

Ionomics and the Study of the Plant Ionome

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Abstract

The ionome is defined as the mineral nutrient and trace element composition of an organism and represents the inorganic component of cellular and organismal systems. Ionomics, the study of the ionome, involves the quantitative and simultaneous measurement of the elemental composition of living organisms and changes in this composition in response to physiological stimuli, developmental state, and genetic modifications. Ionomics requires the application of high-throughput elemental analysis technologies and their integration with both bioinformatic and genetic tools. Ionomics has the ability to capture information about the functional state of an organism under different conditions, driven by genetic and developmental differences and by biotic and abiotic factors. The relatively high throughput and low cost of ionomic analysis means that it has the potential to provide a powerful approach to not only the functional analysis of the genes and gene networks that directly control the ionome, but also to the more extended gene networks that control developmental and physiological processes that affect the ionome indirectly. In this review we describe the analytical and bioinformatics aspects of ionomics, as well as its application as a functional genomics tool.

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CONCEPTS OF THE IONOME AND IONOMICS

The ionome was defined as all the mineral nutrient and trace elements found in an organism (47). This definition extended the previously used term metallome (61, 90) to include biologically significant nonmetals (77). The ionome also includes both essential and nonessential elements. The concept of the ionome has been applied to *Saccharomyces cerevisiae*; the mineral nutrient and trace element profile of 4385 mutant strains from the *Saccharomyces* Genome Deletion collection were recently quantified (19). This work in *S. cerevisiae*

represents the first full genome-wide scan for genes involved in the regulation of the ionome in any organism.

The ionome can be thought of as the inorganic subset of the metabolome, a term originally used to describe the global metabolite pools in *Escherichia coli* and *S. cerevisiae* (60, 85) and has now been generalized to describe the “quantitative complement of all the low-molecular weight molecules present in cells in a particular physiological or developmental state” (for a review see Reference 29). Further drawing on these parallels with the metabolome and its study of metabolomics or metabonomics (58), the study of the ionome, called ionomics, is defined here as the quantitative and simultaneous measurement of the elemental composition of living organisms and changes in this composition in response to physiological stimuli, developmental state, and genetic modifications.

This definition captures and highlights several critical concepts in the study of the ionome. Firstly, the study of the ionome is predicated on the fact that it should provide a snapshot of the functional status of a complex biological organism; this information is held in both the quantitative and qualitative patterns of mineral nutrients and trace elements in the various tissues and cells of the organism. Such a concept rests heavily on the early work of Pauling & Robinson (for a review see Reference 74), in which they developed the notion that a quantitative metabolite profile can be indicative of a particular physiological or disease state. To capture this information contained in the ionome, the precise and simultaneous quantification of as many of the components of the ionome as possible is necessary. Secondly, the power of ionomics lies in its ability to precisely capture information about the functional state of an organism under different conditions. These conditions may be driven either by genetic differences or developmental differences or by biotic or abiotic factors.

The underlying cause of an alteration in the ionome may be either direct or

indirect. For example, alterations in the mineral nutrient levels in the soil or the loss of function of an important ion transporter would be expected to directly affect the ionome. Conversely, alterations in cell wall structure or acidification of the apoplast, for example, might be expected to indirectly affect the ionome. Given the relatively high throughput and low cost of ionomic analysis (hundreds of samples per day at approximately \$10/sample), compared with metabolomic or proteomic analysis (tens of samples per day at approximately \$100/sample), ionomics has the potential to provide a powerful and relatively low cost approach to not only the functional analysis of the genes and gene networks that directly control the ionome, but also to analysis of the more extended gene networks that control developmental and physiological processes that indirectly affect the ionome.

HISTORY OF IONOMICS

The inception of ionomics occurred with the blending of ideas from both metabolomics and plant mineral nutrition. The conviction held by Pauling & Robinson (for a review see Reference 74) in the late sixties and early seventies that the metabolite profiles of an organism contain a rich source of information that is reflective of the physiological status of an organism has been confirmed many times since their pioneering work, and there are now many recent reviews of metabolomics that provide excellent overviews of this topic (for example, see Reference 80). The ability to explore the information content of an organism's metabolite profile only became truly feasible with the advent of reliable small-molecule profiling technologies such as gas-liquid chromatography-mass spectrometry (GC-MS) and proton-nuclear magnetic resonance ($^1\text{H-NMR}$). Similarly, our understanding of the basic biology of mineral nutrient homeostasis in plants has very much paralleled our analytical capability to quantify these mineral nutrients. However, only with the development of reliable inductively cou-

pled plasma (ICP) technologies and the ability to simultaneously analyze all the significant elemental components of a plant was it possible to fuse the early ideas of Pauling and Robinson and others on metabolite profiling with our broad understanding of plant mineral nutrition. This fusion gave rise to the concepts of the ionome and ionomics, and occurred at a time when both bioinformatics tools and genetic tools such as sequenced genomes, DNA microarrays, and gene deletion collections were being developed. Given this milieu, we feel that ionomics is coming of age as a tool to both help unravel the functional complexities of genomes and probe the physiologies of plants and animals, and the future for discoveries in ionomics is bright.

Below we describe the analytical and bioinformatics tools required to perform ionomics, and discuss how ionomics can be applied to advance our knowledge and understanding of biological systems.

ANALYTICAL TECHNOLOGY REQUIRED FOR IONOMICS

To achieve the key analytical requirements of ionomics, the "quantitative and simultaneous measurement of the elemental composition of living organisms," requires choosing specialized instrumentation and sample preparation protocols on the basis of various selection criteria. These criteria include sample throughput, dynamic quantification range, sensitivity, elements to be measured, sample size availability, reliability, cost, portability, and the need to measure the ionome in either a bulk sample (e.g., dried and milled whole maize plant) or with either low spatial resolution (1–10 mm, e.g., sample of leaf or root tissue) or high spatial resolution (10–100 μm , e.g., elemental map of a trichome or *Arabidopsis thaliana* seed) in either two or three dimensions. Furthermore, the optimum analytical solution must always be balanced with the financial support available for the project. Because most ionomic analyses are generally comparative (for example, did the

ionome change when gene *x* was deleted), precision is important analytically, not accuracy. To clarify, precision is a measure of how consistently a result is determined by repeated measurements. Precision is critical if you want to establish that an observed alteration in the ionome is due to the perturbation the experimenter applied to the system rather than due to uncontrolled analytical or environmental error. Conversely, accuracy is a measure of how close a measurement is to the true absolute value. High accuracy in ionomics is required only if the experimenter wants to make conclusive statements about the absolute concentration of particular elements in the ionome. An example of such a statement is “the minimal quota for this element is 2×10^5 atoms of zinc per cell” (61). The need for precision, accuracy, or both has numerous implications for the analytical methodology chosen to perform ionomics, some of which are discussed below.

Methods for elemental analysis fall into two groups: techniques that utilize the electronic properties of an atom (emission, absorption, and fluorescence spectroscopy) or techniques that utilize its nuclear properties (radioactivity or atomic number). Below we review some of the most common methods for the simultaneous quantification of multiple elements (see **Table 1** for summary), along with various other analytical considerations such as sample preparation, standardization, and normalization. We also discuss other auxiliary analytical methods for the acquisition of both spatial ionomics and chemical speciation information.

