# Executive-Summary: To understand the molecular mechanism of tocopherol action and on this basis to be able to plan better clinical intervention studies and Tissue and cellular distribution of 3 human tocopherol associated proteins (hTAPs)

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***Introduction:*** α-Tocopherol (vitamin E) has in addition to its anti-oxidative abilities also non anti-oxidative activities, which can influence gene expression and cellular signaling cascades. In the past few years, the mechanisms involved in the uptake of vitamin E from the diet and the proteins involved in these processes have been characterized. For the regulation of the plasma concentration of vitamin E by the liver, the α -tocopherol transfer protein, α -TTP, has been identified. However, the mechanisms of the intra-cellular distribution of vitamin E are still unknown. We have identified three tocopherol associated proteins (TAP1, 2, 3) in human cells as candidate proteins that may be responsible for the intra-cellular transport of vitamin E and its non anti-oxidative activities.

***Results and Discussion:*** Three genes were cloned from human lung tissues, which code for the three human SEC14-like proteins (TAP1, 2, 3). These proteins are closely related with SEC14p from *Saccharomyces cerevisiae*, and also with the tocopherol transfer protein (α -TTP) and the cellular retinaldehyde protein (CRALBP). In the amino-terminus, these proteins have a SEC14-like domain to which hydrophobic ligands can bind. At the carboxy-terminus, these proteins possess an additional GOLD domain, which in other proteins (GCP60, PAP7) serves as an adaptor domain that binds to the Golgi giantin or the peripheral benzodiazepine receptor from mitochondria, respectively.
First, the three TAP proteins were cloned with a carboxy-terminal hemagglutinin-tag and after transfection into cell lines, the TAP proteins were localized. Within the cells the TAP proteins are present in vesicles, which are predominantly concentrated at the Golgi and the endoplasmic reticulum. In part, there is also localization at the plasma membrane visible. When the GOLD domain is deleted, the distribution of the TAP proteins in the cell is changed.

The three TAP proteins bind *in vitro* various tocopherols, as well as specific phospholipids (phosphatidylcholine, phosphatidylinositol, and phosphatidylglycerol). The tocopherols modulate the phosphatidylinositol-dependent kinase in a TAP dependent manner.

Three novel polyclonal antibodies against peptides derived from the three TAP proteins were generated and tested on western blots. It was found that depending on the tissue the TAP proteins are differentially expressed. In addition to that, the expression pattern of the three TAP proteins was assayed on tissue arrays that contained 30 different human tissues. In the tissues tested the TAP proteins are mainly located to the Golgi and the endoplasmic reticulum. These results suggest that the three TAP proteins are most likely involved in the intra-cellular distribution of tocopherols and phospholipids. A correct distribution is possibly important for the further metabolic conversion of these ligands, or for the formation of storage or secretory vesicles.

***Conclusions:*** The human TAP proteins are expressed ubiquitously in most tissues, however the intensity of expression is tissue-dependent. The three TAP proteins possibly regulate the amount and distribution of cellular tocopherols and phospholipids. By the specific transport of tocopherols and phospholipids to enzymes and organelles, the TAP proteins may influence the activity of lipid-dependent cellular processes. A constant and adequate distribution of tocopherol within the cells is important to prevent cardiovascular diseases like atherosclerosis, as well as neurodegenerative diseases like Alzheimer's, Parkinson's or ataxia. Vitamin E deficiency leads mainly to neuro-degenerative symptoms, and the function of TAP in the brain may also be to mediate an efficient vitamin E transport. The TAP proteins could also, depending on the bound hydrophobic ligand, be involved in signal transduction processes. However, additional experiments are required to determine the exact cellular function of the TAP proteins.