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Association of a multi-synthetase complex with translating ribosomes in the archaeon *Thermococcus kodakarensis*

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ABSTRACT

In archaea and eukaryotes aminoacyl-tRNA synthetases (aaRSs) associate in multi-synthetase complexes (MSCs), however the role of such MSCs in translation is unknown. MSC function was investigated in vivo in the archaeon *Thermococcus kodakarensis*, wherein six aaRSs were affinity co-purified together with several other factors involved in protein synthesis, suggesting that MSCs may interact directly with translating ribosomes. In support of this hypothesis, the aminoacyl-tRNA synthetase (aaRS) activities of the MSC were enriched in isolated *T. kodakarensis* polysome fractions. These data indicate that components of the archaeal protein synthesis machinery associate into macromolecular assemblies in vivo and provide the potential to increase translation efficiency by limiting substrate diffusion away from the ribosome, thus facilitating rapid recycling of tRNAs.

Structured summary of protein interactions:
LeuRS physically interacts with **DNA methylase 115, flagellin 118, acetylpolyamine aminohydrolase 140, Pyruvoyl-dependent arginine decarboxylase 154, RecJ-like exonuclease 476, acetyl-CoA acetyltransferase (mevanolate Pathway) 110, glutamine amidotransferase, class I 437, GMP synthase subunit B 184, pyridoxine biosynthesis protein 1183, quinolinate synthetase 206, L-aspartate oxidase 322, uridylate kinase 164, putative molybdenum cofactor biosynthesis protein C 127, bifunctional carboxypeptidase/aminoacylase 214, aspartate racemase 166, serine/threonine protein kinase 111, SAM-dependent methyltransferase 144, GTP cyclohydrolase 398, DNA topoisomerase VI subunit A 209, DNA topoisomerase VI subunit B 192, Type A Flavoprotein 911, NAD(P)H:rubredoxin oxidoreductase (Fatty acid metabolism) 120, NAD(P)H:rubredoxin oxidoreductase 120, cofactor-independent phosphoglycerate mutase 909, bis(5'-adenosyl)-triphosphatase 205, thiamine monophosphate kinase 179, pyruvate formate lyase family activating protein 298, 3-hydroxy-3-methylglutaryl-CoA reductase (mevanolate), N(2), N(2)-dimethylguanosine tRNA methyltransferase 145, N2, N2-dimethylguanosine tRNA methyltransferase 170, putative 5-methylcytosine restriction system, GTPase subunit 947, D-aminopeptidase 540, calcineurin superfamily metallophosphoesterase 118, rubrerythrin-related protein 317, 30S ribosomal protein S12P 161, DNA-directed RNA polymerase subunit beta 373, protein disulfide oxidoreductase 139, 30S ribosomal protein S27e 178, ribonuclease Z 122, 2-oxoglutarate ferredoxin oxidoreductase subunit gamma 352, 2-oxoglutarate ferredoxin oxidoreductase subunit alpha 407, methylmalonyl-CoA mutase, N-terminus of large subunit 172, AP endonuclease (base excision repair pathway) 365, CTP synthetase 105, PBP family phospholipid-binding protein 272, lipoate-protein ligase A, C-terminal section 234, peptide chain release factor 1 331, 30S ribosomal protein S15P 143, NADH oxidase 432, Putative oxidoreductase 538, NAD(P)H-flavin oxidoreductase 471, ferredoxin–NADP(+) reductase subunit alpha 471, Lrp/AsnC family transcriptional regulator 378, glycine dehydrogenase subunit 2 255, glycerol kinase 257, phosphomannomutase-related protein 321, ribose-5-phosphate isomerase A 107, phosphate transport regulator 193, isopentenyl pyrophosphate isomerase (mevanolate Pathway) 500, amino acid kinase 203, NADH:polysulfide oxidoreductase 203, 5'-methylthioadenosine phosphorylase 158, 30S ribosomal protein S9P 171, DNA-directed RNA polymerase subunit D 302, cytidylate kinase 305, adenylate kinase 109, 30S ribosomal protein S8P 180, 30S ribosomal protein S17P 131, serine–**

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glyoxylate aminotransferase, class V (transferasetransaminase), **predicetd ATPase 204**, **metallo-beta-lactamase superfamily hydrolase 134**, **metallo-beta-lactamase superfamily hydrolase 134**, **metal-dependent hydrolase 253**, **putative RNA-associated protein 167**, **proteasome subunit alpha 174**, **tRNA-modifying enzyme 172**, **sugar-phosphate nucleotydyltransferase 108**, **cytidylyltransferase 128**, **N-acetylchitobiose deacetylase 124**, **transcriptional regulator 364**, **glutamine synthetase 120**, **N6-adenine-specific DNA methylase 194**, **ArsR family transcriptional regulator 113**, **5'-methylthioadenosine phosphorylase II 280**, **DNA repair and recombination protein RadA 323**, **30S ribosomal protein S6e 106**, **pyruvate ferredoxin oxidoreductase subunit beta 282**, **cysteine desulfurase 521**, **hydrogenase maturation protein HypF 235**, **iron-molybdenum cofactor-binding protein 192**, **ATPase 260**, **4Fe-4S cluster-binding protein 254**, **phosphopyruvate hydratase 650**, **fructose-1,6-bisphosphatase 140**, **aspartate carbamoyltransferase catalytic subunit 158**, **Bipolar DNA helicase 448**, **bipolar DNA helicase 448**, **molybdenum cofactor biosynthesis protein A 182**, **proteasome-activating nucleotidase 474**, **deoxycytidylate deaminase 163**, **cell division protein FtsZ 821**, **ribulose bisophosphate carboxylase 1767**, **chaperonin beta subunit 460**, **DEAD/DEAH box RNA helicase 175**, **30S ribosomal protein S10P**, **elongation factor 1A**, **elongation factor 2**, **cysteinyl-tRNA synthetase**, **translation-associated GTPase**, **prolyl-tRNA synthetase**, **translation initiation factor 2B subunit beta**, **tyrosyl-tRNA synthetase**, **glycyl-tRNA synthetase**, **methionyl-tRNA synthetase**, **30S ribosomal protein S3Ae**, **30S ribosomal protein S19e**, **translation initiation factor 2**, **30S ribosomal protein S2**, **30S ribosomal protein S11P**, **30S ribosomal protein S4**, **30S ribosomal protein S5P**, **30S ribosomal protein S4e** and **30S ribosomal protein S19P** by pull down (View interaction).