Inductively Coupled Plasma

The goal of ICP is to ionize analyte atoms for their detection by either optical emission spectroscopy (ICP-OES) [also known as atomic emission spectroscopy (ICP-AES)] or mass spectrometry (ICP-MS). The ICP is designed to generate a plasma, a gas in which atoms are present in the ionized state. To generate a plasma a silica torch is used, situated

within a water- or argon-cooled coil of a radio frequency generator (RF coil). Flowing gas (plasma gas) [typically argon (Ar)] is introduced into the plasma torch and the radio frequency field ionizes the gas, making it electrically conductive. The plasma is maintained by the inductive heating of the flowing gas. The plasma, at up to 8000 K, is insulated both electrically and thermally from the instrument, and maintained in position by a flow of cooling argon gas (coolant gas). The sample to be analyzed, as an aerosol, is carried into the plasma by a third argon gas stream (carrier gas). Generally, elements to be analyzed by ICP must be in solution (there are exceptions to this, discussed below under Laser Ablation ICP). An aqueous sample is preferred, and particulates should be avoided because they can clog the instrument. A nebulizer in the instrument transforms the aqueous sample into an aerosol. The sample is pumped into the nebulizer via a peristaltic pump where it is converted into an aerosol, which passes into the spray chamber with the carrier argon gas. In the spray chamber the finest sample droplets are swept into the plasma while the large sample droplets settle out and run to waste. Various nebulizer designs allow sample flow rates to range from less than 20 μL per minute to more than 2 mL per minute, and some designs can accommodate some fine particulate matter as well. The optimal flow rate is determined by the transfer efficiency, sample size, and plasma matrix effects. A system with good transfer efficiency will get more analyte ions to the detector per volume of sample introduced, which allows for a smaller sample size and reduces matrix effects within the plasma. Desolvation systems improve transfer efficiency by increasing the analyte/solvent ratio before the sample enters the plasma. Two methods of desolvation are commonly employed: porous membrane and heating/cooling. On introduction into the plasma atoms in the sample are ionized, generally into singly charged positive ions. Once ionized the analyte atoms are detected using either an optical emission spectrometer or a mass spectrometer.

Table 1 Comparison of various elemental analysis techniques with potential application in ionomics

Technique	Name ^a	Sensitivity ^b	Analysis time ^c	Sample Prep	Matrix Effects	Quantification	Cost	Elements detected	Summary	Notes
ICP-MS	Inductively coupled plasma mass spectro-metry	PPT to PPB; PPQ (10E-15 mass fraction) with sector instruments	1 to 3 minutes; depends on number of elements measured, sample uptake, and washout	Simple acid digestion or microwave digestion	Extensive, but can be corrected for using equations or reduced with collision cells or a sector instrument	NIST standards available for calibration; somewhat appropriate SRMs available for plants	\$10 to \$60 per sample; lowest prices for efficient in-house work; instrument price \$125 to \$400k	Most elements, but sensitivity varies; can measure individual isotopes	Possible to run hundreds of samples daily with excellent sensitivity	Both ICP-MS and ICP-OES depend on having a consistent supply of high-quality argon
ICP-OES	Inductively coupled optical emission spectro-metry	PPB	1 to 2 minutes; depends mainly on uptake and washout times	Simple acid digestion or microwave digestion	Extensive, can be partly corrected for using equations	NIST standards available for calibration; somewhat appropriate SRMs available for plants	Costs similar to ICP-MS, although purchase prices of instruments are lower	Most elements, but sensitivity varies; in practice fewer elements than ICP-MS due to lower sensitivity	Can achieve higher throughput than ICP-MS at the cost of some elements and sensitivity	Gains some sensitivity compared with ICP-MS by running more concentrated samples
XRF	X-ray fluorescence	sub-PPM	30 seconds to several minutes; may depend on number of elements measured	Extensive; samples are either powdered and compressed into a wafer or fused into a glass	Amount of fluorescence is very sample dependent; samples must be "known" for accurate determinations	Standards may need to be prepared by user; not as accurate as ICP methods	\$10 to \$150 per sample or more, depending on number of elements and type of instrument	Difficult to measure elements with atomic numbers lower than sodium	Versatile system for measuring PPM levels of many elements in solid samples	Instrument forms vary widely and impact type of data obtained; high-end instruments may be used to localize elements
NAA	Neutron activation analysis	10E-15 mass fraction for some elements	Hours to days; requires a neutron source such as a reactor or an accelerator	Sample may be in various states; sample is placed in polyethylene vial; simple prep	Present but correctable; relatively unimportant compared to other methods	Used as a primary analytical method to test other instrumental techniques	Expensive; \$500 to \$450 per sample	Nearly 70% of the elements; more than 30 elements possible simultaneously	Costly, slow, very sensitive, accurate; useful for checking other techniques	Getting all desired elements may be impossible or push the analysis into the highest cost per sample

^aProton-induced X-ray emission possible as an alternative.

^bSensitivity is element dependent for every technique.

^cAssumes sample prep is not limiting.

Abbreviations: PPT, parts per trillion; PPB, parts per billion; NIST, National Institute of Standards and Technology; SRM, standard reference materials.

Optical Emission Spectroscopy

When ionized analyte atoms in the ICP plasma fall back to ground state they emit photons at a wavelength characteristic of a given element. For quantification of these analytes the light from the plasma, representing a summation of emitted light from all the atoms introduced into the plasma from the sample, can be focused and passed through optical slits into a spectrometer. Within the spectrophotometer an optical filter is used to separate the collected photons by wavelength, and a charge injection device (CID) detector simultaneously measures the intensities of photons at multiple wavelengths. By comparing these energy intensities to reference standards a quantitative measurement of each element in the sample can be obtained.

Inductively Coupled Plasma Mass Spectrometry

The ability of ICP to ionize atoms makes it an effective ionization source for detection by mass spectroscopy. Ions from the ICP pass through sampling and skimmer cones (typically made of nickel) before being focused by a series of ion lenses into the quadrupole mass analyzer. Ions are transmitted through the quadrupole on the basis of their mass to charge ratio and detected by an electron multiplier. Quantification is achieved by comparison with reference standards for each element of interest.

One critical advantage of ICP-MS over ICP-OES is that it allows for a smaller sample size owing to its greater sensitivity. A small sample size has numerous benefits for ionomic analyses. A small sample size usually requires less sample preparation time, which in turn may be faster and easier. Small samples also allow more samples to be loaded into an autosampler, allowing longer analytical runs. The small sample size required for ICP-MS also makes nondestructive sampling of small plants possible, a prerequisite in a classical genetic screen where interesting mutants need

to be saved and not destroyed by sampling. In the deficit column, smaller samples require cleaner conditions, are harder to weigh accurately, and may be more difficult to handle. One additional advantage of ICP-MS over ICP-OES is that individual isotopes may be measured, which introduces the possibility of isotopic spiking for pulse-chase-type experiments and isotope dilution procedures for improved analytical accuracy and precision.

One of the main drawbacks of ICP-MS is that the formation of polyatomic ionic species in the plasma can interfere with the measurement of particular elements; e.g., $^{40}\text{Ar}^{16}\text{O}^+$ interferes with the determination of ^{56}Fe . These polyatomic species are generated from ions derived from the plasma gas or sample components. The cold/cool plasma approach, which uses a reduced plasma temperature to reduce the formation of argon-based interferences, is effective at minimizing some of these interferences, but is difficult to optimize, and it is time consuming to move back and forth between hot and cold plasma. However, use of the collision/reaction cell alleviates the problems associated with the cold/cool plasma approach by removing polyatomic species from the plasma before they enter the mass analyzer. In this configuration, ions from the ICP enter the mass analyzer interface as normal, where they are now extracted into the collision/reaction cell positioned before the quadrupole. One or two collision/reaction gases such as hydrogen and methane are fed into the cell, where, under the influence of a radio frequency field, the introduced gas(es) collide and react with ions from the sample. Through a number of ion-molecular collisions and reactions polyatomic interfering ions like $^{40}\text{Ar}^{16}\text{O}^+$ are eliminated. The collision/reaction gases used are chosen on the basis of the interfering polyatomic species to be removed. For example, such technology allows for the use of ICP-MS to measure Se at its most abundant isotope, 80 (49.6%), which is normally covered by Ar_2 , by a reaction of the $^{40}\text{Ar}^{40}\text{Ar}^+$ dimer with hydrogen gas. An even more biologically important element, Fe, can be measured at its most

abundant isotope, 56 (91.7%), by reacting the interfering $^{40}\text{Ar}^{16}\text{O}^+$ ion with methane. A complementary approach to the collision/reaction cell is to use a desolvating sample introduction system.

