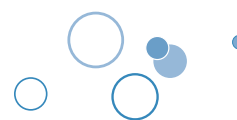


## RNA Finds a Groove in ERA to Act as a GAP



**IN SHORT >** Making new proteins is one of the most fundamental functions of any cell, from bacteria to humans. All living things use DNA to create messenger RNA (mRNA) that contains the code for making the proteins of life from their component amino acids. Ribosomes are complexes of protein and RNA and are responsible for using the mRNA code to assemble proteins. Bacterial ribosomes consist of a complex of proteins and RNA that form two particles, the large 50S particle and the smaller 30S particle. The 30S particle contains 21 ribosomal proteins and a 1,540-nucleotide ribosomal RNA (16S rRNA). Before proteins can be made, this complex must be assembled, a process that is regulated by about 20 protein cofactors. Some of these cofactors, named GTPases, use the energy of hydrolysis of the GTP nucleotide as a molecular switch to check that each step occurs in the proper order before moving on to the next. One of these GTPases, ERA, is an essential protein in bacteria that is required for the final processing of the 16S rRNA and assembly of the 30S ribosomal subunit. Structural studies performed at two U.S. Department of Energy synchrotron x-ray light sources, including the SER-CAT 22-BM beamline at the APS, have now shown how the interaction of ERA with 16S rRNA affects its GTPase activity and function as a key regulator of protein translation.

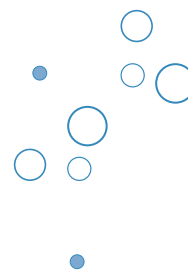
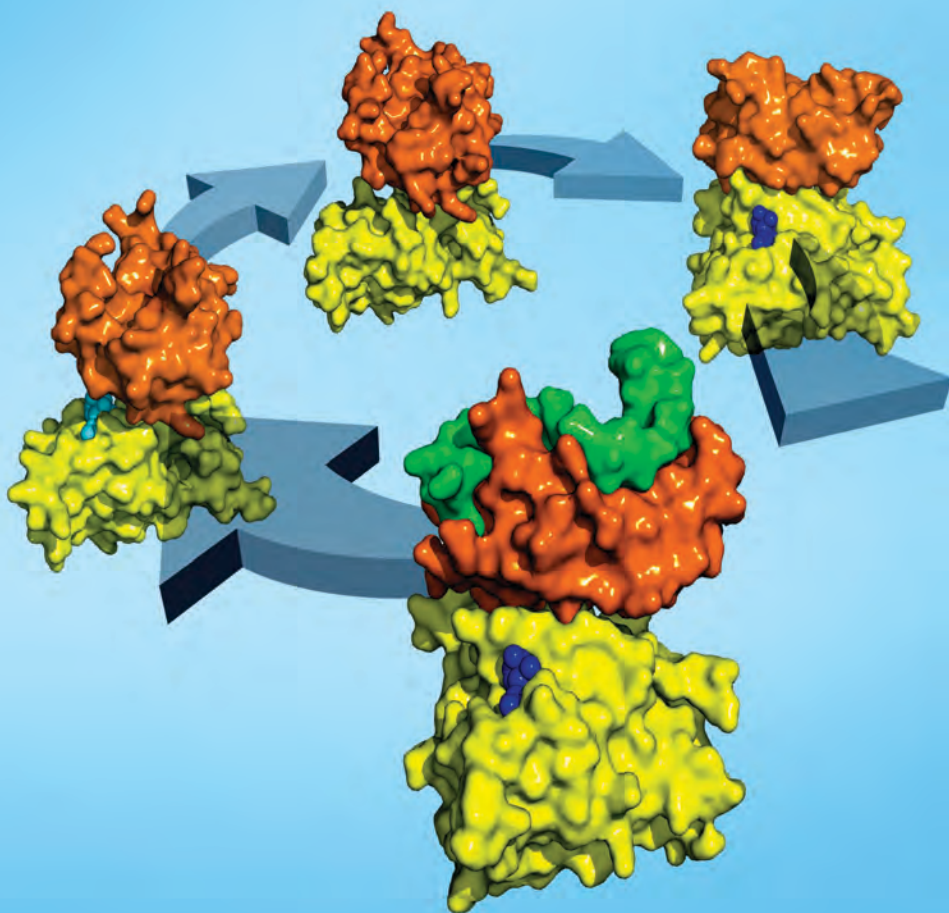
**MORE >** Bacterial ERA contains an N-terminal GTPase domain connected by a 17 amino acid linker to a KH domain that binds to RNA. It has been shown to bind to both the 16S rRNA and to components of the 30s ribosomal subunit. However, the interaction of the two domains with each other and with RNA was unknown. In this work, carried out at the SER-CAT beamline and

the X9B beamline of the National Synchrotron Light Source at Brookhaven National Laboratory, researchers from the National Cancer Institute of the National Institutes of Health solved two structures, one of ERA bound to GDP and the other of ERA bound to a GTP analog, GNP, plus the 3' end of the 16S rRNA,  $_{1531}\text{AUCACCUCCUUA}_{1542}$ , which is thought to be the binding

*> Fig. 1 The functional cycle of ERA is composed of four states, including apo-ERA, ERA-GTP, ERA-GTP-RNA and ERA-GDP. The GTPase domain (in yellow) and KH domain (in orange) of ERA and the 3' end of 16S rRNA (12 nucleotides, in green) are shown as molecular surfaces, while the GTP (in blue) and GDP (in cyan) molecules are represented as atomic spheres.*

site of ERA. The AUCA of this RNA sequence is conserved among bacteria, archaea, and eukaryotes. The CCUCC is conserved among prokaryotes and is known as the anti-Shine-Delgarno sequence essential for translation start site recognition.

The GDP-bound form of ERA adopted the same conformation as a previously solved structure for ERA with no nucleotide, called apo-ERA. The ERA-GNP-RNA complex adopted a different conformation that was the same as the conformation previously shown for ERA-GNP without RNA and showed that RNA binding to ERA does not affect the structure of the protein. However, GTP binding does appear to have a large effect on the structure of the protein and also makes a big difference to the way RNA can bind. In the ERA-GNP-RNA structure, RNA lies within a positively-charged groove of the KH domain making contact through the first 9 nucleotides of the RNA. In the ligand-free and GDP-bound structures, this groove is occluded by the movement of a negatively charged helix, effectively



See > Chao Tu, Xiaomei Zhou, Joseph E. Tropea, Brian P. Austin, David S. Waugh, Donald L. Court, and Xinhua Ji\*, "Structure of ERA in complex with the 3' end of 16S rRNA: Implications for ribosome biogenesis," Proc. Nat. Acad. Sci. USA **106**(35), 14843 (September 1, 2009). DOI:10.1073/pnas.0904032106

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blocking the binding of negatively charged RNA.

RNA binding does not affect the structure of ERA but it does affect the function. The team showed that RNA binding affects the rate of GTP hydrolysis of ERA by speeding it up, acting as a GTPase-activating protein or GAP. Further investigation showed that both the AUCA and the CCUCC were required for this GAP activity. However, the effect on GTPase activity was modest, only about a 6-fold increase, compared to  $10^5$  in other GTPases, leaving open the possibility that ERA's GTPase

activity might be affected by other ribosomal proteins as well.

Based on their discoveries, these researchers propose a functional cycle for ERA activity including its GTP hydrolysis cycle and RNA binding (Fig. 1). Now, to fill in the gaps, the team will focus on identifying the impact of each nucleotide in the RNA on ERA binding and activity and on learning more about its function by studying the mouse and human forms of ERA.

—Sandy Field

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> 22-BM • SER-CAT • Life science  
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