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Nitrate, NO and ROS signaling in stem cell homeostasis

Aakanksha Wany[1], Christine H Foyer[2]*, Kapuganti Jagadis Gupta[1]*

[1]National Institute of Plant Genome Research, Aruna Asaf Ali Marg, 110067, New Delhi, India

[2]Centre for Plant Sciences, School of Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK

*Correspondence: jgk@nipgr.ac.in (K.J. Gupta); C.Foyer@leeds.ac.uk (C.H.Foyer)

Corresponding author websites

Dr Jagadis Gupta Kapuganti

Website: http://www.nipgr.res.in/research/dr_jagadis.php

Twitter: https://twitter.com/DrJagadisNIPGR

Prof Christine H. Foyer

Website: https://www.fbs.leeds.ac.uk/staff/profile.php?tag=Foyer_CH

Twitter: https://twitter.com/c_foyer

Abstract

Shoot and root growth is facilitated by stem cells in the apical meristems (SAM and RAM). Recent reports have demonstrated a close link between nitrogen nutrition, nitric oxide (NO) and reactive oxygen species (ROS) in the regulation of SAM and RAM functions in response to nitrogen availability.

The shoot and root apical meristem (SAM and RAM) contains a group of proliferating stem cells, which play an important role in organogenesis. Several hormones and other signaling molecules fulfil important functions in SAM and RAM regulation. A recent study has demonstrated that SAM activity responds to soil nitrate availability through the expression of WUSCHEL (WUS), which is a key regulator of stem cell homeostasis [1]. Nitrate sensing pathways regulate cytokinin precursors, which function as long-range signals to modulate WUS expression and control of SAM homeostasis. However, nitrate-dependent signaling is likely to be more complex than can be explained solely in terms of cytokinin signaling. Given the demonstrated roles of nitric oxide (NO) and ROS in stem cell functions [2], these crucial redox signals may fulfil key roles in the regulation of SAM activity responses to soil nitrate availability.

NO and ROS play central roles in plant and animal stem cell homeostasis. For example, NO donors accelerate the differentiation of mouse and human embryonic stem cells [3]. Moreover, NO and nitrate reductase (NR) activity influence root stem cell niche homeostasis [4]. The observation that nia1nia2 mutants, which are NO as well as NR-deficient, have small root meristems with abnormal cell divisions supports the notion of a role for nitrate dependent NO pathway in the regulation of stem cell functions. Moreover, the levels of WUS related homeobox 5 (WOX5) transcripts are decreased in the nia1nia2 mutants [4].

Extensive crosstalk exists between NO, ROS and the signaling pathways associated with phytohormones such as auxin, ethylene and cytokinin. Together with these hormones, ROS and NO participate in the control of organ development [1,4,5]. Several mutants that have low levels of NO such as nia1nia2, noa1 show increases in auxin levels [4]. Similarly, NO treatment induces auxin accumulation [4].

Mitochondrial metabolism and signaling play a central role in the control of meristem activity. While the molecular mechanisms that regulate mitochondrial processes and associated signaling in the RAM remain poorly understood, two genes, RETARDED ROOT GROWTH (RRG) and PROHIBITIN3 (PHB3), have been shown to regulate both mitochondrial functions and stem cell activity. RRG encodes a mitochondria-localized protein that is required for cell division in the RAM [6]. Loss of PHB3 functions not only impairs mitochondrial functions, but also slows cell division rates in the meristem. Mutants defective in PHB3 show a constitutive ethylene response, suggesting that PHB3 plays a role in ethylene signaling [7]. Recently, Kong et al. [8] demonstrated that prohibitin proteins maintain the root stem cell niche (SCN). Loss of prohibitin functions compromised the mitotically-active quiescent center (QC) cells and the suppressed surrounding actively-dividing stem cells. This regulation was achieved through the ROS-responsive transcription factors ERF115, ERF114, and ERF109 (Figure 1).

PHB3 is not only essential for maintenance of the SCN via the inhibition of cell proliferation in the QC and activation of cell division in the proximal meristem (PM) (Fig 1), but it is also required for NO production during hydrogen peroxide (H_2O_2)-mediated stress responses [9]. Redox homeostasis is important in SCN maintenance. Phb3 mutants accumulate more ROS and show increased expression of AOX1a, AOX1c, NDA1, NDB2, NDB3 and NDB4, which are involved in mitochondrial redox homeostasis. These findings suggest that PHB plays a role in the control of mitochondrial ROS production and signaling. Greatly increased ROS accumulation can cause root meristem defects [8]. For example, Arabidopsis thaliana mutants that accumulate high levels of glutathione disulfide (GSSG) and low levels of reduced glutathione (GSH) are defective in root meristem functions [10]. Low nitrate availability can increase ROS accumulation via reduced levels of antioxidants/NO [11]. NO can influence cellular redox homeostasis by regulation of glutathione synthesis and hence, ROS removal and also by increased expression of the mitochondrial alternative oxidase (AOX), which limits ROS production in mitochondria.

