DOI 10.18699/BGRS/SB-2020-217

Keeping the gate closed: WOX5 supports the balance between the proximal and distal root meristems via auxin biosynthesis in *Arabidopsis thaliana* L.

Maria S. Savina
ICG SB RAS, Novosibirsk, Russia
savinams1991@gmail.com

Taras Pasternak
Institute of Biology II/Molecular Plant Physiology University of Freiburg, Freiburg, Germany
taras.p.pasternak@gmail.com

Victoria V. Mironova
ICG SB RAS, Novosibirsk, Russia
Novosibirsk State University
kviki@bionet.nsc.ru

Nadezda Omelyanchuk
ICG SB RAS, Novosibirsk, Russia
nadya@bionet.nsc.ru

Viktoriya V. Lavrekha
ICG SB RAS, Novosibirsk, Russia
vvl@bionet.nsc.ru

Abstract — The functioning of the root stem cell niche depends on the auxin maximum formed and maintained by auxin transport and local biosynthesis. The auxin maximum is located in the quiescent center (QC), which separates the mitotically active proximal and distal root meristems. Transcription factor WUSCHEL HOMEOBOX (WOX5) expressed in QC controls stem cell maintenance. In WOX5-overexpression line 35S::WOX5-GR we recorded increase in auxin biosynthesis through TAA1 upregulating and changes in PIN-mediated auxin transport. Altogether it leads to auxin redistribution between proximal and distal root meristems. We show with mathematical modeling that increasing TAA1 expression is sufficient to trigger auxin redistribution. Numerical simulation results reproduce wild type and WOX5 overexpressing columella phenotypes. Key role of auxin biosynthesis was support with L-kynurenine (the competitive inhibitor of TAA1 activity) treatment for 48h, which partially restored the wild type phenotype in 35S::WOX5-GR line. Our model predicts formation of "quiescent domain" by actively dividing columella stem cells and columella stem cell daughters that was proved by 3D image analysis of root tips with traced cell cycle events.

Keywords — RAM, auxin, WOX5, mitotic activity, mathematical model, image analysis, iRoCS toolbox, EdU

INTRODUCTION

Root apical meristem contains a stem cell niche where a quiescent center (QC) promotes maintenance of the adjacent stem cells (initials) [1]. The distal part of the root stem cell niche consists of the columella stem cells (CSCs) producing daughters (CSCDs) further differentiating into columella (central root cap) cells (DCCs). WOX5 (WUSCHEL-RELATED HOMEOBOX 5) transcription factor is the QC signal maintaining the columella stem cells as a part of the distal meristem [2]. Auxin concentration maximum in the QC is also required for maintenance of the adjacent stem cells, including CSCs, and for differentiation of CSC daughters [3]. Tian and co-authors proposed and provide some evidence that WOX5 modulates auxin biosynthesis in the QC [4]. Recent work shows that WOX5 acts mainly in the QC and WOX5 mobility was not required to inhibit CSC differentiation [5]. However, mechanisms underlying this modulation and impact of this regulation on stem cell functions are not fully understood. Here we demonstrate that WOX5 activates the first step of auxin biosynthesis and this is very important for functioning of root stem cells, because disturbance in this regulation (e.g. in the case of WOX5 overexpression) violates root stem cell renewing and differentiation.

METHODS

We create 1D hybrid computation model of auxin distribution in columella cells. In the model cell growth, active and passive auxin transport between cells, and substances degradation are described by continuous functions, but transitions between the cell cycle states are discrete events, which are depend on auxin concentration in the cell.

Recently, iRoCS toolbox was developed [6] for annotation of the root tip organization in three dimensions. Together with the refined experimental procedure for detection of cell cycle progression [7], it provides an unprecedented potential to study the mechanism of cell cycle regulation in an entire organ. Here, using these techniques, we analysed the distributions of the key cell cycle events – DNA replication and mitosis in the root tips of *35S::WOX5-GR* line.

RESULTS

We observe in *35S::WOX5-GR* overexpression transgene upon DEX treatment that columella stem cell daughters do not undergo normal differentiation. We notice that during 48 hours of DEX treatment almost all rows of differentiated columella cells are detached but small cells in the enlarging of upper columella part do not have starch granules and look similar to the wild type CSCs. This means that CSC descendants occurred just after DEX activation of WOX5 expression do not undergo differentiation, but activate mitotic cell cycle progression.

We show that WOX5 modulates TAA1-mediated auxin synthesis and PIN-mediated auxin transport in root apical meristem. We hypothesize that modulation TAA1-mediated auxin synthesis results in observed changes in columella phenotype of WOX5 overexpression line.

We developed 1D computational model of auxin distribution in columella with growth and division of cells. Using the mathematical modeling approach we showed that WOX5-dependent upregulation of TAA1-mediated auxin synthesis are sufficient to reproduce all other changes observes in the *35S::WOX5-GR* plant roots. Incubation of L-kynurenine (the competitive inhibitor of TAA1 activity) provides partially recovery the wild type columella phenotype of WOX5 overexpression line. So, we can conclude that the main role of WOX5 gene for auxin distribution is modulation of TAA1-mediated auxin synthesis.

Moreover, numerical calculations predict that active cell divisions occur only in the lower part of columella while the

cells in the upper part of columella remain quiescent in *35S::WOX5-GR* transgenic line. 3D image analysis of cell cycle events distribution in the root tip confirms the model prediction.

ACKNOWLEDGMENT

The work was supported by the Russian Foundation for Basic Research (19-54-12013).

REFERENCES

[1] Jiang K, Feldman LJ (2005) Regulation of root apical meristem development. Annu Rev Cell Dev Biol 21:485–509.

[2] Zhou, J. J., & Luo, J. (2018). The PIN-FORMED auxin efflux carriers in plants. International journal of molecular sciences, 19(9), 2759.

[3] Pi, L., Aichinger, E., van der Graaff, E., Llavata-Peris, C. I., Weijers, D., Hennig, L., ... & Laux, T. (2015). Organizer-derived WOX5 signal maintains root columella stem cells through chromatin-mediated repression of CDF4 expression. Developmental cell, 33(5), 576-588.

[4] Tian, H., Wabnik, K., Niu, T., Li, H., Yu, Q., Pollmann, S., ... & Friml, J. (2014). WOX5–IAA17 feedback circuit-mediated cellular auxin response is crucial for the patterning of root stem cell niches in Arabidopsis. Molecular Plant, 7(2), 277-289.

[5] Berckmans, B., Kirschner, G., Gerlitz, N., Stadler, R., & Simon, R. (2020). CLE40 signalling regulates the fate of root stem cells in Arabidopsis. Plant Physiology.

[6] Schmidt, T., Pasternak, T., Liu, K., Blein, T., Aubry-Hivet, D., Dovzhenko, A., ... & Ronneberger, O. (2014). The iRoCS T oolbox–3 D analysis of the plant root apical meristem at cellular resolution. The Plant Journal, 77(5), 806-814.

[7] Pasternak, T., Tietz, O., Rapp, K., Begheldo, M., Nitschke, R., Ruperti, B., & Palme, K. (2015). Protocol: an improved and universal procedure for whole-mount immunolocalization in plants. Plant Methods, 11(1), 50.