Role of a conserved pseudouridine in U2 snRNA on the structural and electrostatic features of the spliceosomal pre-mRNA branch site

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Abstract

A pseudouridine (ψ) residue in a phylogenetically conserved position of U2 snRNA that pairs with the intron to form the pre-mRNA branch site helix of *S. Cerevisiae* has been shown to induce a dramatically altered architectural landscape compared with that of its unmodified counterpart. In the ψ-dependent structure the branch site adenosine in an extrahelical position, with the nucleophilic 2'OH positioned at the surface of the widened major groove. Clustering of electronegative functional groups and kinking of the backbone in the modified structure also result in a region of exceptional negativity in the region of the 2'OH. These features may assist in recognition and activity of the branch site during the first step of splicing. This is the first case in which a native ψ has been shown to induce a major alteration in structure. However, it is likely that other conserved modification sites in the spliceosome and ribosome may impact structurally on assembly and function.

1 Introduction

1.1 The spliceosome

Following transcription, precursor messenger (pre-m)RNA molecules undergo a series of processing reactions prior to translation of their message into protein. Among these essential reactions is the removal of introns, or noncoding regions of the pre-mRNA, and the ligation of exons, the flanking coding regions. In some cases, such as Group I and Group II introns, the catalytic power resides entirely within the RNA component. In eukaryotes, the splicing reaction utilizes the same chemical mechanism as the Group II intron, but is catalyzed by the spliceosome, a dynamic ribonucleoprotein machine requiring both small nuclear (sn)RNA and protein components (Moore et al. 1993). Although the RNA components only make up a small percentage of the total spliceosomal mass, evidence is accumulating to support the hypothesis that the RNA fraction is the catalytic agent.

The cyclic nature of spliceosome activity requires that at least some of the components must reassemble around each pre-mRNA substrate. The dynamic and highly specific nature of spliceosome activity makes details of its assembly and

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the molecular basis of recognition among components a particularly fascinating and challenging target of study.

1.1.1 Role of snRNAs in splicing chemistry

Among the five spliceosomal U snRNAs, U2 and U6 snRNA are the only two to be involved in both the first and second transesterification reactions of splicing, and they exhibit the greatest phylogenetic conservation. Multiple segments of pairing interactions between U2 and U6 snRNA (Fig. 1) are critical for splicing activity (Madhani and Guthrie 1992). Evidence for the direct role of U2 and U6 snRNA in splicing comes from experiments by Valadkhan and Manley (2001, 2003), which showed that a protein-free complex of these snRNAs, along with an intron strand and divalent metal ions, was sufficient to catalyze covalent products.

1.1.2 The pre-mRNA branch site

In addition to essential pairing interactions between U2 and U6 snRNA, a segment of U2 snRNA identifies and pairs with a consensus sequence of the intron (Fig. 1). This sequence is absolutely conserved in yeast, but greater flexibility is seen in high eukaryotes; however, the relative position of purines and pyrimidines, as well as the identity of several residues, is strictly conserved. The U2 snRNA-intron pairing forms a short complementary helix (seven base pairs in yeast) with an invariant single unpaired adenosine residue on the intron strand. The 2'OH of this adenosine is the nucleophile in the first cleavage reaction; it is known as the branch site because of the 2'-3'-5' branched product. In addition to its catalytic role, specific recognition of the pre-mRNA (*i.e.* prior to the splicing reaction) branch site region is likely to assist in recruitment of other components of the active spliceosome. The structural features contributing to positioning of the 2'OH for nucleophilic activity and subsequent activation, and/or recognition by other components of the catalytic core, are major issues in understanding the structural biology of RNA splicing.

1.2 Modified bases in structural RNAs

Shape and charge are two major criteria in predicting interaction between biomolecules. RNA molecules, through formation of loops bulges, base mismatches, presents varied topological and electrostatic landscape to potential ligands. As a mechanism to increase opportunities for folding and recognition, to enhance ion binding affinity, and/or increase thermal stability, structural/functional RNA molecules (e.g. tRNAs, rRNAs, snRNAs) expand the limited vocabulary of four bases by undergoing site-specific post-transcriptional chemical modification of selected bases. Such modifications alter the local electrostatic and topological landscape of regions important for intra- or intermolecular interaction and, therefore, have the potential to augment opportunities for RNA-ligand recognition.