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# VALYL-TRNA SYNTHETASE INTERACTS WITH β-SUBUNIT OF THE EUKARYOTIC TRANSLATION ELONGATION FACTOR COMPLEX eEF1B

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Valyl-tRNA synthetase (VRS) catalyzes specific valine attachment to the cognate tRNAs. In higher eukaryotes, VRS forms high-molecular-weight complex with eEF1B group of translation elongation factors [1]. Recently, a quaternary organization of the eEF1B complex has been reported [2], however, the binding subunit(s) of valyl-tRNA synthetase in this complex remained unknown.

***Aim*** of our work was to test the interaction of the VRS N-terminal domain (VRS-Nt) with α, β and γ subunits involved into the eEF1B complex.

***Methods.*** Recombinant subunits eEF1Bα, eEF1Bβ, eEF1Bγ and the N-terminal domain of VRS were expressed in *E.coli* and purified to homogeneity by affinity, ion-exchange and/or size-exclusion chromatography. Complex formation between isolated eEF1B subunits and VRS-Nt was tested by analytical gel filtration and by *in vitro* pull-down assay using the later as a bait. Eluted fractions were analyzed by SDS-PAGE.

***Results and Discussion.*** Recombinant eEF1Bα and eEF1Bγ subunits do not form a stable complex with VRS-Nt as judged by analytical gel filtration approach. Incubation of recombinant eEF1Bβ with VRS-Nt resulted in precipitate formation which made gel filtration impossible to perform. *In vitro* pull-down experiment showed that only recombinant eEF1Bβ subunit was able to bind Co-agarose with immobilized VRS-Nt, but not Co-agarose resin alone: fractions eluted by high imidazole concentration contained both proteins as judged by SDS-PAGE.

***Conclusions.*** We conclude that the only eEF1Bβ subunit is responsible for VRS attaching to the eEF1B complex. The N-terminal domain of valyl-tRNA synthetase is necessary and sufficient for this interaction.

***Kew words:*** valyl-tRNA synthetase, eukaryotic translation elongation factors, protein complexes, protein-protein interactions.

*Authors' contribution.* NTK and VFS performed all experiments, VFS and BSN analyzed bibliography and proposed the experimental conception. All authors interpreted the obtained results.

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## REFERENCES

1. *Bec G., Kerjan P., Zha X.D., Waller J.P.* Valyl-tRNA synthetase from rabbit liver. I. Purification as a heterotypic complex in association with elongation factor 1. *The Journal of biological chemistry*. 1989, 264(35):21131–7. https://doi.org/10.1016/S0021-9258(19)30056-0.
2. *Bondarchuk T.V., Shalak V.F., Lozhko D.M., Fatalska A., Szczepanowski R.H., Liudkovska V., Tsuvariev O.Y., Dadlez M., El'skaya A.V., Negrutskii B.S.* Quaternary organization of the human eEF1B complex reveals unique multi-GEF domain assembly. *Nucleic Acids Res.* 2022, 50(16):9490–9504. https://doi.org/10.1093/nar/gkac685.