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Transcriptional activity of chicken W-linked genes at the lampbrush chromosome stage based on RNA-seq and RNA-FISH data

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Motivation and Aim: In the domestic chicken, the W chromosome is annotated with 28 protein-coding genes, all of which have corresponding homologs on the Z chromosome. The expression of these genes has been documented in various tissues [1]. Notably, female germinative stem cells exhibit an abnormal increase in the expression of 21 Z-W gene pairs, implying the operation of distinct sex determination mechanisms [2]. The aim of this study is to investigate whether the expression of W chromosome genes persists in growing oocytes during the diplotene stage of prophase I of meiosis.
Methods and Algorithms: Transcription of W chromosome genes was examined by analysing RNA sequencing data, aligned to the *GGswu1* genome assembly, from both nuclear and cytoplasmic total and poly(A) RNA fractions extracted from chicken lampbrush stage oocytes [3]. The nuclear and cytoplasmic RNA-seq profiles were visually inspected in the Integrative Genome Viewer to assess the transcriptional activity of Z-W gene pairs. Transcriptional activity was estimated by read coverage along exons in the cytoplasmic RNA fraction, and also by read coverage along exons and introns in the nuclear RNA fraction. To verify the transcription of W chromosome genes, we performed RNA fluorescence *in situ* hybridisation (RNA-FISH) on isolated lampbrush chromosome preparations. Probes for RNA-FISH were prepared by PCR with primers for specific regions of the selected W chromosome genes.
Results: We demonstrated that the most Z-W gene pairs are expressed in chicken oocytes at the lampbrush chromosome stage according to RNA-seq data of oocyte cytoplasmic total and poly(A) RNA. Nuclear RNA-seq profile along exons and introns allows differentiation of transcripts originating from W-linked genes from their homologues on chromosome Z. For example, the *hnRNPK* gene is transcribed from both the Z and W chromosome loci, the full-length *RASA1* transcript is synthesised from the Z chromosome, *HINT1* gene is transcribed from Z chromosome, whereas the multicopy gene *HINT1W* (as a part of tandemly repeated sequence sate-HINT1) is not transcribed, and finally the *TCF4* gene appears to be transcriptionally silent on both sex chromosomes. The transcription of several genes on chicken lampbrush chromosome W was confirmed by RNA-FISH. A novel approach has been developed to synthesise a set of PCR-derived probes, eliminating the need for BAC clone-based probes. As a result, we clarified the RNA sequencing data for a number of W chromosome genes, such as

hnRNPK. Finally, we analysed the transcriptome of embryos at an early developmental stage (EGK.III) and revealed the inheritance of spliced mRNA from the Z-W gene pairs. *Conclusion*: In the current study, we found that the transcriptional activity of W chromosome genes observed in germinative stem cells is maintained in chicken oocytes at the lampbrush chromosome stage. The subsequent detection of spliced mRNA for Z-W gene pairs in early embryogenesis suggests their potential role in development as maternal factors. Remarkably, despite the extensive expansion of repetitive sequences in over 85 % of the W chromosome and enrichment of heterochromatin markers such as DNA hypermethylation, a number of W-linked genes are transcribed at the lampbrush chromosome stage of oogenesis.

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