An alternative approach to the removal or reduction of interfering polyatomic ions is to utilize a single collector magnetic sector high-resolution ICP-MS (HR-ICP-MS). Because of the increased resolution of the mass analyzer of these instruments it is possible to simply resolve the interfering polyatomic ions from the analyte ions of interest. HR-ICP-MS provides the gold standard for the resolution of interfering polyatomics. However, HR-ICP-MS instruments are currently more expensive than quadrupole ICP-MS instruments (though the differential of approximately \$150,000 is rapidly shrinking), and scan speeds for detection of multiple elements are lower (though not dramatically).

In summary, ICP-OES or ICP-MS can both be used effectively for ionomics. ICP-OES has the advantage of lower cost and simplicity, whereas ICP-MS has an edge in sensitivity and the ability to detect different isotopes of the same element. Although ICP-OES is less sensitive than ICP-MS, some of this sensitivity is won back by the robustness of ICP-OES in more concentrated sample matrices. Whereas ICP-MS struggles with sample matrices with greater than about 0.1% solids, ICP-OES can handle up to about 3% dissolved solids. Both ICP-OES and ICP-MS have been used successfully for large-scale ionomics projects; Eide and colleagues (19) used ICP-OES to measure approximately 10,000 samples over two years in yeast, and other researchers used ICP-MS to measure approximately 80,000 samples between 2001–2007 in *A. thaliana* (10, 47, 76).

By coupling laser ablation for sample introduction to ICP-MS (LA-ICP-MS), information about the ionome can be acquired without sample preparation and with two-dimensional spatial resolution. Generally, a UV laser light is focused on the sample; spot sizes can be varied from 5–300 μm . At the

point of focus the UV laser ablates material from the sample, and this ablated material is introduced into the plasma of the ICP-MS via an argon carrier gas that flows over the sample being ablated. LA-ICP-MS analysis can be carried out on a single spot on the sample, rastered over several spots (e.g., **Figure 1**), or scanned continuously across the sample to develop a two-dimensional elemental map of the sample. By using the laser to sample a single spot over time an elemental depth profile of the sample can also be developed. LA-ICP-MS is well suited for the ionomic analysis of specific tissues or small regions of samples, such as root tips, vascular tissue, etc. This technique would also be useful for the tissue localization of ionomics changes that had previously been identified in bulk samples of leaves, for example. Furthermore, LA-ICP-MS should be applicable to high-throughput ionomic screening of arrayed samples. LA-ICP-MS has been used extensively for the analysis of geological samples, human artifacts, and bone, teeth, and hair. However, LA-ICP-MS has currently had only limited application for plant samples; for example, Punshon and colleagues (70) analyzed U and Ni on the surface of leaves. The limited availability of cooled sample chambers required for the analysis of hydrated samples and difficulties in quantification of elements using LA-ICP-MS may explain this limited application. However, we feel that this technique holds great potential for ionomic analysis.

X-Ray Fluorescence

X-ray fluorescence (XRF) is the emission of secondary or fluorescent X-rays from an atom that has been excited by the absorption of high energy X-rays or gamma rays. The emitted fluorescence X-rays have energies characteristic of the atom from which they were emitted, and therefore can be used to detect and quantify specific elements in a complex mixture. For the primary excitation of the sample, radiation of sufficient energies is required to allow for the removal of tightly held electrons

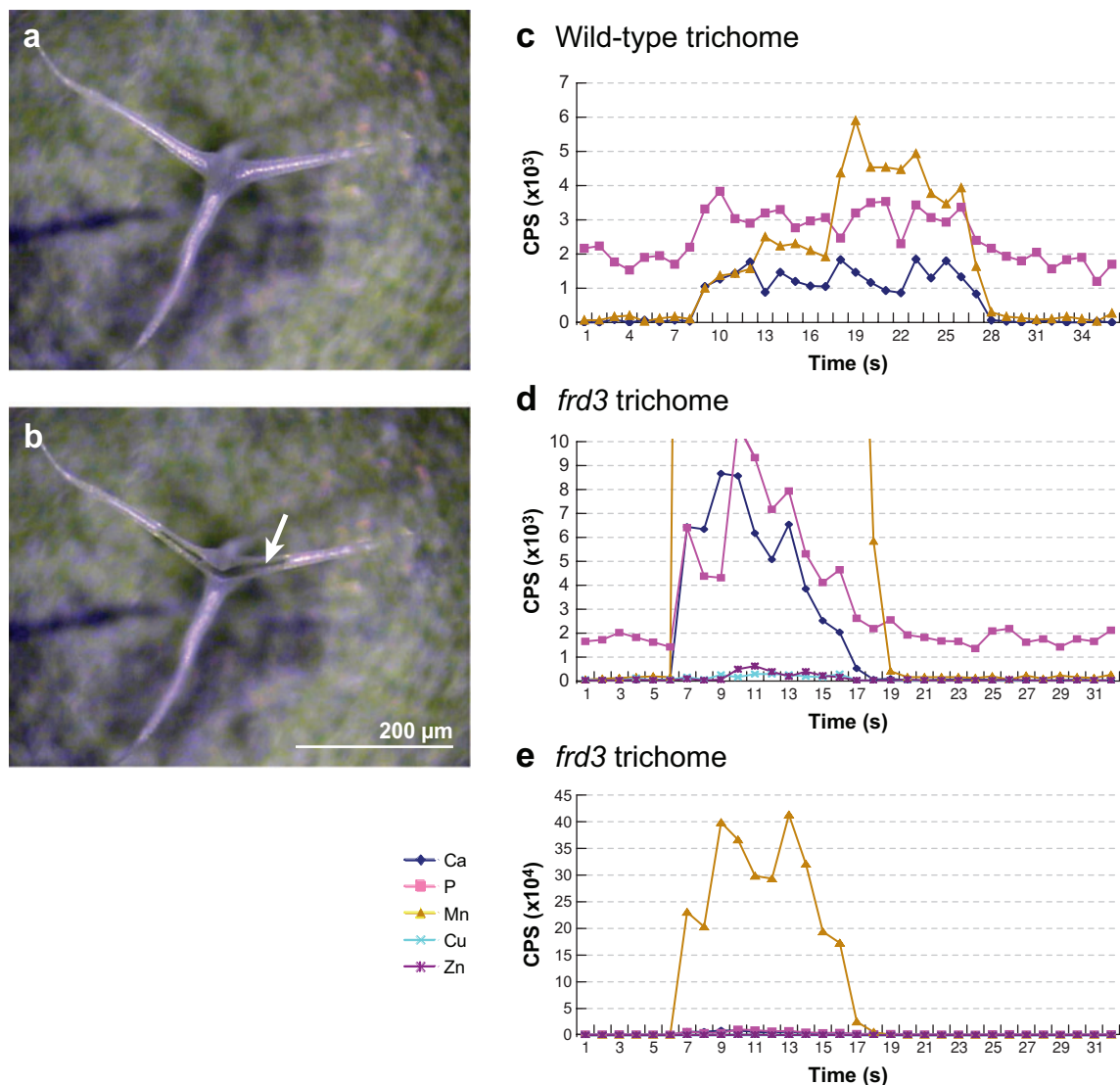


Figure 1

Laser ablation ICP-MS. *Arabidopsis thaliana* trichome analyzed by in situ laser ablation inductively coupled plasma mass spectrometry (ICP-MS). (a) Trichome before ablation. (b) Trichome after ablation (arrow). (c) ICP-MS data for trichome on a wild-type leaf showing levels of Ca, P, and Mn. (d,e) ICP-MS data for trichome of a mutant (*frd3*) leaf, with different y-axis scales, showing elevated accumulation of Ca, P, Mn, Cu, and Zn. Image and data courtesy of Brett Lahner, Luke Gumaelius, and David E Salt. CPS, counts per second.

