Supplemental Materials

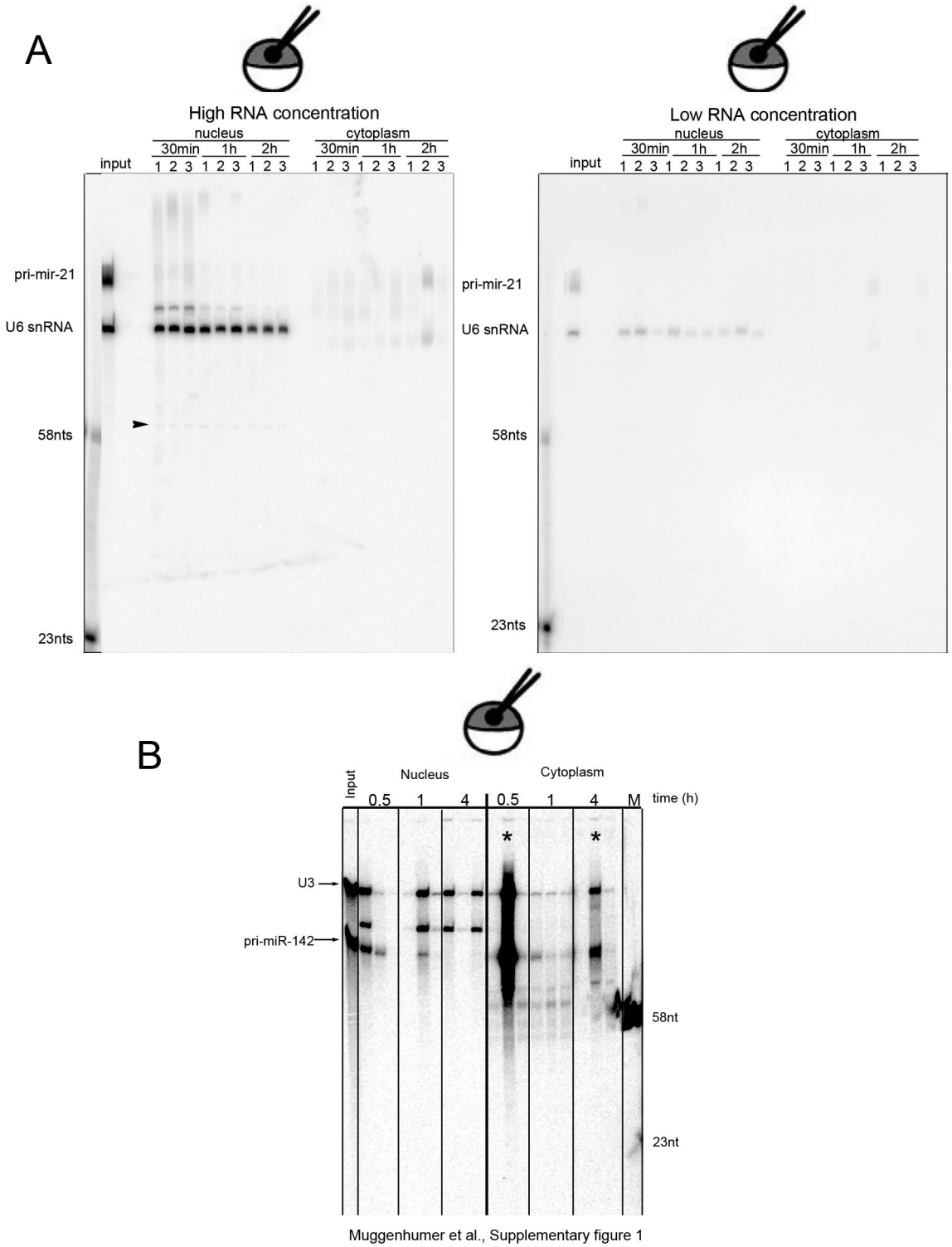
Molecular Biology of the Cell

Muggenhumer et al.

