Nitrogen-responsive transcription factor kinetics meter plant growth

Garo Z. Akmakjian[a] and Julia Bailey-Serres[a,1]

The wealth of data provided by large-scale -omics studies empowers the discovery of gene regulatory networks and molecular mechanisms that underlie specific phenotypes. Most transcriptomic studies provide a snapshot of how organisms interact with their environment, recording change after a given time or in response to a single environmental variable. Organisms have not evolved in binary states of treatment vs. control and instead experience a continuum of environmental states, from optimal growth conditions to mild and ultimately severe stress. Organismal phenotypes vary along this continuum, but how molecular responses are metered to influence these phenotypes is poorly understood. In PNAS, Swift et al. (1) provide a deeper understanding of plant adaptations to nitrogen (N) availability by investigating how plant root transcriptomes change in response to varying N concentration over time. Their work on *Arabidopsis thaliana* not only provides a more nuanced understanding of how plants sense and appropriately adjust their metabolism to different N levels, it establishes a predictive framework for identifying critical components of N responses that can be targets for crop improvement.

N is an essential nutrient found in many macromolecules. Plant roots acquire bioavailable N as nitrate or ammonium and assimilate N from these inorganic molecules into amino acids for use in protein synthesis, as well as the synthesis of nucleic acids, chlorophyll, and other secondary metabolites throughout the plant (2). N, however, is a commonly limiting nutrient in crop production, leading to the widespread amendment of soil with mineral N fertilizers. Nearly 120 Tg of N as ammonium fertilizers is produced annually (3), and their application has greatly improved crop yield and food security (4). N fertilizers, however, are both costly and significant environmental pollutants as their application generates greenhouse gas emissions, promotes acid rain, contaminates groundwater reservoirs, and causes eutrophication of freshwater and estuarine ecosystems (5). N fertilizers are also an environmental threat because of fossil fuel used in their synthesis, with up to 2% of the world's energy used for their production (6). Their cost is often prohibitive to economically disadvantaged farmers and in marginal production environments, reducing yield potential (7). There is thus a significant need for innovations that lessen reliance on N fertilizers (8), including the development of crops with greater nitrogen-use efficiency (NUE) to maximize growth and yield.

A critical requirement to improving NUE is understanding how plants 1) sense N availability and 2) respond to varying N availability. Plant growth is influenced by the type and quantity of N provided, with increasing N availability resulting in more robust vegetative growth (9, 10). The mechanisms responsible for this N dose–response, however, are unknown. Plant responses to N deficiency have been extensively studied, and components of N uptake, metabolism, and signaling have been manipulated in attempts to improve NUE (11). In the field and in nature, however, plants experience varying levels of N availability, while many strategies to improve NUE in crops have improved growth and yield at either low or high N availability, but not both (11). It is imperative to better understand plant responses to a wide range of N availability to improve crop growth regardless of field conditions.

In PNAS, Swift et al. (1) use high-throughput RNA-sequencing to evaluate how plant root transcriptomes adjust to varying levels of N over time. The authors identified thousands of genes whose expression not only changed in a temporal manner but also changed as a function of the quantity of bioavailable N provided (which they term N dose), indicating that the expression of these genes is sensitive to the amount of N available to the plant. Interestingly, for many of these genes, the relationship between N availability and gene expression could be explained by Michaelis–Menten kinetics (Fig. 1*A*), a classical, century-old model originally devised to describe enzyme reaction rates as

[a]Center for Plant Cell Biology, Botany and Plant Sciences Department, University of California, Riverside, CA 92521

Author contributions: G.Z.A. and J.B.-S. wrote the paper.

The authors declare no competing interest.

See companion article, "Nutrient dose-responsive transcriptome changes driven by Michaelis–Menten kinetics underlie plant growth rates," 10.1073/pnas.1918619117.

[1]To whom correspondence may be addressed. Email: serres@ucr.edu.