in the inner shells of atoms. High-energy electrons from outer shells replace these lost electrons, and in the process release fluorescence X-rays, which can be detected for elemental quantification. This high-energy primary ex-

citation radiation can be supplied from a conventional high-voltage X-ray tube to produce a range of X-ray energies, allowing excitation and quantification of a broad range of atoms. However, gamma-ray sources can also

be used to produce an overlapping range of energies, which allows for multielement detection. Such sealed gamma-ray sources are inherently radioactive, and therefore do not require large power supplies and can be used in small portable XRF instruments, which are useful for rapid ionomic analyses in the greenhouse or the field. For example, plant tissue and soil could be analyzed directly in the field, and decisions about collection of fresh tissue for genotyping of a segregating mapping population can be made. Such experiments could be very valuable for the identification of quantitative trait loci (QTL) involved in ionomic adaptation to particular soil conditions. Given that XRF is generally a nondestructive procedure, ionomic analysis by XRF can be performed on living plant specimens without compromising their viability. However, because uniform samples produce more precise analytical data, samples are generally prepared as flat discs thick enough to allow the sample to absorb all the energy of the primary excitation beam.

Synchrotron radiation is a third source of excitation energy for XRF. Here X-rays are focused into a very intense beam with a small cross-sectional area, allowing analysis of both the distribution and concentration of multiple elements with trace-level detection limits and micrometer to submicrometer spatial resolution. Such microXRF techniques can be applied for elemental analysis of a single region of a sample, or be used for sample scanning in two dimensions, without any sample preparation. Furthermore, XRF microtomography and confocal XRF imaging can be used to map the elemental content of a sample in three dimensions. However, given the intensity of the excitation energies used with these synchrotron-based X-ray beams, these procedures can damage the sample, and will quite possibly be detrimental to the viability of living organisms. A related elemental analysis method, based on the same principles as XRF, is particle (or proton) induced X-ray emission (PIXE). In this method a beam of protons is generally used to excite atoms in the sample,

which are then detected by their emission of fluorescent X-rays as in XRF.

In the early 1990s, before the ionome or ionomics had been defined, Delhaize and coworkers (18) applied XRF for the successful multielement screening of more than 100,000 mutagenized *A. thaliana* seedlings to identify mutants with altered ionomes. This screen identified three mutants: *pbo2*, which accumulates threefold higher P in leaves compared with wild-type; *pbo1-2*, which accumulates reduced P in leaves [and was previously identified by Poirier and colleagues (69) in a colorimetric screen for P mutants] (17); and *man1* (now known as *frd3*), which overaccumulates a range of metals in leaves, including manganese, for which it was originally named (16). Owing to the lack of molecular genetics tools for *A. thaliana* when these mutants were first identified, it took several more years before the loci underlying these mutant phenotypes were identified (6, 9, 26, 75). Interestingly, one of the first mutants to be identified in a truly ionomic screen by Delhaize, *frd3* (formally *man1*), was used as the positive control in the second large-scale ionomic screen performed by Salt and coworkers (47), and was in fact rediscovered in this second ICP-MS-based screen.

A second novel application of XRF to ionomics is the recent use of synchrotron-based microXRF as a rapid screening tool for the possible identification of *A. thaliana* seeds with mutant ionomic phenotypes (94). In this proof-of-concept work, *A. thaliana* seeds were arrayed in 5×3 blocks, and each block was scanned with a focused X-ray beam to quantify the relative content of Mn, Fe, Ni, Cu, Zn, K, and Ca of each seed in the block. Such a microXRF-based ionomics methodology holds great promise for the rapid screening of many thousands of seeds or other tissue samples. However, a practical application of the method awaits further optimization work.

Synchrotron-based microXRF has also been used successfully both for ionomic analysis of small samples and for two-dimensional imaging of the ionome in several different

biological samples, including intact Se-tolerant and Se-sensitive diamondback moths (22), intact zooplankton (20), the growth front of mussel shells (46), and mycorrhizal plant roots (96). Recently, XRF microtomography and confocal imaging were also used for quantitative imaging of the three-dimensional distribution of multiple elements in various plant samples (32, 35, 38, 55) (e.g., XRF microtomography, **Figure 2**). The recent insightful combination of microXRF imaging with nanogold immunolocalization offers the unique opportunity for the simultaneous colocalization of known cellular structures, such as the mitochondria and

chloroplast, with particular features revealed by microXRF elemental mapping (50). Such colocalization information could be very important for the biological interpretation of XRF imaging.

Although these XRF imaging techniques do not have the throughput required for ionomics screens, they are proving to be powerful tools for understanding the fundamental biological processes that underlie the ionome. For example, Kim and coworkers (38) recently used XRF microtomography to quantitatively map the three-dimensional distribution of various elements in intact *A. thaliana* seeds (**Figure 2**). Using this technique they were

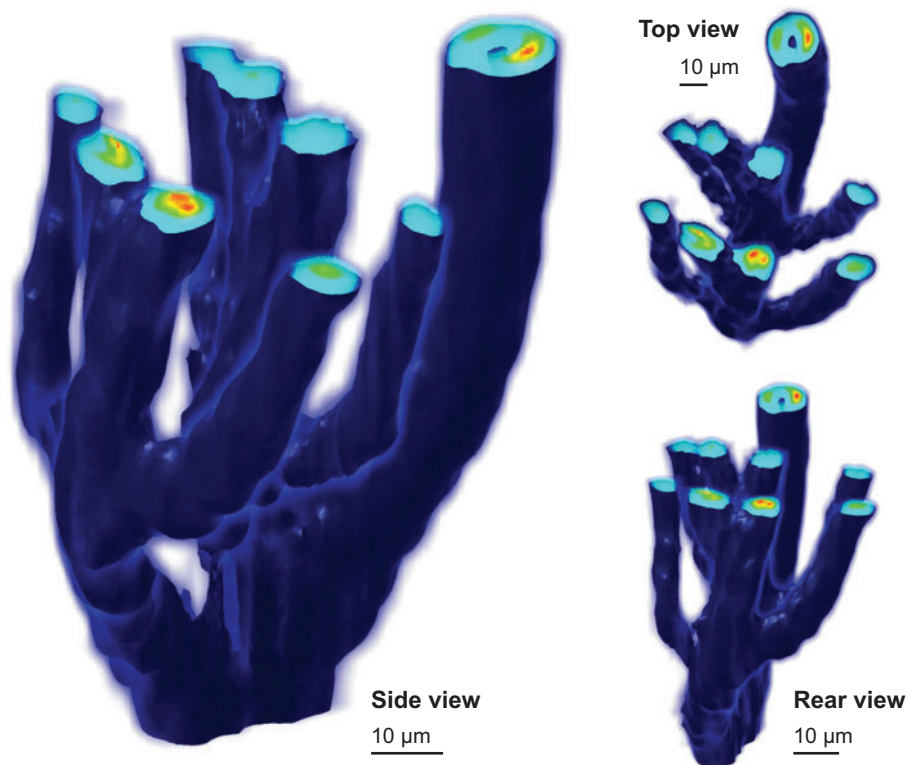


Figure 2

X-ray microtomography. Three-dimensional rendering of Fe K-alpha fluorescence in an *A. thaliana* (Col-0) seed, with in silico transaxial sectioning (z axis, upper 50% removed), showing the Fe localization to a discrete cell layer surrounding the embryonic provasculture. Three views are shown: side (*main panel*), top (*upper inset*), and rear (*lower inset*) views. The branched structures are the Fe surrounding the provasculture of the cotyledons, and the unbranched structure is the provasculture of the radicle. Warmer colors correspond to higher Fe fluorescence. Image courtesy of Tracy Punshon, Tony Lanzirotti, and Mary Lou Guerinot.

able to determine that the vacuolar Fe uptake transporter VIT1 is directly involved in storage of Fe in the provascular strands of the *A. thaliana* embryo, and without such localized stores of Fe *vit1-1* seedlings fail to thrive.

