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New plant protein involved in cell death.

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Although the yield of natural and recombinant proteins could reach high levels in plant cells, the mechanism by which tolerance to overproduced proteins is achieved remains unclear. We previously showed on transient expression system that rapid protein synthesis can result in leaf necrosis with the appearance of moribund and dead cells [1]. To understand the mechanisms by which protein overproduction can lead to cell death, we selected and isolated a gene encoding protein with 50% homology to Kunitz trypsin inhibitor (KTI) and later named it plant death factor (PDF). Biologically, PDF mRNA increase is likely to accompany cell death and reduce mRNA and/or protein accumulation. To identify the correct outcome, we created genetic constructs encoding *N. benthamiana* PDF and *Arabidopsis thaliana* KTI under control of 35S promoter in the binary vectors. Point mutations were inserted in the PDF sequence resulting in the substitution of key amino acids of the putative active site (PDFmut). Polyclonal mice antibodies were obtained for detection of the corresponding proteins in plant extracts. Mice were immunized with recombinant PDF and KTI, produced in *E.coli*. Agroinjection experiments with PDF sequence in antisense polarity (asPDF) showed that host PDF gene knockdown resulted in an increase of viral vector-directed protein production. We believe that PDF constrains excessive mRNA and protein synthesis and its knockdown leads to the increased protein accumulation.

The synthesis of PDF mRNA may reflect the level of biosynthetic activity of the plant tissue. To obtain experimental confirmation of this hypothesis, we found that in contrast to the leaves, the root system of the plant, and, mainly, root border cells have an increased level of PDF mRNA synthesis. Moreover, it appeared that the amount of PDF mRNA in root tissues of flowering tobacco plants was up to 400-fold higher than in roots of young seedlings. We concluded that the level of PDF gene expression in root cells reflects and characterizes the age of the plant.

Литература

1. T. Komarova, E. Sheval, and Y. Dorokhov. Uncontrolled protein overexpression leads to plant cell autophagy. FEBS Journal, 276:206–206, 2009.