INTRACELLULAR TRANSPORT AND TOXICITY OF A TYPE I RIBOSOME-INACTIVATING PROTEIN

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Ribosome-inactivating proteins (RIPs) are potent inhibitors of protein synthesis that accumulate in different tissues of many plant species. These proteins are N-glycosidases that are able to remove a specific adenine present in a universally conserved region of 23S/25S/28S rRNA, thus blocking protein synthesis. Several of these proteins have been shown to be endowed with antiviral and/or antifungal activity. According to the local cell death hypothesis, compartmentalized RIPs would reach the cytosol upon viral infection causing inactivation of host cell ribosomes and thus blocking virus replication and spreading through the plant. Several attempts have been therefore made to exploit RIPs for the production of transgenic plants resistant to viral or fungal infection. However, constitutive expression of these proteins has often been found to be toxic toward host cells.

Here we have studied the intracellular trafficking of saporin (a type I RIP from *Saponaria officinalis*) when expressed in tobacco protoplasts, and characterized the mechanism of toxicity toward host cells. We find that saporin expression is extremely toxic to tobacco protoplasts, causing a drastic reduction in protein synthesis. By expressing active site mutants we could determine that saporin behaves as a secretory protein, accumulating in the incubation medium of transfected tobacco protoplasts. Still, the toxicity associated with saporin expression indicates that a fraction of the synthesized polypeptides accumulates in the cytosol. This could be due either to inefficient targeting to the endoplasmic reticulum, to retrotranslocation from the endomembrane system, or to re-uptake of the toxin from the incubation medium. Our data indicate that toxicity is not due to endocytosed saporin and that the signal peptide has the potential of controlling the toxicity of any saporin precursor that fails to be targeted to the endoplasmic reticulum. In addition, we find that mutations interfering with signal peptide cleavage reduce the toxicity associated with saporin expression. All together these data indicate that toxicity is due to the release in the cytosol of polypeptides that have transiently been exposed to the action of signal peptidase and suggest potential mechanisms by which RIP-expressing cells may control the access of these enzymes to the cytosol upon viral infection.