🔴 **QNAS**

QnAs with Gloria M. Coruzzi

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For plants, nitrogen is a key nutrient responsible for growth and development. Farms throughout the world rely on fertilizers rich in the element to increase production. However, fertilizer use carries a high environmental impact, and reducing fertilizer use remains a challenging goal, especially as the global population grows. Uncovering the genome-wide regulatory networks that control nitrogen use efficiency has been the research focus of Gloria M. Coruzzi, the Carroll and Milton Petrie Professor of Biology at New York University's (NYU) Center for Genomics and Systems Biology. Coruzzi also helped develop VirtualPlant (http://virtualplant.bio.nyu.edu/cgi-bin/vpweb/), a software platform that allows scientists to study the interaction of gene networks in several plant species, including the model plant *Arabidopsis thaliana* as well as food crops. PNAS recently spoke to Coruzzi, who was elected to the National Academy of Sciences in 2019, about her current research.

PNAS: In your Inaugural Article (1), you demonstrate that Michaelis–Menten kinetics, which have been used to describe the kinetics of enzymatic reactions, drive plants' transcriptional response to the amount of nitrogen received. Why is this significant for plant biology, and what does this discovery mean?

Coruzzi: How organisms sense and respond to changes in nutrient dose is a basic unanswered question in biology with special relevance to agriculture. Studies by us and others have shown that nitrogen nutrient sensing enables plants to reprogram their genome-wide expression and alter metabolism and development. However, the molecular mechanisms that allow nitrogen dose to inform gene-expression levels and how they lead to dose–responsive changes in plant biomass have remained unknown.

To answer these questions, we exposed *Arabidopsis thaliana* seedlings to a matrix of nitrogen doses and monitored genome-wide expression changes as a function of time. Our mathematical modeling of gene expression by quantitative mRNA-sequencing revealed that the dynamics of transcriptomic responses to nitrogen dose mirror the simple enzyme kinetics described by Michaelis–Menten (MM) in 1913 (2, 3). In our study (1), a gene whose expression is significantly fit by the MM model allowed us to estimate the maximum rate of transcript change, known as V_{max}, as well as K_m, the dose of nitrogen at which half of V_{max} was achieved. Using these parameters, we were able to determine that the MM kinetics model can explain the expression of 30% of nitrogen dose–responsive genes.

PNAS: The Michaelis–Menten model was developed for enzymatic reactions, not for genomic transcription. How were you able to adapt it for this discovery?

Coruzzi: According to the MM kinetics model, changing enzyme abundance will impact the maximum rate of reaction possible (V_{max}). Because transcription factors establish the rates at which transcription takes place, they can be directly analogized to the catalytic enzymes in the MM model.

We assessed the robustness of our MM models by perturbing specific transcription factors that mediate nitrogen dose–responsive gene expression. We found that altering transcription factor abundance *in planta* could perturb nitrogen dose transcriptomic responses including V_{max}. In addition, we showed that the over-expression of a master transcription factor, called TGA1, not only led to an increase of V_{max} of nitrogen dose–responsive mRNAs, but also to an acceleration of nitrogen-dependent plant growth rates. In this way, our study of the basic mechanisms that underlie the transcriptome kinetics that respond to changes in nitrogen dose has the potential to enhance plant growth and improve nitrogen use efficiency—placing it squarely in Pasteur's Quadrant—the scientific space where basic discoveries ultimately benefit society.

PNAS: How does this work fit within the broader context of your research?
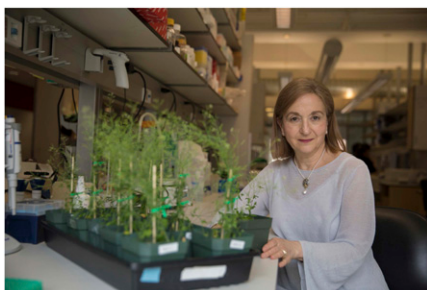
Gloria M. Coruzzi in her laboratory at New York University's Center for Genomics and Systems Biology. Image credit: ©Moree; courtesy of New York University Photo Bureau.

This is a QnAs with a member of the National Academy of Sciences to accompany the member's Inaugural Article on page 12531.

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Coruzzi: Our studies exploit genomic and systems biology approaches to generate and test hypotheses for how plants sense and respond to a changing nutrient environment. For example, we know that changes in nutrient dose have dramatic effects on gene expression and development. However, an outstanding question is whether organisms respond to changes in absolute nutrient amount, moles, versus concentration relative to water, molarity. This gap in our basic knowledge is well-suited to plant studies, as soil drying or drought can alter nutrient concentration without changing its absolute amount. The results also have practical implications for agriculture.

To answer this question, in a recent study (4) we exposed rice to a factorial matrix, varying nitrogen dose and water combinations, and quantified transcriptome and phenotype responses. Using linear models, we identified distinct dose–responses in gene-expression or phenotypes, to either nitrogen-moles, water-volume, nitrogen-molarity (N/W), or a synergistic interaction (N×W). Importantly, genes whose expression patterns are best explained by nitrogen-dose and water interactions (N/W or N×W) were associated with crop outcomes in replicated field trials conducted in collaboration with scientists at the International Rice Research Institute in the Philippines. Thus, the nitrogen-by-water response genes we identified may assist future efforts to develop crops resilient to increasingly arid, low nutrient soils.

PNAS: How did you decide to focus on genomics and systems biology?

Coruzzi: Our current and future work exploits time-series data to uncover the mechanisms of transcriptional response networks in plants. Interestingly, in the original Michaelis–Menten paper (2)—the inspiration for our Inaugural paper (1)—the authors emphasized the importance of time in their opening paragraph, saying: "…achieving the final aim of kinetic research; namely, to obtain knowledge of the nature of the reaction from a study of its progress." As an aside, I was surprised to learn that Menten of the 1913 Michaelis–Menten article was a woman biochemist—a rare event for those days.

Our studies exploit time—the fourth and largely unexplored dimension of transcription—to uncover the temporal transcriptional logic of gene regulatory networks involved in nitrogen signaling in plants. There is a pressing but unmet need in the field to experimentally refine such gene regulatory network models. To this end, we developed a high-throughput method to validate transcription factor–target interactions in isolated plant cells. This has allowed us to experimentally refine the temporal transcriptional logic for about 150 transcription factors in the nitrogen-signaling response in *Arabidopsis*.

PNAS: Where do you see your work headed in the future?

Coruzzi: Currently, we are scaling up our transcription factor validation assays to cover all of the 70 transcription factor families in *Arabidopsis*. We will also be using this large validated dataset as "priors" to train a new time-based network inference method, in collaboration with colleagues at NYU's Courant Institute of Mathematical Sciences. More broadly, the time-based experimental and machine-learning approaches we are developing can be applied to uncover the temporal transcriptional logic for any signaling response system in biology, agriculture, or medicine.

1 J. Swift, J. M. Alvarez, V. Araus, R. Gutiérrez, G. M. Coruzzi, Nutrient dose-responsive transcriptome changes driven by Michaelis–Menten kinetics underlie plant growth rates. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 12531–12540 (2020).
2 L. Michaelis, M. L. Menten, Die Kinetic der Invertinwirkung. *Biochemisch Zeitschrift* **49**, 333–369 (1913).
3 K. A. Johnson, R. S. Goody, The original Michaelis constant: Translation of the 1913 Michaelis–Menten paper. *Biochemistry* **50**, 8264–8269 (2011).
4 J. Swift, M. Adame, D. Tranchina, A. Henry, G. M. Coruzzi, Water impacts nutrient dose responses genome-wide to affect crop production. *Nature Comm.* **10**, 1374 (2019).