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Monogenic and multigenic transcription loops in lampbrush chromosomes of birds

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Motivation and Aim: Genome expression can be regulated by the speed of transcription. The rate of nascent RNA synthesis is unusually high in lampbrush chromosomes. This is most likely achieved by the tight coverage of transcribed genes by elongating RNA polymerases [1]. Recently, we established that at the lampbrush chromosome stage, avian oocytes accumulate enormous amounts of mRNAs, long non-coding RNAs and small non-coding RNAs that are synthesised in the oocyte nucleus [2]. We concluded that in avian oocytes, hypertranscription on the lateral loops of giant lampbrush chromosomes is a primary mechanism to synthesise large amounts of maternal RNAs for each of the transcribed genes. Since we detected unspliced or partially spliced nuclear transcripts for many genes, we aimed to detect nascent transcripts and characterize the organision of transcription units *in situ*.
Methods and Algorithms: Here, we used an RNA-FISH protocol that allows us to map nascent gene transcripts on the lateral loops of lampbrush chromosomes [3]. FISH probes were prepared from the chicken BAC-clone library CHORI-261 (https://bacpacresources.org/chicken261.htm) to the selected genomic regions. BAC-clone based probes were labelled by nick-translation as described in [4]. Lampbrush chromosomes were isolated microsurgically from the nuclei of chicken diplotene oocytes. FISH on lampbrush chromosome preparations was performed according to DNA/RNA and DNA/DNA+RNA hybridisation procedures. Control experiments were carried out to assess the quality of spreads and the integrity of nascent transcripts on lampbrush chromosome preparations [3]. RNA-seq data for chicken lampbrush stage cytoplasmic and nuclear RNA fractions are available at NCBI Sequence Read Archive (SRR23800660-SRR23800671) [2].
Results: By RNA-FISH we visualised transcription units of a number of protein-coding genes on chicken lampbrush chromosomes. Specifically, we described transcription loops for RBFOX1, RBMS3, CCSER1, GRID2, INPP5A and PARD3B genes, some of them being involved in embryo development and maternal RNA stability. For all studied examples, we observed complete correspondence of transcript detection by nuclear RNA-seq and by RNA-FISH with nascent transcripts. Genomic regions that are non-transcribed according to RNA-seq data were found within chromomeres. Such genomic regions often lack annotated genes (so-called gene deserts) and may contain untranscribed genes. Interestingly, a lateral loop inserted into a chromomere may contain several individual transcription units separated by untranscribed chromatin knots.
Using RNA-FISH with probes at the beginning, middle and end of the transcribed gene, we demonstrated that introns are removed from lampbrush transcripts before the end of the transcription unit. Visualisation of co-transcriptional splicing in case of RBFOX1

and RBMS3 genes by light microscopy is accompanied by detection of correctly spliced maternal mRNAs deposited in the oocyte cytoplasm. We also conclude that the length of the transcription loops depends on the length of the gene, particularly the length of introns. In the late oocyte nucleus, transcriptional output tends to decrease while the boundaries of transcription units are maintained. Consistently, RNA-FISH data revealed nascent gene transcripts on the drastically shortened lateral loops in late stage oocytes.
Conclusion: We argue that nuclear RNA-seq profile allows predicting the chromomere-loop pattern of lampbrush chromatin domains. Genomic regions containing a number of actively transcribed genes would form a transcription loop, while genomic regions without transcription units would be packed into chromomeres or chromatin knots. We observed both monogenic and multigenic transcription loops organised as follows: (1) a single transcribed gene on a relatively long lateral loop, (2) a long gene with lengthy introns on an extremely long lateral loop with multiple RNP matrix gradients, (3) many genes of different polarity transcribed in a single lateral loop and separated by chromatin knots. We conclude that lampbrush chromosomes provide an especially useful system for studying thousands of transcription loops as well as the packaging of transcriptionally active chromatin and nascent RNAs. Since hypertranscription is not unique to the oocyte nucleus, lampbrush chromosomes are a valuable model for studying the mechanisms of global transcriptome upregulation.
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