

Alternative Splicing in Bone Following Mechanical Loading

Sara M. Mantila Roosa, Graduate Student at Purdue University

It is estimated that 80% of human genes express multiple mRNA transcripts due to alternative splicing, including genes that are important in bone biology: FOSB, IGF1, and RUNX2. The goal of this study was to determine the extent of alternative splice variant expression in a bone subjected to mechanical loading. We used the rat forelimb loading model, in which the right forelimb was loaded axially for 3 minutes, while the left forearm served as a non-loaded control. Animals were subjected to loading sessions every day, with 24 hours between sessions. Ulnae were sampled at 11 time points, from 4 hours to 32 days after beginning loading. The time points are referenced to the number of hours or days after the first bout of bone loading was applied. RNA was isolated and mRNA abundance was measured at each time point using Affymetrix exon arrays (GeneChip[®] Rat Exon 1.0 ST Arrays). An ANOVA model was used to identify potential alternatively spliced genes across the time course, and five genes with significant alternative splice *p*-values were validated with qPCR: *Akap12*, *Fn1*, *Pcolce*, *Sfrp4*, and *Tpm1*. The number of alternatively spliced genes varied with time, ranging from a low of 68 at 12h to a high of 992 at 16d. The functions of the primary isoform of the alternatively spliced genes were variable, but many with a known function in bone were identified. We conclude that alternative splice variants play an important role in loading-induced bone formation.