Ribosomal Proteins S5 and S12 Interact with the Central Core of the Small Ribosomal Subunit.

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Introduction:

The ribosome is one of the most important and complex molecular machines in living cells. The small subunit of the ribosome (SSU) plays a crucial role in decoding messenger RNA (mRNA). We have re-determined SSU domain architecture based on secondary and three-dimensional structures, and have provided a coherent structural scheme for understanding SSU function and evolution. Our revised domain architecture contains a central domain (domain A) which establishes a central hub of the SSU.

Domain A is an integrated and independent structural unit, which we have isolated from the rest of the 16S rRNA. Domain A is the core from which all other SSU domains radiate. We refer to isolated domain A, severed from the rest of the 16S rRNA, as "Domain A^{ISO}". Domain A^{ISO} is an experimental model of domain fused to stem loops to form a single piece of RNA.

In native ribosome, ribosomal proteins S5 and S12 interact with domain A. We have tested the binding properties of ribosomal proteins S5 and S12 to domain A^{ISO} model *in vivo* and *in vitro*. Our yeast-three hybrid assay results reveals that S5 and S12 are not binding to domain A^{ISO} RNA *in vivo* in that these proteins contains many positively charged amino acid residues leading to their unspecific binding affinity to nucleic acids. Furthermore, we have shown that S5 fusion protein binds to different RNAs including domain A^{ISO}, P4-P6 domain of group I intron, and a 12 mer duplex RNA *in vitro* unspecifically. Ladder effect is observed in these interactions suggesting that multiple proteins bind to one RNA molecule. Moreover, selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE) and circular dichroism (CD) experiments revealed that binding of S5 fusion protein to domain A^{ISO} RNA causes a disruption in the tertiary structure of domain A^{ISO} RNA, more specifically in the central pseudoknot. Our results support the assembly map by Nomura[1] (Mizushima & Nomura, 1970) which indicates that S5 and S12 are tertiary binding proteins. Tertiary binding proteins require primary and secondary binding proteins to first bind 16S rRNA before they can be bound. We hypothesized that the positively charged residues of S5 are breaking up the tertiary structure of the central pseudoknot. Since the central pseudoknot is not very stable due to its flexibility, high positive charges can cause disruption of the structure. In other words, folding of the central pseudoknot is affected by positively charged ribosomal protein S5. We believe that primary binding of these proteins would retard the 16s rRNA folding by destabilizing the central pseudoknot in 30S assembly. This hypothesis is also consistent with the fact that domain A is buried in the core of 16S rRNA, requiring other proteins to bind and stabilize the overall 16S structure prior to binding of highly basic proteins S5 and S12.

References:

[1] Nomura M. and Mizushima S. (1970) *Nature*, **226**, 1214-1218