A recent study [1] reports that stem cell homeostasis was modified under conditions of high nitrate nutrition. However, this treatment would also favour NO production via increased NR activity and the generation of the intermediate nitrite [12]. Hence, the increased NO production under high nitrate may influence stem cell regulation. Moreover, the possibility of increased NO production under low nitrogen nutrition

cannot be excluded. In addition to NR, the mitochondrial electron transport chain can utilize nitrite as a substrate to produce NO via complexes III and IV [12]. Cellular, nitrite levels depend on NR activity and the concentration of nitrate. Hence, even mitochondrial NO production depends on nitrate availability.

The daughter stem cells in animal proliferation systems have a larger population of mitochondria [13]. It is therefore, possible that NO production is increased in actively proliferating mitochondria via the mitochondrial reduction of nitrite to NO [12]. Several mechanisms of stem cell regulation via NO signaling might contribute to the findings described by Landrein et al. [1]. Firstly, NO mediates cytokinin-triggered activation of the cell cycle gene, CYCD3, which induces cell proliferation and meristem maintenance [14]. Hence, nitrate could modulate stem cell maintenance via NO production. Secondly, H_2O_2 promotes stem cell differentiation [15] and NO is likely to play a role in this process. For example, nitrate or cytokinin-induced NO can contribute to shoot stem cell differentiation via H_2O_2 production. NO can also increase H_2O_2 production via the induction of superoxide dismutase, which converts superoxide (O_2^-) into H_2O_2. Thirdly, low NO production under low nitrate availability might enhance O_2^- production [12] leading to increased WUS expression to maintain stem cell homeostasis.

Nitrate-derived NO production may therefore, have multifaceted roles in stem cell homeostasis. Although, Landrein et al. [1] provided an excellent model for nitrate-mediated regulation of stem cell homeostasis, we consider that the close link between nitrogen nutrition and NO dictates an integrated view of the role for NO signaling in the regulation of stem cell activity in response to nitrogen availability. Further investigations of the roles of NO in the SCN are required in order to gain deeper insights into how this signaling molecule regulates stem cell homeostasis.

In the natural environment, N exists in various forms e.g. nitrate, ammonium, or a combination of both. However, the relative composition of these N forms and their relative concentrations depends on many factors, including soil properties and management, such as the rotation of crops and presence of other plant types e.g. legumes. Moreover, soil N levels often fluctuate due to factors such as microbial nitrate reduction, denitrification and ammonium oxidation. We consider that the close link between nitrogen nutrition and NO production implicates this signaling molecule in the

nitrate-dependent regulation of stem cell activity. Defining the precise roles of NO in meristem organisation will provide further insights into stem cell homeostasis and its regulation. The study by Landrein et al. [1] laid the foundation of a new concept linking primary processes and metabolism to stem cell functions. Defining the precise roles of NO in this process will increase our understanding of the relationships between nutrient availability and the whole plant signaling that regulates growth and organogenesis.

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References

1. Landrein, B. et al. (2018) Nitrate modulates stem cell dynamics in Arabidopsis shoot meristems through cytokinins. Proc. Natl. Acad. Sci. U. S. A. 115, 1382-1387
2. Foyer, C.H., et al. (2018) Redox regulation of cell proliferation: Bioinformatics and redox proteomics approaches to identify redox-sensitive cell cycle regulators. Free Radic Biol Med. DOI: 10.1016/j.freeradbiomed. 2018.03.047)
3. Mujoo, K., et al. (2008) Role of nitric oxide signaling components in differentiation of embryonic stem cells into myocardial cells. Proc. Natl. Acad. Sci. U. S. A. 105, 18924–18929
4. Sanz, L. et al. (2014) Nitric oxide plays a role in stem cell niche homeostasis through its interaction with auxin. Plant Physiol. 166, 1972–1984
5. Ponce, G. et al. (2005) Auxin and ethylene interactions control mitotic activity of the quiescent centre, root cap size, and pattern of cap cell differentiation. Plant Cell Environ 28, 719–732
6. Zhou, X. et al. (2011) The Arabidopsis RETARDED ROOT GROWTH gene encodes a mitochondria-localized protein that is required for cell division in the root meristem. Plant Physiol. 157, 1793–1804
7. Christians, M.J. and Larsen, P.B. (2007) Mutational loss of the prohibitin AtPHB3 results in an extreme constitutive ethylene response phenotype coupled with partial loss of ethylene-inducible gene expression in Arabidopsis seedlings. J. Exp. Bot. 58, 2237–48

