Synthesis and trafficking of the tonoplast potassium channel AtTPK1

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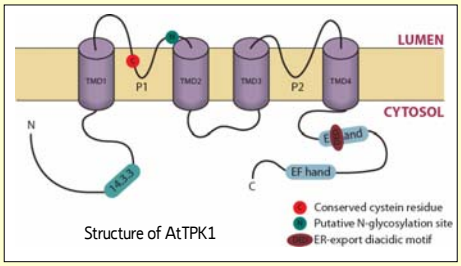
The tandem-pore potassium channel AtTPK1:

AtTPK1 is a K^+ selective, voltage-independent channel[1]. It has been shown to be activated by cytosolic Ca^{2+} and regulated by GRF6, a 14-3-3 protein[1,2]. Tonoplast localisation has been observed in transient expression systems, but never confirmed *in planta*.
Many potassium channels form functional complexes enclosing 4 pore domains. The ability of AtTPK1 to form homo-oligomers has been demonstrated[3], however the exact stochiometry of this complex has still to be unravelled.

Targeting motif for tonoplast proteins:

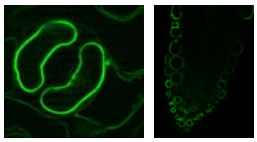
Previous studies mainly focused on α-TIP aquaporin. They demonstrated that α-TIP reaches the tonoplast through a Golgi-independent route[4]. They also showed the importance of the 6th transmembrane domain and the cytosolic C-terminus for tonoplast targeting, finally identifying a necessary but not sufficient PIEPPPHH targeting motif[5]. Similarly, the cytosolic longin domain of the SNARE AtVAMP711 has also been shown to contain tonoplast targeting information[6].



Structure of AtTPK1

Confirmation *in planta* of the tonoplast localisation:

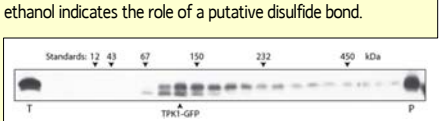
Fluorescence observations
TPK1-GFP in transgenic *Arabidopsis* plants



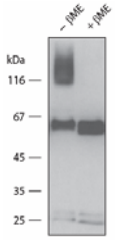
Subcellular fractionation on isopycnic sucrose gradient.
TPK1-GFP is mainly present in the tonoplast fractions (7 to 11)

Dimerisation:

Dimerisation was confirmed using velocity gradient centrifugation. The sensitivity of the dimer to β-mercapto-ethanol indicates the role of a putative disulfide bond.



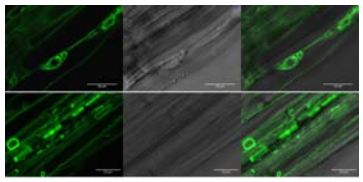
TPK1-GFP is able to form homodimers
In native conditions, TPK1-GFP migrates through a velocity sucrose gradient as a ~130kDa protein, the mass of a homodimer.



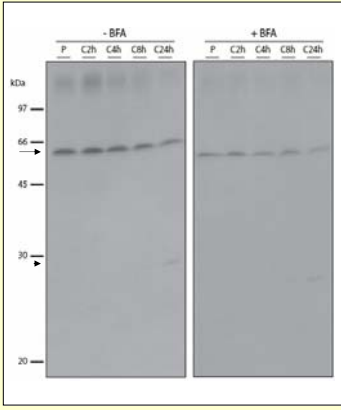
Involvement of a disulfide bond
The dimer, detectable under oxidizing conditions (-), disappears under reducing conditions (+).
βME = betamercaptoethanol

Involvement of the Golgi apparatus in TPK1-GFP trafficking:

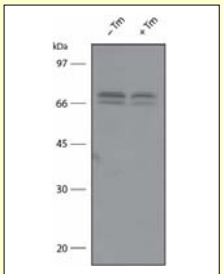
Inhibition of Golgi-mediated secretory protein traffic (by Brefeldin A treatment) influences TPK1-GFP localisation *in planta*, indicating an involvement of the Golgi apparatus in TPK1-GFP traffic, unlike the Golgi-independent pathway determined for α-TIP.
Pulse-chase analysis indicates that TPK1-GFP is a rather stable protein and BFA does not affect its turnover.
Glycan modification activities of the Golgi apparatus could not be used to trace TPK1 traffic, as the potential *N*-glycosylation site (N131) is not used *in vivo*.



