Solving the puzzle of Fe homeostasis by integrating molecular, mathematical and societal models

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Short title: Training Models in Plant Cell Biology

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Highlights

- Recent studies highlight the complexity of cellular responses to iron deficiency
- Simulation based inference (SBI) is a mathematical approach that can facilitate our understanding of stress responses by formally integrating prior knowledge and new observations, whether they be qualitative or quantitative.
- Collaborative application of SBI to biological phenomena requires effective communication, which is the cornerstone of inclusion in science and beyond

Keywords: Iron homeostasis, Simulation based inference (SBI), Inclusivity

Abstract

To ensure optimal utilization and bioavailability, iron uptake, transport, subcellular localization, and assimilation are tightly regulated in plants. Herein, we examine recent advances in our understanding of cellular responses to Fe deficiency. We then use intracellular mechanisms of Fe homeostasis to discuss how formalizing cell biology knowledge via a mathematical model can advance discovery even when quantitative data is limited. Using simulation based inference to identify plausible systems mechanisms that conform to known emergent phenotypes can yield novel, testable hypotheses to guide targeted experiments. However, this approach relies on accurate encoding of domain-expert knowledge in exploratory mathematical models. We argue that this would be facilitated by fostering more "systems thinking" life scientists, and that diversifying your research team may be a practical path to achieve that goal.

Introduction

Existing in multiple oxidation states and a wide redox potential range, iron (Fe) can readily donate and accept electrons, making it an excellent cofactor for primary metabolic processes including DNA synthesis and repair, respiration, and photosynthesis. In excess, Fe can react with oxygen, causing the formation of damaging reactive oxygen species.[1] However, deficiency in Fe availability, sensing, and response inhibit growth and development. Decreased Fe availability is perceived initially in the shoot and signaled to the root, inducing epigenetic regulation, and massive signaling, transcriptional, metabolic changes that lead to dynamic changes in Fe uptake and transport mechanisms within the root epidermis[2-6]. Herein, we discuss recent advances that shed light on these changes, and the ongoing challenges associated with making sense of these dynamic, multiscale physiological phenomena. We then address how a subset of relevant regulatory phenomena can be viewed through the lens of a formal modeling framework. This approach allows for leveraging of the powerful mathematical approach of simulation-based inference, which can be used to formally test and refine mechanistic hypotheses by integrating heterogeneous data. As this approach requires accurate encoding of domain-expert knowledge in exploratory mathematical models, we close by suggesting that this interdisciplinary work would be facilitated by intentional efforts to cultivate "systems thinking" amongst plant cell biologists. Systems thinking cell biologists are life scientists with an aptitude for translating biological knowledge into clearly delineated agents, organized into well-specified systems, with articulated interactions between parts of the whole. We propose that inclusive lab environments can provide opportunities for serendipitous cross-disciplinary conversations that foster systems-thinking outside the confines of didactic cross-training.

I. Recent advances in the cell biology of plant Fe response

Fe deficiency prompts reprogramming of the leaves

Iron deficiency (-Fe) alters chloroplast and thylakoid structure, resulting in decreased electron transport and reduced photosynthetic capacity – one of the most evident, and economically relevant, signs of -Fe [7-9]. Recently, Przybyla-Toscano et al. generated a manually curated gene atlas of over 1000 Fe-containing proteins in *A. thaliana*, categorized as Fe-S, Haem, and non-FeS/non-haem Fe proteins, which exhibit distinct subcellular localization patterns and evolution ages [10]. In response to Fe deficiency these, and other Fe-response proteins, as well as Fe itself, can relocalize within cells to facilitate Fe uptake and use efficiency [11,12]. By calculating the net CO_2 assimilation rate to Fe content in barley, Saito et al. have recently shown that Fe is relocalized to the thylakoid membrane in barley leaves to increase Fe use efficiency [13]. Fe is a cofactor in all major photosynthetic protein complexes, and chloroplastic Fe accounts for 60-80% of Fe in photosynthetically active leaves [14]. Consequently, another -Fe response is to decrease photosynthesis and the synthesis of associated proteins while increasing photoprotective mechanisms [7] and / or repressing Fe assimilation into Fe-proteins [15,16]. One of the key enzymes involved in Fe-assimilation is Sulfur Utilization Factor B (SUFB), a critical component of the plastid iron–sulfur (Fe–S) assembly pathway that is down-regulated early after Fe deficiency. Using inducible SUFB RNAi lines as a tool to distinguish between the impact of -Fe versus the loss of Fe assimilation into critical photosynthesis machinery, Kroh and Pilon recently found surprisingly little similarity in transcriptomic response of sufb mutants under Fe sufficiency and WT plants under -Fe, despite similar photosynthetic response [17]. Decreased accumulation of Fe-S proteins in *sulfb* mutants suggests that -Fe causes a decrease in SUFB, which then coordinates decreased Fe-S clusters with decreased photosynthetic transport. Thus, it is the lack of Fe in leaves – not changes in Fe utilization – that triggers reprogramming of the leaf transcriptome [17].

