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# What the transcriptome does not tell — proteomics and metabolomics are closer to the plants' patho-phenotype

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The proteome and metabolome of the plant provide a wealth of additional information on plant–microbe interactions since they not only represent additional levels of regulation, but often they harbor the end products of regulatory processes. Proteomics has contributed to our understanding of plant–microbe research by increasing the spatial resolution of the analysis within the infected tissue, because components of the basal immunity were uncovered in the apoplast. Metabolomics has developed into a powerful approach to discover the role of small molecules during plant–microbe interactions in non-model plants since it does not depend on the availability of genome or transcriptome data. Moreover, novel molecules involved in systemic acquired resistance and the precursors for the formation of molecules that provide physical barriers to prevent spreading of pathogens were identified.

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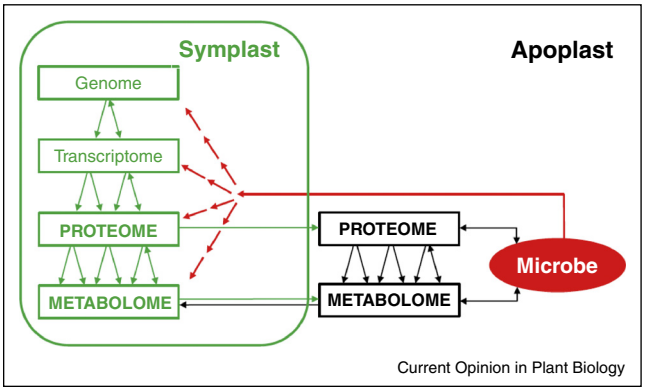
## Introduction

When plants interact with microbes, their metabolic and often morphological phenotype is drastically changed. To understand the complexity of the underlying mechanisms, it is necessary to apply methods that permit a complete assessment of the plant's responses at different hierarchical levels. Rapid advances in plant systems biology have been accomplished by the development and improvement of 'omics' technologies that encompass a set of tools primarily for the determination of the transcriptome, proteome and metabolome as well as to analyze the interactions between these different sets of data forming the interactome [1•].

Till now, transcriptomics is the most frequently applied technique because of relatively low cost and simple handling compared with proteomics or metabolomics [2]. This goes along with developments in increasing the spatial resolutions of the techniques. So far most data focus on the whole-plant, organ or tissue level. However, without proteomics and metabolomics, only a partial understanding of the systems response can be achieved, because not all genes that are transcribed are also translated into functional gene products [3]. Furthermore, temporal scales of translation, protein turnover and metabolite formation may strongly diverge. In response to environmental cues the transcription of genes is induced or switched off leading to changes in the transcriptome. All subsequent processes affecting the proteome and metabolome may be delayed or may even follow different temporal regimes as has been shown for central metabolism [4]. Because the disappearance of the existing proteins and the accumulation of newly produced proteins does not only depend on their transcriptional and translational rates, but also on their degradation rates, which differ between different protein species, changes in the transcriptome are not congruent with changes in the proteome [5]. Furthermore, proteins and their multiple interactions control the biosynthesis of compounds that are required for growth or defense. The metabolome, therefore, adds a further level of complexity to plants' adaptive reactions that furthermore may regulate the proteome and/or the transcriptome (Figure 1).

The proteome and metabolome of the plant do not just mirror transcriptional changes in response to environmental changes but provide a wealth of additional information on the performance of the plant's immune system since they often represent the end products of regulatory processes [6]. Upon plant–microbe interactions existing proteins are activated or deactivated by phosphorylation or the reversible binding of other side groups, for example, phosphorylation, glycosylation, acylation, etc. [7,8••] and thereby trigger signaling cascades that may eventually confer tolerance or susceptibility. In addition to inducible formation of defense compounds, the immune system comprises constitutive components. Proteomics and metabolomics are the methods of choice to study the qualitative and quantitative composition of the constitutive system. Our knowledge of the constitutive defenses is still incomplete because antimicrobial proteins and metabolites, which are already in place and protect the plant from their ubiquitous enemies, have attracted much less attention than the changes set off by distinct pathogens. Sophisticated and

**Figure 1**




Scheme depicting the flow of information and hierarchy of regulatory processes during plant–microbe interactions at different spatial and hierarchical levels. Green: plant-derived levels; red: microbe-derived levels; black: system levels that derive either from the plant or from the microbe or from both.

highly sensitive detection methods have uncovered an incredible diversity of secondary metabolites and a huge variation between different plant species, but so far only a minor fraction of these compounds has been unequivocally chemically identified even with respect to their cellular localization.

