

Physiology and metabolism

Two old grannies catch fire in the new millennium

Editorial overview

John Browse* and Gloria Coruzzi†

Addresses

*Institute of Biological Chemistry, Washington State University, Pullman, Washington 99164-6340, USA; e-mail: jab@wsu.edu

†Department of Biology, New York University, 1009 Main Building, 100 Washington Square East, New York, New York 10003, USA; e-mail: gloria.coruzzi@nyu.edu

Current Opinion in Plant Biology 2000, **3**:179–181

1369-5266/00/\$ – see front matter

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Abbreviation

EST expressed sequence tag

In the biological lexicon, there are few words that do more to conjure up the musty past than physiology and metabolism! The transfer of auxin action effected with small agar blocks; the symptomology of nutrient deficiencies; little punnets of seedlings tipped up on edge to reveal the effects of gravity and light; even the thought of separating the compounds labeled from radioactive CO₂ fails to engender much excitement. The terms ‘physiology and metabolism’ are so redolent of the slow and painstaking ways of the old order that we are not the first editors to consider whether a more upbeat title was needed for this issue. This millennium issue, ‘Physiology and metabolism 2000’, attempts to highlight examples that demonstrate how misleading these stereotyped images have become. The truth is that these two areas of plant biology have been more radically changed than any other area by the new approaches and techniques that have swept through modern biology. The reason for this is that those decades of agar-block and clinostat research have provided a legacy of careful observations that demand explanations at the molecular level. This wealth of phenotypic analyses also makes physiology and metabolism natural playgrounds for modern genetic approaches. For example, nutrient deficiency expands upon molecular, genetic and physiological dissection into a complex but logical set of acquisition imperatives and allocation decisions that affect, and are influenced by, almost all branches of plant metabolism. This complexity of regulation cries out for new genomic and proteomic treatments. Finally, we will see examples in which these new molecular-genetic and genomic treatments have led to a collapse of barriers between the traditional biological disciplines of ‘physiology and metabolism’ versus development. Instead they provide an increased appreciation of the highly integrated nature of metabolism and of physiological processes with growth and development.

This issue attempts to highlight examples that have helped to ignite a new and exciting expansion of physiology and metabolism as the new millennium begins. The

thematic approach we have taken is to highlight topics in which the use of molecular biology, molecular genetics, and new and emerging technologies such as genomics, have revealed that ‘boring’ metabolites and physiological processes are important components that control ‘hot’ areas such as signal transduction, defense, and development. Molecular-genetic tools have enabled huge advances in our understanding of the mechanisms controlling nutrient uptake and have uncovered a role for nutrients as signals in plants. Molecular-genetic and genomic technologies have also revealed yet more previously unknown biochemical pathways. At the same time, new and improved biochemical techniques (e.g. better X-ray and electron crystallography, and sophisticated spectroscopy) and electrophysiological techniques have led to a more detailed understanding of fundamental processes such as photosynthesis and stomatal function, respectively. Future advances in the fields of physiology and metabolism will very much be impacted by new and developing technologies including protein engineering and mathematical modeling, the final topics covered in this issue.

Nutrient uptake and signaling

Nutrient uptake and signaling is a fine advertisement for ways in which new molecular genetic approaches have led to rapid advances in our understanding of this important area of plant physiology and metabolism. Transporters for most macro- and micronutrients have been cloned using a variety of techniques including microbial complementation, expressed sequence tags (ESTs), and *Arabidopsis* mutant isolation. These studies have begun to define the molecular basis for the uptake and transport of many of the macronutrients (including NH₄, NO₃, K⁺, Ca²⁺, H₂PO₄, Cl⁻, SO₄²⁻ and Mg) and micronutrients (including Zn, Mn²⁺, Fe³⁺ and Cu²⁺) for which genes encoding transport proteins have been cloned. Expression of these transporter proteins in heterologous systems has greatly enriched our knowledge of their physical properties (e.g. substrate affinity and capacity) and the significance of these properties to their *in vivo* function. For example, Nico von Wirén *et al.* (pp 254–261) make the point that low-affinity transport often correlates with high capacity, a crucial parameter for maintenance of large influx at high external availability. Biological complexity is introduced by the existence of gene families, interaction of subunits in heteromeric complexes, and cell-specific gene expression. Thus, an understanding of the physical interactions involved in transporter function *in vivo*, will require knowledge of where and when the genes encoding transporters are expressed. These topics are covered in several of the reviews in this issue.

