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Overview of genes transcribed in the chicken lampbrush-stage oocyte and their key transcriptional regulators

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Motivation and Aim: Chromosomes become lampbrushy due to an exceptionally high rate of nascent RNA synthesis. However, the set of genes transcribed on avian lampbrush chromosomes was not characterised. Recently, we performed nuclear and cytoplasmic transcriptome profiling of chicken oocytes at the lampbrush chromosome stage of oogenesis [1]. Here we have undertaken a functional characterisation of the genes expressed in chicken oocytes, tracing their expression levels during early embryogenesis and predicting their transcriptional regulators.

Methods and Algorithms: Gene set enrichment analysis to find over-represented gene ontology terms, key transcription regulators and histone modifications was performed using ENRICHR software. Tissue-specificity index (tau) was obtained from [2] and was used as a measure tissue-specificity of gene expression. K-means clustering and heatmap plotting were performed using Phantasus software. Visualisations were done with R software, version 4.2.2.

Results: A total of 77 and 95 % of all reads from the chicken oocyte nuclear and cytoplasmic total RNA libraries, respectively, were mapped within annotated genes. Transcripts for 10697 protein-coding genes and 2488 long non-coding RNA genes in the oocyte nucleus and 11402 protein-coding genes and 910 lncRNA genes in the oocyte cytoplasm were detected. The oocyte nucleus contains nascent transcripts (characterised by the presence of reads corresponding to introns) for more than 85 % of the protein-coding genes whose transcripts are found in the oocyte cytoplasm. In order to perform a functional characterisation of genes expressed in the oocytes, we traced their expression level during early embryogenesis, identified seven clusters of co-expressed genes and found associated biological processes. For that, chicken oocyte transcriptome data was combined with chicken embryos expression dataset [3]. Next we compared RNA repertoire in the oocyte nucleus and cytoplasm with the transcription profiles in different chicken organs. The identified co-expression clusters can be divided into 4 groups – those where the maximum expression is in oocytes, or in early embryos before zygotic genome activation, or in the embryos after genome activation, or in differentiated organs. The data suggest that the set of genes transcribed in lampbrush stage oocytes is enriched in genes with a broad expression pattern (housekeeping genes). Genes for lncRNAs were enriched in two clusters – the cluster of nuclear retained RNAs, suggesting their function in intranuclear events, and the cluster of genes not transcribed in the oocyte, consistent with their role as tissue-specific regulators activated at later stages of development.

Enrichment analysis was used to find key transcription regulators from ChEA 2022 database, which are associated with subsets of genes with the highest and the lowest levels in chicken oocyte. Hypertranscription on lampbrush chromosomes can be activated by predicted transcriptional regulators MYC, YY1, E2F1, KDM5B, ZFX, and others. At the same time, repression of the inactived genes in avian lampbrush chromosomes can be controlled by epigenetic mechanisms involving Polycomb complexes PRC1 (RNF2, BMI1, CBX2, PHC1) and PRC2 (SUZ12, JARID2, EZH2, EED, MTF2).

Conclusion: Strong overlap between sets of genes whose transcripts were found in the oocyte nucleus and cytoplasm indicates transcription within the oocyte nucleus at the lampbrush stage. The total number of genes transcribed in the oocyte itself is comparable to that in other tissues. Genes expressed in the oocyte nucleus are characterised by a broader expression profile compared to genes whose transcripts were absent in the oocyte. Clusters of genes whose high transcript levels correlate with each other in the oocyte nucleus and cytoplasm are mainly associated with essential cellular processes including transcription, mRNA catabolism, piRNA metabolism, DNA repair, RNA splicing, protein synthesis, DNA replication, cell cycle progression and mitochondria functions. We assume that maternally deposited transcripts derived from the oocyte nucleus are required for the cellular processes associated with oogenesis and early embryogenesis.

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