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**Oral Communication Abstract – 2B.05**

# ONLY A MINORITY OF INTEGRAL MEMBRANE PROTEINS WITH COMPLEX N-GLYCANS RESIDE ON THE TONOPLAST

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About one third of the secretory proteins of the cell are N-glycosylated and their glycans are very often modified when proteins travel throught the Golgi complex. Tonoplast proteins can reach the membrane either passing or bypassing the Golgi. To investigate on the extent of Golgi involvement in the traffic of tonoplast proteins, we therefore analyzed the subcellular distrubution of Arabidopsis proteins with complex glycans. Plant N-glycans can be modified by Golgi enzymes, acquiring Golgi-specific complex sugars (by addition of b1,2 Xylose, a1,3 and a1,4 Fucose). Intact vacuoles were purified from Arabidopsis leaves. Peripheral membrane proteins were removed before sub-fractionation by centrifugation to separate tonoplast microsomes form the vacuolar content. As a control, total microsomes and soluble proteins were purifed from protoplasts. The samples were subjected to SDS-PAGE and protein blot using antisera against organelle markers (BiP, GRP96, gTIP) to verify the purity of fractions or antiserum against plant complex glycans, which recognizes plant Golgi-modified glycans containing Fuc or Xyl. Total microsomes were also prepared from Arabidopsis leaves and fractionated by sucrose density gradient at the equilibrium. The origin of microsomes in the fractions were determined using antisera against marker proteins from plasma membrane (PIP2) or tonoplast (TIP). Our results demonstrate that: i) the bulk of the proteome with complex N-glycans is constituted by soluble proteins; ii) among membrane proteins, the majority is at the plasma membrane, while only a minor proportion of tonoplast proteins has complex glycans. Analysis using concanavalin A, which binds high-mannose (unmodified) N-glycans is under way to determine whether the results reflect a minor contribution of N-glycoproteins to the tonoplast proteome or a major tonoplast traffic route bypassing the Golgi complex. The biological implication of these data will be discussed.

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