Intracellular precursors and secretion of alkaline extracellular protease of Yarrowia lipolytica

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S Matoba, J Fukayama, R A Wing, and D M Ogrydziak

Institute of Marine Resources, University of California, Davis 95616.

Abstract
Processing and secretion of the alkaline extracellular protease (AEP) from the yeast Yarrowia lipolytica was studied by pulse-chase and immunoprecipitation experiments. Over half of newly synthesized AEP was secreted by 6 min. Over 99% of AEP activity which was external to the cytoplasmic membrane was located in the supernatant medium. Polypeptides of 55, 52, 44, 36, and 32 kilodaltons (55K, 52K, 44K, 36K, and 32K polypeptides) were immunoprecipitated from [3H]leucine-labeled cell extracts by rabbit antibodies raised against mature, secreted AEP (32K polypeptide). Experiments with tunicamycin and endoglycosidase H indicated that the 55K, 52K, and 44K polypeptides contained about 2 kilodaltons of N-linked oligosaccharide and that the 36K and 32K polypeptides contained none. Results of pulse-chase experiments did not fit a simple precursor-product relationship of 55K----52K----44K----36K----32K. In fact, maximum labeling intensity of the 52K polypeptide occurred later than for the 44K and 36K polypeptides. Secretion of polypeptides of 19 and 20 kilodaltons derived from the proregion of AEP indicated that one major processing pathway was 55K----52K----32K. The gene coding for AEP (XPR2) was cloned and sequenced. The sequence and the immunoprecipitation results suggest that AEP is originally synthesized with an additional preproI-proII-proIII amino-terminal region. Processing definitely involves cleavage(s) after pairs of basic amino acids and the addition of one N-linked oligosaccharide. Signal peptidase cleavage, dipeptidyl aminopeptidase cleavages, and at least one additional proteolytic cleavage may also be involved.