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Additional Methods

RiboTag IP/RNAseq

Pools of oocytes from RiboTag\textsuperscript{fl/fl} : Zp3-Cre mice primed with 5U PMSG (National Hormone and Peptide Program) were retrieved 45 hrs after injection and incubated for 0,2,4,6 hrs in maturation medium. After harvesting, RNase free PBS-1% PVP and immediately transferred to 300 μl of fresh supplemented homogenization buffer (S-HB) (50 mM Tris pH 7.4, 100 mM KCl, 12 mM MgCl\textsubscript{2}, 1% NP-40, 1 mM DTT, Protease inhibitors, 40U RNAsin (Promega - N2115), 40U RNAseOUT, 100 μg/ml Cyclohexamide and 1 mg/ml Heparin (Sigma - H3393). Cells were then freeze/thawed 3 times in this lysis buffer and homogenates centrifuged at 15,000 rpm in table top refrigerated centrifuge. 3 μg of anti-HA (Covance - MMS-101R) or mouse IgG (Abcam - ab37355) were added to the oocyte lysate and incubated for 4 hours at 4 °C on a rotor. 25 μl of pre-washed protein G magnetic Dynabeads (Invitrogen - 10007D) were added to each lysate and incubated for 5 hours at 4°C on a rotor. Beads were washed with high salt buffer 5 times (50 mM Tris pH 7.4, 300 mM KCl, 12 mM MgCl\textsubscript{2}, 1% NP-40, 1 mM DTT, 40U RNAseOUT, 100 μg/ml Cyclohexamide), Urea to a final concentration of 1 M was included in these washes. RNA was eluted from beads with 200 μl of RTL lysis buffer (RNeasy Micro Plus kit – Qiagen – 74034) supplemented with 1% 2-Mercaptoethanol (Sigma - M6250). RNA extraction was carried out using the RNeasy Micro Plus kit according to manufactures instructions. Total RNA was eluted from column in 9 μl of DEPC treated water.

RNA preparations were tested for quality on Bioanalyzer chips and were used for preparation RNA-seq libraries with ovation RNA-seq system v2 kit (NuGEN). Briefly after a round of amplification (Kurn et al., 2005; Dafforn et al. 2004 ), the RNA template was partially degraded by heating and the second strand cDNA synthesized using DNA polymerase. The double-stranded DNA was then amplified using single primer isothermal amplification (SPIA). Libraries from the SPIA amplified cDNA were made using the Ultralow DR library kit (NuGEN). The RNA-seq libraries were analyzed by Bioanalyzer and quantified by QPCR (KAPA). High-throughput sequencing was done using a HiSeq 4000 instrument (Illumina).

A second set of RiboTag IP samples from oocytes harvested at 0 or 6hr of maturation were processed in a manner similar to that described above except that Urea high stringency washes was limited to the last two washes. Pilot experiments with RiboTag IP/qPCR indicated that the five Urea washes lead to a decrease of non-specific IgG signal but also to a significant loss of the specific IP signal. The other steps and data analysis were the same as those described for the first dataset. Fastq files were subjected to QC and analyzed using different workflows including TopHat and Cufflinks, or HISAT2 and Cufflinks, or HTSeq and DESeq2. Average reads depth for the first dataset was 30 million and second data set 60 million.

DaPars analysis

Bam files derived from the above RNAseq mapping were converted to .bedgraph files and used as an input for the DaPars computation analyses. We have used this algorithm for \textit{de novo} identification and quantification of dynamic APA events between RiboTag IP samples at 0 and 6 hrs of oocyte maturation (Xia et al. 2014). After identification of the distal PAS, the algorithm uses a regression model to infer the exact location of a \textit{de novo} proximal PAS and
related boundary. The presence of a proximal PAS was manually verified for at least 20 transcripts chosen randomly from the list of proximal PAS containing transcripts predicted with DaPars algorithm. The percentage of distal APA usage index (PDUI) was then calculated as a parameter of the relative abundance of the distal transcript; in our case, this is the relative abundance of the transcript with the distal PAS associated with the ribosomes. By comparing changes in PDUI between the two sets of data collected at 0 and 6 hrs, predictions can be made on the preferential ribosome loading of a mRNA that uses the proximal PAS (positive ΔPDUI) or the distal PAS (negative ΔPDUI). The statistical analyses used are the default parameters set by the algorithm, with the exception that we relaxed the stringency including PDUI differences of 0.1. Output files were parsed by removing data with adjusted P value of >0.05.

**Subcloning and sequencing of the anchored PCR products**

Total RNA extracted from 50 oocytes at 0 h was used for Oligo(dT) anchored PCR with Primer A, which initiates before the stop codon of CcnB1, and reverse oligo(dT)-Anchor primer, as described in the previous part. Three bands in the 1.5% agarose gel were cut, purified respectively using Universal DNA Purification Kit (Tiangen, DP214), and inserted to a plasmid pCloneEZ-Blunt-Amp/HC (CloneSmarter Technologies, C5861) for sequencing with M13F/R primer. For sequencing full-length of the Long form, we also used another primer synthesized, the sequence of which is CCATTTTCCAGTGGTTGCC. The sequencing data were mapped to NM_172301.3 to confirm the autenthicity of the amplified fragment and to confirm the site of polyadenylation.

**RiboTag IP/ qPCR**

RiboTag IP was performed as previously described (Sousa Martins et al. 2016). For qPCR, extracted RNA from input or IP pellet (with HA antibody or control IgG) was used to prepare cDNA using SuperScript III First-Strand Synthesis system (Invitrogen, 18080-051) using random hexamer oligonucleotide primers. qPCR was performed with primers in Table 2 using KAPA SYBR FAST qPCR Kit (Roche, 07959427001). In each experiment, a standard curve was generated by amplification of DNA encompassing the 3’ UTR of Ccnb1. The copy number was calculated from the Ct value using the following equation: Number of copies = (x ng * 6.0221 x 10^{23} molecules/mole)/[(N*660 g/mole) * 1 x 10^9 ng/g)]. Non specific immunoprecipitation was measured by using IgG rather than the HA antibody and the background was subtracted from the HA IP. Estimated copy number values were corrected for the number of oocytes used in the experiments. To derive the copy number for each 3’ UTR, the values obtained with the amplification of the intermediate and long form were subtracted from the copy number of the short form.

**Western blot** The Western blot detecting CCNB1 was performed as previously described (Chen et al., 2011).
**Additional References**