Neutron Activation Analysis

Neutron activation is the process whereby free neutrons are captured by atomic nuclei. This neutron capture results in the formation of new nuclei that are frequently radioactive. These radioactive nuclei will decay with time, and a proportion of the energy is released as gamma radiation. This emitted gamma radiation can be detected, and gamma rays at a particular energy are indicative of the presence of a specific radionuclide. Processing of the gamma-ray spectra released from a neutron-irradiated sample can therefore yield the concentration of various elements in the sample. This type of technique can allow the nondestructive quantification of multiple major and trace elements in a sample. Because neutrons have no charge they can penetrate and pass through most samples, activating the whole sample. Gamma rays released from the activated sample are also very penetrating and can escape the sample for efficient detection. Therefore, elemental analysis by neutron activation analysis (NAA) in general does not suffer from errors introduced due to the sample matrix. NAA can be performed on a sample directly, without sample preparation. In this configuration the analysis is termed instrument neutron activation analysis (INAA), and it is this approach that is most useful for the large sample numbers required for ionomic analysis. Unfortunately, NAA does require access to a nuclear reactor as a source of neutrons to perform the initial activation step, which could limit the usefulness of NAA for a long-term ionomics project.

Elemental analysis of plant samples by NAA dates back to at least the mid-1960s. Analysis was initially focused on single elements, but by the early 1970s researchers were performing simultaneous analysis of macro,

micro and trace elements in plants by NAA (54). The power of elemental profiling using NAA was rapidly applied to environmental toxicology, where the transport of potentially toxic trace elements from the environment, through crop plants and livestock to humans, has been monitored using NAA (15, 23, 33, 83). Multielement analysis of plant samples by NAA was also used to identify and monitor areas of heavy metal pollution across broad geographical areas and multiple years (8, 25). Interestingly, NAA was also used to perform multielement quantification on plant samples collected within and across broad phylogenetic groupings, for the identification of trends in mineral nutrient and trace element accumulation in plants across taxa (62, 87). However, even though the use of NAA for plant samples goes back more than forty years, to our knowledge NAA has not yet been used as a high-throughput elemental analysis tool for ionomic analyses, as defined here. For example, unlike ICP-(OES & MS) and XRF, NAA has not been used as a screening tool for the identification of ionomic mutants. This is surprising given the high throughput and minimal sample preparation required for INAA. We do note that NAA has been applied to perform ionomics in the study of breast cancer (24, 57), colorectal cancer (3, 81), and brain cancer (2); in these studies the ionome was shown to be perturbed in the diseased tissues or organisms.

ANALYTICAL STANDARDIZATION

Because ionomics generally involves the comparison of two nearly identical organisms across an experiment that can last between hours to years, standardization across these timescales can set the lower limit on the discovery and determination of the differences between these two organisms. The sharpest comparison occurs when they are colocated as much as possible both spatially and temporally, starting from the growth stage and continuing through the chemical analysis. In

this way the effect of instrument drift (i.e., changes in the instrument's analytical responsiveness with time) is reduced. The effect of drift can be further minimized by the use of internal standards, and by recalibrating as frequently as necessary. For comparing samples in separate runs, or even from different labs, external standards [National Institute of Standards and Technology (NIST) traceable] and standard reference materials (SRMs, available from NIST) become useful. Because the matrix matching of samples is very important, reference material must sometimes be prepared from the same type of material as that under analysis. Note that because differences between these very similar organisms are often due to environmental factors, this careful standardization across time and distance is helpful for elucidating these factors. Environmental factors can also be gauged by the essential inclusion of positive control plants in every group of samples.

The precision of the determination of the amount of material in each analytical sample can be limiting in the ability to discern differences in the ionome if the samples are small or unwieldy. One way to avoid this problem is to normalize samples to their analytical signal and the included matrix-matched reference standards. This method is based on the assumption that all the samples have identical compositions. If, after the calculation is done, a line is found to be ionomically different, then the affected elements are removed from the data set, and the calculation iterated (47). An added advantage of this technique is that it saves the time of determining the sampled amount by the standard method, such as weighing.

An important, often overlooked part of the data analysis is the necessity of presenting the data in a form that is most useful to the end users. Because gigabytes of data may be produced with current technology, and many people with varied backgrounds may need to understand the significance of the results, today's challenge is to reduce the data to easy concepts like percentage change and p-values

and make it available through simple and efficient interfaces.

SAMPLE HARVESTING AND PREPARATION

Harvesting is a crucial element in plant ionomics. Getting the equivalent portion of each plant is necessary because plant tissue is far from homogeneous. Even leaves just a week apart in age can show significant differences in their ion profiles. Particularly when using ICP-OES or ICP-MS as the measurement technique, getting the same amount of sample each time can improve the results dramatically. This effect is due to two factors: First, the sample matrix affects the plasma in ways that may not be corrected for by the internal standards. Second, a very small or large sample may be outside the linear dynamic range of some of the calibration curves, or even below the detection limit. Harvesting is also the stage where sample contamination can either be reduced by a good washing technique or introduced from utensils or hands.

Sample preparation for the ICP techniques is typically acid digestion and dilution. Open air or microwave digestion can be used. Both require a fume hood that can handle acid fumes. If laser ablation is used as a sample introduction technique, then sample preparation consists of fixing the sample to a flat surface and possible drying or freezing of the sample. Sample chambers for laser ablation are now available that can accommodate a 96-well plate, which could facilitate the automation of the targeting process for appropriate samples, e.g., seeds. NAA does not require extensive sample preparation. The sample must be sealed in a small vial and can be solid or liquid (or gas). All that matters is the amount of analyte within the vial. XRF has the most difficult preparation; the samples typically must be homogenized and pelletized. However, a large screen has already been undertaken using XRF in which leaf samples were merely pressed onto flat surfaces (18); as noted above,

microXRF techniques require only proper mounting.

AUXILIARY IONOMIC METHODOLOGIES

A critical aspect of ionomics is the necessity of follow-up studies to understand the biology that underpins the observed ionic changes. Such experiments often require different types of analytical approaches than those used to make the initial ionic observation. A mutant with an altered leaf ionome is a powerful tool for the identification of genes involved in regulating the ionome. However, a better understanding of both the distribution and chemical speciation of the elements altered in the mutant could provide important clues to the function of the gene underlying the mutant phenotype.

We discussed above the use of both LA-ICP-MS and microXRF as methods for the determination of spatial distribution changes in the ionome. However, both these techniques reveal nothing about the chemical environment or oxidation state of the elements of interest. To address these questions of localization and speciation, X-ray spectroscopy and liquid chromatography coupled to ICP-MS and electrospray ionization mass spectrometry (ESI-MS) have been used successfully on plant samples and are discussed further here.

X-Ray Absorbance Spectroscopy

Previously, we discussed the use of XRF as a method for multielement quantification in plant samples. Such an approach involves the excitation of atoms across a broad range of excitation energies, allowing the detection of multiple elements. However, X-ray absorbance spectroscopy (XAS) relies on the excitation of a specific element of interest. The sample is scanned with X-rays of a narrow range of energies, chosen to exclusively excite the element of interest, and the X-ray absorption spectrum is recorded.