1. Introduction

Aminoacyl-tRNA synthetases (aaRSs) attach amino acids (aa) to their cognate tRNAs to form aa-tRNA, which is then delivered to the ribosome for protein synthesis as a ternary complex with translation elongation factor 1A (EF1A) and GTP [1]. AaRSs have been found in a variety of complexes with each other and with other factors, potentially expanding the functions of aaRSs both within and beyond translation [2]. In mammalian cells nine aaRSs (ArgRS, AspRS, GlnRS, GluRS, IleRS, LeuRS, LysRS, MetRS and ProRS) associate with three non-synthetase protein factors (p18, p38, and p43) to form a large multi-aminoacyl-tRNA synthetase complex (MSC). The accessory proteins are important for the formation and stability of the complex, promote binding of tRNAs to the complex, and also play other roles outside translation [3–6]. In addition to the MSC, another aaRS, ValRS forms a complex with the human multi-subunit translation elongation factor 1H (EF1H), which increases the catalytic efficiency of $tRNA^{Val}$ aminoacylation [7]. In lower eukaryotes, including *Saccharomyces cerevisiae,* complexes have been characterized between GluRS, MetRS and Arc1p, and between SerRS and the peroxisome biosynthesis factor Pex21p, both of which enhance tRNA binding to the respective aaRSs [8–10].

Although many of these associations were first described in eukaryotic cells, numerous multi-enzyme complexes containing aaRSs have also been identified in both Bacteria and Archaea. In Bacteria, complexes comprised of one aaRS and a second non-aaRS protein have been implicated in diverse cellular functions including editing of misacylated tRNAs, indirect synthesis of aa-tRNA and metabolite biosynthesis [11–13]. In Archaea, aaRS-containing complexes were first described in *Haloarcula marismortui*, with many aaRSs purified in one or possibly two large complexes, and in *Methanocaldococcus jannaschi* where ProRS was found to interact with components of the methanogenesis machinery [14–16]. In another archaeal methanogen, *Methanothermobacter thermautotrophicus*, one complex composed of LeuRS, LysRS, ProRS and EF1A was identified while another contained SerRS and ArgRS. In both cases the formation of MSCs was found to improve the catalytic efficiency of tRNA aminoacylation by the aaRSs present in the corresponding complexes [17–21].

Previous studies on aaRS subcellular localization led to the proposal that MSCs directly channel aa-tRNAs to EF1A without dissociation in the cytoplasm [18,22]. This channeling could potentially provide a sequestered pool of aa-tRNAs specifically for utilization in protein synthesis, although a direct interaction between the ribosome and aaRSs in the MSC has not been demonstrated. The mammalian MSC (MARS) has been shown to interact with polysomes, but whether this reflects substrate channelling during protein synthesis is unclear given the presence of three essential aaRS-interacting factors that also function outside the complex [6,23]. MSCs have been identified in Archaea that do not require aaRS-interacting factors for assembly and function, potentially providing suitable systems to investigate MSC interactions with other components of the translation machinery. Previous attempts to characterize, purify and reconstitute archaeal MSCs have met with some limited success, in part due to the comparative instability of the complex [19]. Here we describe a more systematic investigation of an archaeal aaRS interactome, and define a polysome-associated MSC in the archaeon *Thermococcus kodakarensis*, providing evidence for the interaction of aaRSs with the mRNA translation machinery and consistent with substrate channeling during protein synthesis.

2. Materials and methods

2.1. Strain construction and protein purification

Construction of shuttle vector $pHis_6$-HA LeuRS and its use to transform *T kodakarensis* strain KW128 were performed as previously described [24,25]. N-terminally tagged intein fusion derivatives of LeuRS, ProRS and EF1A, and N-terminally His_6 tagged TyrRS were produced in *Escherichia coli* using standard procedures (see Supplementary data for details of construction of the corresponding plasmids). Intein tagged LeuRS, ProRS and EF1A were produced by transforming *E. coli* BL21(DE3)pLysS (Stratagene) with pTYB11 vectors containing the respective genes. Protein was produced by first incubating a starter culture at 37 °C (240 rpm) until mid log phase was reached and then using this to inoculate a larger culture (1L). The larger culture was grown to an OD_{600} of 0.2 at 37 °C and then transferred to 18 °C (190 rpm) for 90 min. Protein expression was induced with 0.5 mM IPTG for 12 h. Cell-free extract was produced by sonication of cells in buffer A [20 mM Tris–HCl [pH 8.0], 500 mM NaCl and 10% glycerol] containing a protease inhibitor mixture tablet (Complete Mini, EDTA-free; Roche Applied Science) followed by centrifugation at 150,000×*g* for 45 min. The resulting supernatant was loaded onto a chitin column, washed extensively with buffer A, and cleavage of the intein tag was induced by incuba-