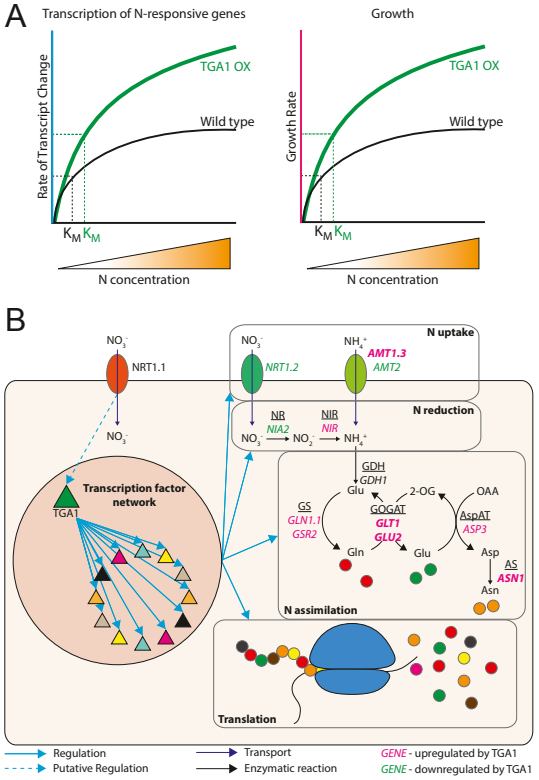

Fig. 1. Role of the transcription factor TGA1 in coordinating proportional transcriptional, metabolic, and growth responses to N availability in roots of *A. thaliana*. (*A*) TGA1 target genes fit the Michaelis–Menten model (wild type; black line) as a function of N availability. TGA1 overexpression (TGA1 OX; green line) increases the rate of change of N-responsive gene mRNAs across N concentrations but also raises K_m. Plant growth rates also fit the Michaelis–Menten model, with the growth kinetic changes of TGA1 overexpressing plants mirroring the changes observed for transcripts of TGA1 target genes. (*B*) Schematic of regulation of N uptake, N reduction, N assimilation, and translation of mRNAs produced by the TGA1 transcriptional network, which is putatively regulated by the nitrate transceptor NRT1.1. TGA1 directly regulates 92 transcription factors in a network that regulates thousands of downstream genes. Enzyme classes are underlined, whereas TGA1-regulated genes are shown in italics and are color coded according to up-regulation (magenta) or down-regulation (green) in the network. Genes shown in bold are direct targets of TGA1. Abbreviations for enzymes: AS, asparagine synthetase; AspAT, aspartate aminotransferase; GDH, glutamate dehydrogenase; GOGAT, glutamine synthase; GS, glutamine synthetase; NIR, nitrite reductase; NR, nitrate reductase. Abbreviations for molecules/metabolites: Asn, asparagine; Asp, aspartate; Gln, glutamine; Glu, glutamate; NH_4^+, ammonium; NO_2^-, nitrite; NO_3^-, nitrate; OAA, oxaloacetate; 2-OG, 2-oxoglutarate.

a function of substrate concentration (12). This has two significant implications. First, both transcriptome and N concentration-dependent growth changes fit the Michaelis–Menten model, indicating that we can expect that targeted breeding to adjust these gene regulation kinetics would have a proportional impact on plant growth. Second, we can infer that the N sensor in plants functions as a rheostat rather than a switch, allowing precise metering of gene regulation to maximize growth under any given N application rather than simply responding to low vs. high N.

To better understand the regulatory mechanisms that contribute to N dose responsiveness, Swift et al. (1) searched for transcription factor binding sites that are enriched in the promoters of genes that fit the Michaelis–Menten model as well as for transcription factors whose transcript levels change significantly within 15 min of increased N availability. Their analysis identified the transcription factor TGACG SEQUENCE-SPECIFIC BINDING PROTEIN 1 (TGA1), previously implicated in N responses (13), and genetic analysis demonstrated that TGA1 is required for the transcriptional regulation of the genes that fit the Michaelis–Menten model. Increasing the expression of *TGA1* with a near-constitutive promoter (overexpression) not only raised the rate of maximum rate of transcript change (V_{max} in Michaelis–Menten terms) but also raised the V_{max} of plant growth with varying N concentration (Fig. 1*A*), demonstrating that manipulation of *TGA1* results in molecular changes that translate into, and can be predictive of, agronomically important traits. N-regulated genes orthologous to *Arabidopsis TGA1* are present in rice and predicted to control a similar response network (14). Genetic variation in *TGA1* regulation or function could thus be a breeding target to improve NUE in crops.

The Michaelis–Menten modeling of Swift et al. (1) allows predictions of additional avenues for further improving NUE. Whereas *TGA1* overexpression raised V_{max}, it also raised the Michaelis constant K_m, or the N concentration at which the rate of change of N-responsive transcripts reaches 1/2 V_{max}. In terms of enzymatic kinetics, this result is unexpected as raising the enzyme concentration (or here, the transcription factor concentration and presumably its transcriptional output) would increase V_{max} without altering K_m, as K_m is inversely proportional to the enzyme's affinity for its substrate and should thus be insensitive to enzyme concentration. Because there is an increase in K_m, however, one can predict that additional regulatory mechanisms are present that dampen the impact of TGA1 overexpression. Future work that identifies and manipulates these regulators could have a synergistic effect with TGA1 to further improve NUE.

