Supplementary Figure S1.
Relative abundance (in %) of transcripts from DNA transposons in oocytes and embryos at the stages indicated. Data derive from RNA sequencing data from 2 independent biological replicates and at 2 technical replicates. Note that the relative abundance of Mer1-type transcripts peaks at the 2-cell stage, whereas the relative abundance of Tip100 shows the opposite behavior. Data were processed as in figure1.
Supplementary Figure S2.
Optimisation of chromatin immunoprecipitation for low cell numbers.

a-b. ChIP performed on ES using a standard protocol (a) and the protocol adapted for reduced amount of cells (b). ChIP analysis performed using 30µg of chromatin for each IP is shown in a. H3K9me3 is enriched at IAP loci, this enrichment is less pronounced at LineL1 and SineB2, H3K4me4 is close to undetected levels in agreement with data published by Martens and colleagues (Martens JH, EMBO J. 2005 Feb 23;24(4):800-12). The same profile for H3K9me3 and H3K4me3 enrichment (b) is obtained using the protocol that we optimised to work with low amount of cells (500 cells for 3 IPs). We were unable to perform Re-ChIP with such low amount of cells and therefore we cannot ascertain whether the two methylation sites are present on the same nucleosome.

Note that comparison of these data with those from figure 2b suggests that RE have an ‘atypical’ configuration with a high enrichment of H3K4me3 at the beginning of development, which seems to be resolved into a more ES-type at the 8-cell stage.

c. Diagram depicting the detailed steps of the optimised ChIP protocol.
Supplementary Figure S3.

a- b. Heterochromatin marks are remodelled after fertilisation. Immunostaining with H4K20me3 (a) and H3K64me3 (b) antibodies reveals a global reduction of these constitutive heterochromatin marks from the 2-cell stage. The male and female pronuclei are indicated in the zygote and the polar body is indicated by an arrowhead. Note that both H4K20me3 and H3K64me3 are undetectable from the 2-cell stage. Shown are full projections of representative embryos of at least 10 analysed per stage. Scale bar is 10 mm.

c. The antisense Line L1 17nt RNA is forming triple helix significantly less efficiently than the sense RNA. Triple helix assay was performed as in figure 4b except that here the dsDNA was radioactively labelled (and not the ssRNA). Forward ssDNA radioactively labelled and annealed with reverse ssDNA was incubated with either sense (lanes 2-5, ssRNA FW) or antisense (lanes 7-10, ssRNA RV) in 1x, 2x, 10x or 600x-fold excess and separated by PAGE. The expected position of the triplex on the gel is indicated on the left. Note that the gel was ran for a shorter time than the gel shown on figure 4b.
Supplementary Figure S4

a. **Specificity of the anti-poly(dA).poly(rU).poly(rU) antibodies** measured by their reactivity to different polynucleotide conformations. Curves were obtained by plotting the logarithm of antibody concentrations (ab.conc.) used in the assays (X axis) and the absorbance values (abs. 405 nm) from the ELISA data (Y-axis). Data is presented as in Gorab et al (Chromosome Res. 2009;17(6):821-32).

b. **dA.2rU reactivity in nuclei of late 2-cell embryos is sensitive to RNase A and RNase V1.** Embryos were permeabilised and treated with RNase A or RNase V1, fixed and processed for immunostaining with the anti-triplex antibody. Shown are confocal sections representative 2-cell nuclei of 7/7 and 10/14 embryos analysed for RNase V1 and RNase A treatment, respectively. Scale bar is 5 µm. The dotted line marks the contour of the nuclei in each panel.

c. **Microinjection of the short 17nt-long RNA from Line L1s promotes transcriptional activation of Line L1s but not of IAPs.** Quantification of IAP nascent transcripts sites (mean intensity) and their intensity (max intensity) detected by RNA-FISH as shown in figure 4f. Quantification was done using ImageJ. Each dot corresponds to individual nuclei of the 3 experimental groups: non-injected, Line RNA and scrambled RNA.
Supplementary Figure S5.

a. **Relative distribution of the abundance of Line L1 transcripts** derived from Tf (L1spa, AF016099 Genbank), Gf (AC021631 Genbank, positions 62222…67691) or Af (AY053455 Genbank consensus) families. The number of reads aligned to each family was analysed with maqv0.7.1. allowing 3 mismatches. Only uniquely mapped reads were considered for the analysis. Shown is the average relative abundance for each of the 3 families from 3 (oocyte) or 2 (2-cell and 8-cell stage) independent biological replicates and S.E.M. Note that the transcript abundance is calculated based on uniquely mapped reads to one single consensus sequence of each of the three families.

