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大鼠Notch 1胞质段的克隆及其表达

Cloning and Expression of the Intracellular Cytoplasmic Domain of Rat Notch 1 $$\operatorname{Receptor}$$

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中文摘要:

Notch 1信号途径参与决定细胞命运,其水解后产生的活性片段——胞质段(Notch 1 intracellular cytoplasmic domain, NICD)能被转运进核,激活下游靶基因的表达.Notch 1参与细胞的增殖、分化、程序性死亡、发育过程中的形态发生和器官形成等许多重要过程.为了获得重组的NICD,以大鼠脑cDNA文库为模板,用PCR方法扩增出编码NICD的基因片段,克隆至谷胱甘肽-S-转移酶(GST)融合表达载体pGEX-KG中,并在大肠杆菌中获得较高水平的表达.表达的融合蛋白GST-NICD分子质量约为120 ku左右,以包涵体和可溶性两种形式存在,易于亲合层析纯化.为制备抗体和进一步的功能研究奠定了基础.

革文摘要.

Notch proteins are involved in cell-fate selection throughout development. Signalling through the transmembrane receptor Notch is trigg ered by ligand binding, which induces the proteolytic cleavage of the Notch protein. This cleavage generates an intracellular fragment of the Notch protein (Notch-ICD), which translocates into the nucleus and modifies transcription of target genes through its association with the CS L familily of DNA binding protein (where CSL stands for CBF1, Su (H), Lag-1). Notch activity affects the implementation of differentiation, p roliferation, and apoptotic programs, providing a general developmental tool to influence organ formation and morphogenesis. To obtain recomb inant rat NICD, a long template and high fidelity PCR was used to clone NICD (1744V-2530K) DNA fragment from rat brain cDNA library. The cloned NICD fragment was confirmed by sequencing and then subcloned into glutathione -S-transferase (GST) fusion protein expression vector pGEX-K G. The GST-NICD fusion proteins were expressed in *E. coli* JM109 after inducing by IPTG. The fusion proteins were purified by affinity chromato graphy on glutathione Sepharose 4B.

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