Communicating Fe deficiency to and across the root

To trigger Fe uptake, leaves must also communicate the -Fe condition to the root. The phloem mobile signal IRON MAN (IMA), a family of Fe-binding peptides that is expressed in the phloem, may control this process [18]. However, many questions remain about how the shoot -Fe signal is perceived and transduced from the vasculature, through the endodermis and cortex, and into the epidermis. One possible mechanism is the activity of iron-responsive mobile transcription factors such as (POPEYE) PYE and (IAA-LEUCINE RESISTANT3) ILR3 [19,20]. PYE and ILR3 appear to form a heterodimer that negatively regulates -Fe response, which is disrupted by (BTS) BTS, a vasculature specific Fe-binding E3 ligase [21]. When ILR3 and PYE are mobilized to the epidermis and cortex, ILR3 interacts with close homologs to positively control expression of binding partners of the master bHLH regulator FER-like Iron-deficiency-induced Transcription factor (FIT) [22,23]. Once -Fe is perceived by the epidermis, FIT is activated to regulate the expression of Fe uptake genes such as membrane localized Ferric Reduction Oxidase2 (FRO2), membrane localized Ferric reductase [24], and IRT1, a broad-spectrum transporter of a range of metals including ferrous Fe [25,26]. This process is facilitated by the membrane localized proton pump, Arabidopsis plasma membrane H+-ATPase isoform 2 (AHA2), which lowers the rhizosphere pH, increasing ferric Fe solubility, and secretion of Fe-mobilizing coumarins [27]. Together, FRO2, IRT1 and AHA activity constitute the Strategy 1 response, a conserved molecular mechanism in dicots that increases Fe content while resulting in excess non-Fe uptake [28]. IRT1, FRO2, and AHA2 actually form a complex in the outer plasma membrane of root epidermal cells to facilitate Fe uptake in the absence of Fe and presence of excess non-Fe metals [29]. IRT ubiquitination is strongly elevated by the presence of non-Fe metals, and IRT1 also appears to be subjected to endocytosis in the absence of Fe and presence of non-Fe metals. Phosphorylation of IRT results in the dissociation of the IRT1, FRO2 and AHA complex, suggesting that this complex generates a region of Fe uptake at the root surface that is dissociated when IRT1 is phosphorylated in the presence of excess non-Fe metals [26]. Excess non-Fe metals, rather than Fe, control IRT1 by binding to IRT1 at specific

histidine resides and triggering its phosphorylation by another kinase, CBL-Pnteracting Protein kKnase23 (CIPK23). IRT1 phosphorylation then creates a docking site for the E3 ligase IRT1 Degradation Factor1 (IDF1), which facilitates IRT1 ubiquitination. FYVE1, a Fab1, YOTB, Vac1 and EEA1 (FYVE)-domain containing phosphatidylinositol-3-phosphate-binding protein then interacts with IRT1 and Ubiquitin and recruits them to the late endosomes for subsequent degradation within the lytic vacuole [25,26,30-32].