Supplementary Figures

Supplementary Figure 1. *Analysis of pri-miRNA processing in oocyte nuclei*

A) To test whether the efficiency of pri-miRNA processing was dependent on the concentration of injected substrate, different concentrations of pri-miR-21 were injected into oocyte nuclei. At a concentration of 10ng / nucleus (left panel) a faint band at the height of the expected pre-miR-21 was detectable (arrowhead at ~60nt) while no processing of pri-miR-21 was detectable when only 2ng of pri-miR-21 were injected per nucleus (right panel). B) Stage VI oocyte nuclei were injected with radiolabeled pri-miRNA-142 together with nuclear retained U3 snoRNA. At different time points after injection nuclei and cytoplasms were manually separated, the RNA was extracted and analyzed by autoradiography after Urea-PAGE electrophoresis. Pri-miRNA-142 was degraded in nuclei while U3 snoRNA was stable. When the RNA mixture was misinjected into the cytoplasm (asterisk) both U3 and pri-miRNA-142 were relatively stable. Each lane represents RNA from a single oocyte nucleus or cytoplasm. The RNA was monitored for up to 4 hrs after injection. Injected material is loaded (input) as well as molecular weight markers corresponding to the predicted sizes of pre- and mature miR-142



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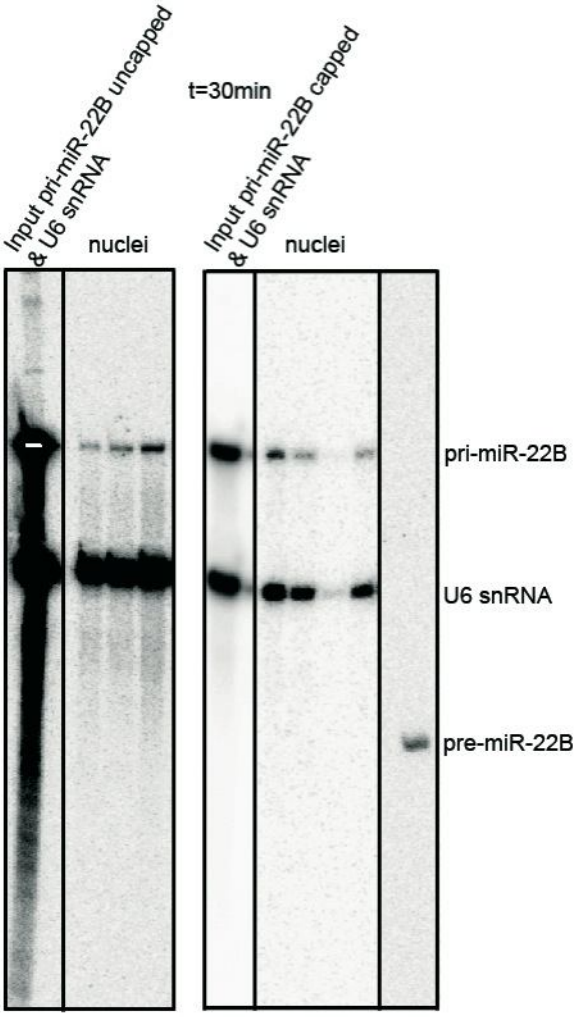
Supplementary figure 2. *Nuclear stability of pri-miRNAs is not dependent on their capping status.*

Uncapped (left panel) and capped (right panel) pri-miRNA-22B was injected together with U6 snoRNA as a nuclear injection control. The input lane shows an aliquot of the injected mixture. After 30 minutes of incubation, nuclei were extracted and the RNA was analyzed on an Urea-PAGE. Both capped and uncapped pri-miRNAs are degraded at similar rates in nuclei. This is consistent with the fact that the many endogenous miRNAs in *Xenopus* are derived from introns and will thus lack a cap structure (Tang and Maxwell, 2008).