8. Kong, X. et al. (2018) PHB3 maintains root stem cell niche identity through ROS responsive AP2/ERF transcription factors in Arabidopsis. Cell Rep. 22, 1350-1363
9. Wang, Y. et al. (2010) The Arabidopsis prohibitin gene PHB3 functions in nitric oxide-mediated responses and in hydrogen peroxide-induced nitric oxide accumulation. Plant Cell. 22, 249–59
10. Innocenti, G. et al. (2007) Glutathione synthesis is regulated by nitric oxide in Medicago truncatula roots. Planta 225, 1597-602
11. Shin, R. et al. (2005) Reactive oxygen species and root hairs in Arabidopsis root response to nitrogen, phosphorus and potassium deficiency. Plant Cell Physiol. 46, 1350-7
12. Planchet, E. et al. (2005) Nitric oxide emission from tobacco leaves and cell suspensions: rate-limiting factors and evidence for the involvement of mitochondrial electron transport The Plant Journal 41, 732–743
13. Katajisto, P. et al. (2015) Stem cells. Asymmetric apportioning of aged mitochondria between daughter cells is required for stemness. Science 348, 340–343
14. Shen, Q. et al. (2013) Nitric oxide mediates cytokinin functions in cell proliferation and meristem maintenance in Arabidopsis. Mol. Plant 6, 1214-25
15. Zeng, J. et al. (2017) Redox regulation of plant stem cell fate. EMBO J. 36, 2844-2855

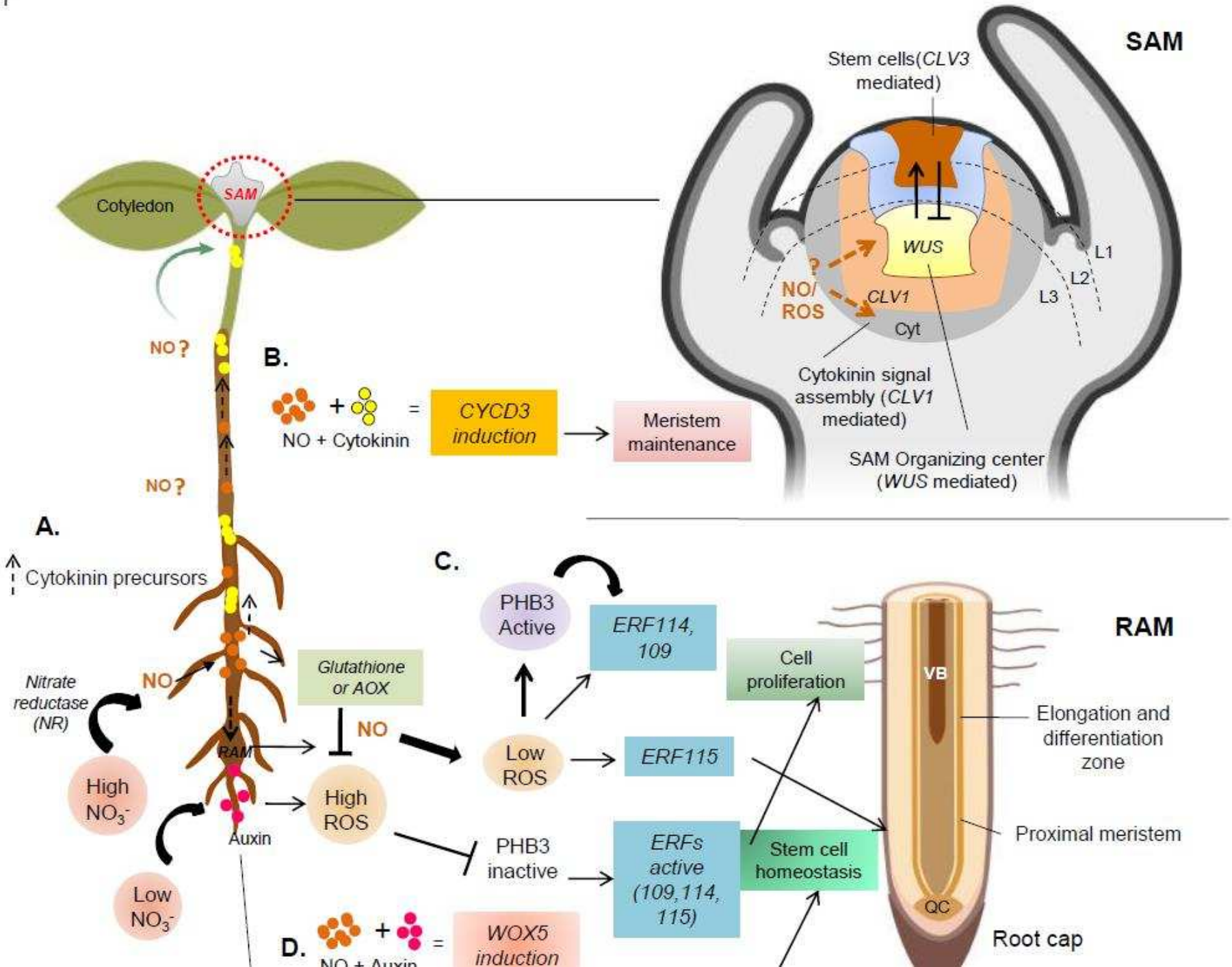
Figure legend:

