-culture retrotransposition assay [4]. TEs have a capacity to cause deleterious mutations and they are often viewed as harmful parasites [5]. Consistent with this idea is that numerous mechanisms operate in animals to silence TEs that include transcriptional silencing mediated by DNA methylation [6–8] and chromatin changes [9,10].

In invertebrates, repression of mobile elements occurs post-transcriptionally by an RNA interference (RNAi)-like mechanism. RNAi refers to the selective degradation of mRNA induced by double-stranded RNA (dsRNA), and is one of the mechanistically related RNA silencing pathways (reviewed in [11]). It is viewed as a form of defense against viruses and other parasitic sequences and one of the proposed roles for RNAi in the germ-line in metazoa is to inhibit TEs [12–14]. Whether this role extends to mammals is not clear.

RNAi operates in mammalian cells and appears to be a major pathway responding to long dsRNA in germ cell lineage-competent cells such as oocytes, early embryos, and undifferentiated embryonic stem (ES) cells [15–17]; these cells lack an interferon response [15,18]. Because mammalian TEs apparently can generate dsRNA [19–21]

* Corresponding authors.
E-mail addresses: rschultz@sas.upenn.edu (R.M. Schultz), antoine.peters@fmi.ch (A.H.F.M. Peters).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.06.106

and expression of several retrotransposons occurs in mammalian oocytes and early embryos [21–23], a likely function of RNAi in oocytes and early embryos would be to constrain expression of TEs, thereby limiting their activity in germ-line cells. Indeed, inhibiting Dicer in preimplantation mouse embryos or ES-cells results in an increased abundance of mRNAs of retrotransposons L1, IAP, and MuERV-L [20,24].

To address post-transcriptional silencing of TEs in mammals, we analyzed the stability of TE-derived RNAs in mouse oocytes using microarrays and RT-PCR. Oocyte growth is accompanied by the cessation of transcription in the fully grown oocyte [25]; transcription resumes by the late 1-cell stage/2-cell stage [26]. *In vitro*, this window of transcriptional quiescence can efficiently be extended by treatment with the phosphodiesterase (PDE) inhibitor milrinone, which blocks the resumption of meiosis. Thus, milrinone treatment provides an opportunity to assess mRNA stability without interfering with developmental processes such as resumption of meiosis. The underlying assumption is that, under these conditions, TEs would exhibit greater instability due to RNAi-mediated degradation. Results of the experiments described here indicate that the abundance of transcripts of several (but not all) mobile elements is markedly reduced in oocytes inhibited from undergoing maturation *in vitro*. These elements include mVL30 and IAP, retrotransposons exhibiting the highest transcript abundance in the oocyte.

Materials and methods

Oocyte and embryo collection and culture. Oocytes for microarray and RT-PCR analyses were isolated from superovulated 6- to 8-week-old CF1 or CD1 females, respectively. Fully grown germinal vesicle (GV)-intact oocytes were collected 46 h after eCG injection (5 IU) from cumulus cell–oocyte complexes. Metaphase II-arrested eggs were collected from eCG- and hCG-primed mice. One-cell embryos were harvested from eCG- and hCG-primed females that were mated.

Oocytes were pooled and one-half was immediately frozen in lysis buffer for RNA isolation at a later time. The other portion was cultured in CZB containing 1 mM glutamine and 2.5 μM milrinone for 48 h at 37 °C in a humidified atmosphere of 5% CO_2 in air. MII eggs and 1-cell embryos were collected in FHM/Hepes 20 and 21 h post-eCG injection, respectively. For all stages, oocytes and embryos were pooled from several mice and RNA was isolated from batches of 50 oocytes/embryos.

RNA isolation and RT reaction. RNA was isolated using the "Absolutely RNA Nanoprep Kit" (Stratagene). Briefly, cells were transferred to lysis buffer and stored at −80 °C. After thawing, 100 ng of *Escherichia coli* rRNA was added to each sample as carrier. RNA was purified according to the manufacturer's instructions. RNA was eluted using two separate elution steps resulting in a total volume of 20 μl (i.e., 2.5 oocyte-equiv/μl). Reverse transcription was performed from total RNA corresponding to 20 oocytes or embryos using random primers (200 ng) and SuperScript II RNase H Reverse Transcriptase (Invitrogen) according to the manufacturer's protocol.

PCR. For PCRs, cDNA corresponding to 0.2 embryos or oocytes was used as a template. Primer sequences are listed in Table 1. Amplifications were carried out using *Taq* DNA polymerase (Qiagen). Thermocycling was performed in a Bio-Rad iCycler using the following PCR conditions: 1 cycle at 95 °C for 5 min; the indicated number of cycles at 95 °C for 45 s, 55 °C for 45 s, and 72 °C for 1 min; 72 °C for 10 min and a final hold at 4 °C. For MT 22 cycles were used, for ActB, Mos, Oct4, Plat, and Zp3 28 cycles, for Orr1 30 cycles, for IAP, L1, mVL30, RLTR1B, SINE B1, SINEB2 32 cycles, for Ezh2 35 cycles and for Etn 38 cycles, respectively. PCR products were resolved on a 2% agarose gel and afterwards the gels were stained with SYBR green I (Molecular Probes, 1:10,000). Fluorescence was detected on a Typhoon 9400 scanner (Amersham Biosciences).

