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Poster Sessions

Localization of AGN-1, which binds to protein phosphatase 6, with tau alternative splicing variants in U1spliceosome

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We cloned AGN-1 that interacted with protein phosphatase 6 as a molecule highly expressed in nephritic rat kidney. Our previous studies showed that AGN-1 was co-localized with U1-70K, one of U1snRNP, and splicing factor SC35, which was found to participate in the synthesis of tau alternative splicing variants, 4R-tau and 3R-tau. In addition, AGN-1 siRNA treatment resulted in alteration of 4R-tau/3R-tau mRNA ratio in neuronal cells in culture. In this study, we investigated co-localization of AGN-1 and tau alternative splicing variants in U1 spliceosome, composed of U1-70K and U1-A. RNA immunoprecipitation was carried out using nuclear extract prepared from Neuro2a cells and anti-phosphorylated SC35 antibody, followed by RT-PCR to detect tau pre-mRNA/mRNA bound to phosphorylated SC35. To confirm co-localization of SC35, AGN-1, U1-70K, and U1-A, protein immunoprecipitation was also performed with anti-phosphorylated SC35 antibody. RNA immunoprecipitation showed PCR fragments derived from 4R-tau and 3R-tau. Protein immunoprecipitation revealed co-localization of U1-70K, U1-A, AGN-1, and phosphorylated SC35. These results suggest that in U1 spliceosome, AGN-1 may localize with tau alternative splicing variants, 4R-tau and 3R-tau.