The essential pre-mRNA splicing factor U2AF^{65} accommodates divergent nucleotides at the central position of the polypyrimidine 3´ splice site signal

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Inherited mutations in polypyrimidine (Py) signals that mark the major class of 3´ splice sites are associated with a subset of human genetic diseases (e.g. www.hgmd.org). The essential pre-mRNA splicing factor U2AF^{65} recognizes the Py tract and promotes association of the U2 spliceosomal particle with the pre-mRNA. Natural human Py tracts often are interrupted with purines, which may contribute to differential regulation by alternative splice site choice. U2AF^{65} is known to prefer binding and splicing of poly-uridine tracts, yet adapts to variations of the consensus splice site sequence. Prior structures have shown that two tandem RNA recognition motifs (RRM) of the pre-mRNA splicing factor U2AF^{65} recognize the Py tract[1] and further reveal nucleotide-contacts with the inter-RRM linker[2]. Despite this progress, the nucleotide-specificity of the U2AF^{65} inter-RRM linker, and the relationship between the structurally-observed U2AF^{65} – nucleotide-binding sites and natural Py tract sites, remain unknown.

Here, we determined four structures of U2AF^{65} bound to oligonucleotides derived from a prototypical Adenovirus Major Late Promoter (AdML) Py tract. We vary the sequence of the central nucleotide of the Py tract, which interacts with the inter-RRM linker of U2AF^{65}. The structures reveal RNA flexibilities, rather than protein conformational changes, accommodate purine substitutions at the central Py tract position. The splicing efficiencies of corresponding AdML variants in human cells agree with the structural observations and *in vitro* binding affinities. We further confirm the relevance of U2AF^{65} – splice site interactions by assessing the impact of structure-guided U2AF^{65} mutations for splicing of representative endogenous transcripts in cells.

Altogether, these results are a step towards relating natural 3´ splice site sequences to molecular recognition by U2AF^{65} in the initial stages of spliceosome assembly.

[1]PDB ID's 2G4B, 3VAF, 3VAG, 3VAH, 3VAI, 3VAK, 3VAL, 3VAM, 3VAJ, 4TU7, 4TU8, 4TU9
[2]PDB ID's 5EV1, 5EV2, 5EV3 and 5EV4