Affymetrix microarray hybridization and analysis. Total RNA from four replicates of each treatment was used for linear, two-round amplification by *in vitro* transcription and target cRNA preparation according to the Affymetrix Small Sample Prep Technical Bulletin (www.affymetrix.com). Total RNA from each replicate was reverse-transcribed using the Affymetrix cDNA synthesis kit and cRNA was produced by *in vitro* transcription (IVT) by T7 RNA polymerase using the Affymetrix IVT kit as per the manufacturer's instructions. Twenty micrograms of biotinylated cRNA was fragmented by heating with magnesium (as per Affymetrix's instructions) and 15 μg of fragmented cRNA was serially hybridized to MOE430 2.0 GeneChips and then processed according to the manufacturer's instructions (GeneChip Analysis Technical Manual, www.affymetrix.com) at the FMI Microarray Facility. Microarray analysis was performed as described in [27] and the resulting data from this study are available at the Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo). Briefly, GC-RMA algorithm from Bioconductor was used to estimate probe set expression values. The expression values were then exported as a text file and imported into GeneSpring 7 (Silicon Genetics) with default per-chip normalization to the 50th percentile and per-gene normalization to the median. The per-chip and per-gene normalized data are referred to as

Table 1
Primers used for RT-PCR

Name	Forward	Reverse	References
Actb	5′-TGGGAATGGGTCAGAAGGACT-3′	5′-GGGTCATCTTTTCACGGTTGGC-3′	
Ezh2	5′-AGCCTTGTGACAGTTCGTGC-3′	5′-TTTAGAGCCCCGCTGAATG-3′	
Pou5f1	5′-GGAGAAGTGGGTGGAGGAAG-3′	5′-GGGAAACCCTGTAGCCTCATAC-3′	
Zp3	5′-AAGCTCAACAAAGCCTGTTCG-3′	5′-TATTGCGGAAGGGATAC AAGG-3′	
Mos	5′-CCATCAAGCAAGTAAACA-3′	5′-AGGGTGATTCCAAAAGAGTA-3′	[16]
Plat	5′-CATGGGCAAGAGTTACACAG-3′	5′-CAGAGAAGAATGGAGACGAT-3′	[16]
IAP	5′-GCACCCTCAAAGCCTATCTTAT-3′	5′-TCCCTTGGTCAGTCTGGATTT-3′	[20]
mVL30	5′-CCTTTGTTGCCCAGGTAAGTC-3′	5′-CACTGTAGCCAGTTGTGACCAG-3′	
L1	5′-TTTGGGACACAATGAAAGCA-3′	5′-CTGCCGTCTACTCCTCTTGG-3′	[9]
RLTR1b	5′-TCCTTCCCTTTGCCCTATTT-3′	5′-GGCTGGAACTGGTGAGATGT-3′	[21]
ETn	5′-CAGGCTTTGGAGACAATAGGG-3′	5′-TCTCTCAGGGAACTCAGAAACG-3′	
ORR1	5′-CTTTAGTTGATGGCCCAGGA-3′	5′-CCAACTCTGCCCTCTGTAGC-3′	[21]
MT	5′-ATGTCTTGGGGAGGACTGTG-3′	5′-AGCCCCAGCTAACCAGAACT-3′	[21]
SINE B1	5′-GTGGCGCACGCCTTTAATC-3′	5′-GACAGGGTTTCTCTGTGTAG-3′	[9]
SINE B2	5′-GAGATGGCTCAGTGGTTAAG-3′	5′-CTGTCTTCAGACACTCCAG-3′	[9]

"raw" and "normalized" expression values, respectively. Because we expected down-regulation of an unknown number of genes during the milrinone treatment, we generated a list of 23,989 probe set for further analysis from probe sets present in all four control replicates (GV-oocyte, $t = 0$; raw signal value >50).

Results and discussion

General characterization of the microarray analysis

We first compared fully grown GV-intact oocytes freshly isolated from the ovary, with oocytes cultured in the presence of 2.5 μM milrinone for 48 h (Fig. 1); additional information can be found in the Supplemental Material. The profile of raw signal from MOE430 2.0 arrays was uniform (Fig. 1A). The average number of probe sets with present signal (raw signal value >50) per chip was 23,577 (±314). It should be noted here that the number of probe sets on the microarray is higher than the number of expressed genes because the array is partially redundant.

We performed several clustering analyses of the microarray data using various gene lists. In each case replicates of each experimental condition clustered together (Fig. 1B). For most genes, raw signal and normalized values did not vary much between replicates. Of note is the presence of many genes whose relative abundance was decreased in oocytes following culture when compared to their freshly isolated counterparts, as evidenced by the appearance of blue bands.