A FITting response to marshal the agents of Fe uptake

As a master regulator of Strategy I -Fe response, FIT has been the focus of intense study [33,34]. When Fe is resupplied to the roots, FIT transcription is turned off, and degradation of FIT by BTSLike (BTSL) proteins in the epidermis results in decreased expression of Fe uptake genes [35]. Consequently, both BTS and BTSL provide tight regulation of Fe uptake across the entire root [23]. FIT has been recently shown to form a regulatory loop with General Regulatory Factor11 (GRF11), a 14-3-3 regulatory protein that is controlled by Non-Response To Fe-Deficiency2 (NRF2) through Histone H3 lysine4 trimethylation [5]. FIT also interacts with CBL-Interacting Protein Kinase (CIPK11), a serine-threonine protein kinase that interacts with calcineurin B-like (CBLs) calcium-binding proteins. *In silico* analyses followed by *in vitro* kinase assays indicate that CIPK11 phosphorylates FIT at Ser272. A non-phosphorylatable version of FIT shows increased nuclear localization compared to that of phosphomimicking FIT, decreased capacity for self-transcriptional regulation, and decreased -Fe response. Thus, FIT phosphorylation by CIPK11 facilitates nuclear localization and transcriptional regulatory function, likely in response to changes in Ca^{2+} fluctuations, which, as this study shows, occurs in the root vasculature in response to -Fe [36]. While the role of FIT in the vasculature was not

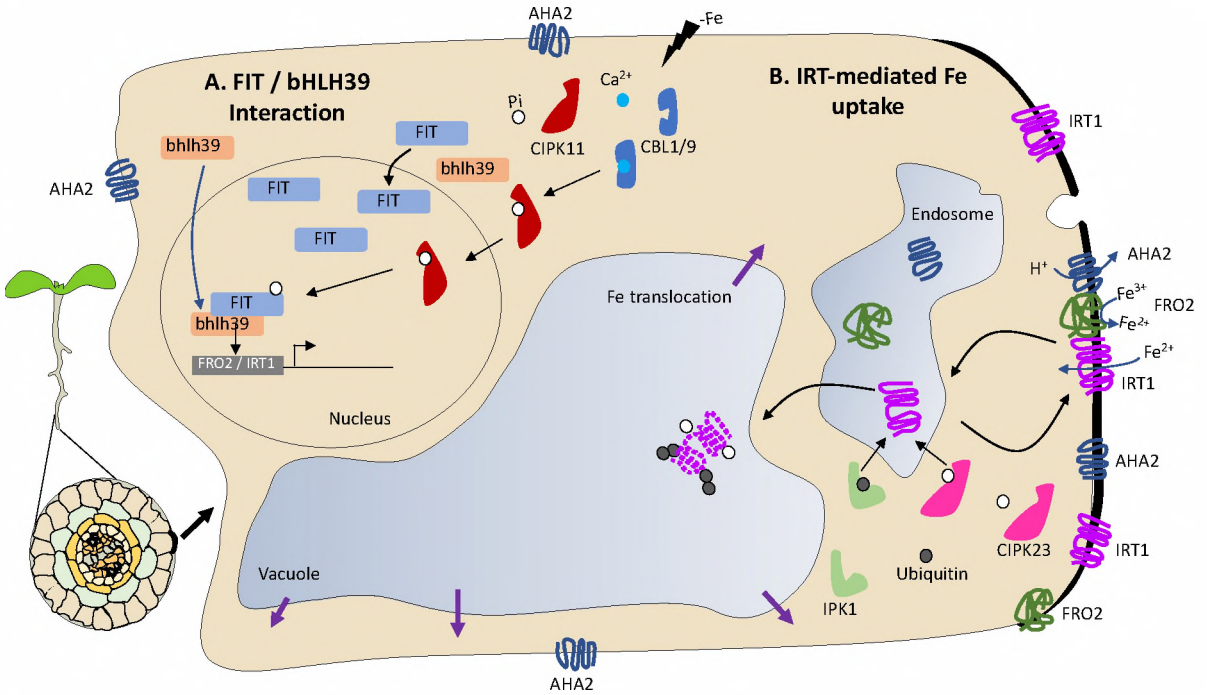

Figure 1. Recent advances in our understanding of cellular responses to -Fe. A. Ca^{2+}-dependent FIT phosphorylation and FIT presence in the nucleus are critical for bHLH39 accumulation in the nucleus. bHLH39 heterodimerization with FIT is then required for FIT transcriptional activity. **B.** In the presence of Fe, IRT cycles between the endosome and the PM. However, in response to -Fe IRT1, FRO2, and AHA2 form a poplar localized complex that generates a region of Fe uptake at the root rhizosphere. In the presence of excess non-Fe metals, IRT is phosphorylated, ubiquitinated, and endocytosed.

