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.