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Sucrose-Induction of Ornithine Decarboxylase is Mediated by MAP Kinase Activity in Tobacco Suspension Cultured Cells

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MAP kinase cascade is involved in transduction of various signals including mitogens and stresses. In plants several MAP kinases have been characterized and cloned. In animal cells MAP kinase activity is required for induction of ornithine decarboxylase (ODC), a rate limiting enzyme in polyamine biosynthesis, and sugar activates MAP kinase in *Catharanthus roseus*. Thus we examined the role of MAP kinase in regulation of ODC by sucrose in suspension cultured BY2 cells. Subculturing of stationary cells with fresh medium resulted in re-entering into cell cycle and rapid increase in ODC activity. ODC transcript level showed just slight and transient increase 1 to 3 h after subculture. 42 kDa MAP kinase activity measured by in-gel myelin basic protein (MBP) phosphorylation assay, was also increased showing the same kinetics as ODC activity. In sucrose-free medium the re-entering into cell cycle and increase in ODC activity were not observed. 42 kDa MAP kinase activity also failed to increase without sucrose. ODC transcript level was not affected by sucrose deficiency. U0126 a potent and specific inhibitor of MAP kinase kinase (MEK) abolished the increase in ODC activity by sucrose and did not affect the level of ODC transcript. Inhibition of 42 kDa MAP kinase activity by this inhibitor was confirmed by in-gel MBP phosphorylation assay. In post M phase synchronization system, 42 kDa MAP kinase activity was increased along with the progression of cell cycle into G1 through S phase. U0126 inhibited ODC activity and DNA replication completely in post M phase-synchronized cells, and consequently was likely to block the escape from S phase, abolishing G1 peak of ODC transcript level. These results suggest that the sucrose-induction of ODC activity is mediated by MAP kinase activity and that the detected 42 kDa MAP kinase activity is required for the completion of S phase for which ODC activity would play a certain role.

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Overexpression of a *Chlamydomonas* Catalase Gene in Tomato Increases Protection against Oxidative Stress Caused by Paraquat and H_2O_2

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Catalase (H_2O_2:H_2O_2 oxidoreductase), which converts hydrogen peroxide into water and molecular oxygen, is a tetrameric heme-containing enzyme found in nearly all aerobic organisms. In plants, a catalase is primarily located in peroxisomes and is involved in the detoxification of active oxygen species, which are generated during cellular processes such as photorespiration and beta-oxidation of fatty acids or by different environmental stresses. A recent report that catalase activity is inhibited by salicylic acid also indicates its involvement in systemic acquired resistance to pathogens. To evaluated the defense system in plant cells to oxidative stress caused by paraquat and H_2O_2 treatments, we have cloned a cDNA encoding a catalase (Crcat) from a unicellular green alga, *Chlamydomonas reinhardtii* and constructs containing the cDNA encoding a Crcat were introduced in the sense

orientation into the tomato genome. We have studied the protection of plant against paraquat and H_2O_2 treatments in transgenic tomato plant.

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Proteolytic Processing of E. coli-Expressed P1/HC-pro Complex in Soybean Mosaic Virus

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Soybean mosaic virus (SMV) proteinases P1 and helper component (HC-Pro) participate in the proteolytic processing of viral polyproteins. The P1 proteinase catalyzes proteolysis between itself and HC-Pro. The each gene product and fused P1/HC-Pro complex were synthesized by reverse transcription-polymerase chain reaction (RT-PCR) using the combination of primers derived from 5′ and 3′ ends of P1 and HC-Pro, respectively, and their sequences have been determined. To define the junction region between the two partners responsible for processing, single amino acid replacement of serine by phenylalanine was introduced into the junction site by RT-PCR using a primer derived from the mutated 3′ end of P1. We inserted each fused gene construct containing a conserved and a mutated cleavage site into pET vector. After production, the corresponding protein was cleaved in vivo by the proteinase, that is encoded by the plasmid harboured by a specialized E.coli host. The mutation at the junction site resulted in no proteolytic processing of the fused recombinant protein. The released protein remains soluble and can be purified from cell extracts by means of an affinity tag (a poly His group). Cleavage at the junction between P1 and HC-Pro occurs during expression, respectively producing 30 kDa and 45 kDa on the SDS-PAGE. We purified the P1 and HC-Pro by detergent extraction of cell lysate and FPLC. The purified each protein was active in a mildly-denaturing gelatin SDS-PAGE. We examined the dependence of proteinase activity on pH, temperature and presence of inhibitors, using proteinase assay by gelatin or casein as a substrate.

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Enhanced resistance to dehydration in trehalose-producing transgenic tobacco plants

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Six lines of transgenic tobacco plants harboring *E. coli* TPS (trehalose-6-phosphate synthase) gene (*ots* A) were generated by leaf disc transformation. Transgenic plants were identified by PCR, Northern hybridization, and trehalose synthesis. All lines of transgenic plants manifested stunted growth and extended generation time in varying degree. In addition, transgenic plants typically exhibited some morphological changes including altered leaf morphology and increased number of leaves with more vestigial branches along the main stem. Leaves became longer and thinner to form lancet-shaped and some leaves were severely irregular-shaped with curls and wrinkles. The stunted growth was not accompanied with early senescence or necrosis.

Homozygous plants obtained in F_2 generation were used to test their responses against dehydration. All transgenic plants showed enhanced tolerance to dehydration as shown by improved retention in fresh weight upon air-drying both in detached leaves and whole plants. In addition,