IRT is phosphorylated, ubiquitinated, and endocytosed.

explored in this study, it will be critical to explore this further as we consider how -Fe signals are perpetrated across the various root cell types.

Phosphorylation is not the only phenomenon that impacts FIT activity. FIT activity also requires interaction with binding partners, such as basic helix-loop-helix transcription factor, bHLH39 [37]. While both FIT and bHLH39 are cytosolic and nuclear localized, in WT plants, a recent study has shown that, in the absence of FIT, bHLH39 becomes primarily cytosolic [38]. A similar mechanism has been observed in rice, in which OsbHLH156, an -Fe responsive FIT ortholog, positively regulates -Fe response in rice and interacts with bHLH IB ortholog (IROn-related bHLH transcription factor 2) IRO2, which is also a master regulator of Fe response. OsbHLH156 also requires the presence of IRO2 for nuclear localization [39]. Thus, Fe uptake in plants is tightly regulated by a myriad of conserved transcriptional, post-transcriptional, and cellular processes that we are only beginning to fully understand (Figure 1).

FITting together the puzzle pieces of Fe homeostasis: a role for formal modeling

Given the need to (i) sense Fe status in the leaves, (ii) mobilize a signal that communicates to root, and (iii) stimulate a response that is cell-type specific and cascades from the vasculature to the epidermis – in order to release Fe stores, increase Fe uptake and transport Fe to the shoot – much work remains in order to achieve a predictive understanding of the emergent phenomenon of Fe homeostasis. With dynamic regulatory events happening at the transcriptional and post-translational levels, and transcriptional regulators moving from between cells of differing phenotype and from shoot to root, multi-scale mathematical models could play a critical role in advancing our state of knowledge. These formal testable models can integrate *in vivo* and *in vitro* data on enzyme kinetics, protein stability and localization, emergent Fe response phenotypes, and -omics data to explore the dynamic implications of these findings.

Ideally, relevant modeling approaches should maximally leverage both extant and new data in the field – thereby allowing us to assess the limits of our current understanding and to progressively update that knowledge. The field of simulation-based inference offers just such a possibility. Unlike purely data-driven modeling, simulation-based inference explicitly invokes existing knowledge about regulatory mechanisms, along with hypothetical candidate mechanisms, to enable an efficient search for models and model parameters that plausibly match our biological observations. The first step in applying this approach is to adopt a simulatable knowledge representation for our current biological models. In the following section, we use the example of FIT/bHLH39 interactions to outline how the pictorial model shown in Figure 1 can be translated into a formal model representing the explicit logic of known and hypothetical mechanisms of FIT regulation. Expressing our domain expert knowledge in this way is the first step to leveraging extensive empirical observations within the complementary knowledge-discovery framework of mathematical modeling.

II. Outlining a FIT-for-purpose model

Figure 1 depicts currently understood FIT and bHLH39 interactions in pictorial form. Figure 2 depicts a formal model of these same biological phenomena, with circles indicating pools of proteins of interest and squares indicating the biological events that impact each pool. In creating this diagram, we have made explicit the known and hypothesized mechanisms that contribute to the emergent phenomenon of bHLH39 nuclear accumulation – along with their explicit interdependencies. To begin with, FIT and BHLH39 are transcriptionally regulated in the nucleus, with synthesis of the proteins resulting in cytoplasmic accumulation. We chose to represent the combination of transcription and translation as a single event (numbers 1 and 2),

a simplification that we are assuming is appropriate, given that the relevant literature does not indicate critical post-transcriptional regulatory phenomena. This kind of simplification is frequently made in the early stages of modeling, with the assumption being a candidate for rejection if the model fails to be adequately predictive.

