

Calnexin from *Pisum sativum*: Cloning of the cDNA and characterization of the encoded protein

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Abstract: A full-length cDNA of 1951 bp encoding a calnexin (CNX) protein was cloned from a *Pisum sativum* expression library. The open reading frame (ORF) within this cDNA encodes a 551-amino acid protein with a calculated molecular mass of 62.47 kDa that exhibits extensive homology with the CNX proteins from soybean (80%), *Arabidopsis thaliana* (70%), maize (70%), and dog (39%). The characteristic CNX signature motifs, KPEDWDE and GXW, generally found in molecular chaperones, are present in pea CNX (PsCNX), along with putative sites for Ca^{2+} binding and phosphorylation. In PsCNX, a signal sequence and a single transmembrane domain are also present at the N- and C-terminal ends, respectively. The PsCNX protein is expressed constitutively at the RNA level in vegetative and flowering tissues, as was evident from Northern analysis. Expression of PsCNX was light independent. In vitro translation of PsCNX cDNA yielded a 75-kDa precursor, which, in the presence of canine microsomal membranes, was cotranslationally processed into a 72.5-kDa product and was imported and localized to the endoplasmic reticulum. Trypsin treatment of the in vitro translated PsCNX in the presence of canine microsomes generated a further processed 67-kDa intraluminal form. The results with PsCNX also showed that the plant protein is a phosphoprotein containing phosphoserine residues, as evidenced by immunoprecipitation of PsCNX with anti-phosphoserine antibody. The PsCNX protein was also phosphorylated by endogenous kinases of pea microsomes.

Index Keywords: calnexin; arabidopsis; article; calcium binding; dog; endoplasmic reticulum; maize; molecular cloning; molecular weight; nonhuman; open reading frame; phytochemistry; priority journal; protein analysis; protein binding; protein expression; protein localization; protein phosphorylation; sequence homology; Amino Acid Sequence; Animals; Base Sequence; Calcium-Binding Proteins; Calnexin; Cloning, Molecular; DNA, Complementary; Dogs; Endoplasmic Reticulum; Gene Expression; Microsomes; Molecular Chaperones; Molecular Sequence Data; Peas; Phosphoproteins; Protein Processing, Post-Translational; Rabbits; Sequence Homology, Amino Acid; Arabidopsis; Arabidopsis thaliana; Canis familiaris; Embryophyta; Glycine max; Pisum sativum; Sativum; Zea mays

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