Figure 1. A model showing the multilevel regulatory role of nitrate, NO and ROS in stem cell homeostasis in SAM and RAM. High nitrate can induce cytokinin precursors to activate SAM that leads to increased meristem size and triggers organogenesis. Nitrate is a substrate for NO production via nitrate reductase (NR). Hence, NO (orange dots) produced by NR can play a vital role in stem cell homeostasis in both shoot and root apical meristem via modulation of cytokinins and ROS production. (A) Under high nitrate nutrition, NO production is induced in roots [12] via high NR activity and accumulation of the intermediate, nitrite. Nitrate, cytokinin-induced NO or NO-induced cytokinins can activate precursors (shown by yellow dots) in the vascular region. NO-induced cytokinin may play a role in enhancing SAM activity. Coordinated activation of the WUS-CLV system modulates stem cell homeostasis in the SAM. Cytokinin precursors signals in the SAM regulates WUS expression. In turn, WUS activates CLV3, which binds to CLV1 and inhibits WUS expression. Redox regulation of stem cell homeostasis [15] is also mediated by regulated ROS production. (B) NO mediates cytokinin functions in cell proliferation and meristem maintenance [14] via CYCD3 cell cycle gene expression. (C) In the RAM, both high and low nitrate nutrition differentially regulate ROS production. Low nitrate can induce ROS that can inactivate prohibitin (PHB3) to activate ERFs (ERF109, 114, 115), which allows cell proliferation and differentiation [8]. But the NO produced under high nitrate can reduce ROS via

induction of glutathione and AOX (antioxidant) levels to keep ROS to minimum. The reduced ROS can activate PHB3 to inhibit ERFs (ERF115) for stem cell niche maintenance at QC and induce ERF109 and 114 to induce cell proliferation at elongation and differentiation zone of RAM. PHB3 plays an important role in the maintenance of stem cell niche by inhibiting the cell proliferation at the QC of RAM and simultaneously stimulating cell division at proximal meristem. (D) NO also interacts with auxin to modulate the expression of WOX5 to maintain stem cell niche homeostasis [4].

----- = black dashed lines indicates a mechanism in action, ----- = orange dashed lines indicates a mechanism in question with a possible cause, ? =orange question mark indicates a molecule might have a role in the mechanism, L1 = layer 1, L2= layer 2, L3= layer 3 of the SAM, Cyt= cytokinin, ⊣ = inhibitory action, VB= vascular bundle and QC= quiescent centre, orange dots= NO, yellow dots = cytokinin /cytokinin precursors, pink dots = auxins

Figure 1


SAM
Stem cells(CLV3 mediated)
WUS
L1
L2
L3
NO/ ROS
CLV1
Cyt
Cytokinin signal assembly (CLV1 mediated)
SAM Organizing center (WUS mediated)
Cotyledon
SAM
NO ?
NO ?
B.
NO + Cytokinin
CYCD3 induction
Meristem maintenance
A.
Cytokinin precursors
Nitrate reductase (NR)
NO
RAM
High NO3-
Low NO3-
Auxin
Glutathione or AOX
NO
High ROS
C.
PHB3 Active
ERF114, 109
Low ROS
ERF115
PHB3 inactive
ERFs active (109,114, 115)
Cell proliferation
Stem cell homeostasis
D.
NO + Auxin
WOX5 induction
RAM
VB
QC
Elongation and differentiation zone
Proximal meristem
Root cap