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Heterologous Expression of Yeast Prepro- α -Factor in Rat Pituitary GH₃ Cells

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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 α f was heterologously expressed in rat pituitary GH₃ cell. In GH₃ 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 pp α f was accurately processed to the mature α -factor with an efficiency of $\approx 20\%$. 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 pp α f in GH₃ rat pituitary cells results in the secretion of mature α -factor and unprocessed p α f 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 pp α f 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

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담배거세미나방(*Spodoptera litura*)의 6령 유충의 복강에 micro syringe를 사용하여 약 10^6 세포의 *Salmonella Typhimurium*을 주입시켜 항균활성단백질을 유도 합성시킨 후 ion-exchange column, gel filtration 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.