Features within the recorded X-ray absorption spectrum reveal useful information about the chemical speciation of the element being probed. Such methods require minimal sample preparation, and therefore provide *in vivo* chemical speciation information. XAS is generally divided into X-ray absorption near edge spectroscopy (XANES) and extended X-ray absorption fine structure analysis (EXAFS). Both approaches provide complementary information. XANES provides information about the oxidation state and overall ligand environment of the element of interest. EXAFS provides detailed information about the bond lengths between the element of interest and the element(s) it is coordinated with, along with qualitative information on the identity of the ligating atoms. These approaches were first applied to plants in the mid-1990s by Salt and colleagues (78) and Krämer and colleagues (44) for the chemical speciation of Cd and Ni in bulk plant tissues, respectively. Since this initial work, XAS has been used by many investigators to probe the speciation of numerous different elements in plants, including As, Cr, Cu, Mn, S, Se, Pb, U, and Zn. Using microfocused X-ray beams, quantification and chemical speciation can also be performed in small localized areas of a sample. For example, Freeman and coworkers (22) used microXANES (μ XANES) in an elegant study to probe Se speciation in target tissues of diamondback moth larva. Here, Se was chemically speciated in the thorax, hindgut, and abdominal deposits of the moth, and differences were observed between Se-tolerant and Se-sensitive ecotypes.

The use of microfocused X-ray beams has also been developed further, to allow quantitative chemically specific imaging. Pickering and coworkers (68) initially applied an approach of this type to the quantitative localization and chemical speciation of Se in leaves and roots of the natural Se hyperaccumulator *Astragalus bisulcatus*. In a follow-up study the imaging resolution was increased to 5 μ m, allowing, for example, quantification and speciation of Se in the vascular tissue

of the plants (67). High-resolution quantitative chemically specific imaging was also used to determine the localization and chemical speciation of As in the As-hyperaccumulating fern *Pteris vittata* (66). This type of imaging revealed in exquisite detail the amount and localization of arsenite, arsenate, and As(III) coordinated with thiol ligands in several tissues in the sporophyte and the gametophyte of *P. vittata*.

As is clear from these examples, the data obtained from XAS can provide important leads in the search to uncover the underlying biology reflected in a change in the bulk properties of the ionome.

Liquid Chromatography–ICP–MS

By coupling chromatographic separation of the chemical species of interest (e.g., arsenate from arsenite) with detection using ICP–MS, specific chemical forms of elements of interest can be quantified. Because coelution with standards is not considered sufficient evidence for molecular identification, several researchers have run liquid chromatography (LC) separation with parallel ICP–MS and ESI–MS (71). However, because extraction is required to prepare the sample for chromatography, great care must be taken when using this technique to avoid the artifactual formation of new chemical species or changes in the quantities of existing species. That said, this approach has been applied successfully to the quantification and speciation of various elements in plant samples, including As and Se (42, 72).

BIOINFORMATICS INVOLVED IN IONOMICS

In any large-scale ionomics project, where many hundreds or thousands of samples are to be analyzed over an extended period of time, it will be critical to implement an information management system to control all aspects of the process. This system will include the management of plant growth, harvesting, sample

preparation, elemental analysis, and data processing. These workflow tools will allow, for example, scheduling and tracking of samples within the ionomics pipeline and generation of automated reminders when plants are ready for harvesting or data have been analyzed and stored in the database. Workflow tools can also be developed to control sample submission to the ionomic analysis pipeline from outside laboratories. The Purdue Ionomics Information Management System (PiiMS) is a working example of such a workflow control system (10), in which the ionomics workflow has been broken down into four stages: planting, harvesting, drying, and analysis. Each stage is represented by a portal through which information about the stage is collected, curated, and displayed (Figure 3).

Critically, workflow tools also provide for the logical organization of the workflow in the ionomics pipeline into discrete processes, providing a logical framework for the capture of contextual information (metadata such as plant genotype, growth conditions, date planted, etc.) necessary to fully describe the experiment. To implement an efficient system of metadata collection it is critical to develop a standardized nomenclature to describe “objects” within the system. This nomenclature can be a controlled vocabulary in which terms describing each object in the database are predefined (e.g., soil mix 1 = Sunshine Mix LB2, Carl Brehob & Son, Indianapolis, IN or SALK_132258_homo = a homozygous *A. thaliana* line with a T-DNA insert in gene *At2g19110*). This type of controlled vocabulary helps unify and standardize terminology and avoids duplication and contradiction. However, controlled vocabularies generally do not capture relations between the objects and concepts the vocabulary describes, thus limiting their use for data retrieval, data integration, and knowledge extraction. To overcome these limitations of controlled vocabularies, information management systems for ionomics, and other high-throughput omics technologies, should be based on a comprehensive ontology that describes all the entities

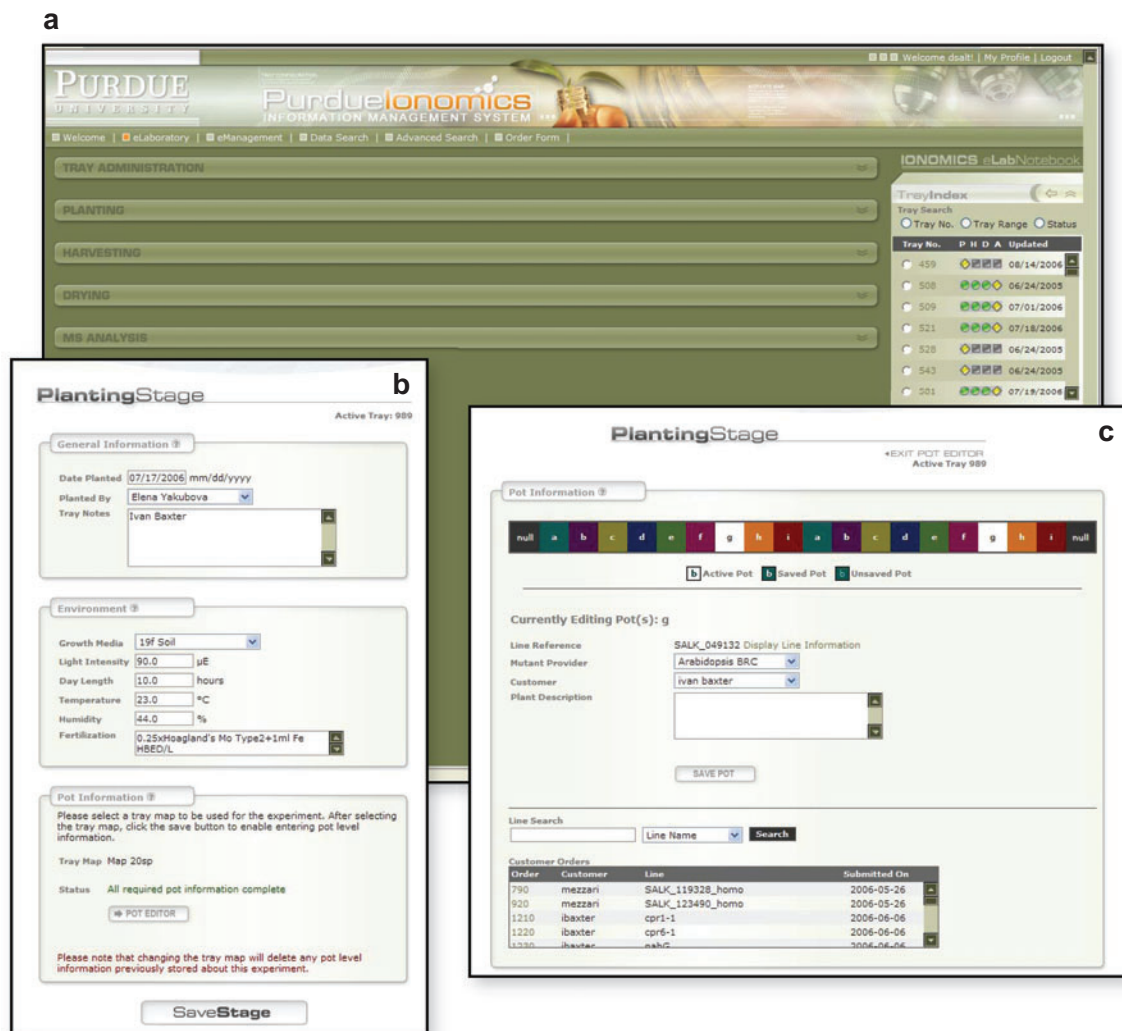


Figure 3

Information Management eLaboratory portal used to control workflow in the Purdue Ionomics Information Management System (*a*), with examples of modules accessed through this portal that are used to collect metadata during the planting stage. These include modules to collect experiment level information (*b*) and information that defines each line and its physical location in the planting tray (*c*). Courtesy of Reference 10.

