Mechanism of action and physiological function of DbpA: a ribosomal RNA chaperone

DEPARTMENT OF CHEMISTRY & CHEMICAL BIOLOGY

DbpA is an ATP-dependent RNA helicase that possesses two N-terminal RecA-like domains, which perform the RNA unwinding, and a C-terminal RNA-binding domain that mediates specific tethering of DbpA to hairpin 92 of the Escherichia coli 23S ribosomal RNA. Hairpin 92 is located near the site of peptide bond formation; thus, DbpA acts in a highly conserved region of the ribosome. Previous data using model substrates containing hairpin 92 showed that the substrates support the DbpA protein's double helix unwinding activity, provided that the double helix had a 3'end single-stranded region. The 3'end single stranded region was suggested to be the start site of the DbpA catalytic core double helix unwinding. Our data show that the 3'end single-stranded region is not required for the DbpA protein's unwinding activity and that the DbpA protein unwinds a double-helix by directly loading on it. This conclusion has important implications for the role of DbpA during ribosome assembly in vivo: it suggests that during the ribosome assembly process, DbpA could unwind substrates, which are separated from hairpin 92 by space or other macromolecules, by directly loading on those substrates. Moreover, previous studies showed that the expression of helicase inactive R331A DbpA produced the accumulation of a large subunit intermediate particle. We identified two novel large subunit intermediates accumulated in cells expressing R331A DbpA. In addition, we characterized the intermediates structures and kinetics of conversion to 50S large subunit. Our experiments demonstrate the intermediates belong to three different stages of the large subunit ribosome assembly and they rearrange to form the 50S large subunit via three parallel pathways. This is the first time that the existence of multiple pathways of large subunit ribosome assembly was observed experimentally. Last, our RNA structural experiments demonstrate that formation of peptidyltransferase center is the last step of large subunit ribosome assembly, preventing the formation of malfunctioning ribosomes. This conclusion agrees with the data previously gathered on the bacteria and eukaryotic mitochondria ribosome.

A COLLOQUIUM BY:
Eda Koculi

February 12, 2018
Clark 101
4:00 p.m.