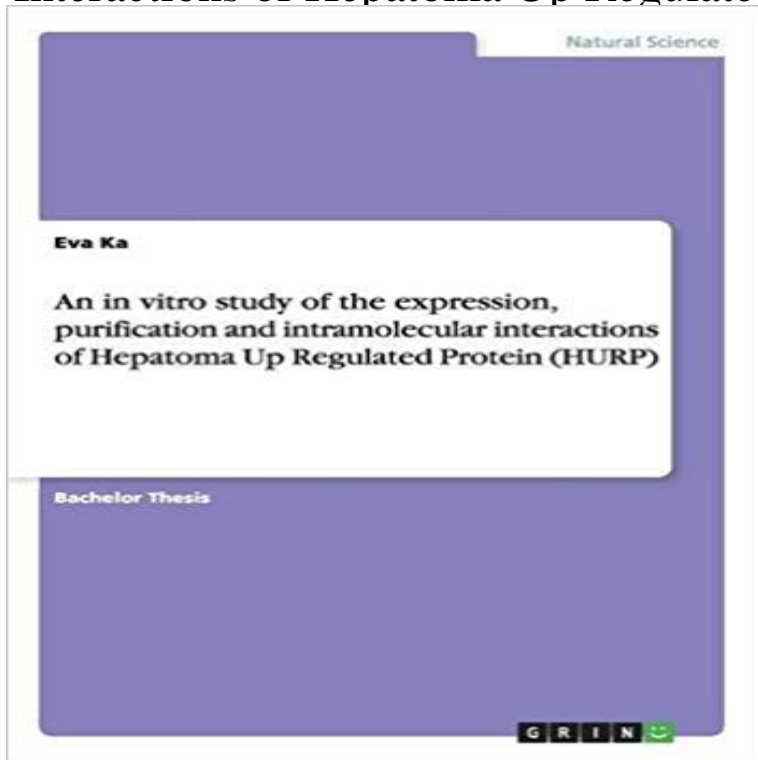


An in Vitro Study of the Expression, Purification and Intramolecular Interactions of Hepatoma Up Regulated Protein (Hurp) (Greek Edition)



Bachelor Thesis from the year 2011 in the subject Biology - Micro- and Molecular Biology, grade: A+ or 10.0, Democritus University Of Thrace (Molecular Biology and Genetics), language: Modern Greek, abstract: This manuscript is a diploma undergraduate thesis written in Greek; Research was conducted at the University of Thrace, at the Department of Molecular Biology and Genetics. Hepatoma Up Regulated Protein (HURP) is a microtubule-associated protein essential for the assembly of the mitotic spindle. HURP binds microtubules via its N-terminal domain, which is inhibited by HURPs C-terminal tail. The kinase Aurora A activates HURP by phosphorylating its C-terminus, which dissociates from the N-terminal microtubule binding domain. This diploma thesis explores how the autoinhibitory interaction between the N- and C-termini of HURP affect binding to proteins with key functions in spindle assembly (e.g., the mitotic motor Eg5 and the microtubule associated proteins TPX2 and XMAP125). I used molecular cloning techniques to generate recombinant GST- and His tagged chimeras of HURPs N- and C-termini. I optimized conditions for the expression of these proteins in bacteria and their purification by liquid chromatography methods using an FPLC system. I performed pull-down assays by incubating the recombinant proteins with HeLa cell extracts and ran western blots to examine the interactions of HURP with Eg5, TPX2 and XMAP125.

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