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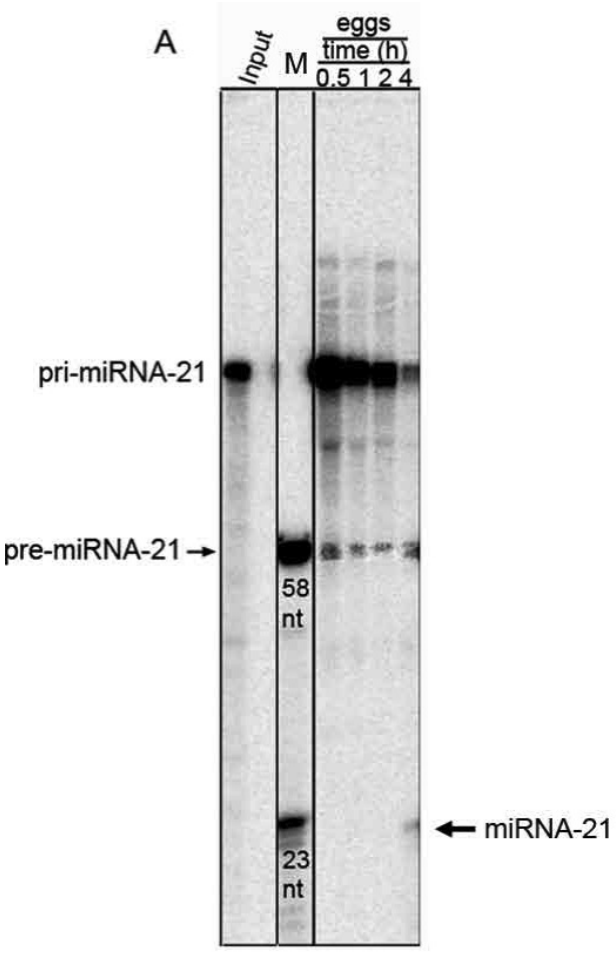
Supplementary figure 3. *Processing of pri-miRNA-21takes place in matured eggs.* Pri-miRNA-21 was into eggs. RNAs were extracted at indicated time points from individual eggs and loaded