and their logical relations in both planned and ongoing experiments. Work is progressing in the development of general biological ontologies (4, 53), those for plants (31), and those focused on high-throughput data acquisition technologies (34, 88). However, much still remains to be done to integrate these ontolo-

gies into a cohesive whole that can describe all facets of ionomics or other omic technologies.

The preprocessing of elemental profile, or ionomic data, is a critical step in the ionomic workflow before data can be analyzed for the extraction of knowledge (see section above

on Analytical Standardization). Because such data preprocessing is best done by the analyst that collected the data, tools to accomplish such data preprocessing must be incorporated into the workflow at the stage that the analyst interacts with the information management system. Metadata describing how the preprocessing is performed also should be collected at this stage, so that such preprocessing steps can be recreated at a later date. These types of integrated tools have been implemented in the PiiMS (10).

For an information management system to be useful it must provide tools that allow for the retrieval, display, and download of the ionomics data and associated metadata that it stores. Furthermore, access to integrated and flexible data analysis tools would be a great asset to such an information management system. One example is the ability to select ionomic data from across a series of experiments, format the collected data and merge data from external sources (other databases or user supplied data), and submit it for normalization, statistical tests, clustering, and classification. Ideally, the researcher could build their own custom analysis workflows and upload new analysis algorithms for inclusion in the custom workflow. Such workflows should allow the results of one analysis to become the inputs of another. To the authors' knowledge, integration of such analysis tools into an existing high-throughput data management platform does not yet exist, although several groups are working on developing such systems to facilitate this type of integration using BioMOBY and Taverna (see below).

To further enhance and enrich the ability to extract knowledge from large ionomic datasets it is also critical to be able to incorporate information from other existing databases. National and international efforts have funded the development of a wide array of information and computational resources. Although these provide a rich set of genotypic, phenotypic, and analytical resources, considerable expertise is required to find and use appropriate resources, and integration across

datasets is very difficult. Web Services (14, 56) provide a means to knit together disparate resources without requiring the very complex task of integrating the information into the core ionomics database. Currently, the PiiMS (10) is compatible with the Web Services branch of BioMOBY (89), and the development of PiiMS as a client for interaction with the MOBY-S framework for exchange of data and analytical services is currently under development.

Taverna is a recently developed scripting language and set of software tools to facilitate the easy construction of custom analysis workflows and use of distributed computing resources (30). The use of Taverna, integrated with BioMOBY, is one possible solution to the integration of high-throughput ionomic data generation platforms with other external data and computing resources (36). Such a set of tools should provide the ability to develop custom data analysis workflows for hypothesis testing and knowledge generation from large ionomic data sets.

Open access to large publicly funded functional genomics datasets is not only an obligation to the funding agency, but is also critical if the datasets are going to be efficiently mined for valuable biological information. This is as true for ionomic datasets as it is for genome sequence or transcriptomics data, for example. Open access to "raw" data sets would allow multiple investigators to carefully analyze data from many different perspectives, which would help to develop and refine robust biological knowledge. To facilitate this process for ionomics it will be critical to make ionomics data, and its associated metadata, freely available by the deployment of search and retrieval tools, which preferably should be Web Service-enabled to allow integration with systems such as BioMOBY and Taverna, as described above.

To promote rapid knowledge generation about the ionome and the genes and gene networks that underpin it, it will be critical that information obtained through experimental or bioinformatic approaches be retained

within this information management system. Such annotation will allow the iteration of ionomic discovery. Currently, systems to allow researcher-driven annotation of genes with biological knowledge are very limited across all current biological models. With the rapid growth in the number of sequenced genes, functional data, and their interrelationship, data systems allowing for such systematized annotation are in great demand.

Finally, because of the complex, interdisciplinary nature of any large-scale ionomics project, the research is almost inevitably going to be collaborative. Collaborative projects such as these, almost by definition, are going to occur across various principle investigators' laboratories, institutions, and field sites. To facilitate such distributed activities it will be important for the information management system designed for the project to be web-enabled and contain tools for the distributed input and output of data, coordination of experiments, and sharing of biological insights.

APPLICATIONS OF IONOMICS

As defined in this review, a central theme of ionomics is the study of changes in the ionome in response to "physiological stimuli, developmental state, and genetic modifications." It is in this context that we discuss the application of ionomics to the discovery of gene function (functional genomics) and the assessment of the physiological status of plants.

Functional Genomics

With genotyping, including sequencing and polymorphism identification, rapidly becoming routine, the identification of phenotypic variation, and its association with genotypic variation, is limiting the leveraging of genomic information for knowledge generation. As a high-throughput phenotyping platform, ionomics offers the possibility of rapidly generating large ionomics data sets on many thousands of individual plants. Utilization of such a phenotyping platform to screen mapping

populations with available modern genetic tools provides a very powerful approach for the identification of genes and gene networks that regulate the ionome.

Genetic variation for these screens can either be artificially induced using various mutagens, including ethyl methanesulfonate (EMS), X-rays, fast neutrons (FN) (41), T-DNA (7, 45) and transposable elements such as Dissociation (Ds) (64), or be derived from natural populations. The probability of identifying a plant harboring a mutation in a gene that affects the trait of interest, in this case the ionome, is dependent on both the mutation frequency and size of the gene(s). Mutation frequency varies between mutagens; FN and EMS produce on average 30–60 mutations per diploid genome (41), compared with 1.4 mutations for T-DNA (21). To perform a saturation screen using an EMS- or FN-mutagenized population would therefore require phenotyping of approximately 10,000–20,000 M2 plants, whereas the same screen with T-DNA would require 200,000–400,000 M2 plants. Clearly, even when using an EMS- or FN-mutagenized population the screening system used to identify plants with an altered ionome needs to be relatively high throughput to achieve saturation.

EMS- and FN-mutagenized populations have been successfully used for the identification of various ionomic mutants, including mutants with perturbations in single elements, such as P (17, 69) and Na (59, 76), and mutants with multiple ionomic changes (16, 47). With the efficient identification of ionomics mutants, mapping the causal locus becomes the limiting factor in gene identification. One approach to overcome this potential bottleneck is to use a mutagen, such as T-DNA, that has an easily identifiable sequence tag. This sequence tag can be used for the rapid identification of the mutagenic insertion site using PCR. The main drawback of these technologies is that their relatively low mutation frequencies make screening to saturation impractical with current ionomic throughput. However, the high mutation frequencies of

EMS and FN make them more favorable mutagens for saturation ionic screens. Unfortunately, identification of the causal locus for a particular mutant phenotype is laborious when using EMS and FN because the mutation is not tagged.