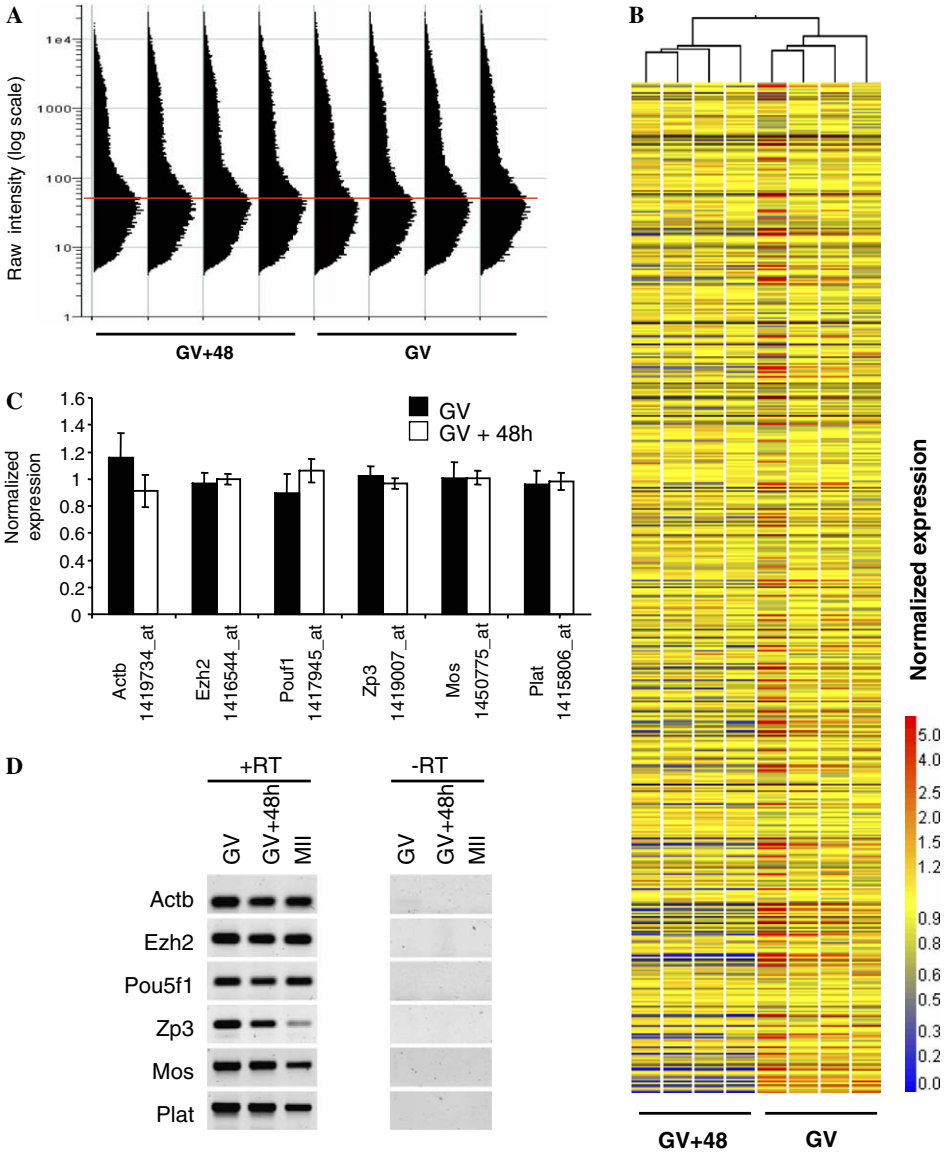

Fig. 1. Evaluation of microarray data. (A) Hybridization signal profile on all microarrays. *X*-axis shows relative abundance of probe sets with raw intensities depicted on the *Y*-axis. (B) Conditional clustering analysis. Displayed are all differentially expressed probe sets (ANOVA, $p < 0.05$) with a raw signal >50 in the GV samples. (C) Mean normalized value for a set of six transcripts, which did not show significant down-regulation during milrinone treatment. Error bar = standard deviation. (D) Analysis of the same genes by RT-PCR. MII, metaphase II-arrested egg. +RT, samples were reverse-transcribed; −RT, samples were not reverse-transcribed.

To address further the reliability of the microarray data, we analyzed the relative abundance of six transcripts by RT-PCR, which showed little, if any, changes in their mean normalized expression values (Fig. 1C and D). These transcripts included: the dormant maternal mRNAs *Mos* and *Plat*, which are stored during oocyte growth and not translated until the resumption of meiosis and which are stable following extended culture of fully grown GV-intact oocytes [16]; the oocyte-specific gene *Zp3*; the ubiquitously expressed housekeeping gene *Actb*; and genes with regulatory functions *Pou5f1* (transcription factor Oct-4) and a polycomb gene *Ezh2*. This analysis yielded a good correlation between the microarray data and RT-PCR data, providing further support for the robustness of the microarray results (Fig. 1C and D).

In the absence of transcription, the majority of probe sets that display a change should show a decrease in relative abundance, and such was the case (Fig. 2A and B). A scatter plot of 23,989 probe sets (raw value >50) where per-gene normalized values of milrinone-treated oocytes are plotted against control oocytes and using a 2-fold cut-off revealed a population of transcripts whose relative abundance decreased. There were 27 probe sets exhibiting up-regulation above the 2-fold cut-off in the milrinone treatment but the majority of probe sets showing more than 2-fold-change were down-regulated (1091). The 2-fold cut-off is a default for many microarray experiments. We have addressed the problem of fold-change cut-off in more detail because linear amplification likely introduced more variability into the analysis resulting in a higher rate of false positive changes. Fig. 2B shows relationship between fold-change cut-off and numbers of up-regulated and down-regulated transcripts. These results indicate that even at the 2-fold cut-off, the number of up-regulated transcripts is fairly small (27) and there are only three transcripts found up-regulated at the 3-fold cut-off. Only six of the 2-fold up-regulated probe sets (none of 3-fold) pass the *t*-test ($p < 0.05$).

As mentioned above, 1091 probe sets were found to be down-regulated more than 2-fold, which represents 4.35% of all transcripts; Fig. 2C shows the distribution of the fold down-regulation across the 1091 probe sets down-regulated more than 2-fold. Most of the probe sets detected a smaller down-regulation but there were also transcripts showing a strong reduction in abundance (up to ~52-fold). The initial fold-change filter reduces the number of genes considered for statistical validation, which is an important step for minimizing the false discovery rate. For the statistical analysis we applied a one-way ANOVA ($p < 0.05$) reducing the 2-fold list from 1091 to 424 probe sets, while most of the removed probe sets showed lower fold-changes (see Supplemental Material for gene lists). Although the number of actual transcripts is somewhat smaller because the probe sets are redundant, we estimate that the total number of affected transcripts does not exceed a few percent of all expressed genes. In particular, we rarely observed down-regulation of abundant transcripts, i.e., transcripts whose

