



NPAS4, a typical bHLH-PAS transcriptional factor, and its engagement with 14-3-3 proteins beyond canonical recognition motifs

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Abstract

Background: NPAS4 (Neuronal PAS domain containing protein 4) a member of the bHLH-PAS (basic helix-loop-helix-Per-ARNT-Sim) is well known as a transcriptional factor serves as master regulator of neural circuit homeostasis, memory formation, and synaptic plasticity. On the other side 14-3-3 are scaffolding protein in brain and binds with phosphorylated client proteins and modulate their localization and activity. Some studies had shown that interaction of 14-3-3 with some bHLH family members influences on their localization and activity. However, to date no any data had shown whether NPAS4 interacts with 14-3-3 proteins and this interaction would be new clue to understand pathways of neurodegeneration development involving Tau (Microtubule associated protein).

Methods: To address this, our study is aimed to set out to test whether 14-3-3 and NPAS4 actually interact. Using Scansite 4.0 and ELM for in silico prediction, we identified potential 14-3-3 binding sites within NPAS4. Strikingly, these predicted motifs fell inside intrinsically disordered regions (IDRs). As a first experimental step, we performed co-localization studies in neuronal cells using confocal microscopy.

Results: Our results revealed that fluorescently tagged NPAS4 and 14-3-3 co-localize in transfected N2a cells, hinting at a possible interaction. This leads us to propose that 14-3-3 proteins may control NPAS4's subcellular location and activity. To move forward, we plan to study this interaction in living neuronal cells using pull-down assays. Importantly, we are also trying to achieve for the first time full length NPAS4 expression in *Pichia pastoris* primarily data will be present.

Keywords: NPAS4, Interactions, Prediction, 14-3-3, bHLH-PAS