However, the advent of high-throughput genotyping methodologies and the availability of genome-wide knockout collections has started to relieve some of these difficulties. For example, DNA microarray-based technologies are being used to perform bulk segregant analysis (BSA) (51) for the rapid localization of causal loci to within a few cM in mapping populations with no predetermined genetic markers (12, 91). DNA microarray-based genotyping is also being used for the identification of causal deletions underlying phenotypes of interest (12, 27). Deletion detection using DNA microarray-based techniques is limited by the size of the deletion and the number of features on the DNA microarray relative to the genome size. The current version of the Affymetrix *A. thaliana* array contains ~1.6 million features, which provides a probe spacing of ~35 bp across the genome and allows for reliable detection of deletions of >300 bp in length. Furthermore, new massively parallel sequencing, using systems like 454's GS Flex, Illumina's Solexa, and ABI's SOLID, is set to revolutionize the identification of causal mutations by allowing the rapid sequencing of large regions of DNA to identify polymorphisms between mutant and wild-type plants. To illustrate the power of this new sequencing technology, it recently took only two months, using the 454 sequencing technology, to sequence James Watson's entire genome, the "DNA behind the DNA". This contrasts with the 13 years it took to sequence the first human genome, completed in 2003. With several companies and technologies competing, the cost and speed of genotyping should continue to rapidly decrease, removing this step as a roadblock to identifying causal loci.

Once identified, candidate genes can be rapidly tested by screening for the ionic

phenotype of interest in various types of sequence-indexed insertion lines carrying a mutant allele of the gene of interest. Such collections currently exist for various plants, including *A. thaliana* (T-DNA, Ds, and dSpm), rice (T-DNA, *tos17*, and Ds) and maize (UniformMu). Where sequence-indexed insertion collections do not exist, targeting induced local lesions in genomes (TILLING) (28) is an alternative strategy for the identification of mutant alleles of candidate genes.

Genetic variation among and within natural populations can also be used as a tool for gene discovery, and has been applied extensively in *A. thaliana* (1, 40, 63), maize (79, 84), and rice (5, 39), for example. Further, genetic studies in model organisms can provide a critical bridge between a molecular gene-based approach to analyzing function and an evolutionary investigation of adaptive and natural selection (52, 82).

Via the use of immortalized mapping populations known as recombinant inbred lines (RIL), derived from a variety of natural accessions, researchers have identified QTL for several ionic traits in various species, including *A. thaliana*, rice, and maize. In *A. thaliana* these traits include phosphate accumulation in seed and shoot (11), shoot Cs accumulation (65), shoot selenate accumulation (97), seed K, Na, Ca, Mg, Fe, Mn, Zn, and P accumulation (86), and sulfate accumulation (49). In rice and maize these traits include P, Si, Na, and K accumulation (43, 48, 73, 92, 93). Once ionomics QTL have been identified, genomic tools available for *A. thaliana* and to some extent rice and maize can be used to locate the genes that underlie these QTL and thus describe the traits at a molecular level (for a review see Reference 13). Such an approach was recently taken to identify the genes responsible for QTL that control Na in rice and *A. thaliana* (73, 76); interestingly, the responsible gene was found to be the Na-transporter HKT1 in both species. Loudet and coworkers (49) recently identified the gene that controls a major QTL for sulfate accumulation in *A. thaliana*; this gene

encodes adenosine 5'-phosphosulfate reductase, a central enzyme in sulfate assimilation. Researchers are also well on the way to identifying the gene that controls a major QTL for seed P content in *A. thaliana*, which has currently been narrowed down to only 13 open reading frames (11).

Many species have thousands of natural accessions collected in stock centers that can be used for association mapping (95). Advances in high-throughput genotyping have enabled the genome-wide coverage necessary to use this technique to identify small chromosomal regions containing loci that are associated with a given trait. Although this technique requires the phenotyping of thousands of plants in a single experiment, the causal loci is likely to be within approximately 20 kb in *A. thaliana* (37, 98). The fine resolution of this technique should be extremely useful for gene discovery, and will make it the technique of choice where populations and genotypes are available. Phenotyping platforms like ionomics, which analyze many phenotypes simultaneously on the same sample, are poised to efficiently utilize the resources necessary for association mapping.

The availability of the homozygous full-genome knockout collection for *A. thaliana* from The *Arabidopsis* Information Resource was imminent when this review was written. With this collection in hand loss-of-function alleles can be screened for every gene in the *A. thaliana* genome for ionomic phenotypes. Taking such a reverse genetics approach needs no postscreen gene identification, and would require screening of approximately 52,000 individuals (with two alleles for each gene). At a throughput of 1000 samples per week (the current throughput of the Purdue Ionomics Facility) this screen would take approximately 1–2 years. A similar reverse genetics approach is currently underway, but because homozygous lines are limiting until the full collection is available, as of when this article was written ionomic data were available on only approximately 1500 unique genes

(<http://www.purdue.edu/dp/ionomics>; described by Reference 10).

Given the rapid expansion of genetic tools for the established genetic model organisms, including *A. thaliana*, rice, and maize, the future for ionomic gene discovery looks very bright. Extensions of such genetic resources, such as sequenced genomes, mapping populations, and stable transformation systems into other plant species with interesting phenotypic characteristics, will further expand the horizons for ionomics. These new horizons will include new opportunities to understand the regulation of the ionome in other organisms and in relation to their adaptation, ecology, and natural selection.

Assessment of Physiological Status

The ionome of a plant is controlled by a summation of multiple physiological processes, starting in the rhizosphere and ending with evapotranspiration and phloem recycling to and from the shoot. Alterations in any of these processes that are involved in the transport of inorganic ions from the soil solution to the shoot could possibly affect the ionome. Because of this, the shoot ionome is likely very sensitive to the physiological state of the plant, and different ionomic profiles may reflect different physiological states. Such characteristic ionomic profiles, if they exist, could be useful as biomarkers for the particular physiological condition with which they are associated. In plants, ionomic biomarkers may be a simple way to determine if a plant has entered a particular physiological or biochemical state, e.g., cold or drought stress, disease, perturbed cell wall or wax biosynthesis, etc. Ionomic biomarkers may also allow the screening of individual plants for increased susceptibility to particular stresses, or alterations in processes that are not easily measured in high throughput, such as changes in root architecture, cell wall structure, etc. Our recent identification of an ionomic mutant in which low shoot Ca is driven by perturbations in root

cell wall structure (I. Baxter, B. Lahner, A. Rus & D.E. Salt, unpublished observations) supports such an approach. To date little work has been done in plants to establish ionomics biomarkers; however, the concept holds promise.

CONCLUSION

With the \$1000 genome sequence a rapidly approaching reality, high-throughput phenotyping platforms will be critical for the association of genotype with phenotype for the process of gene discovery. Here we discussed the development and application of ionomics as a

high-throughput phenotyping platform, with the capacity to analyze approximately 1000 samples/week with a single analytical instrument. Because the ionome of a plant is the summation of many biological processes, a high-throughput ionomics platform offers a viable system for probing the multiple physiological and biochemical activities that affect the ionome, in tens of thousands of individuals. Ionomics, in combination with other phenotyping platforms such as transcript profiling, proteomics, and metabolomics, therefore offers the potential to close the growing gap between our knowledge of genotype and the phenotypes it controls.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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NOTE ADDED IN PROOF

Association mapping of an ionic trait was recently used for the first time in the identification of a novel mitochondrially localized transporter responsible for regulating molybdenum accumulation in *Arabidopsis*. This was recorded in the following article by Baxter et al.:

Baxter I, Muthukumar B, Park HC, Buchner P, Lahner B, et al. 2008. Variation in molybdenum content across broadly distributed populations of *Arabidopsis thaliana* is controlled by a mitochondrial molybdenum transporter (MOT1). *PLoS Genet.* 4:e1000004. doi:10.1371/journal.pgen.1000004



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