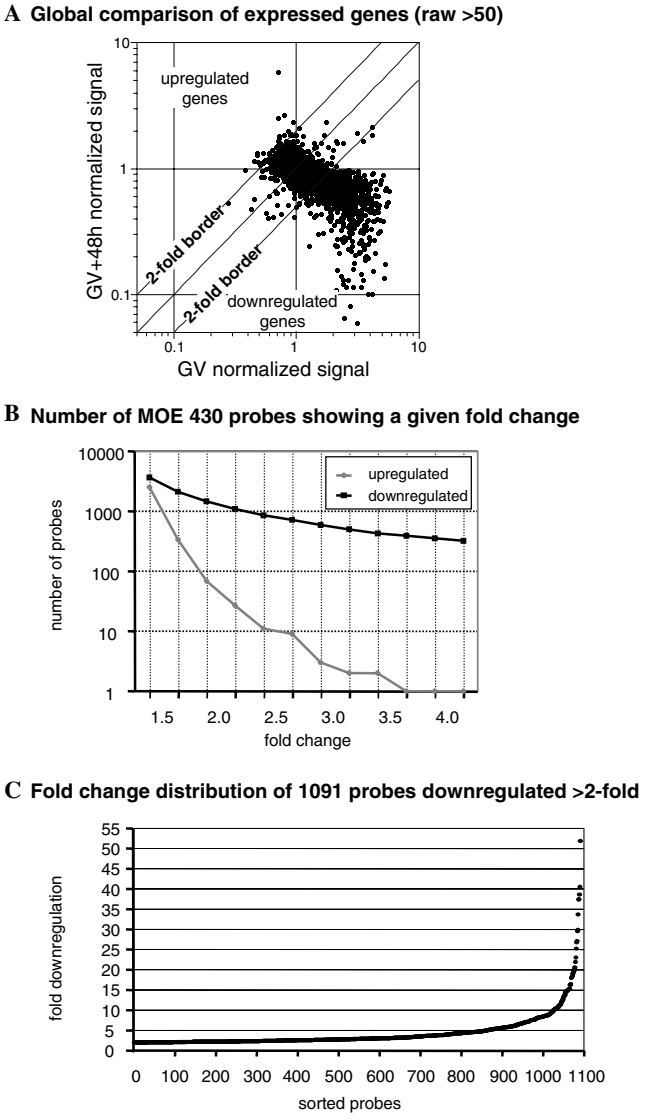

Fig. 2. Global view of differentially regulated transcripts. (A) Scatter plot showing comparison of per-gene normalized expression levels of 23989 transcripts detected present in the fully grown oocyte (GV). Each gene is represented by a dot. (B) Number of up-regulated and down-regulated transcripts as a function of fold-change cut-off. *Y*-axis (logarithmic scale) shows number of gene probes, which pass a fold-change cut-off depicted on the *X*-axis. (C) Distribution of fold-change for 1091 probe sets down-regulated more than 2-fold. Probe sets (*X*-axis) were sorted according to their increasing fold-down-regulation, which is displayed on the *Y*-axis.

raw expression value was higher than several thousands. Therefore, maternal mRNAs, which accumulate during oocyte growth, are highly stable in the fully grown GV-oocyte. This result is consistent with results of previous experiments which indicated that oocyte mRNA is stable during the growth phase [28,29].

The cause of the observed instability of down-regulated mRNAs is unknown. Down-regulated transcripts are not strongly enriched for AU-rich or other known mRNA-destabilizing sequences (data not shown). It should be noted that known mechanisms degrading maternal mRNAs in the oocytes are associated with resumption of meiosis or other developmental transitions [30], which do not occur

in our model system. It is possible that a fraction of the down-regulated mRNAs represents microRNA (miRNA) targets. It has been recently shown that post-transcriptional regulation by miRNAs may involve mRNA degradation even in the absence of extensive base paring to their targets [31,32], possibly as a consequence of relocalization of repressed mRNAs to cytoplasmic domains known as P-bodies (reviewed in [33,34]). There are hundreds of known mammalian miRNAs [35]. Each mammalian cell studied so far expressed numerous miRNAs and oocytes are likely not an exception. Studies of miRNA targets estimate that up to 30% of mammalian genes could be targeted by miRNAs [36]. However, it is not known how many genes are regulated by miRNAs in specific cell types. Given the limited information about what makes a functional miRNA target site, prediction algorithms generally estimate that hundreds of mRNAs are targeted by individual miRNAs [37–41]. A recent study of *Drosophila* cells depleted of the miRNA pathway components indicates that miRNAs may down-regulate up to several percent of cellular transcripts [42]. If a similar fraction of transcripts would be targeted in the mouse oocytes, a majority of down-regulated mRNAs could be miRNA targets. However, this issue cannot be directly addressed until technical problems with cloning miRNAs from very small samples will be solved and miRNA pathway-deficient oocytes will be available.

How the down-regulation of hundreds of transcripts would affect developmental competence of mouse oocytes is not known. It is interesting to note, however, that culturing porcine oocytes for 22 h in the presence of IBMX, another phosphodiesterase inhibitor, which has a comparable effect to milrinone in preventing resumption of meiosis, does not negatively affect developmental competence as measured by culturing *in vitro* matured and fertilized eggs to the blastocyst stage [43]. Whether the developmental competence to term is affected is not known.

Stability of mRNA of repetitive elements

As mentioned above, several mobile elements in invertebrates were described as endogenous targets for RNAi [12,13,22,44] but there is presently little evidence supporting this role for RNAi in mammals [20,24]. If RNAi-mediated degradation of repetitive elements exists in the mouse oocyte, the targeted transcripts should be found among the mRNAs down-regulated after milrinone treatment.

To this end we conducted a systematic survey of the stability of transcripts derived from repetitive elements. We generated a list of murine repetitive elements highly similar to or perfectly matching MOE 430 2.0 probe sets (see Supplemental Material). We then extracted and analyzed raw and normalized data for these repetitive elements in Genespring 7. Although the majority of the Affymetrix probes for various repetitive elements appeared stable, several probes (perfectly matching intracisternal A particle (IAP) and mVL30 retrotransposons) showed a robust down-regulation (Fig. 3A). Instability of IAP and mVL30 transcripts was further confirmed by RT-PCR with primers amplifying a specific region upstream of the 3′ long terminal repeats (Fig. 3B). RT-PCR analysis of other repetitive elements revealed down-regulation of L1 transcripts (which show a low hybridization signal and a weak down-regulation on microarrays (Fig. 3A)) and of the RLTR1b element (absent on the microarrays).

