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Sex-biased mouse liver lincRNAs co-localize to Topologically Associating Domains (TADs) with sex-biased protein-coding genes.

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Long intergenic non-coding RNAs (lincRNAs) have increasingly been found to play a regulatory role in a broad range of biological systems, affecting diverse physiological processes and disease states. We recently characterized ~5,000 liver-expressed lincRNAs with respect to their gene structures, species conservation, chromatin accessibility and epigenetic states, including many lincRNAs that are differentially expressed between male and female mouse liver (Melia *et al* (2016) *Molecular and Cellular Biology*, PMID: 26459762). A subset of these sex-biased lincRNAs is likely to regulate chromatin states associated with sex differences in liver gene expression. Here, we extend this work to include anti-sense and other long non-coding RNAs, as well as lincRNAs with low levels of poly-adenylation by analysis of male and female mouse liver RNA-seq datasets representing ribosomal RNA-depleted nuclear liver and total liver RNA, as well as poly-adenylated nuclear liver and total liver RNA. These analyses confirmed 103 previously discovered sex-biased liver lincRNAs and identified 112 novel sex-biased liver lincRNAs showing significant, >4-fold, sex-differential expression. Further analysis identified 87 of the 215 sex-biased liver lincRNAs as candidates for transcriptional regulation of sex-biased protein coding genes in *cis*, based on their co-localization to the same genomic Topologically Associating Domain (TAD). Thus, 87 sex-biased liver lincRNAs and 104 sex-biased genes co-localized within 57 TADs spanning 17 of the 21 mouse chromosomes, including the X chromosome. Furthermore, 57 of the 87 sex-biased liver lincRNAs showed significant nuclear enrichment. Striking examples of sex-biased, nuclear-enriched lincRNAs associated with sex-biased genes within the same TAD include: (1) a 4-fold female-biased, nuclear-enriched lincRNA associated with a cluster of six male-biased *Ces* family genes; (2) an 8-fold male-biased, highly nuclear enriched lincRNA associated with two strongly (≥250-fold) female-specific genes, *Hsd3b1* and *Hao2*, *and* two more moderately (4 to 9-fold) male-biased genes, *Hsd3b2* and *Hsd3b5*; and (3) a strong (120-fold) male-specific, nuclear-enriched lincRNA associated with a cluster of 18 male-biased *Mup* genes. The presence of sex-biased lincRNA–protein-coding gene pairs of both the same and the opposite sex bias within the same TAD suggests both positive and negative regulatory roles for the sex-biased liver lincRNAs. Complementary technologies, including single molecule fluorescent in situ hybridization (smFISH), are currently being implemented to visualize the sex-differential expression and intracellular localization of these lincRNAs and help assess their functional significance. Supported in part by NIH grants R01 DK33765 (to DJW) and Biomolecular Pharmacology Training Grant T32-GM008541.