Besides their biophysical properties, molecular studies have uncovered a second level of biological regulation imposed on nutrient transporters; namely transcriptional regulation in response to nutrient starvation. For example, phosphate transporters are shown to be transcriptionally regulated by phosphate deficiency (see review by KG Raghothama, pp 182–187). Moreover, the physical properties of transporters and their metabolic regulation appear to be intricately linked. In the case of ammonium transporters, N deficiency turns on the gene for the ammonium transporter (*AtAMT1;1*) with the highest substrate affinity (see von Wiren *et al.*). Understanding the mechanisms of nutrient sensing and signal transduction is a new challenge in the field of metabolic regulation. Are the nutrients themselves or their downstream metabolites sensed? For example, there is evidence that glutamine might be the feedback signal for repression of ammonium influx and *AtAMT1;1* expression (see von Wiren *et al.*).

Understanding whether the nutrients themselves act as signals or whether their metabolites are sensed, and uncovering the signaling pathways leading to gene regulation is a new challenge in the field of plant physiology. Progress in this area has been enabled by molecular-genetic approaches. In the review of sulfur uptake by Kazuki Saito (pp 188–195), we learn that our understanding of the molecular basis for sulfur allocation and signal transduction is still limited. Analysis of a Met over-producing *Arabidopsis* mutant revealed, however, that the stability of the mRNA for a Met-biosynthetic enzyme (cystathionine γ -synthase) is negatively autoregulated by an amino-acid stretch in its translational product in the presence of Met or its metabolites. Thus, a novel mechanism for post-transcriptional control by metabolites has been newly uncovered as a result of molecular-genetic studies of sulfur metabolism in *Arabidopsis*.

Certain nutrients have long been recognized as small molecules that affect signaling and development (e.g. Ca^{2+}), and the understanding of how Ca^{2+} regulates stomatal opening has risen to exquisite detail (see Blatt, pp 196–204). Nevertheless, putative roles for other macro- or micronutrients in regulating physiology and development are only recently beginning to emerge as a result of molecular-genetic studies. An excellent example covered in this issue includes the recently discovered role that copper plays in ethylene signaling (Ed Himelblau and Richard Amasino, pp 205–210). Genes encoding copper-trafficking proteins (i.e. *COPPER CHAPERONE* and *RESPONSIVE TO ANTAGONIST1*) were uncovered as a result of the characterization of ethylene-signaling mutants. Moreover, yeast expressing *ETR1* revealed that copper is required for high-affinity ethylene binding by the ethylene receptor. These are prime examples in which molecular-genetic studies have uncovered the molecular basis by which a nutrient may regulate growth and development.

Plant response to environment

A rather different challenge in nutrient signaling is described in the review by Mike Blatt. The regulation of solute and water fluxes in leaf guard cells requires the integration of multiple inputs to provide the optimal stomatal aperture for a given set of conditions. Genetic approaches have made contributions in this area but new discoveries were initially more critically dependent on biochemical tools developed for studies of Ca^{2+} signaling in animals as well as biochemical and biophysical discoveries about the control of Ca^{2+} channels in plants. Indeed, you will not find a nicer example of the convergence of biophysics, biochemistry and genetics than the one described in Mike Blatt's article.

The flip side of the nutrient issue is the requirement that plants have to protect themselves from the acquisition of poisonous heavy metals from the soil. Understanding the mechanisms of heavy-metal detoxification is an important consideration in plant husbandry and a prerequisite to enhancing plants as agents of phytoremediation. In his review (pp 211–216), Chris Cobbett describes the successful use of mutation genetics, map-based cloning and yeast expression to identify genes encoding phytochelatin synthase and other proteins required for the chelation and sequestration of cadmium in plants. This is a fine example of the way in which molecular genetic approaches have led to rapid advances in areas that were previously intractable and to the knowledge and genes necessary to usefully manipulate the metabolism and physiology of plants.

