Symplastic connections in Arabidopsis root tips

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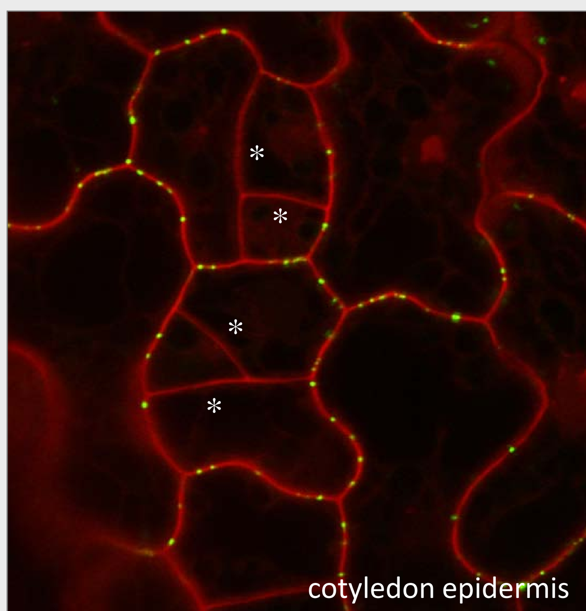
Research Question

Plant cells exchange solutes and certain macromolecules via plasmodesmata. For selected cells in leaves and roots and for embryos it has been shown that the size exclusion limit of plasmodesmata differs depending on the cell type and/or developmental stage of the tissue. We studied the symplastic connections in the root tip of *Arabidopsis thaliana*.

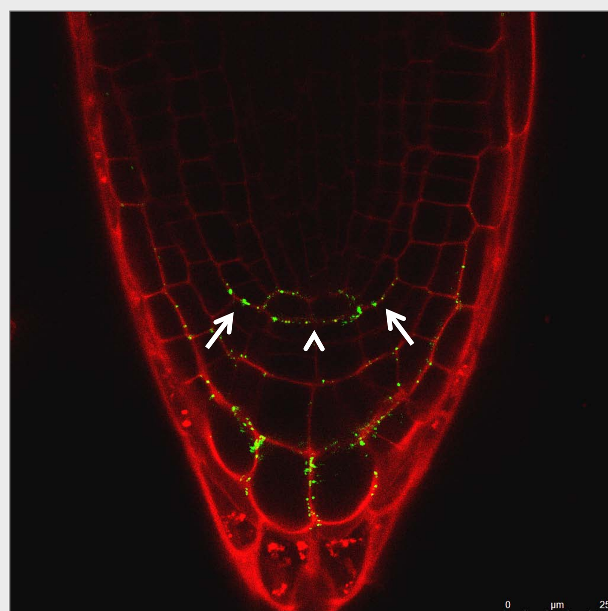
MP17-GFP Marker

Using MP17-GFP protein, that binds to secondary plasmodesmata, we could show that in the root tip some cell walls contain many secondary plasmodesmata. The strongest labeling was identified in the cell walls of quiescent centre, cortex/endodermis initials and in the columella.

