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Heterologous Expression of Yeast Prepro-α-Factor in Rat Pituitary GH_3 Cells

이명애*[,1], 한상열[1], 박상대[1], 홍승환[1,2]
서울대학교 자연대학 [1]분자생물학과, [2]유전공학 연구소

Yeast pheromone α-factor is a 13-amino acid peptide hormone that is synthesized as part of a larger precursor, prepro-α-factor, consisting of a signal peptide and a proregion of 69 amino acids, which contains three potential glycosylation sites. The carboxy-terminal half of the precursor contains four tandem copies of mature α-factor, each preceded by spacer peptides of six or eight amino acids (variations of Lys-Arg-Glu-Ala-Asp-Ala-Glu-Ala), which are excised to produce mature α-factor. To investigate the molecular basis for intracellular sorting, proteolytic processing, and storage of peptide hormone precursors, yeast pp αf was heterologously expressed in rat pituitary GH_3 cell. In GH_3 cells, as in yeast, the nascent polypeptide is efficiently targeted to the ER, where it undergoes cleavage of its amino-terminal signal peptide and core glycosylation to form glycosylated pro-α-factor. Subsequently, this species rapidly disappears from cells with a half life of ≅30 min, and are secreted to the medium. In these cells ppαf was accurately processed to the mature α-factor with an efficiency of ≅20%. However, only 10% of the newly synthesized mature α-factor and unprocessed precursor were stored intracellularly, whereas 90% was sorted to the constitutive pathway and secreted rapidly into the medium with kinetics identical to endogenous growth hormone. We demonstrated that expression of yeast ppαf in GH_3 rat pituitary cells results in the secretion of mature α-factor and unprocessed pαf into the medium, suggesting that this wild type-prohormone could transit through the mammalian secretory pathway as endogenous protein. Our results show that signal peptide of yeast ppαf does direct it into the ER, and proregion does to the distal elements of the Golgi apparatus, respectively, but processing in putative cleavage site are not efficient.

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담배거세미 나방(*Spodoptera litura*)의 유충으로부터 유도합성된 항균활성 단백질 Spodopsin IA, IB, IC의 정제 및 특성

최충식*, 여성문[1], 채권석[2], 김학렬
고려대학교 생물학과, 단국대학교 생물학과[1],
기초과학지원연구소 생명과학부[2]

담배거세미나방(*Spodoptera litura*)의 6령 유충의 복강에 micro syringe를 사용하여 약 10^6 세포의 *Salmonella Typhymurium*을 주입시켜 항균활성단백질을 유도 합성시킨 후 ion-exchange column, gel filteration column과 마지막으로 reverse phase column을 이용하여 항균단백질을 정제한 다음 그 특성을 조사하였다. 정제된 항균단백질들 중 그람음성세균인 *E. coli*나 그람양성세균인 *M. luteus*등에 대해 강한 항균활성을 나타내는 약 4kDa의 분자량을 갖고있는 단백질을 Spodopsin IA, IB, IC 라고 명명하고 이들 각각을 tricine electrophoresis와 mass spectrometry 그리고 amino acid sequencer를 이용하여 정확한 분자량과 아미노산 서열을 조사하였으며 pI를 비롯한 각 단백질의 성질을 비교조사하였다.