The finding that transcriptional activation of N-responsive genes varies with N dose indicates that plants can perceive N availability along a continuous range, suggesting that the N sensor in plant root cells functions as a rheostat. The results of Swift et al. (1) further suggest that an N sensor modulates *TGA1* expression (and consequently TGA1 activity) proportionally to the amount of available N. Although the sensor remains unidentified, this behavior allows plants to appropriately tune their metabolism to N-resource availability. To better understand how TGA1 influences N responses, the authors identified direct transcriptional targets of TGA1 using a combination of chromatin immunoprecipitation, nascent messenger RNA (mRNA) sequencing, and the TARGET (Transient Assay Reporting Genome-wide Effects of Transcription factors) method, a transcription factor manipulation assay used to distinguish direct and indirect transcriptional targets (15). Prior work used the TARGET method to identify 580 TGA1 direct targets that are both N responsive and change over time following N application, as well as to construct gene regulatory networks for 32 additional N-regulatory transcription factors (16). Of the 584 direct targets of TGA1 identified in the current study, 92 encoded transcription factors, suggesting that TGA1 activates a transcriptional cascade responsible for the expression of 2,280 indirect targets, the majority of which fit the Michaelis–Menten model. TGA1 targets are enriched in genes involved in processes such as N assimilation and protein translation (Fig. 1*B*); increasing TGA1 activity at higher N concentrations thus meters use of available N. The targets of TGA1 also significantly overlap with the downstream targets of NITRATE TRANSPORTER 1.1 (NRT1.1), the plasma membrane transporter for NO_3^- uptake in root cells. NRT1.1 acts as a transceptor to regulate the N deficiency response

across a wide range of N concentrations (11) and is thus a likely factor in sensing N availability. TGA1 and NRT1.1 may work in the same pathway to regulate N-responsive genes. How NRT1.1 perception of N might translate into a response proportional to the concentration of N is unknown.

Swift et al. (1) have laid the groundwork to achieve a more complete understanding of plant N responses over a range of environmental conditions. By incorporating both time and N dosage into their analysis, this work identifies a transcription factor that not only influences N use and growth during low N conditions but also at intermediate and high (subinhibitory) levels of N. The authors' strategy thereby identifies TGA1 as both a critical component of regulating N assimilation as well as an attractive breeding target for improving crop NUE. This work also demonstrates that not all N-responsive genes fit a classical Michaelis–Menten model, suggesting that additional layers of regulatory complexity underlie N responses that must be understood to even further improve NUE.

1 J. Swift, J. M. Alvarez, V. Araus, R. A. Gutierrez, G. M. Coruzzi, Nutrient dose-responsive transcriptome changes driven by Michaelis–Menten kinetics underlie plant growth rates. *Proc. Natl. Acad. Sci. U.S.A.*, 10.1073/pnas.1918619117. (2020).
2 A. Krapp, Plant nitrogen assimilation and its regulation: A complex puzzle with missing pieces. *Curr. Opin. Plant Biol.* **25**, 115–122 (2015).
3 D. Fowler *et al.*, The global nitrogen cycle in the twenty-first century. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **368**, 20130164 (2013).
4 R. N. Roy, A. Finck, G. J. Blair, H. L. S. Tandon, *Plant Nutrition for Food Security* (Food and Agriculture Organization of the United Nations, 2006).
5 X. Zhang *et al.*, Managing nitrogen for sustainable development. *Nature* **528**, 51–59 (2015).
6 J. G. Chen *et al.*, Beyond fossil fuel-driven nitrogen transformations. *Science* **360**, eaar6611 (2018).
7 P. L. Pingali, Green revolution: Impacts, limits, and the path ahead. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 12302–12308 (2012).
8 J. Bailey-Serres, J. E. Parker, E. A. Ainsworth, G. E. D. Oldroyd, J. I. Schroeder, Genetic strategies for improving crop yields. *Nature* **575**, 109–118 (2019).
9 G. G. McNickle, J. S. Brown, When Michaelis and Menten met Holling: Towards a mechanistic theory of plant nutrient foraging behaviour. *AoB Plants* **6**, plu066 (2014).
10 R. P. Lana *et al.*, Application of Lineweaver–Burk data transformation to explain animal and plant performance as a function of nutrient supply. *Livest. Prod. Sci.* **98**, 219–224 (2005).
11 Y.-Y. Wang, Y.-H. Cheng, K.-E. Chen, Y.-F. Tsay, Nitrate transport, signaling, and use efficiency. *Annu. Rev. Plant Biol.* **69**, 85–122 (2018).
12 L. Michaelis, M. L. Menten, K. A. Johnson, R. S. Goody, The original Michaelis constant: Translation of the 1913 Michaelis–Menten paper. *Biochemistry* **50**, 8264–8269 (2011).
13 J. M. Alvarez *et al.*, Systems approach identifies TGA1 and TGA4 transcription factors as important regulatory components of the nitrate response of *Arabidopsis thaliana* roots. *Plant J.* **80**, 1–13 (2014).
14 M. Obertello, S. Shrivastava, M. S. Katari, G. M. Coruzzi, Cross-species network analysis uncovers conserved nitrogen-regulated network modules in rice. *Plant Physiol.* **168**, 1830–1843 (2015).
15 B. O. R. Bargmann *et al.*, TARGET: A transient transformation system for genome-wide transcription factor target discovery. *Mol. Plant* **6**, 978–980 (2013).
16 M. D. Brooks *et al.*, Network Walking charts transcriptional dynamics of nitrogen signaling by integrating validated and predicted genome-wide interactions. *Nat. Commun.* **10**, 1569 (2019).