No down-regulation was observed for ORR1, MT, and SINE B1 and B2 elements (Fig. 3B). It should be noted that a recent study reported that many gene transcripts expressed in oocytes are chimeric, containing LTR class III retrotransposon sequences fused to their 5′ ends [21]. The function of these transcripts in preimplantation development remains unresolved. The MOE430 2.0 chip does not contain a perfectly matching probe set for MT-like retrotransposons and our data do not discriminate between these chimeric transcripts and their *bona fide* counterparts because the array probes used are biased towards the 3′ end of the transcript. In any case, RT-PCR with MT primers showed no change in their mRNA levels after milrinone treatment (Fig. 3B).

Interestingly, RT-PCR analysis of repetitive transcripts down-regulated during milrinone treatment revealed variable behavior during normal development (Fig. 3B). The RT-PCR analyses of unfertilized and fertilized eggs (Fig. 3B) are consistent with previously reported expression data [21,23,27]. Fig. 3B illustrates differences between regulation of individual mobile elements. For example, IAP shows a strong down-regulation of mRNA during meiotic maturation while mVL30 and RLTR1B transcripts appear relatively stable. This latter observation does not necessarily contradict the milrinone result as milrinone treatment of GV-oocytes lasted for 48 h while the MII and zygote samples where isolated about 20 h after induction of meiotic maturation *in vivo*. Thus, mRNA degradation may not be apparent within this shorter time period and could explain why mVL30 and RLTR1B do not show a clear down-regulation. Alternatively, the mechanism degrading certain repetitive transcripts in the fully grown GV-oocytes may not efficiently work during meiotic maturation. The differential behavior of IAP illustrates however the diversity in post-transcriptional regulation of repetitive elements. The strong down-regulation of IAP mRNA levels in the MII eggs supports the idea that meiotic maturation enhances the degradation process of IAP mRNA possibly by incorporating one of the known mechanisms degrading maternal mRNAs upon resumption of meiosis [30].

The IAP retrotransposon is one of the most aggressive parasitic sequences known in the mouse genome and present in approximately 1000 copies per haploid [45,46]. IAP is highly expressed in the oocyte and early embryos [23]. It is estimated that there are ~13,000 transcripts in the oocyte, which are reduced ~10-fold in the ovulated egg and then increased up to ~150,000 transcripts between the one-cell and the blastocyst stages [23]. Microarray data also confirmed that IAP mRNA is an abundant transcript;

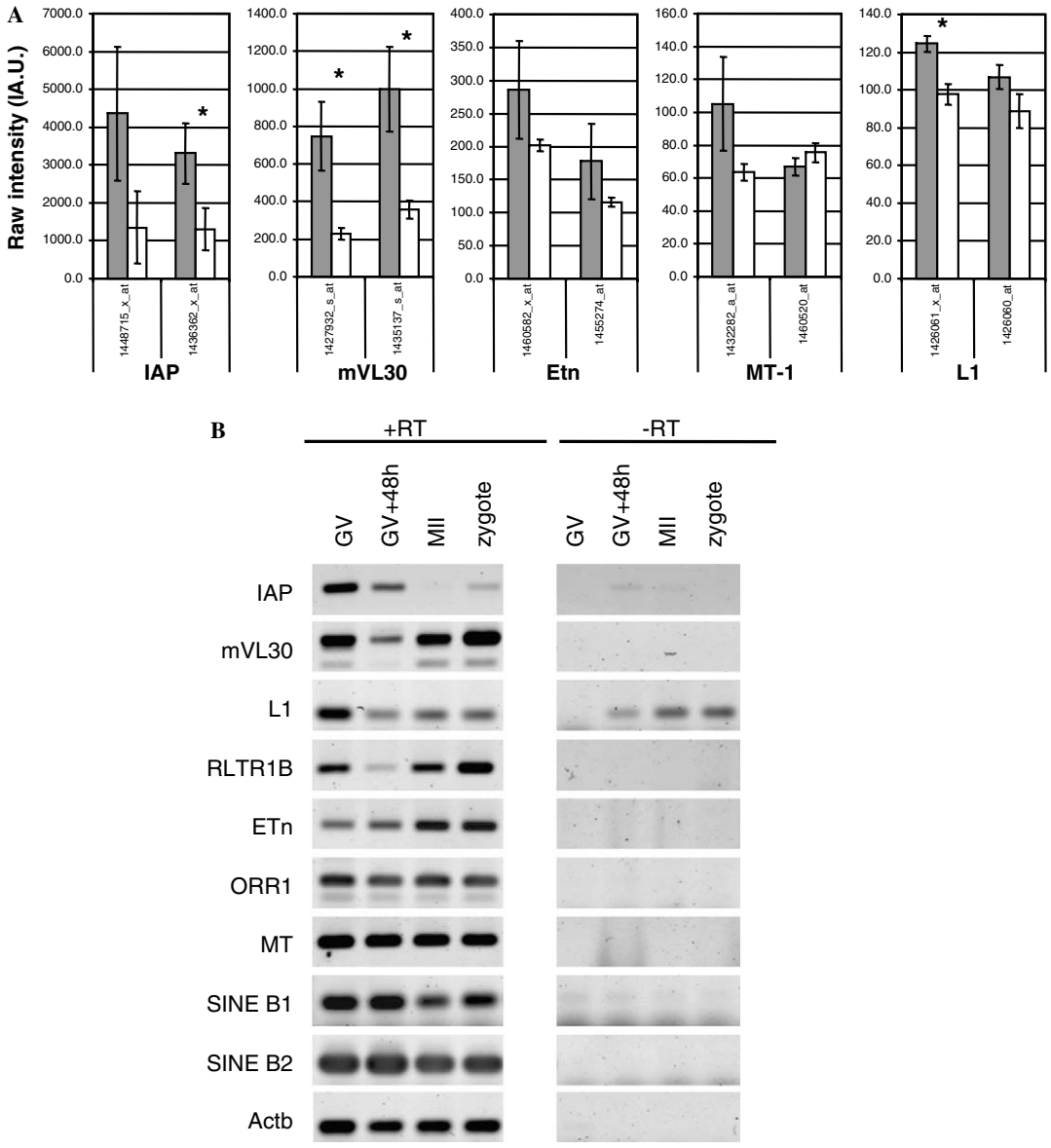

Fig. 3. Relative changes of transcript abundance for several different mobile elements. (A) Effect of milrinone treatment on expression of different repetitive elements. For IAP, mVL30, L1 and Etn, expression of two most specific Affymetrix MOE 430 2.0 probe sets with the highest hybridization signal (Supplemental Table 2) is displayed as a mean raw expression value (*Y*-axis) for control (GV) and milrinone-treated oocytes (GV + 48 h). * indicates *t*-test *p*-value < 0.05. (B) RT-PCR analysis of expression of repetitive elements performed on the same material as shown in Fig. 1D.

