Received: 18 May 2020 | Revised: 29 July 2020 | Accepted: 26 August 2020

DOI: 10.1111/jnc.15178

ORIGINAL ARTICLE

Journal of Neurochemistry JNC The Official Journal of the International Society for Neurochemistry WILEY

Bicuculline regulated protein synthesis is dependent on Homer1 and promotes its interaction with eEF2K through mTORC1-dependent phosphorylation

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Funding information
South Australian Health and Medical Research Institute; Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro, Grant/Award Number: Research fellow E-26/202.966/2017 and sediadas E-26/010.101037/2018; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Grant/Award Number: PDSE - 88881.188435/2018-01 and Ph.D. fellow 88882.456039/2019-01; Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: research fellow from CNPq 303101/2015-6 and universal 426687/2018-3

Abstract

The regulation of protein synthesis is a vital and finely tuned process in cellular physiology. In neurons, this process is very precisely regulated, as which mRNAs undergo translation is highly dependent on context. One of the most prominent regulators of protein synthesis is the enzyme eukaryotic elongation factor kinase 2 (eEF2K) that regulates the elongation stage of protein synthesis. This kinase and its substrate, eukaryotic elongation factor 2 (eEF2) are important in processes such as neuronal development and synaptic plasticity. eEF2K is regulated by multiple mechanisms including Ca^{2+}-ions and the mTORC1 signaling pathway, both of which play key roles in neurological processes such as learning and memory. In such settings, the localized control of protein synthesis is of crucial importance. In this work, we sought to investigate how the localization of eEF2K is controlled and the impact of this on protein synthesis in neuronal cells. In this study, we used both SH-SY5Y neuroblastoma cells and mouse cortical neurons, and pharmacologically and/or genetic approaches to modify eEF2K function. We show that eEF2K activity and localization can be regulated by its binding partner Homer1b/c, a scaffolding protein known for its participation in calcium-regulated signaling pathways. Furthermore, our results indicate that this interaction is regulated by the mTORC1 pathway, through a known phosphorylation site in eEF2K (S396), and that it affects rates of localized protein synthesis at synapses depending on the presence or absence of this scaffolding protein.

KEYWORDS
eEF2K, Homer1, mTORC1, protein synthesis, synapse

Abbreviations: BDNF, brain-derived neurotrophic factorBic: bicuculline; Ca^{2+}-CaM, calcium–calmodulin complex; CAMKII, Ca2+-CaM-dependent kinase II; CHX, cycloheximide; eEF2, eukaryotic elongation factor 2; eEF2K, eukaryotic elongation factor 2 kinase; mGluR, metabotropic glutamate receptor; mTOR, mammalian/mechanistic target of rapamycin; mTORC1, mTOR complex 1.