onto denaturing acrylamide gels. Processing of pri- to pre and further on to mature miRNAs can be followed. The injected pri-miRNA is loaded in the input lane.



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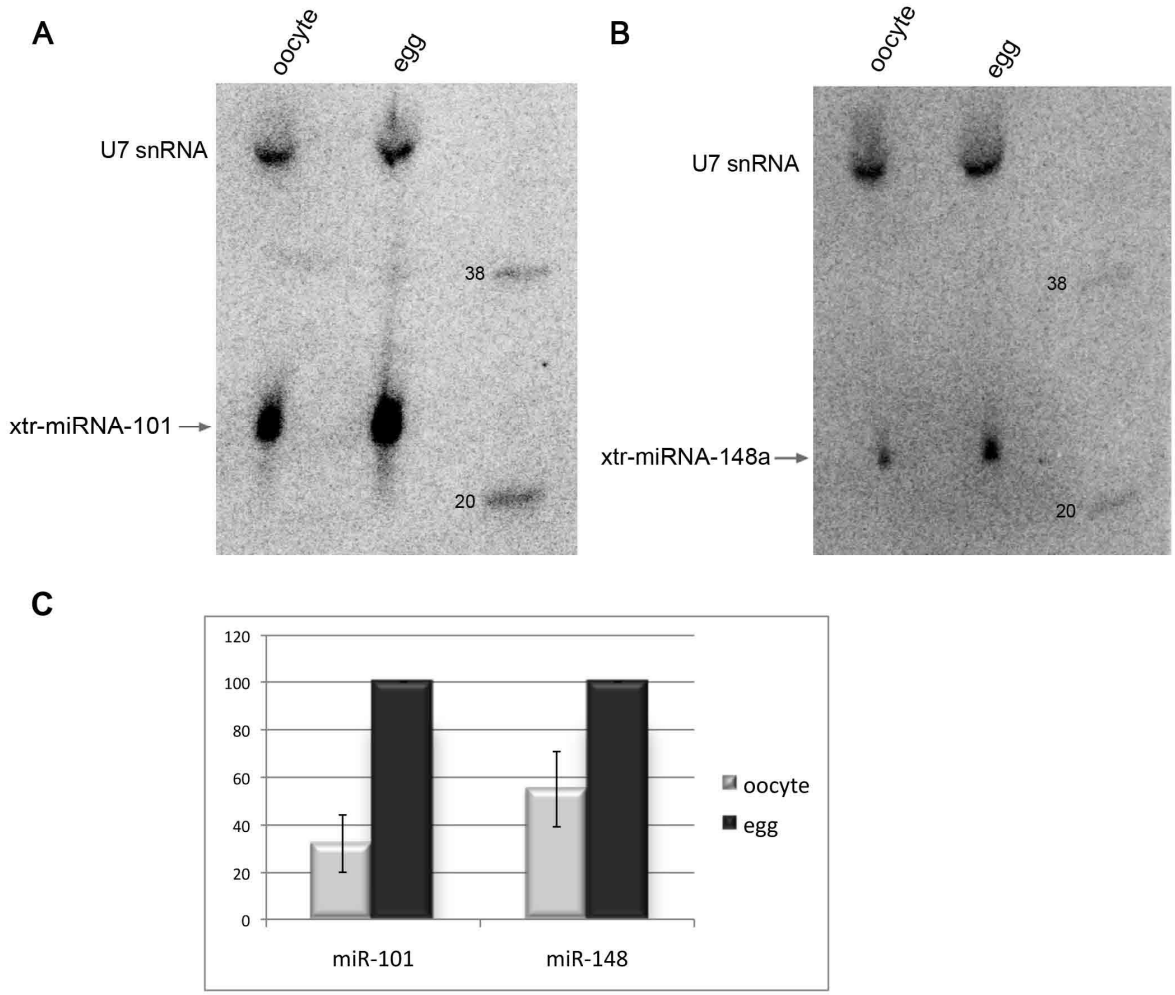
Supplementary figure 3

