Annotation of the chicken W sex chromosome using RNA-seq reveals candidate female-determining genes

Summary

Birds have a ZZ male: ZW female sex chromosome system and while the Z-linked DMRT1 gene is necessary for testis development, the exact mechanism of sex determination in birds remains to be fully resolved. Of particular interest is the female-specific W sex chromosome, which is poorly characterised. Few genes have been mapped to the avian W, and little is known of their expression and function. We used RNA-seq to produce a detailed profile of gene expression in the chicken blastoderm and in the embryonic gonad prior to sexual differentiation. We found robust sexually dimorphic gene expression in both tissues pre-dating overt sexual differentiation. A total of 3235 genes were differentially expressed between the sexes, including many novel W-linked genes expressed in females. The data support the emerging view that sexual identity is established in avian cells early in embryogenesis, long before gonadal sex differentiation and the development of sexual phenotype. We used a novel combination of genome guided and de novo transcriptome assembly to annotate the chicken W sex chromosome, expanding the list of confirmed W-linked genes from several to forty. Notably, we assembled full-length transcripts for most of these genes, which were hitherto known only as short putative W fragments or fragments within contigs of unknown genomic location (the “Unknown-random” chromosome). FISH mapping and PCR confirmed W-linkage of a representative sample of these genes, and quantitative RT-PCR validated female-restricted expression. Sequence comparisons of these assembled W genes with their Z-linked homologues, together with their expression profiles, identified novel potential female sex determinants. The data significantly enhance our understanding of the avian W sex chromosome and its gene content. In contrast to previous assumptions, this study indicates that the chicken W chromosome is highly transcriptionally active.