FIT is known to be activated by phosphorylation (event 3), and the FITp form of the protein translocates into the nucleus (event 4). BHLH39 also translocates into the nucleus (event 5). While activated FIT and bHLH39 are known to form a heterodimer, the explicit representation of these proteins in both cytoplasmic and nuclear sub-compartments forces us to ask where the dimerization occurs. One possibility is that dimerization only occurs in the nucleus, where the proteins are known to co-regulate other Fe-deficiency response proteins. We produce a candidate regulatory model by incorporating that possibility in our diagram as event 6. As we have posited this event without direct empirical evidence, we must be conscious of it as an assumption that requires validation. FITp is known to augment its own transcription, modifying the likelihood of event 1. All proteins have their respective turnover events, preventing unrealistic unbounded accumulation of any one species.

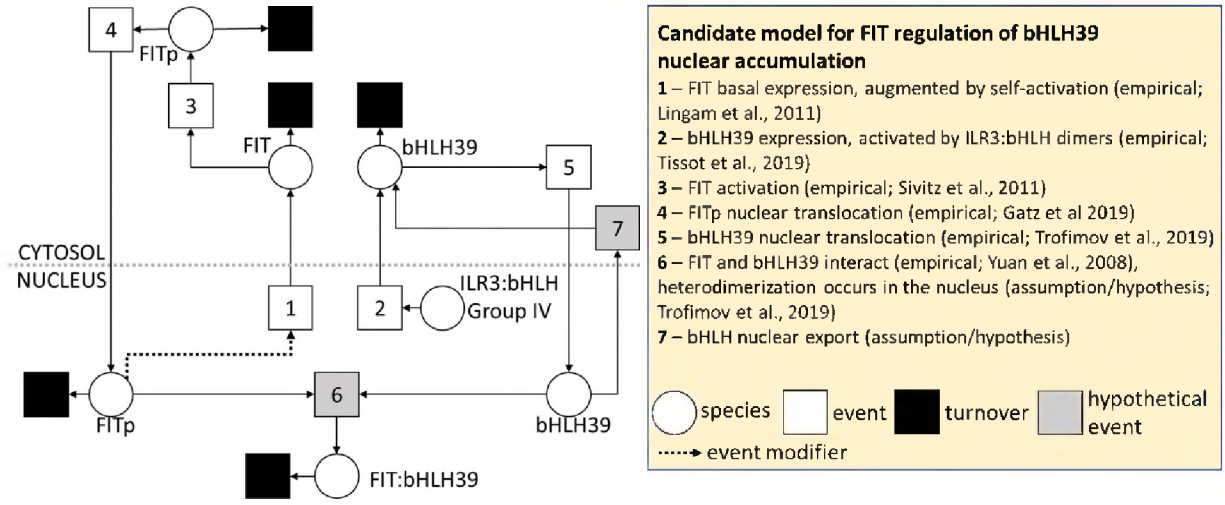

Figure 2. Model of biological phenomena associated with FIT/bHLH39 interaction

Finally, event 7 represents nuclear export of bHLH39. Trofimov et al. observed augmented accumulation of bHLH39 in the presence of active FIT. The authors offer a few possible mechanisms to explain this phenomenon, including the possibility that heterodimerization prevents nuclear export of bHLH39 [38]. We depict this possibility by allowing for export of the bHLH39 monomer. Thus, events 6 and 7, heterodimerization and nuclear export, compete for the available pool of nuclear bHLH39. Accumulation of FITp in the nucleus could shift that balance in favor of dimerization and, consequently, augment the nuclear retention of bHLH39. That will only happen if the overall system dynamics result in sufficient FITp accumulation. If this model fails to be predictive, one could consider alternative mechanisms such as dimerization being required prior to nuclear transport. Determining whether the model is predictive requires data and a suitable mathematical framework for estimating model parameters and evaluating model plausibility. While one might wish to leverage the sophisticated tools of modern biotechnology to directly observe the specific events captured in the model, it is important to recognize that we may be able to perform initial model selection just by combining candidate formal models with a clearly articulated set of expected emergent behaviors of the system. The set of computational approaches known as simulation-based inference (SBI) make this possible.

