Universal Alternative Splicing of Noncoding Exons

The human transcriptome is so large, diverse, and dynamic that, even after a decade of investigation by RNA sequencing (RNA-seq), we have yet to resolve its true dimensions. RNA-seq suffers from an expression-dependent bias that impedes characterization of low-abundance transcripts. We performed targeted single-molecule and short-read RNA-seq to survey the transcriptional landscape of a single human chromosome (Hsa21) at unprecedented resolution. Our analysis reaches the lower limits of the transcriptome, identifying a fundamental distinction between protein-coding and noncoding gene content: almost every noncoding exon undergoes alternative splicing, producing a seemingly limitless variety of isoforms. Analysis of syntenic regions of the mouse genome shows that few noncoding exons are shared between human and mouse, yet human splicing profiles are recapitulated on Hsa21 in mouse cells, indicative of regulation by a deeply conserved splicing code. We propose that noncoding exons are functionally modular, with alternative splicing generating an enormous repertoire of potential regulatory RNAs and a rich transcriptional reservoir for gene evolution.