Biochemistry: An Indian Journal

BioChemistry
ISSN: 0974-7427

Commentary | Vol 15 Iss 2

Ancient Protein KsgA and its Functions

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Received: February 03, 2021; **Accepted:** February 08, 2021; **Published:** February 22, 2021

Commentary

Ribosome biogenesis is a multistep process that occurs in all organisms. The processing of ribosomal RNA (rRNA) from a primary transcript, as well as the modification of rRNAs and ribosomal proteins (r-proteins) and the connection of r-proteins with rRNA, necessitates a high level of coordination and a slew of other components. The result of this process is the formation of a functional ribosome from two distinct ribosomal subunits that join together during translation initiation. In general, a simplified ribosome biogenesis strategy seems to be indicative of the process throughout evolution. The nucleotide modification level of ribosome biogenesis indicates significant differences in the ribosome maturation process between prokaryotes, eukaryotes, and archaea. Only three rRNA changes, in particular, are retained across all three kingdoms. In large ribosomal subunit (LSU) rRNA, one example is the conversion of U1958 (Escherichia coli numbering) to pseudouridine. While the alteration itself is universal, the machinery and thus the technique by which it is performed vary by kingdom and organism. The dimethylation of two adjacent adenosines (A1518 and A1519 (*E. coli* numbering)] in the universally conserved 3′ terminal helix of the small ribosomal subunit (SSU) rRNA are the other changes preserved throughout evolution. The generally conserved Dim1p/KsgA enzyme family catalyzes these dimethylation changes, which are found on almost all known ribosomes except for two organelles. As a result, these two methylations, as well as the enzyme family that causes them, are unrivaled in terms of their presence in SSU biosynthesis, and they constitute an intriguing challenge in determining the evolutionary and functional importance of this rRNA modification system. Following the identification of E. coli strains resistant to the aminoglycoside antibiotic kasugamycin due to a lack of methylation of A1518 and A1519, ksgA was discovered. Dim1p was discovered as the yeast orthologue of ksgA through complementation investigations in *E. coli*. These experiments required and took advantage of Dim1p's ability to methylate E. coli SSU rRNA in vivo, confirming the enzyme family's exceptional conservation at the catalytic level. Because of an extra 18S rRNA-processing role, Dim1p is necessary. Lack of dimethylation of helix 45 did not affect cell growth when a mutant allele of dim1 (dim1-2) was used. Despite the nearly universal retention of this methyltransferase system, methylation of the two adenosines is not required in *E. coli* or Saccharomyces cerevisiae. These findings suggested that there might be another purpose that could explain the survival of these genes from a distant ancestor. Dim1p function in S. cerevisiae has received more attention in terms of cellular relevance and molecular knowledge than KsgA function in prokaryotes. Many earlier studies of KsgA function have relied on a small number of kasugamycin-resistant strains that have been demonstrated to lack dimethylation of helix 45 but are otherwise uncharacterized, making it difficult to interpret the results. In vitro experiments, on the other hand, revealed that treating precursor SSU particle components with KsgA before in vitro reconstitution improved their activity in a polypeptide synthesis assay when compared to their untreated counterparts. Methylation of mature reconstituted SSUs showed no effect on their performance in the same assay, implying that the methylation system is more critical in biogenesis than in translation. Furthermore, despite the extreme conservation of both the sequence and modifications at A1518 and A1519, neither changes in the sequence nor methylation appear to affect SSU incorporation to 70S ribosomes in vitro; however, the absence of methylation at A1518 and A1519 in helix 45 has a subtle impact on a read-through of nonsense and frameshift mutations in vivo. KsgA has also been genetically related to several variables involved in ribosome function, with overexpression alleviating growth problems and genetic deletion worsening growth defects in already damaged strains. We recently presented a model for KsgA's interaction with us, and based on these in vitro data, we indicated that KsgA plays a role in restricting SSU access to IF3 and 50S subunits. However, it was unclear how such a role for KsgA might express itself in the context of the SSU biogenesis and translational pathways' complicated cellular milieu. It was also unknown whether such a function would be conserved in the KsgA family of proteins. We employed

Citation: Kizaki N. Ancient Protein KsgA and its functions. Biochem Ind J. 2021; 15(2):149.

an E. coli strain with a precise deletion of KsgA to better understand the function of the protein. We show that the ksgA genotype causes cold sensitivity and changed ribosome profiles in the 70S ribosome, as well as a shift in the typical populations of free SSUs and SSUs. Furthermore, while KsgA deficiency is not fatal, it does cause SSU rRNA processing errors similar to those seen with Dim1p depletion, whereas LSU rRNA processing is unaffected. Overexpression of archeal (*Methanocaldococcus jannaschii*) and, to a lesser extent, eukaryotic (*S. cerevisiae*) homologs of KsgA (referred to as Dim1p) can suppress this phenotype, which results in the cold-sensitive phenotype. As a result, it appears that KsgA is a genuine SSU biogenesis factor with a conserved role. A methyltransferase-deficient variant of KsgA was studied to determine the role of methylation by KsgA in biogenesis in vivo. Surprisingly, this did not dissociate the biogenesis and modification activities; in both wild-type and ksgA strains, the catalytically inactive KsgA has a deleterious effect on growth and affects ribosome formation. As a result, the presence of KsgA in a form that is unable to methylate SSUs is more harmful to ribosome synthesis than the absence of KsgA entirely. This mutant version of KsgA is found to be persistently linked to SSUs produced in vivo, implying a mechanism to explain the symptoms. Our findings imply that KsgA is a late-stage ribosome biogenesis component that is released from the assembled subunits when methylation occurs. Thus, methylation may regulate KsgA release from the newly developed SSU, which is accompanied by conformational rearrangements that allow ultimate maturation and entry into the translation cycle. We developed a model that explains the roles of KsgA in SSU biogenesis as well as the ramifications of having no KsgA or a catalytically inactive KsgA form present. Given that modification of the two neighboring adenosines in SSU rRNA is not required, this novel functional role for KsgA and probably its homologs provides a functional mechanistic explanation for the KsgA/Dim1p enzyme family's exceptional conservation.