Simulation based inference can guide you to the best FIT

SBI has been exploited in multiple biological fields. For example, in the last 2 years, the field of ecology and evolutionary biology has produced >40 publications leveraging these approaches [40-43]. While examples exist, [44-49] uptake in cell biology and related fields is comparatively limited. One challenge is that SBI is an evolving field in its own right – rarely providing an off-the-shelf solution for modeling needs [50-56]. For this reason, a technical description of SBI is beyond the scope of this review. We wish instead to highlight key features of the approach that can be leveraged to achieve model selection, model parameterization, and iterative integration of new data as part of a model-driven research strategy. SBI methods allow one to perform a search for models and parameters that are consistent with both prior knowledge and empirical data. This is achieved through a framework in which a candidate model is simulated (i.e., translating the formal model in Figure 2 into a system of differential equations, a stochastic rule-based model, or other appropriate mathematical formalism), and the resulting predictions are evaluated against empirical observations to determine their validity. Scored predictions inform an approximated likelihood of the observations, given the model. As scoring can encompass multiple objectives (quantitative data or qualitative constraints), this makes it possible to invoke heterogeneous data types in model evaluation. The commonly employed SBI approach of approximate Bayesian computation uses this framework in a cycle of sampling, simulating, evaluating, and updating to refine – or signal the need to reject – a candidate model [45,46,48,53]. More recent SBI implementations leverage developments in the field of machine learning to reduce performance trade-offs between efficiency and accuracy and adopt improved sampling strategies via active learning [50,51,54,55].

Applying SBI to a biological problem where data is limited or qualitative means making a mental shift from asking whether you can create the right model for the biological problem in question and toward asking whether you can computationally pre-screen candidate mechanisms for plausibility and then identify model predictions that would most readily discriminate between multiple plausible mechanisms. This computational pre-screening can point to non-heuristic, testable mechanistic hypotheses to guide a targeted experimental strategy. It can also provide a framework for iteratively incorporating new observations into model design and evaluation. All one needs to get started is a candidate model, sampled parameter values, and a solver to run the simulation. The diagram in Figure 2 is an ideal starting point for a modeling effort, as each square represents a single kinetic event that should be translated into a mathematical rate expression. Each arrow entering a square makes explicit that the rate expression should depend quantitatively on the abundance of the species serving as input. The diagram formally connects the biological possibilities to the elements of the mathematical formulation and visually connects the knowledge of the biologist to the language of a mathematical modeler.

For the next generation of scientists, the growing wealth of cross-training educational opportunities will continue to create individuals equipped to bridge the “modeler” and “biologist” perspectives with native facility. However, when each contributing discipline entails its own deep innovative discovery, we limit advances in the field if we expect these hybrid modes of discovery to be solely the domain of didactally cross-trained scientists. On the other hand, with human, financial, and time resources in the typical lab already stretched thin, it can be challenging for experimental scientists to identify a practical path to leveraging computational approaches in biological discovery. Likewise, to ensure their models adequately reflect current and emerging knowledge in the field, the computational scientist who has a desire to explore the wondrous complexity of biological systems faces the challenge of learning the fundamentals of each new biological problem they encounter. For a plant cell biologist, embarking on this kind of

interdisciplinary journey, we would like to suggest that you can lay the groundwork for relevant collaborations by fostering "systems thinking" within your lab, and that diversifying your research team may be a practical path to achieving that goal.

III. Rethink who's sitting at the bench: diversity can bring the FIT required to advance systems-thinking in plant cell biology

Systems thinking cell biologists are life scientists with an aptitude for translating biological knowledge into clearly delineated agents, organized into well-specified systems, with articulated interactions between parts of the whole. Systems thinking also entails careful communication of whether agents and their interactions were drawn from empirical observations, domain expertise, assumptions, or the spectrum of biological possibilities presented by considering multiple model organisms. Creating the formal model in Figure 2 was a collaborative effort between the plant biologist, computational scientist, and cross-training postdoctoral scholar who have together authored this piece. A typical conversation within this team involves careful consideration of the nature and origin of the biological knowledge. The biologist may assert that one protein activates the other. In drafting the formal model, the computational scientist wonders what, precisely, does this mean? Do the proteins interact directly? Is this conclusion based on *in vitro* or *in vivo* data (impacting boundary conditions, initial conditions, or relevant mechanisms)? Was the interaction observed directly or inferred as an interpretation of a particular assay? Are the proteins expressed in the same cellular compartment or is trafficking required? Or is one protein required for transcription of another? Depending on the level of abstraction chosen, each of these mechanisms could map to a distinct model. Thus, the translation from biological observations to an appropriate formal model requires that a biologist and modeler engage in a conversation that addresses not just what they know but also how they know it and what the alternative interpretations could be. All parties involved must be conscious of communicating across cultures – in this case, bridging disciplinary and epistemological diversity [57].