b. **In vitro assay for triple helix formation on the IAP polypurine stretch identified on position 871.** IAP consensus sequence contains 3 continuous polypurine stretches of ≥ 14nt at positions 871, 2988 and 6736. Triplex assay was conducted with the stretch at position 871 (aaaaaagggagaagaaaa) as in Figure 4: radioactively labeled ssRNA was incubated with dsDNA in 1x, 10x or 20x-fold excess and separated in PAGE. The expected running size of the triplex is indicated on the left and the different combinations of double and triplex are shown schematically on the right. Increasing RNase H concentrations were used to distinguish between Hoogsteen bonding between ssRNA and DNA as opposed to Watson-Crick pairing. Shown is a representative of 3 different experiments.

c. **Schematic representation of murine IAP, Line L1 and Sine B2 and the position of the primers used in this study.** Diagram of a typical IAP, LineL1 and SineB2 full length elements with the position of the primers used for ChIP and RT-PCR analysis. gag, group antigens; pol is the reverse transcriptase; TSD stands for target site duplication, ORF1 and ORF2 for open reading frame 1 and 2 respectively.
Supplementary Figure S6.
Global flowchart of the bioinformatic pipeline implemented for assignment of reads to repetitive elements.
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Supplementary Table S1. Read count per type of RE per stage. The read counts and the relative percentage to the RE transcriptome from the two biological replicates (1 and 2) are shown. Average is the average percentage relative to the total RE transcriptome from the two biological replicates.
**Supplementary Table 2. Primers used in this study**

NCage-Template switching primer: TAGTCGAACTGAAGGTCTCCAGCArGrGrG

NCage-Reverse-transcription random primer: GTACCAGCAGTAGTCGAACTGAAGGTCTCCTCTCTN15

NCage-Semi-suppressive PCR forward primer: TAGTCGAACTGAAGGTCTCCAGC

NCage-Semi-suppressive PCR reverse primer: GTACCAGCAGTAGTCGAACTGAAGGTCTCCTCTCTCT

NCage-Up adapter primer (X3 represents a DNA barcodes of 3 bases): NNX3CTGTGAAACTCTGGAACCTGT

NCage-Down adapter primer (X3 represents a DNA barcodes of 3 bases): ACAGGTTGAGTTCTACAGX3

NCage-Forward PCR primer: CAAGCAGAAGACGGCCACACGATAGTCGAACGGCTGCC

NCage-Reverse PCR primer: AATGATACGCGACCCGCCAGGTTCAAGTCTCAG

Cdx2 FW RT-PCR: 5’AGACAAATACGCGGTGGTGTAGTA

Cdx2 RV RT-PCR: 5’CCAGCTCAGCTTCTCCTCCTGA

ZP3 FW RT-PCR: 5’GGTGGAGTGTTGTCTGGAAGCTGAA

ZP3 RV RT-PCR: 5’GATAGGGTGTTGCTCCTACATTG

Line TFO RNA: AGAAAAAGAGGAAAAAA
SUPPLEMENTARY NOTE

NanoCage and RNA sequencing

The number of oocytes or embryos used for the libraries are the following: oocytes 21 and 24 for the two biological replicates, respectively; 2-cell stage 30 and 39 embryos; 8-cell stage 11 and 16 embryos; blastocyst 10 and 15 embryos.

a. RNA extraction and Reverse transcription. Total RNA was extracted from embryos using the NucleoSpin RnaXS (Macherey-Nagel) following the manufacturers recommendations. Total RNA was mixed with 1 µl of a solution composed with 0.53 M d-(+)-Trehalose dehydrate (T9531, Sigma Aldrich), 2.6 M d-Sorbitol (240850, Sigma Aldrich), 100 µM of Template switching primer, 10 µM of Reverse-transcription random primer with a random pentadecamer tail (all the primer sequences are listed in Supplementary Table S2) and the final volume was reduced to 2 µl using a Speed vac Concentrator (Savant). The samples were then heated at 65°C for 10 min in a thermocycler (Gene Amp PCR System 9700, Applied Byosystems) and chilled on ice immediately. Subsequently, reverse transcription was carried out in the presence of 1.25× First-strand buffer (Invitrogen), 1.3 mM DTT (Invitrogen), 625 µM dNTP mix (Takara), 925 mM Betaine (B2629, Sigma) and 200 units of SuperScript II (Invitrogen) at 22°C for 10 min, 50°C for 30 min and 75°C for 15 min and chilled on ice.

