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Nuclear abundant stable intronic sequences RNA (sisRNAs) produced by a number of transcribed genes in chicken oocyte

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Motivation and Aim: Birds, like many other vertebrates, have a hypertranscriptional type of oogenesis, with an exceptionally high rate of RNA synthesis in the oocyte nucleus. Higher RNA polymerase II velocity directly correlates with the production of newly synthesised circular RNA (circRNAs) [1]. Recently, we have undertaken a detailed characterisation of the nuclear and cytoplasmic transcriptome of chicken (*Gallus gallus domesticus*) oocytes at the lampbrush chromosome stage of oogenesis [2]. Here we aimed to characterise nuclear and cytoplasmic circRNAs including stable intronic sequences RNA.

Methods and Algorithms: Total RNA, including small RNA, was isolated from the nuclei and cytoplasm of growing chicken oocytes using TRIzol reagent, following a previously developed protocol [3]. To degrade linear RNA, total nuclear RNA was also treated with the 3' to 5' exoribonuclease R (RNase R). Next we performed strand-specific high-throughput sequencing of total nuclear and cytoplasmic RNA fractions, poly(A)-enriched RNA libraries, and small RNA fractions from lampbrush-stage oocytes. CIRCexplorer2 was used to identify circRNAs in total and poly(A)-enriched oocyte nuclear RNA samples and in total nuclear RNA after RNase R treatment. The programme "fast_circ.py" with the "de novo" option that comes with CIRCexplorer2 (Version 2.3.8) was used with default parameters for *de novo* prediction of circRNAs.

Results: In the oocyte nucleus, we detected transcripts for 10697 protein-coding genes and 2488 long non-coding RNA genes. In nuclear RNA fraction certain intronic sequences were predominantly abundant within transcribed genes most probably reflecting separated intronic RNAs. A higher coverage of the individual introns in total RNA-seq of nuclear RNA fraction appears as single or multiple peaks. Resistance to RNase R treatment indicates the lariat form of some of the intronic sequences. The RNAs of intronic origin thus represent stable intronic sequences RNA (sisRNAs), which have been previously characterised in *X. tropicalis* oocyte nuclei [4].

The appearance of mature mRNA for the genes that produce sisRNA suggests that they are spliced out of the nascent transcript. In fact, we checked that sisRNAs come from the same strand as pre-mRNAs. Furthermore, only certain intronic sequences are unusually stable, while the other introns of the same primary full-length transcript are degraded upon splicing. Using CIRCexplorer2, we predicted circRNAs *de novo* in RNA samples after RNase R treatment, in total and poly(A) RNA libraries, with low read

counts in poly(A) RNA libraries. Genomic regions covered by the identified circRNAs include 5'UTR, exonic and intronic regions. These data suggest that a number of circRNAs in the oocyte nucleus are also produced by the back-splicing mechanism and do not have an intron-lariat structure. 5'UTR-derived sisRNAs, either in linear or circular form, stored in the oocyte may be involved in regulating host gene expression. The nuclear abundant RNase R resistant sisRNA of the YWHAQ gene is an example of a sisRNA covering the 5'UTR. RNase R resistant, stable intronic lariats bearing snoRNA or scaRNA are also characterized. Such stable intronic lariats bearing snoRNA do not function as guide RNAs for rRNA and snRNA modification in contrast to mature snoRNA.

Conclusion: Comparison of the nuclear and cytoplasmic RNA profiles of chicken oocytes revealed the nuclear retention of certain introns in the form of sisRNAs resistant to RNase R treatment. The high number of certain intronic sequences stored within the oocyte nucleus is due to months of expression of corresponding genes on the lateral loops of lampbrush chromosomes, as well as due to their unusual stability. Notably, sisRNA production generally does not interfere with host gene expression and the appearance of the corresponding mRNA. We propose that nuclear retained sisRNAs, produced by a number of transcribed genes on the lateral loops of avian and amphibian lampbrush chromosomes via a splicing-dependent mechanism, can maintain the continued transcription of their host genes either at the lampbrush stage of oogenesis or during embryogenesis.

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