IAP probe set raw signal reached 4133, which is more than eight times higher than the average raw signal for transcripts on the chip and places the IAP probe set among the 7% of probe sets with the highest signal. Our previous study of IAP expression in the early embryo found that the knockdown of Dicer in the embryo results in approximately 50% increase in the steady-state level of IAP (from ~60,000 to 90,000 transcripts [20]); this increase would likely be greater if maternal Dicer protein was depleted.

Our previous results indicate that ~1000 molecules of dsRNA are required to trigger RNAi-mediated mRNA degradation [16]. Thus, endogenous RNAi likely requires formation of sufficient amounts of dsRNA to elicit efficient transcript targeting. Production of such dsRNA likely correlates with the level of RNA expression. Retroelements can produce dsRNA by a variety of mechanisms [47] that generate complementary strands. New insertions in the genome also increase the probability of generating antisense transcripts. Indeed, expression of antisense RNA has been demonstrated for MuERV-L [20,21] and IAP retrotransposons in the 2-cell mouse embryo [20]. Although there is no solid evidence for base-pairing of endogenous transcripts, it has been shown experimentally that simultaneous expression of longer sense and antisense transcripts can trigger sequence-specific mRNA degradation in mammalian cells [48]. Thus, high expression levels of a mobile element may increase both the probability of successful retrotransposition as well as the risk of pairing with antisense transcripts. Activation of RNAi by forming these dsRNAs would thereby constrain their expression. Repetitive

elements expressed at low levels would have a lower probability to form enough dsRNA and thereby may escape RNAi targeting. A consequence would be that RNAi-mediated targeting of mobile elements would be rather selective, i.e., it would constrain only those elements that produce enough dsRNA to trigger RNAi. IAP is likely the most abundant autonomous TE mRNA in the mouse oocyte and it is likely a target for RNAi because depletion of Dicer in early embryo cells lead to accumulation of IAP transcripts [20,24]. In addition, IAP mRNA instability in the GV-oocyte is also consistent with the hypothesis that IAP is a natural RNAi target in mouse.

Acknowledgments

We thank Herbert Angliker from the FMI microarray facility. Research at the Friedrich Miescher Institute is supported by the Novartis Research Foundation. M.P. acknowledges the Boehringer Ingelheim Fonds for her PhD fellowship and The Company of Biologists for the Development Travelling Fellowship. P.S. is supported by an EMBO long-term fellowship ALTF 2003-199; research in the Schultz laboratory by NIH Grant HD22681 and research in the Peters laboratory by the NoE network "The Epigenome" (LSHG-CT-2004-503433).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2006.06.106.

References

[1] R.H. Waterston, K. Lindblad-Toh, E. Birney, J. Rogers, J.F. Abril, P. Agarwal, R. Agarwala, R. Ainscough, M. Alexandersson, P. An, S.E. Antonarakis, J. Attwood, R. Baertsch, J. Bailey, K. Barlow, S. Beck, E. Berry, B. Birren, T. Bloom, P. Bork, M. Botcherby, N. Bray, M.R. Brent, D.G. Brown, S.D. Brown, C. Bult, J. Burton, J. Butler, R.D. Campbell, P. Carninci, S. Cawley, F. Chiaromonte, A.T. Chinwalla, D.M. Church, M. Clamp, C. Clee, F.S. Collins, L.L. Cook, R.R. Copley, A. Coulson, O. Couronne, J. Cuff, V. Curwen, T. Cutts, M. Daly, R. David, J. Davies, K.D. Delehaunty, J. Deri, E.T. Dermitzakis, C. Dewey, N.J. Dickens, M. Diekhans, S. Dodge, I. Dubchak, D.M. Dunn, S.R. Eddy, L. Elnitski, R.D. Emes, P. Eswara, E. Eyras, A. Felsenfeld, G.A. Fewell, P. Flicek, K. Foley, W.N. Frankel, L.A. Fulton, R.S. Fulton, T.S. Furey, D. Gage, R.A. Gibbs, G. Glusman, S. Gnerre, N. Goldman, L. Goodstadt, D. Grafham, T.A. Graves, E.D. Green, S. Gregory, R. Guigo, M. Guyer, R.C. Hardison, D. Haussler, Y. Hayashizaki, L.W. Hillier, A. Hinrichs, W. Hlavina, T. Holzer, F. Hsu, A. Hua, T. Hubbard, A. Hunt, I. Jackson, D.B. Jaffe, L.S. Johnson, M. Jones, T.A. Jones, A. Joy, M. Kamal, E.K. Karlsson, et al., Initial sequencing and comparative analysis of the mouse genome, Nature 420 (2002) 520–562.