## The proteomic tool box

Proteomic techniques are used to detect and quantify proteins that are involved in the interaction with microbes as well as in the response to pathogens [7,8••,9,10,11,12]. Proteome analysis encompasses the extraction and separation of proteins, cleavage to peptides and detection by mass spectroscopy [13]. This workflow is now well established. During recent years a major leap that enhanced the sensitivity was the introduction of gel-free separation of the peptides by liquid chromatography and detection by high resolution tandem mass spectrometry [13]. For quantification the extracted proteins can be separated in-gel and stained, but these methods are relatively insensitive. More sophisticated approaches now use labeling techniques, for instance stable isotopes or isobaric tags (iTRAQ) which are reporter tags of different molecular masses that are covalently bound to the N-terminus of the peptides. Protein identification relies on the availability of transcriptome data or genome sequences (useful lists: http://world-2dpage.expasy.org/list/). Despite the great technological progress, a drawback of proteomics is that many proteins are present in very low quantities and remain undiscovered or the same protein is located in different organelles of the same cell fulfilling different functions at different locations [14]. To enhance the detection limit and the resolution, specific protein fractions are enriched or subcellular compartments are isolated for more specific analyses. The analyses are supported by data bases for the subcellular compartmentation of proteins, including platforms for extracellular proteins ([15], http://proteomics.ysu.edu/secretomes/plant.php), which are of particular interest for plant–microbe interactions because invading pathogens first encounter the extracellular matrix of the plant before they reach the plasma membrane and the cytosol.

## Spatial organization of the plant's defence system uncovered by proteomics

The complement of all proteins that are exported out of the symplast comprises the plants secretome and encompasses apoplastic, that is, soluble, as well as ionically and cell wall-bound proteins. The classical secretion pathway involves targeting of the proteins by a secretion peptide to the extracellular space via the endoplasmic reticulum (ER) and Golgi apparatus. Because of the target sequence, the localization of these proteins can be predicted and is often used to validate the localization of proteins detected in apoplastic extracts. However, among hundreds of unique extracellular proteins that were identified in major model species such as *Arabidopsis*, rice and poplar [16,17,18,19••], the majority lack a target sequence (reviewed and a useful list in [20]). For example Kim *et al.* [19••] identified 470 unique extracellular proteins in rice of which only 37% carried a targeting sequence. Interestingly, the extracellular localization of many of the remaining 67% leaderless proteins [19••] could be backed by searches in databases for the secretomes of bacteria and animals (http://www.cbs.dtu.dk/services/SecretomeP/). The reason is that proteins can also be unloaded into the extracellular compartment by exocytosis, a process that is well characterized for animal cells, but less in plants [21]. Exocytosis is now receiving increasing attention in plants because it is faster than the ER-Golgi secretion pathway [20,22]. A major finding in that regard was that exocytotic vesicles are guided by exocyst proteins that are functionally important to recruit rapid cell wall appositions in response to fungal attack [23].

During pathogen attack the proteome exhibits changes in protein abundances that are not necessarily matched by changes in transcript levels. For example, in response to *Phytophthora infestans* only about half of differentially affected potato proteins showed corresponding changes in the transcriptome [24•]. Global analysis of transcript and peptide intensities indicated a rapid decline of proteins while the transcriptome increased [24•]. In specific cases, matches between increased transcript levels and increased abundance of apoplastic proteins were reported, but the magnitude of the transcript changes was generally larger than that of the protein levels [19,25]. These examples underline the future need to understand the magnitude and timing of global pathogen defense at both the transcriptomic and proteomic levels.

Proteomic analyses are instrumental for the characterization of constitutive and induced defenses, when a priori