One very satisfying product of the molecular approaches is a detailed appreciation of previously imprecise concepts. Plant physiologists have long considered freezing stress, with its flight of water from the cytoplasm to extracellular ice, to be akin to dehydration stress. Kazuo and Kazuko Shinozaki's review of new discoveries in these two fields (pp 217–223) makes it clear that this is not mere analogy. The pathways that choreograph cold acclimation and drought responses share many components in common and activate overlapping suites of responses. Isolation of cold-regulated genes by differential screening techniques followed by identification of transcription factors, has exposed some of the signaling pathways involved in these two processes. At the same time, the multiplicity and complexity of these signaling pathways revealed by biochemical and mutational approaches, reprise a common theme: Nature is awesomely sophisticated in providing subtle levels of control. Take heart, thought, it will be just a few short months until we start to see what expression profiling and proteomics can make of the cold acclimation and dehydration responses.

Dissecting the old and discovering new pathways

There have been nasty shocks recently for those who thought that metabolic pathways are structures, enzymes and arrows to be learnt from textbooks. New discoveries in

both primary and secondary metabolism are being made on a regular basis. The discovery of the non-mevalonate pathway of terpenoid synthesis in higher plants (see review by Ohlrogge and Benning, pp 224–228) is a stand-out example because the wrong pathway had been ensconced in the chloroplast for so long. Meanwhile, the pathway for ascorbate synthesis has been the subject of debate and research for more than 30 years. Suddenly, good biochemistry, backed by mutant analysis, has provided a convincing answer. This breakthrough is described by Nick Smirnoff (pp 229–235) along with a summary of the importance of ascorbate in both plants and animals.

All biologists appreciate the central importance of photosynthesis to life on earth but understanding the details of the capture and transduction of light energy is, for many of us, a challenge. We find it easy to blame the arcane vocabulary of spectroscopists and structural biologists, but it is these disciplines that will provide the molecular understanding of the hydrogen-abstraction process that energizes our biosphere. Consider the article by Jerry Babcock and colleagues (pp 236–242) as a tractable sermon on the subject, and take it from a fellow sinner, your time taken to understand this clearly written and understandable account will be well rewarded. As well as improving your knowledge of primary photosynthesis reactions, you will gain a new appreciation of how quantum physics within proteins is relevant to our lives as biologists - and, of course, to our existence on earth.

Impact of new and emerging technologies on physiology and metabolism

If it's true that molecular genetics and improved tools in biochemistry and biophysics have lit the fuse on our 'two aged grannies', then pin your ears back because genomics, proteomics and related techniques (which have their own home in the *Current Opinion in Plant Biology* April issue). These new and emerging technologies are primed to give us the excitement every six-year-old feels as the flame and smoke rush skyward. In this issue, John Ohlrogge and Christoph Benning provide an overview of the impact that EST projects have

had in extending understanding of metabolism. We learn that not all EST projects have to be massive to be significant. Shallow EST projects can be powerful (and not labor or cost intensive) if the appropriate species and tissue is selected. An excellent example is that a single-pass sequencing of 150 randomly picked cDNA clones made from a peppermint oil gland (!), was sufficient to discover cDNAs encoding two novel proteins involved in isoprenoid biosynthesis.

Physiology and metabolism have also been impacted by other new and emerging technologies. In his review (pp 243–248), John Shanklin describes how rational design of protein structure as well as 'irrational' combinatorial genetics and high-throughput screening are shouldering common sense aside – "Evolution has optimized enzyme function over the last billion years hasn't it?" – and will soon allow us to rewrite the script for many metabolic pathways in plants as well as to select and optimize new enzyme functions evolved from existing structures.

To finish, we have a wake-up call from Christoph Giersch (pp 249–253) for an emerging new approach to metabolism that most certainly will attract more attention in the next decade. Mathematical modeling is hugely demanding in the extent of biochemical and cell-biological detail that is required to begin even a preliminary experiment. The difficulties that have dogged plant biochemistry and cell biology (e.g. phenolics, the cell wall) leave us well short of the detailed catalogue of parameters needed to model most pathways in plant metabolism. There is, however, no better tool to address the complexity of and interactions within metabolism in a eukaryotic cell than modeling. This has already been recognized in medical and pharmaceutical sciences where modeling approaches are widely used. It will be a painful process to develop the required expertise and fundamental knowledge necessary for mathematical modeling in plants. Heaven knows the proponents will have to put up with their share of tittering philistines. Nevertheless, mark well, this technology will become an important part of the guidance system for our rocket.