primary plasmodesmata are not labelled by MP17GFP



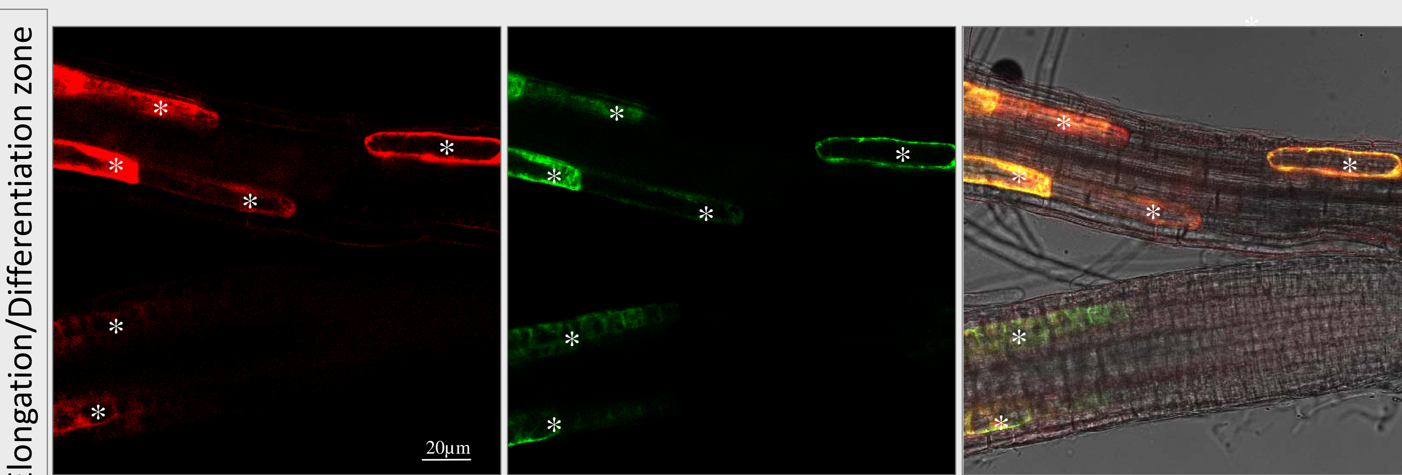
overlay projection of the root tip

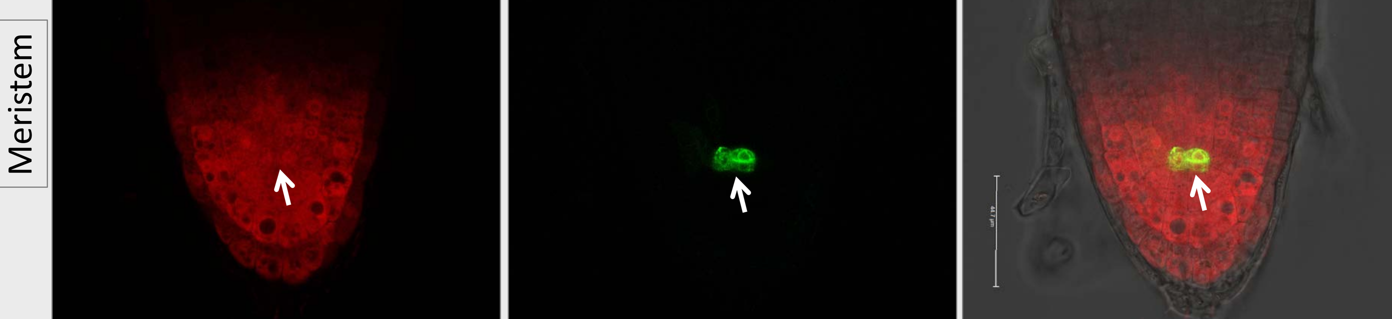


arrow head – quiescent center, arrow – cortex/endodermis initials, asterix – cell plate with primary plasmodesmata (not labelled), red – propidium iodide, green - GFP

Cell Connectivity During Differentiation

All cells of the root meristem are connected by plasmodesmata allowing 1x-mCherry, expressed in quiescent centre, to move cell-to-cell. In the elongation and differentiation zone of the root 1x-mCherry does not move out of cortex cells, which indicates a change in plasmodesmal architecture during differentiation.





arrow – quiescent center, asterix – cortex cells, red – soluble mCherry, green – erGFP

Root hairs are symplastically isolated. Soluble DRONPA activated in neighboring cells cannot move into trichomes.

green – sDRONPA-s, red – propidium iodide stained cell walls, t = minutes after activation

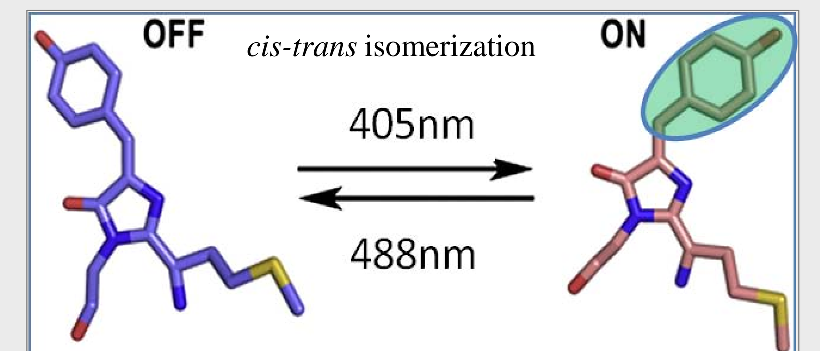
DRONPA-s System

DRONPA-s – synthetic fluorescent reporter protein (27kDa) that allows repeated switching between a fluorescent and a non-fluorescent state to monitor intracellular protein trafficking.