Supplementary figure 4. *Northern blots demonstrate an increase of endogenous Xenopus miR-101 and miR-148a upon oocyte to egg maturation.*

RNAs of equal number of defolliculated oocytes and eggs were isolated and loaded on denaturing urea acrylamide gels. After transfer to a charged nylon membrane the blots were hybridized for with probes for miR-101 (A) and miR-148-a (B) as well as U7 snRNA as a quantification control. (C) Quantification of 3 blots for endogenous xtr-miRNA-101 (left) and xtr-miRNA-148a (right) expression relative to U7 snRNA in oocytes and eggs. Blots were exposed to phosphoimager screens and quantified on a FXPro Phosphoimager (BioRad, CA). Exposures were chosen to avoid saturated pixels that are automatically indicated by the quantification software. Endogenous miRNA expression of xtr-miRNA-101 and xtr-miRNA-148a is strongly enhanced in eggs.



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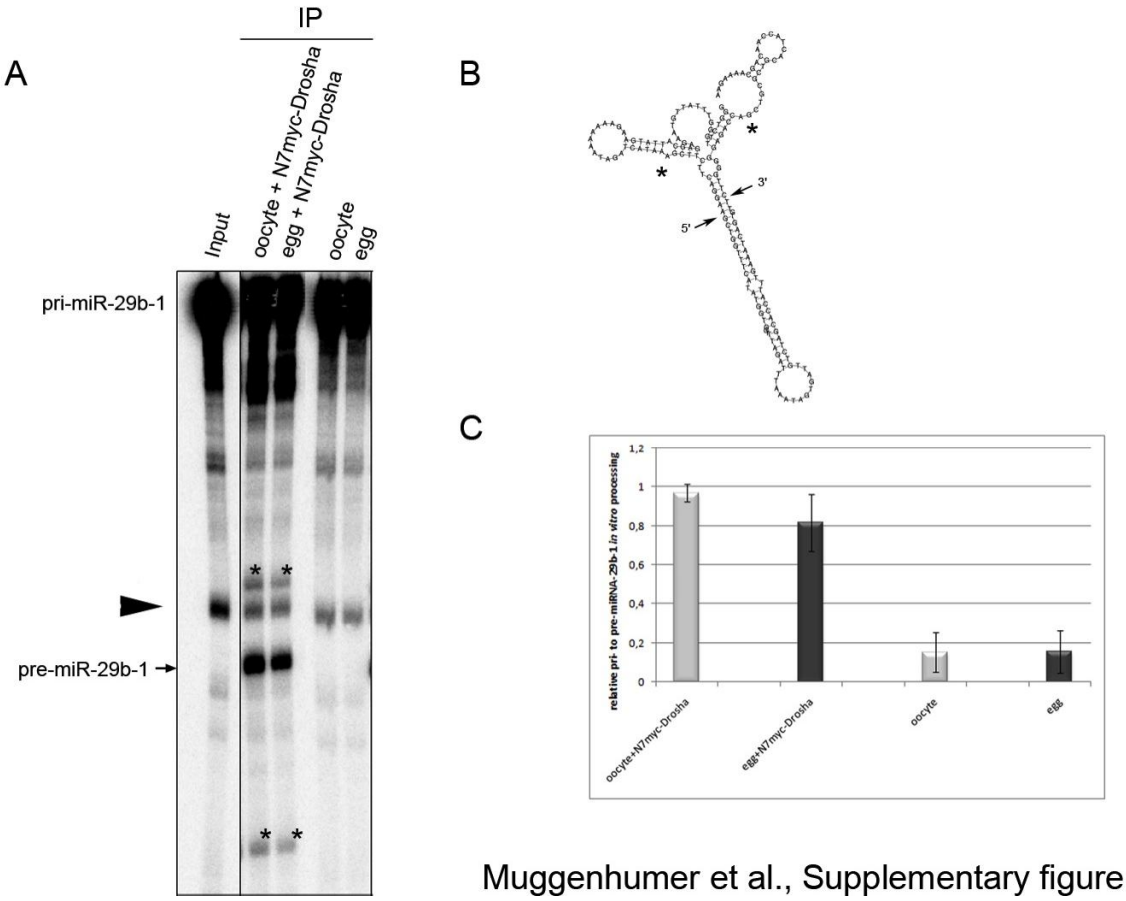
Supplementary figure 5. *Ectopically expressed Drosha expressed in oocytes or eggs is active in vitro*

RNA encoding myc-tagged Drosha was injected in oocytes and half of the cells were matured to eggs. Cells were lysed and lysates were used to immunoprecipitate the translated Drosha protein using α-myc antibody. The precipitated material was used for an in vitro processing assay. (A) The pri-miRNA-29b-1 processing assay reveals activity of recombinant Drosha enzyme purified from oocytes or eggs. The arrow marks the size where pre-miRNA-29b-1 is expected (63nt). Asterisks mark putative cleavage byproducts of 67 and 43 nt respectively. The arrowhead marks a non-specific breakdown band that was already present in the input material. (B) Schematic drawing of the pri-miRNA-29b-1 used in the in vitro processing assay. Cleavage sites are indicated by arrows, side products are labeled by asterisks. Different band intensities reflect different numbers of radiolabeled adenosine residues. (C) Pri- to pre-miRNA-29b-1 ratios were calculated based on absolute intensities . myc-precipitated material from oocytes (column 1) and eggs (column 2) expressing recombinant Drosha do not differ in processing levels. Myc-precipitate from uninjected, control oocytes (column 3) and eggs (column 4) expressing no recombinant Drosha do not exhibit a distinct processing pattern.