While cross-training in relevant disciplines is a way to master both sides of such a conversation, we offer the suggestion that diversifying your research team may be a practical path to cultivating systems-thinking team members. These individuals would be primed to launch effective collaborations with similarly motivated computational colleagues. What is needed is not specific mathematical or computational skills, but rather individuals skilled in a particular type of communication. Indeed, systems-thinking can be viewed as a communication tool – one that is critical to discovery in settings of dynamic complexity and challenging interdependencies. Engaging with scholars outside your typical network is one way to stress-test your "systems" story-telling and engage with scholars who employ systems frameworks across multiple fields.

A scientist who only works with people who think, act, and look like themselves may encounter or create new ideas less frequently than someone who operates in a diverse context [58-65]. Nor would they encounter the challenge of explaining their own ideas to someone with a different set of conceptual priors. A new member of your research group does not simply increment your headcount by one. Bringing them in adds a constellation of first-degree contacts to the group (Figure 3), and it gives group members a chance to practice communicating their science with an outsider. If all members of a research group have similar disciplinary identity and personal backgrounds, they are likely to have a considerable overlap in the sets of individuals with whom they are acquainted, discussing science, and establishing collaborations. If new group members bring with them a breadth of personal and professional identities, then the research group's set of leverageable connections quickly grows, and its shared skill at communicating across boundaries of knowledge is enhanced. As an example, underrepresented students on a university campus are likely to find communities with critical

mass by engaging with societies that span multiple STEM disciplines. When such an individual joins a research group, in addition to their own talents and insights, they bring access to a new and distinct group of scholars and potential collaborators.

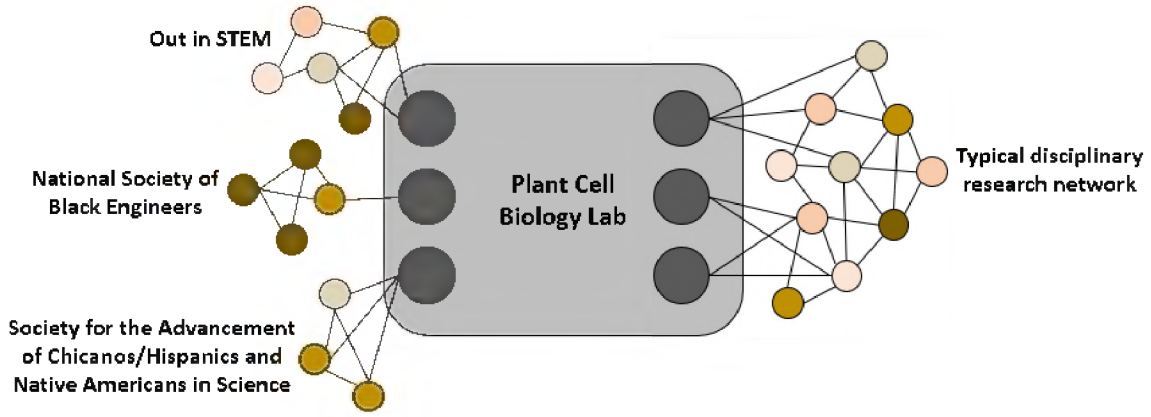

Figure 3. The extended network of a research group grows when diversity is amplified. A typical research group (grey circles around the cartoon table, center) often consists of several individuals with shared membership in professional organizations or institutions like academic departments (right side). However, as diversity in the primary members of a research group increases (left side), the network of professional relations associated with the group is effectively larger. Depicted are several example organizations, but the benefit of diversity is not exclusive to these examples.

Many have described the problems in recruitment, funding, and retention of diversity in science [66-71]. This is to our detriment, as diversity brings new ideas together for conceptual recombination, and diversity is the whetstone on which our communication skills are honed. Moreover, as biology finds itself evolving toward a hybrid discipline [72] perfecting the communication between disciplines and communities will enable us to leverage our collective talents and solve grand challenges in science and beyond.

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