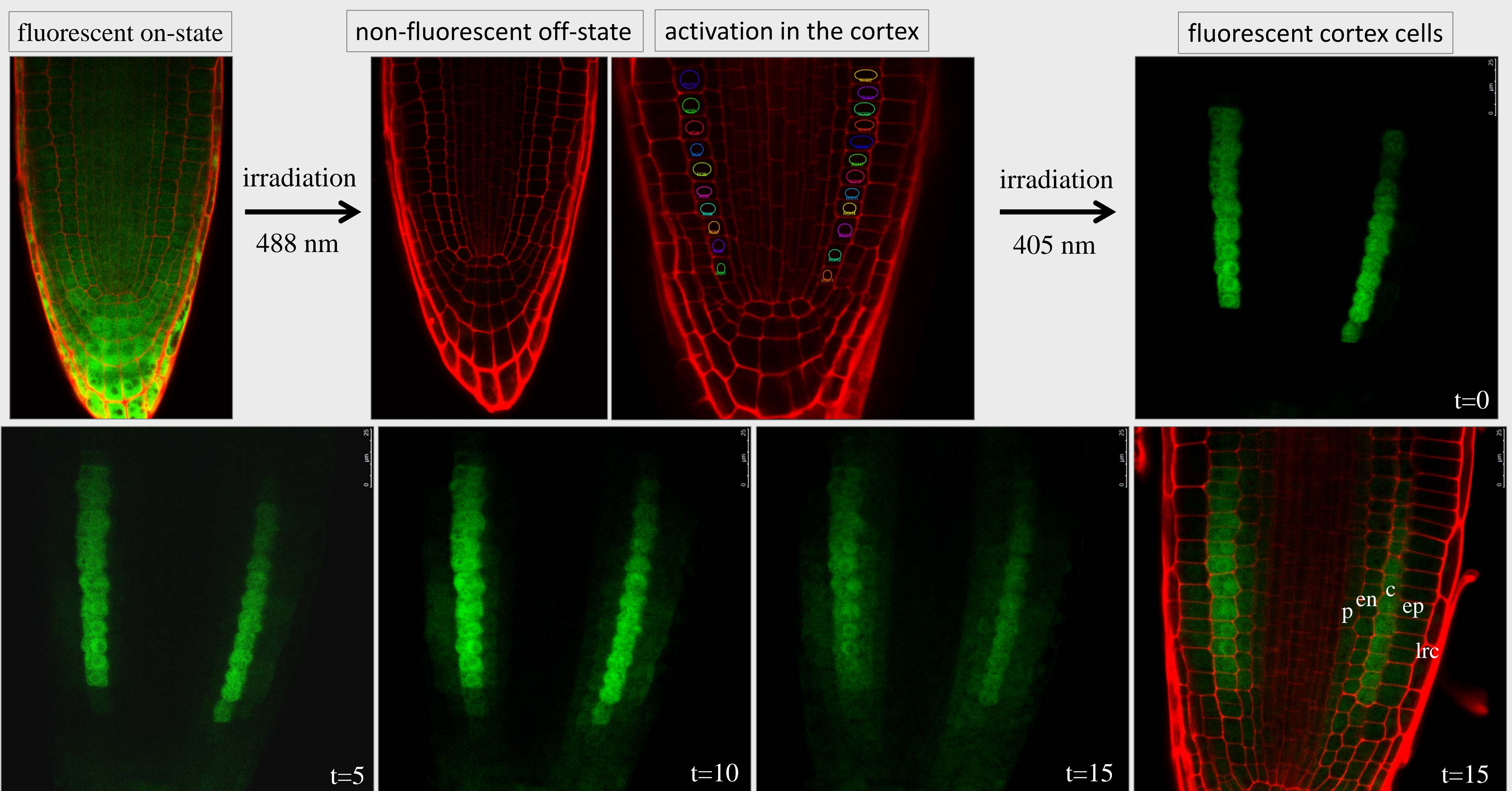
488nm, low laser power -> bright green fluorescence