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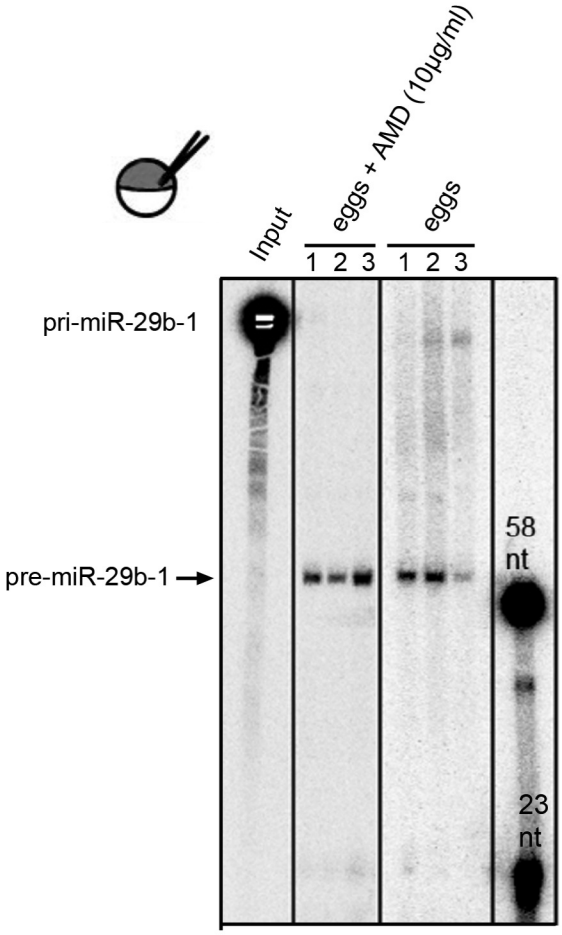
Supplementary figure 6. *Stimulation of Drosha activity does not require ongoing transcription.*

To test for transcriptional activation of Drosha, oocytes were incubated with 10µg/ml Actinomycin D prior to maturation to eggs by progesterone. Eggs were then injected with pri-miRNA-29b-1, incubated for 2h and processing of RNAs isolated from individual eggs (1,2,3) were analyzed on a PAA-Gel. Processing activity could be detected in both the transcriptionally silent cells (eggs+AMD) and untreated control cells (eggs).



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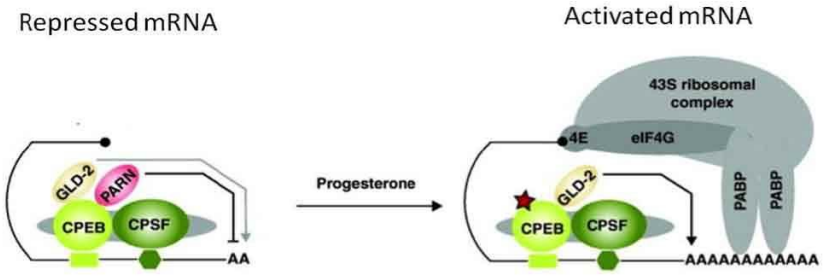
Supplementary figure 6

Supplementary figure 7. *Xenopus tropicalis Drosha* mRNA contains several cytoplasmic polyadenylation signals.

A) In the egg the cytoplasmic polyadenylation signal (light green box) is masked by CPSF. Upon maturation to eggs CPEB is phosphorylated allowing polyA extension (drawing adapted from Villalba, Coll, and Gebauer, 2011).

B) The *Xenopus tropicalis Drosha* mRNA BC157520 contains three putative cytoplasmic polyadenylation signals (light green) preceeding a *bona fide* nuclear poly A signal (dark green).

A



B

Xenopus tropicalis Drosha mRNA (3´end)

…….AGATACATGTCCAGCTGTGTCTGGGGAAACACAAGATAAATCATGCATCTTTTTTTTG
TTTTGTTTTAGGTGTCTTTTTTTTTTTTTAACTTGTCACATTTTAGTGGATTTTATCCATAGCT
GAGCTATTTTC**TTTTTAT**TTAAATACTTTAGTAAATTATTG**TTTTTAT**TATATTG**TTTTTAT**A
A**AATAAA**TGGCAGGTGTGT**AATAAA**AAAAAAAAAAAA

TTTTTAT … cytoplasmatic polyadenylation element (CPE)

AATAAA … polyadenylation hexanucleotide

References:

Tang, G.Q., and Maxwell, E.S. (2008). Xenopus microRNA genes are predominantly located within introns and are differentially expressed in adult frog tissues via post-transcriptional regulation. Genome Res *18*, 104-112.