Seven days old pea (*Pisum sativum* L.) epicotyls were germinated and grown in darkness. Singlet oxygen ($^1O_2$) production was visualized with DanePy (3-[*N*-(β-diethylaminoethyl)-*N*-dansyl] aminomethyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrole) fluorescence. $H_2O_2$ was detected by DAB (3,3'-diaminobenzidine) staining or by transition electron microscopy (TEM) using $CeCl_3$. Hydroxyl radicals (•OH) were detected with spin trapping EPR spectroscopy using POBN or on the basis of HTPA (hydroxyl-terephthalate) fluorescence. Protochlorophyllide (Pchlide) localization was detected by fluorescence microscopy, its monomers/oligomers were identified by low temperature fluorescence emission spectra.

Illumination caused fast turgor loss and wilting in middle segments of the epicotyls accompanied with accumulation of water in the intercellular cavities. During this process, porphyrin-type pigments were gradually bleached, while $^1O_2$ and lipid peroxidation products were detected suggesting a type-II, porphyrin (Pchlide or Chlide) -photosensitized mechanism.

On the other hand, selective assays showed the presence of three different ROS: $O_2^{-\bullet}$, $H_2O_2$ and •OH in the illuminated pea epicotyls, preferentially in the mid-sections. $H_2O_2$ was mainly produced along the radial walls of cells in areas also rich in monomer Pchlide. Although •OH production, which was restricted to the mid-section was light-dependent, the $H_2O_2 \rightarrow$ •OH conversion also occurred without illumination, showing the presence of Fenton-catalysts in this region. These data demonstrate that products of a type-I photoreaction also contribute to disordered water status of the epicotyls and their wilting in light.

## TIP47 protects of mitochondrial membrane integrity and inhibits oxidative stress-induced cell death

E Hocsák[1], Sz Jávor[2], A Szabó[1], B Rácz[1], A Szigeti[3], E Rápolti[3], É Pozsgai[1], F Gallyas Jr.[1], B Sümegi[1]

[1]Department of Biochemistry, University of Pécs, Hungary, [2]Department of Surgical Research and Techniques, University of Pécs, Hungary, [3]Department of Oncotherapy, University of Pécs, Hungary

The intracellular role of tail-interacting protein of 47kDa (TIP47/PP17b) has been controversial, no data are available for its possible role in tumor development, or in regulation of cell death. TIP47 is expressed in almost if not all tissues. During pregnancy, TIP47 serum levels increase; after birth they drop. Although some TIP47 is found on lipid droplets, it is known to be required for the delivery of mannose 6-phosphate receptors from late endosomes to the Golgi, both *in vitro* and in living cells. The protein binds the cytoplasmic domains of the cation-dependent and cation-independent receptors, and is recruited to late endosomes by binding to Rab9 GTPase. The loss of TIP47 destabilizes Rab9 which is also required for proper receptor transport.

The aim of this study was to find an intracellular role of this protein.

The vector containing TIP47, trunceted-TIP47 or the empty pcDNA3.1 vector was transfected into NIH3T3 cells. Cells were treated with $H_2O_2$ and cell viabilities were measured by MTT-viability assay. TIP47 was silenced by diced-siRNA in Hela cells. Mitochondrial membrane potential was monitored on isolated rat liver mitochondria in vitro by fluorescence of Rh123 or on TIP47 transfected and treated NIH3T3 cells in vivo. Depolarization of mitochondria can be visualized *in vivo* by using the membrane potential sensitive dye, JC-1 by fluorescent microscopy. Ratio of apoptosis and necrosis were evaluated after double staining with fluorescein isothiocyanate (FITC)-labeled annexin V and propidium iodide using flow cytometry and fluorescent microscopy.

TIP47 was over-expressed in a cell line normally not expressing it (NIH3T3), or suppressed by small interfering RNA in a cell line that normally express TIP47 (HeLa) at high extent before exposing cells to oxidative stress. Over-expression of TIP47 prevented hydrogen-peroxide induced cell death and the collapse of mitochondrial membrane potential. Suppression of TIP47 synthesis by small interfering RNA technique sensitized the HeLa cells to hydrogen peroxide induced cell death. We proved with both in vitro and in vivo experiments that TIP47 caused hyperpolarisation of mitochondrial membrane and reduced $Ca^{++}$ induced mitochondrial membrane depolarization. In view of the fact that mitochondria may role in both apoptotic and necrotic cell death, we used flow cytometry to determine the percentage of apoptotic and necrotic cells. The results unambiguously justified protective effects of TIP47 protein.

We provided evidence that TIP47/PP17b can bind to mitochondria and can protect mitochondrial membrane integrity, as well as can prevent oxidative stress induced cell death providing the first evidence the possible oncogenic property of TIP47/PP17b.