b. Second-strand synthesis, semi-suppressive PCR amplification and digestion. We performed a small-scale PCR to evaluate the optimal number of cycles needed for second-strand synthesis and amplification. Two µl of first strand cDNA was amplified in a final volume of 100 µl in the presence of 1× ExTaq Buffer (Takara), 200 µM dNTPs (Takara), 100 nM Semi-suppressive PCR forward primer, 100 nM Semi-suppressive PCR reverse primer and 5 units of ExTaq (Takara) with the following cycling conditions: 95°C for 5 min, followed by a total of 40 cycles of [95°C for 15 sec, 65 °C for 10 sec and 68°C for 2 min], and a final incubation at 68°C for 15 min and then paused at 4°C. An aliquot of 10 µl was collected every two cycles (during the elongation step of the PCR) for analysis on 1% agarose gel. We then performed a large-scale PCR amplification using 2µl of the remaining volume of first strand cDNA in 2 reactions of 100 µl with the appropriate number of cycles. The PCR products were then pooled and purified using QIAquick PCR purification kit (Qiagen), and eluted in 50 µl EB buffer. We digested 45 µl of purified PCR product with EcoP15I (NEB) followed by purification of the low-molecular-weight digested fragments using Amicon Ultra-0.5 100K filter device (Millipore) following the manufacturer’s recommendation. The flow-through
containing low-molecular-weight digested fragments was then collected and concentrated using Amicon Ultra-0.5 10K filter device (Millipore).

c. Adapter ligation. Five µl of 1mM of Up adapter primer and of Down adapter primer each were annealed by heating them at 95°C for 2 min followed by a slow cooling to 25°C at 0.1°C/ sec using a TProfessional thermocycler (Biometra). Adapter ligation was carried by incubating 10 µl of the EcoP15I cleavage products with 1 pmol of adapters in a final volume of 20 µl in the presence of 0.5x Mighty DNA ligation mix (Takara) overnight at 16 °C.

d. Tag PCR amplification. We performed a small scale PCR to determine the optimal number of cycles necessary for the amplification of the ligation product. Two µl of ligation product was amplified in a final volume of 100 µl in the presence of 1x LA PCR Buffer II (Takara), 200 µM dNTPs (Takara), 5 µM Forward PCR primer, 5 µM Reverse PCR primer and 5 units of LA Taq (Takara) with the following cycling conditions: 95°C for 5 min, followed by a total of 20 cycles of [95°C for 10 sec, 57 °C for 10 sec and 68°C for 15 sec], and a final incubation at 68°C for 5 min. An aliquot of 10 µl was collected every two cycles (during the elongation step of the PCR) for analysis on 1% agarose gel. We then performed a large-scale PCR amplification using 2µl of the remaining volume of first strand cDNA in 2 reactions of 100 µl with the appropriate number of cycles. For each 100 µl PCR reaction, the excess of primers was digested with 5 units of Exonuclease I (Takara) at 37°C for 30 min and then the enzyme was heat inactivated at 55 °C for 15 min.

f. Gel purification. PCR products were size selected and gel purified from a 6% Novex TBE PAGE gel, (1.0 mm, 10 well, Invitrogen). The band corresponding to the expected size (~120 bp) was cut and the DNA was eluted overnight in the presence of 100µl EB buffer (Qiagen). Gel debris were removed by centrifugation in 5 µm Filter tubes (IST engineering) and the supernatant was ethanol-precipitated in the presence of glycogen and sodium acetate. The pellets were washed with 70% ethanol, dried in a speed vac at room temperature and resuspended in 15 µl EB buffer (Qiagen). The quality of the cDNA libraries was verified and quantified with Agilent High Sensitivity DNA Kit using 2100 Bioanalyzer (Agilent).

g. Sequencing. Libraries were sequenced on the Genome Analyzer IIx (Illumina) as single-end 36 bases reads with custom sequencing primer. The cDNA libraries were loaded in the flowcell at 6pM concentration and clusters were generated following Illumina’s instructions. A total of 13182080 sequences for oocytes, 36638854 for 2-cell stage embryos, 31031219 for 8-cell stage embryos and 2977099 for blastocysts were generated.