The Golgi apparatus is involved in TPK1-GFP traffic.
In BFA treated roots, TPK1-GFP is mislocated in the ER.



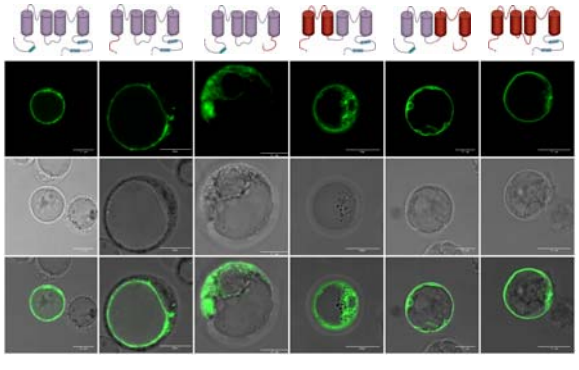
TPK1-GFP turnover is not affected by brefeldin A.
Protoplasts from transgenic plants were subjected to pulse-chase with radioactive Met+Cys and TPK1-GFP was immuno-selected with α-GFP antibodies. TPK1-GFP (arrow) has a half-life time of about 24h. Cleavage of the cytosolic, C-terminal GFP (arrow head) appears to be a slow process. BFA treatment does not affect this phenomenon.

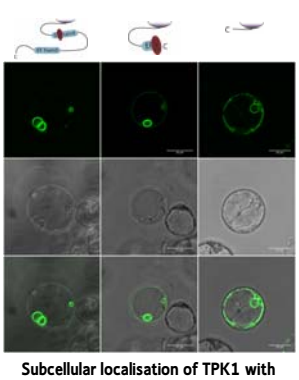


TPK1 is not *N*-gylcosylated
Tunicamycin (Tm), an inhibitor of N-glycosylation, does not influence the apparent mass of TPK1.

Identification of the tonoplast targeting motif:

AtTPK4 is the homologous K^+ channel located at the plasma membrane. We generated several chimeric TPK1-TPK4 constructs to identify which domain of the channel is responsible for its localisation. We concluded (in parallel to Dunkel et al.[7]) that the C-terminus of AtTPK1 is a key domain for tonoplast localisation. We also showed that this domain is sufficient for redirect TPK4-GFP to the tonoplast.
By deleting parts of this C-terminus domain, we confirmed the role of the diacidic ER-export domain identified by Dunkel et al[7].


Subcellular localisation of chimeras between TPK1 (violet) and TPK4 (red)


Subcellular localisation of TPK1 with deletions at the C terminus

Model for AtTPK1 synthesis and traffic to the tonoplast:

- Tonoplast K^+ channel TPK1 forms a homodimers, enclosing 4 pore domains like many functional K^+channels.
- This dimerisation could involve a disulfide bond and be required for correct traffic.
- A diacidic motif, present in the cytosolic C-terminus, is responsible for the exit from the Endoplasmic Reticulum.
- TPK1 uses a Golgi-mediated route to reach the tonoplast, but its half-life is not affected if traffic is blocked by Brefeldin A.
- As the cytosolic C-terminus part of TPK1 is necessary and sufficient for tonoplast localisation, it must contain an additional, positive targeting information.

1. Gobert et al. (2007) PNAS **104**: 10726-10731
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3.Voelker et al. (2006) Plant J. **48**: 296-306
4. Gomez and Chrispeels (1993) Plant Cell **5**:1113-1124
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