488nm, high laser power -> fluorescent off-state

405nm, 2P-laser -> fluorescent on-state

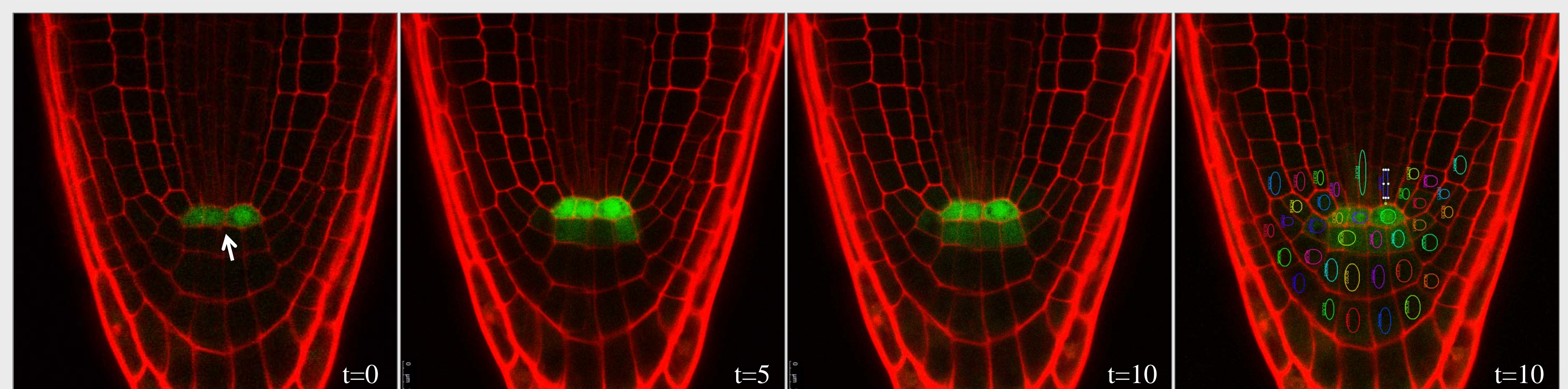


DRONPA flow in the cortex

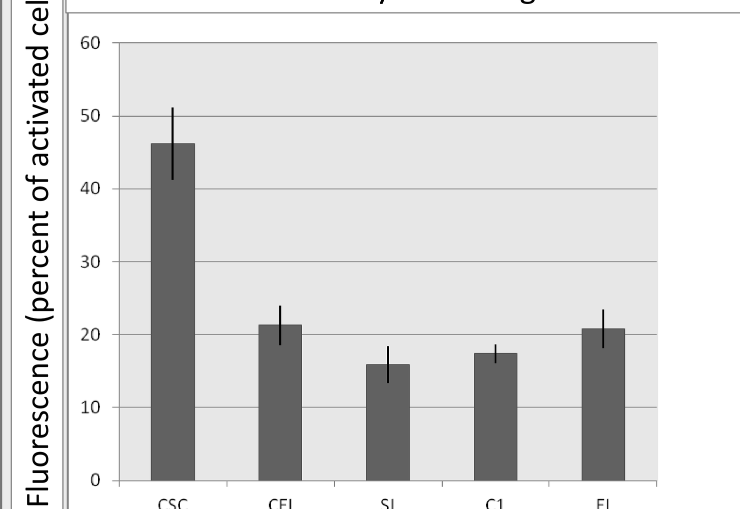


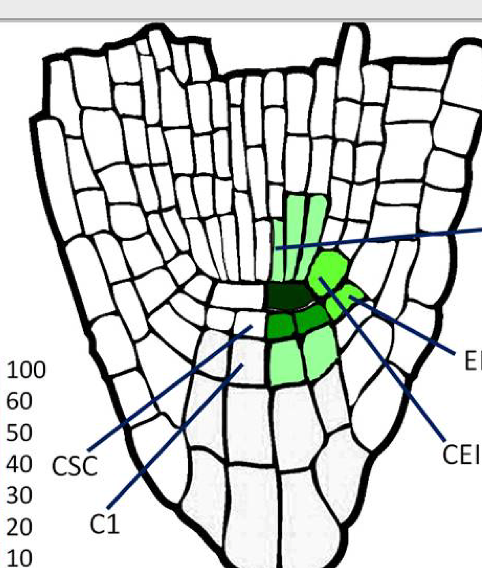
5 min after DRONPA activation in the cortex the fluorescence is detectable in epidermis cells (ep), after 10 min – in the endodermis (en), after 15 min also in the pericylce (p) and the lateral root cap (lrc). Green – DRONPA-s, red – propidium iodide stained cell walls, multicolor circles – activation ROIs, t = minutes after activation.

Quantification of DRONPA flow in the quiescent centre



5 min after DRONPA activation in quiescent centre the fluorescence is detectable in columella stem cells. Green – Dronpa-s, red – propidium iodide stained cell walls, arrow – quiescent centre, multicolor circles – ROIs of fluorescence intensity measurement, t = minutes after activation





The fluorescence intensity of aktivated cell is set to 100 % (dark green)

Best symplastic connectivity between the quiescent centre (qc) and columella stem cells (CSC)

C1 – first columella cell layer,
EI – epidermis initial cell,
SI – stele initials,
CEI – cortex/endodermis initials

Summary

I. Using MP17-GFP lines we could show that the quiescent center, cortex endodermis initials and the columella contain many secondary plasmodesmata.
II. All cells of the root tip are connected by plasmodesmata. The cell-to-cell connectivity is reduced during the process of cell differentiation.
III. The highest symplastic connectivity could be identified between the quiescent centre and columella initials.

Literature: *Hofius et.al. (2001), Lummer et.al. (2001), Ando et al. (2004)*