Summary

The heterogenous nuclear ribonucleoprotein particles (hnRNP) A1 and A2 proteins, are two of the most influential regulators of alternative splicing. Controlling the expression and repression of the correct transcript isoforms, hnRNPA1 and A2 are also highly involved with aberrant splicing due to splicing mutations disrupting the splicing code. Elucidating the binding mechanisms behind these proteins is essential for understanding how to correct splicing errors by manipulating this binding. This thesis will characterize the role of hnRNPA1 and A2 in human disease genes and identify *in vivo* binding sites as novel therapeutic targets.

Utilizing both *in vivo* and *in vitro* methods to characterize RNA-protein interactions, a binding map of hnRNPA1 and A2 eCLIP reads is identified across exons regulated by hnRNPA1 and A2. A central UAG motif is demonstrated as essential for hnRNPA1 and A2 binding, and SPRi is used to demonstrate that the +2A is a critical part of this motif for both proteins. It is also demonstrated that hnRNPA1 and A2 binding is highly enriched directly downstream of the 5'ss of pseudoexons activated during knock down of hnRNPA1 and A2, and that pseudoexon inclusion can be stimulated by blocking the hnRNPA1 and A2 UAG motifs with splice switching oligonucleotides.

Furthermore, a new subgroup of constitutive exons is introduced, that are particularly vulnerable to exonic splicing mutations (ESMs). This thesis describes how these exons are highly dependent on exonic splicing enhancers to be included, and how ESMs are overrepresented in vulnerable exons. It is described how these exons are enriched with hnRNPA1 and A2 eCLIP binding sites, and generally are less well defined than other constitutive exons that are more resilient, and harder to skip by ESMs.

Finally, the autoregulation exhibited by hnRNPA1 and A2 during knock down of the opposite factor is investigated. Using eCLIP data not included in the enclosed manuscripts, it is shown that hnRNPA1 and A2 autoregulates during KD by binding in their respective 3'UTR. The functional importance of hnRNPA1 and A2 as master regulators of RNA metabolism is characterized, and these new findings help further establish the mechanism behind these proteins' role as repressors of splicing.