OFFICIAL ABSTRACT and CERTIFICATION

Intracellular Trafficking of Ajuba in Human Cells

Yujia Li
Great Neck South High School, Great Neck, New York

Cells possess molecular mechanisms that sense DNA damage, leading to DNA repair or cell death. Because cancer cells are often defective in DNA repair and exhibit high genomic instability, understanding DNA repair mechanism is important to discover the early events that can lead to cancer at the cellular level. This study focuses on Ajuba, a protein that is involved in DNA repair control in human cells. Ajuba possesses a complex intracellular trafficking pattern: it enters and exits the nucleus during the S phase. A nuclear export sequence (NES) is found in Ajuba's preLIM region. In addition, no nuclear localization sequence (NLS) has been found. Ajuba is composed of a LIM region, which contains three highly related tandem LIM domains, and a preLIM region. This study sought to gain more understanding of Ajuba's nuclear import and export determinants. It involved transfecting three truncations of the Ajuba DNA, including a preLIM segment, a preLIM without NES segment, and a LIM segment, into the human cell line Hela II and analyzing the expression and intracellular localization of these alleles, in order to map the nuclear import and export determinants of Ajuba. Among the truncations, preLIM without NES segment was obtained using PCR during the project, the other two segments were provided. Leptomycin B was used to block the nuclear export sequence of endogenous Ajuba in Hela II cells. Immunofluorescence microscopy using anti-Ajuba antibodies showed that the preLIM without NES segment of Ajuba is located in the nucleus, and the preLIM segment is located both in the nucleus and the cytoplasm. This suggests the nuclear import determinant is located within the preLIM segment without NES. Blocking nuclear export with Leptomycin B led to accumulation of endogenous Ajuba in the nucleus of Hela II cells, which confirms that the export of Ajuba depends on the NES present in the preLIM region. In addition, the molecular weights of the preLIM and preLIM without NES expression are about the same as shown in Western blot; therefore, the transfected proteins are expressed at the same level between the constructs.

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