[2] E.S. Lander, L.M. Linton, B. Birren, C. Nusbaum, M.C. Zody, J. Baldwin, K. Devon, K. Dewar, M. Doyle, W. FitzHugh, R. Funke, D. Gage, K. Harris, A. Heaford, J. Howland, L. Kann, J. Lehoczky, R. LeVine, P. McEwan, K. McKernan, J. Meldrim, J.P. Mesirov, C. Miranda, W. Morris, J. Naylor, C. Raymond, M. Rosetti, R. Santos, A. Sheridan, C. Sougnez, N. Stange-Thomann, N. Stojanovic, A. Subramanian, D. Wyman, J. Rogers, J. Sulston, R. Ainscough, S. Beck, D. Bentley, J. Burton, C. Clee, N. Carter, A. Coulson, R. Deadman, P. Deloukas, A. Dunham, I. Dunham, R. Durbin, L. French, D. Grafham, S. Gregory, T. Hubbard, S. Humphray, A. Hunt, M. Jones, C. Lloyd, A. McMurray, L. Matthews, S. Mercer, S. Milne, J.C. Mullikin, A. Mungall, R. Plumb, M. Ross, R. Shownkeen, S. Sims, R.H. Waterston, R.K. Wilson, L.W. Hillier, J.D. McPherson, M.A. Marra, E.R. Mardis, L.A. Fulton, A.T. Chinwalla, K.H. Pepin, W.R. Gish, S.L. Chissoe, M.C. Wendl, K.D. Delehaunty, T.L. Miner, A. Delehaunty, J.B. Kramer, L.L. Cook, R.S. Fulton, D.L. Johnson, P.J. Minx, S.W. Clifton, T. Hawkins, E. Branscomb, P. Predki, P. Richardson, S. Wenning, T. Slezak, N. Doggett, J.F. Cheng, A. Olsen, S. Lucas, C. Elkin, Initial sequencing and analysis of the human genome, Nature 409 (2001) 860–921.

[3] J. Jurka, V.V. Kapitonov, A. Pavlicek, P. Klonowski, O. Kohany, J. Walichiewicz, Repbase Update, a database of eukaryotic repetitive elements, Cytogenet. Genome Res. 110 (2005) 462–467.

[4] B. Brouha, J. Schustak, R.M. Badge, S. Lutz-Prigge, A.H. Farley, J.V. Moran, H.H. Kazazian Jr., Hot L1s account for the bulk of retrotransposition in the human population, Proc. Natl. Acad. Sci. USA 100 (2003) 5280–5285.

[5] T.H. Bestor, Sex brings transposons and genomes into conflict, Genetica 107 (1999) 289–295.

[6] D. Bourc'his, T.H. Bestor, Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking Dnmt3L, Nature 431 (2004) 96–99.

[7] K. Hata, Y. Sakaki, Identification of critical CpG sites for repression of L1 transcription by DNA methylation, Gene 189 (1997) 227–234.

[8] C.P. Walsh, J.R. Chaillet, T.H. Bestor, Transcription of IAP endogenous retroviruses is constrained by cytosine methylation, Nat. Genet. 20 (1998) 116–117.

[9] J.H. Martens, R.J. O'Sullivan, U. Braunschweig, S. Opravil, M. Radolf, P. Steinlein, T. Jenuwein, The profile of repeat-associated histone lysine methylation states in the mouse epigenome, EMBO J. 24 (2005) 800–812.

[10] J. Huang, T. Fan, Q. Yan, H. Zhu, S. Fox, H.J. Issaq, L. Best, L. Gangi, D. Munroe, K. Muegge, Lsh, an epigenetic guardian of repetitive elements, Nucleic Acids Res. 32 (2004) 5019–5028.

[11] G. Meister, T. Tuschl, Mechanisms of gene silencing by double-stranded RNA, Nature 431 (2004) 343–349.

[12] T. Sijen, R.H. Plasterk, Transposon silencing in the *Caenorhabditis elegans* germ line by natural RNAi, Nature 426 (2003) 310–314.

[13] A.I. Kalmykova, M.S. Klenov, V.A. Gvozdev, Argonaute protein PIWI controls mobilization of retrotransposons in the *Drosophila* male germline, Nucleic Acids Res. 33 (2005) 2052–2059.

[14] V.J. Robert, N.L. Vastenhouw, R.H. Plasterk, RNA interference, transposon silencing, and cosuppression in the *Caenorhabditis elegans* germ line: similarities and differences, Cold Spring Harb. Symp. Quant. Biol. 69 (2004) 397–402.

[15] S. Yang, S. Tutton, E. Pierce, K. Yoon, Specific double-stranded RNA interference in undifferentiated mouse embryonic stem cells, Mol. Cell. Biol. 21 (2001) 7807–7816.

[16] P. Svoboda, P. Stein, H. Hayashi, R.M. Schultz, Selective reduction of dormant maternal mRNAs in mouse oocytes by RNA interference, Development 127 (2000) 4147–4156.

[17] F. Wianny, M. Zernicka-Goetz, Specific interference with gene function by double-stranded RNA in early mouse development, Nat. Cell Biol. 2 (2000) 70–75.

[18] P. Stein, F. Zeng, H. Pan, R.M. Schultz, Absence of non-specific effects of RNA interference triggered by long double-stranded RNA in mouse oocytes, Dev. Biol. 286 (2005) 464–471.

[19] D.A. Kramerov, M.I. Bukrinsky, A.P. Ryskov, DNA sequences homologous to long double-stranded RNA. Transcription of intracisternal A-particle genes and major long repeat of the mouse genome, Biochim. Biophys. Acta 826 (1985) 20–29.

[20] P. Svoboda, P. Stein, M. Anger, E. Bernstein, G.J. Hannon, R.M. Schultz, RNAi and expression of retrotransposons MuERV-L and IAP in preimplantation mouse embryos, Dev. Biol. 269 (2004) 276–285.

