Structural and functional analysis of SoPIP2;1 add insights into plant aquaporin gating

Anna Alfredsson[1], Maria Nyblom[2], Yi Wang[3], Mikael Ekvall[1], Karin Hallgren[1], Kristina Hedfalk[1], Emad Tajkhorshid[3], Richard Neutze[1], Susanna Törnroth-Horsefield[1]

[1]University of Gothenburg, [2]Chalmers University of Technology, [3]University of Illinois

The aquaporin SoPIP2;1

- Aquaporins allow water to pass through membranes.
- Aquaporins exist in all types of organisms; bacteria, archae and eukaryotes.
- Maintaining the turgor pressure in plant cells requires that the water flux can be regulated in response to water availability [fig. 1].
- SoPIP2;1is a regulated aquaporin. It is the most abundant protein in spinach leaves plasma membranes and is functional as a tetramer.
  - Drought leads to channel closing upon dephosphorylation of Ser 115 and Ser 274.
  - When flooding occurs, the channel closes due to protonation of a conserved histidine.

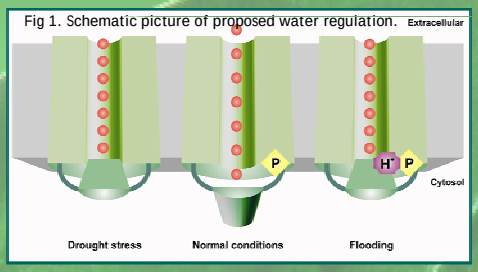

Fig 1. Schematic picture of proposed water regulation.

Previous structures proposed mechanism for regulation

- Crystallized in both closed (2.1 Å) and open (3.9 Å) conformation.
- A Cd^{2+} ion is bound in the closed structure, which is thought to be Ca^{2+} *in vivo*.
  - The ion coordinates a network of ionic and hydrogen bonds which anchors loop D to the N-terminus. Phosphorylation of Ser 115 disturbs this network and release loop D.
  - Residues in loop D create a hydrophobic plug that blocks the pore. Unphosphorylated Ser 274 would cause a steric clash with the hydrophobic plug if the channel tried to open.
  - In this work, phosphomimicing mutants with Ser 115 and Ser 274 replaced by glutamic acid were created to investigate regulation further.

Crystal structures of phosphomimicing mutants show rearrangement in the N-terminus, but the channel remains closed

- The mutation of Ser 115 to Glu disrupts the metal ion binding site, which releases the N-terminus and helix 1 can extend a further half turn into the cytoplasm [fig 3].
- Loop D still remains in its closed conformation, anchored to loop B but the interactions are altered.
- The S274E mutant structure binds Cd^{2+} and looks essentially the same as the closed wild-type structure.
- The closed conformation of the mutants were confirmed with water transportation assays in proteoliposomes.
- The glutamic acid residue does not seem to fully mimic the phosphorylated state. A real phospho-serine would give two negative charges instead of one, and probably release loop D.

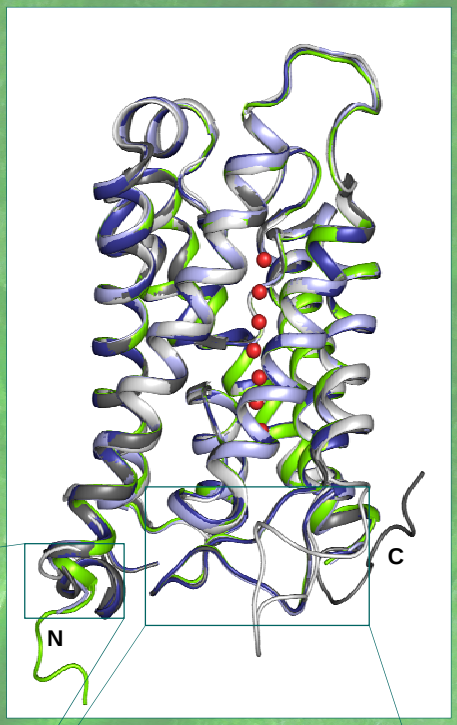

Fig 2. Overlay of all structures. Dark grey=wildtype closed, light grey= wildtype open, green=S115E, dark blue=S274E, light blue=S115E;S274E.

Hypothesis for complete opening of the channel upon phosphorylation of Ser 115

- The open structure [fig 4b] showed an extension on helix 5 compared to the closed structure [fig 4a].
- The S115E mutant is extended on helix 1 [fig 4c].
- We speculate that the structure of SoPIP2;1 phosphorylated at Ser 115 would show both movements [fig 4d].

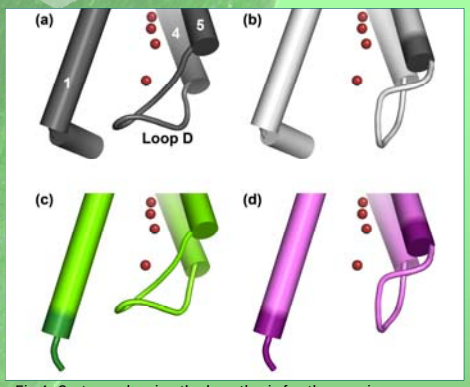

Fig 4. Cartoon showing the hypothesis for the opening mechanism. a) Wild-type closed, b) Wild-type open, c)S115E, d)Hypothetical model of SoPIP2;1 with S115 phosphorylated.

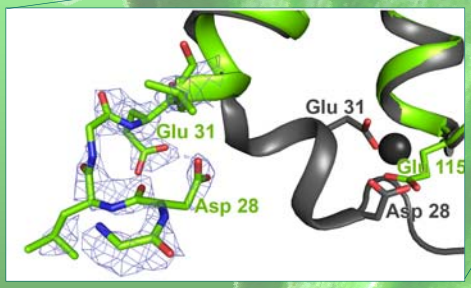

Fig 3. The S115E mutation (green) disrupts the metal ion binding site and the N-terminus is rearranged compared to the closed structure of the wild-type (dark grey).

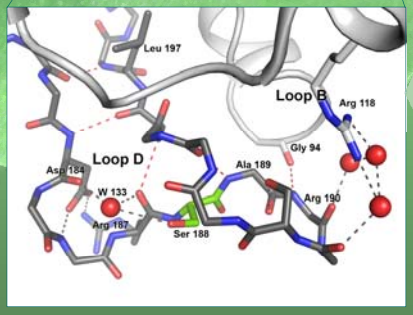

Fig5. Ser 188 (green) sits on a central position in loop D and may contribute to regulation.

Phosphorylation of Ser 188 may also be involved in regulation

- Ser 188 is part of an extensive H-bond within loop D. Phosphorylation at this site may cause this to break down and loop D to swing open [fig 5].
- Ser 188 sits within a protein kinase C phosphorylation site, just like Ser 274.
- Water transportation assays in liposomes showed a significantly higher water permeability for the S188E mutant compared to wildtype.
- Molecular Dynamics simulations indicated that phosphorylated S188 interacts with the C-terminus, causing the channel to open.

Crystallographic data

Structure	S115E	S274E	S115E:S274E
Resolution (Å)	43.44-2.30	34.86-2.95	20.0-2.05
Space group	I4	I4	I4
Completeness (%)	99.9	99.3	99.9
R_{sym} (%)	12.9	15.5	8.4
R-factor (%)	16.5	19.6	15.9
R_{free} (%)	19.3	23.0	18.1

UNIVERSITY OF GOTHENBURG