PROBING FEATURES OF SPLICEOSOMAL PROTEIN p14 WITH RNA LIGANDS

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Introduction

Assembly of the competent spliceosome involves highly specific and ordered RNA-RNA and RNA-protein interactions around the pre-mRNA substrate. At the functional center of the spliceosome is an RNA duplex created by the pairing of a highly conserved region of the intron and the U2 small nuclear (sn)RNA, called the pre-mRNA branch site region. The branch site duplex is surrounded by the U2 snRNP, comprising several protein complexes such as SF3a and SF3b. One of six SF3b subunits, p14, has been shown to interact with the pre-mRNA branch site region [1]. Structures determined by electron cryomicroscopy (cryo-EM) of a ~450kDa SF3b complex [2] and the U11/U12 snRNP of the atac spliceosome [3] show potential RNA binding surfaces. A potential groove for the pre-mRNA is formed by the SF3b complex suggesting locations where p14 and SF3b155 can interact with the pre-mRNA [2,3]. We are studying the interaction of the complex formed between p14 and the pre-mRNA branch site in the eukaryotic spliceosome.

Results and Discussion

^{1}H-^{15}N HSQC experiments of ^{2}H^{13}C ^{15}N-labeled p14 revealed only ~90 of the anticipated 126 crosspeaks (Figure 1, red), suggesting that the protein was partially disordered in solution. With the addition of unlabeled single-stranded (ss) intron RNA, the ^{1}H-^{15}N HSQC experiments of ^{15}N-labeled p14 exhibited ~110 of the anticipated cross peaks (Figure 1, green), some of which were slightly shifted from their previous position, suggesting that addition of ss intron RNA helped p14 become more structured. ^{1}H-^{15}N HSQC spectrum of ^{2}H^{13}C^{15}N-labeled p14 with unlabeled double-stranded (ds) branch site RNA (Figure 2, blue) resulted in ~125 of the anticipated cross peaks, which were further shifted and more well defined. An observation of similar shifts, but of greater extent, upon binding to the dsRNA, suggests intermediate exchange. ^{31}P-NMR spectrum of the ss and ds RNA indicated significant shifts upon binding of p14 to the ds RNA but not the ss RNA, implying specific interaction with the backbone of the dsRNA. This is an important finding, because proteins of the class to which p14 belongs, the RNA Recognition Motif (RRM) proteins, have previously been modeled to recognize the bases of single-stranded RNA only.

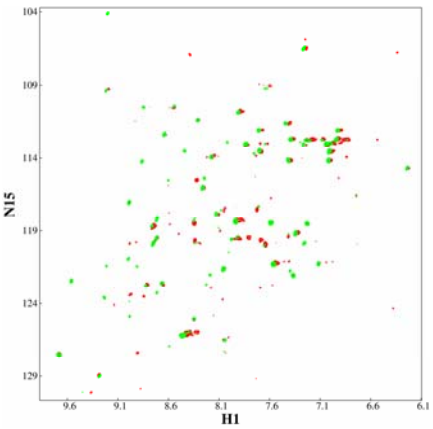

Figure 1. The ^{1}H-^{15}N HSQC spectrum left shows a 200 µM ^{15}N-labeled p14 (red) acquired at 4°C on a 720 MHz Varian spectrometer. The spectrum reveals only ~90 of the anticipated 126 crosspeaks. Addition of intron RNA resulted in ^{15}N-labeled p14 (green), revealing 110 of the anticipated crosspeaks.

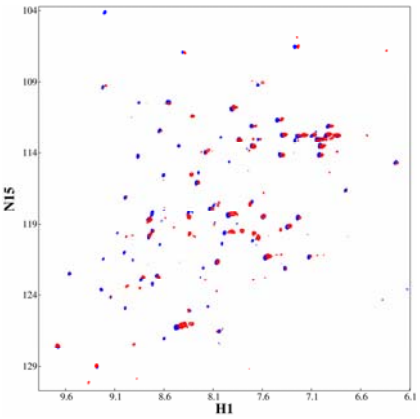

Figure 2. The spectrum left shows a 200 µM ^{15}N-labeled p14 (red) acquired at 4°C on a 720 MHz Varian spectrometer. The ^{1}H-^{15}N HSQC experiment reveals only ~90 of the anticipated 126 crosspeaks. Addition of a double-stranded branch site RNA, resulted in ^{15}N-labeled p14 (blue) revealing ~125 of the anticipated crosspeaks.

Acknowledgements

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References

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[2] Golas, M.M., *et al.* Science, **300**, 980-984 (2003).
[3] Golas, M.M., *et al.* Molecular Cell **17**, 869-83 (2005).