[21] A.E. Peaston, A.V. Evsikov, J.H. Graber, W.N. de Vries, A.E. Holbrook, D. Solter, B.B. Knowles, Retrotransposons regulate host genes in mouse oocytes and preimplantation embryos, Dev. Cell 7 (2004) 597–606.
[22] C.E. Park, M.R. Shin, E.H. Jeon, S.H. Lee, K.Y. Cha, K. Kim, N.H. Kim, K.A. Lee, Oocyte-selective expression of MT transposon-like element, clone MTi7 and its role in oocyte maturation and embryo development, Mol. Reprod. Dev. 69 (2004) 365–374.
[23] L. Piko, M.D. Hammons, K.D. Taylor, Amounts, synthesis, and some properties of intracisternal A particle-related RNA in early mouse embryos, Proc. Natl. Acad. Sci. USA 81 (1984) 488–492.
[24] C. Kanellopoulou, S.A. Muljo, A.L. Kung, S. Ganesan, R. Drapkin, T. Jenuwein, D.M. Livingston, K. Rajewsky, Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing, Genes Dev. 19 (2005) 489–501.
[25] C. Bouniol-Baly, L. Hamraoui, J. Guibert, N. Beaujean, M.S. Szollosi, P. Debey, Differential transcriptional activity associated with chromatin configuration in fully grown mouse germinal vesicle oocytes, Biol. Reprod. 60 (1999) 580–587.
[26] F. Zeng, R.M. Schultz, RNA transcript profiling during zygotic gene activation in the preimplantation mouse embryo, Dev. Biol. 283 (2005) 40–57.
[27] F. Zeng, D.A. Baldwin, R.M. Schultz, Transcript profiling during preimplantation mouse development, Dev. Biol. 272 (2004) 483–496.
[28] R. Bachvarova, V. De Leon, Polyadenylated RNA of mouse ova and loss of maternal RNA in early development, Dev. Biol. 74 (1980) 1–8.
[29] P.T. Brower, E. Gizang, S.M. Boreen, R.M. Schultz, Biochemical studies of mammalian oogenesis: synthesis and stability of various classes of RNA during growth of the mouse oocyte in vitro, Dev. Biol. 86 (1981) 373–383.
[30] Z. Alizadeh, S. Kageyama, F. Aoki, Degradation of maternal mRNA in mouse embryos: selective degradation of specific mRNAs after fertilization, Mol. Reprod. Dev. 72 (2005) 281–290.
[31] L.P. Lim, N.C. Lau, P. Garrett-Engele, A. Grimson, J.M. Schelter, J. Castle, D.P. Bartel, P.S. Linsley, J.M. Johnson, Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs, Nature 433 (2005) 769–773.
[32] S. Bagga, J. Bracht, S. Hunter, K. Massirer, J. Holtz, R. Eachus, A.E. Pasquinelli, Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation, Cell 122 (2005) 553–563.
[33] M.A. Valencia-Sanchez, J. Liu, G.J. Hannon, R. Parker, Control of translation and mRNA degradation by miRNAs and siRNAs, Genes Dev. 20 (2006) 515–524.
[34] R.S. Pillai, MicroRNA function: multiple mechanisms for a tiny RNA? RNA 11 (2005) 1753–1761.
[35] S. Griffiths-Jones, R.J. Grocock, S. van Dongen, A. Bateman, A.J. Enright, miRBase: microRNA sequences, targets and gene nomenclature, Nucleic Acids Res. 34 (2006) D140–D144.
[36] B.P. Lewis, C.B. Burge, D.P. Bartel, Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets, Cell 120 (2005) 15–20.
[37] A. Krek, D. Grun, M.N. Poy, R. Wolf, L. Rosenberg, E.J. Epstein, P. MacMenamin, I. da Piedade, K.C. Gunsalus, M. Stoffel, N. Rajewsky, Combinatorial microRNA target predictions, Nat. Genet. 37 (2005) 495–500.
[38] M. Kiriakidou, P.T. Nelson, A. Kouranov, P. Fitziev, C. Bouyioukos, Z. Mourelatos, A. Hatzigeorgiou, A combined computational-experimental approach predicts human microRNA targets, Genes Dev. 18 (2004) 1165–1178.
[39] B.P. Lewis, I.H. Shih, M.W. Jones-Rhoades, D.P. Bartel, C.B. Burge, Prediction of mammalian microRNA targets, Cell 115 (2003) 787–798.
[40] B. John, A.J. Enright, A. Aravin, T. Tuschl, C. Sander, D.S. Marks, Human MicroRNA targets, PLoS Biol. 2 (2004) e363.
[41] J. Krutzfeldt, N. Rajewsky, R. Braich, K.G. Rajeev, T. Tuschl, M. Manoharan, M. Stoffel, Silencing of microRNAs in vivo with 'antagomirs', Nature 438 (2005) 685–689.
[42] J. Rehwinkel, P. Natalin, A. Stark, J. Brennecke, S.M. Cohen, E. Izaurralde, Genome-wide analysis of mRNAs regulated by Drosha and Argonaute proteins in *Drosophila melanogaster*, Mol. Cell. Biol. 26 (2006) 2965–2975.
[43] T. Somfai, K. Kikuchi, A. Onishi, M. Iwamoto, D. Fuchimoto, A.B. Papp, E. Sato, T. Nagai, Meiotic arrest maintained by cAMP during the initiation of maturation enhances meiotic potential and developmental competence and reduces polyspermy of IVM/IVF porcine oocytes, Zygote 11 (2003) 199–206.
[44] E. Sarot, G. Payen-Groschene, A. Bucheton, A. Pelisson, Evidence for a piwi-dependent RNA silencing of the gypsy endogenous retrovirus by the *Drosophila melanogaster* flamenco gene, Genetics 166 (2004) 1313–1321.
[45] H.H. Kazazian Jr., J.V. Moran, The impact of L1 retrotransposons on the human genome, Nat. Genet. 19 (1998) 19–24.
[46] E.L. Kuff, K.K. Lueders, The intracisternal A-particle gene family: structure and functional aspects, Adv. Cancer Res. 51 (1988) 183–276.
[47] P.M. Waterhouse, M.B. Wang, T. Lough, Gene silencing as an adaptive defence against viruses, Nature 411 (2001) 834–842.
[48] J. Wang, E. Tekle, H. Oubrahim, J.J. Mieyal, E.R. Stadtman, P.B. Chock, Stable and controllable RNA interference: investigating the physiological function of glutathionylated actin, Proc. Natl. Acad. Sci. USA 100 (2003) 5103–5106.