Making new molecules — evolution of structures for novel metabolites in plants
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Secondary metabolites are essential plant fitness within the natural environment by providing defense against attacking and competing organisms including bacteria, fungi, insects, animals and other plants. These compounds’ defensive function is frequently intertwined with specific accumulation in novel developmental structures. While, the biochemical community is making great strides in identifying the genetic and biochemical mechanisms that allow these chemicals to be synthesized there is vastly less progress on understanding the developmental mechanisms that is equally key to their defensive function. In this review, I briefly delve into several novel developmental structures and provide evolutionary hypothesis for how they may have evolved and how they could be unique systems for studying key developmental processes that have heretofore been calcitrant to study.

Introduction
A general discussion of plant metabolism is often centered around two major classes. Primary metabolism is the metabolism which allows a plant to utilize water, carbon dioxide and minerals to create metabolites required to make and maintain cells (sugars, fatty acids, amino acids and nucleic acids) [1]. These chemicals were slotted into a biological function early in the evolution of life as reflected by their synthetic genes being largely conserved across all known plants. Secondary metabolites (also referred to as natural products, phytochemicals, defense chemicals, defense metabolites, specialized metabolites, among others) are the chemicals required for plant interactions with the environment (e.g., pest and pathogen defense compounds, ultraviolet-B sunscreens) [1–4]. This relationship of secondary metabolites with an ever-fluctuating biotic environment imparts an evolutionary pressure upon plants to constantly create new secondary metabolites causing most secondary metabolites to be lineage-specific. Interestingly this lineage novelty does not mean that any given secondary chemical is any less essential to the survival of a plant species within its natural environment than primary metabolites, plants lacking the appropriate secondary metabolism frequently have dramatically reduced fitness if any residual fitness in the field [5]. Where primary and secondary chemicals differ is that in the artificial laboratory environment secondary metabolite deficient plants have sufficient viability to be easily manipulated in any genetic, biochemical or cytological study. This makes systems associated with secondary metabolites frequently easier to study and manipulate than those affiliated with primary metabolites.

While secondary metabolites are essential to a plant’s survival, this is only true under certain conditions when the appropriate pathogen, herbivore or competitor is present [6–8]. In the absence of the appropriate pathogen, herbivore or competitor the compounds are highly expensive to synthesize. In Arabidopsis thaliana, the synthesis of the glucosinolate defense compounds imparts a significant cost in utilizing more than 10% of the energy available in the metabolic network with an associated cost of decreased growth [9–11]. This conditionality of benefit imparts a very complex cost/benefit calculation that the plant species must solve to optimize the benefit of the compound while diminishing its associated costs. Numerous models for this optimization focus on the induction of defense compounds in response to the pathogen or insect [12,13]. Another optimization approach is to developmentally or ontogenically regulate the secondary metabolite so that it is present only in the tissues or life stages when the specific attacking organism occurs [14–18]. Together these regulatory systems can form a complex interaction of genotype, ontogeny, tissue development and environment [19,20]. In addition to these macrolevel concepts, another theorized mechanism for the plant to optimize the cost/benefit calculation is to restrict the localization of secondary metabolites to sites/tissues of optimal activity. This idea is supported by the fact that the accumulation of key secondary metabolites is often limited to specialized tissues such as glandular trichomes, laticifers, secretory cavities, secondary phloem, resin ducts or specialized cell compartments like the cell wall, vacuole or cuticular wax layer [21]. This sequestration from the broader tissues allows the defense chemical to be generated in highly localized pockets of higher concentration, simultaneously increasing the immediate

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Glossary
Definitions of specialized tissues discussed within the text. This is not meant as an exhaustive list of all specialized tissues associated with secondary metabolism in plants.

Laticifer: Elongated cells with altered cytoplasm that accumulate latex and frequently also accumulate defense compounds.

S-cell: Cells within the Brassicaceae that have developmental properties similar to the laticifers but accumulate glucosinolates and not latex.

Extrascular phloem: An additional phloem network within the cucurbits that appears to function for the transport of defense compounds.

Glandular trichomes: Specialized multi-cellular epidermal protrusion that is often optimized as a metabolic factory to produce and store volatile oils and other potential defense compounds.

Secretory cavity: Subdermal structures that produce and accumulate defense compounds. Typically the structure has a storage lumen surrounded by secretory/synthesis cells and an outer layer of parenchymatous cells.

Resin duct: A duct within woody tissues that is lined with glandular epithelium to secrete resin and other defense chemicals.

dose to herbivores and pathogens and decreasing the overall synthetic cost of making the chemical in every cell. Another potential benefit to localized accumulation of secondary metabolites is that this limited distribution can decrease the ability of herbivores and pathogens to evolve resistance to the defense chemistry [22,23].

Thus, an essential component of any secondary metabolite defense is the generation and proper function of the specialized accumulation tissue. Yet very little is known about how these specialized tissues (glandular trichomes, secondary phloem, laticifers, secretory cavities, resin ducts, among others) develop or are regulated. The nescience of these specialized developmental systems has long been ascribed to the common knowledge that these tissues are not present in key model systems and as such not genetically amenable. The advent of new genomics technologies is beginning to erode the model systems technical advantage [24]. However, this antimodel system argument also has an underlying presumption that each specialized developmental system is novel and thus, a unique, independent and different evolutionary event with unique genes, among others. At one point, there was a similar argument about floral development and the generation of novelty but the past two decades have shown that the ABC modular model of floral development simultaneously allows for a common set of gene functions to create an impressive range of novelty using gene duplications, gene losses, neofunctionalization and subfunctionalization events [25,26]. In this review, I would like to posit the argument that this reiteration and novelization of common gene regulatory models for fundamental tissues like vasculature and trichomes could also explain the vast majority of plant secondary metabolite specialized tissues. The key difference being that the complete underlying central developmental models have yet to be found and described in a way to allow for novelization of the tissue for nonfloral tissues. In fact it would be easier to identify these underlying gene regulatory models using the specialized novelty developmental structure that was derived from the original structure rather than the original structure.

Vascular specialization and modification for defense metabolism?
A key specialized tissue for plant secondary metabolism is the laticifer, specialized cell types dominantly defined as having latex [27]. Other key characteristics to laticifers are being highly elongated vascular associated cells with altered cytoplasm [27]. More critically to this review, laticifers are highly polyphyletic throughout the plant kingdom which has suggested that most laticifers represent independent evolutions with an implicit assumption of independent genes for each event. However, the fact that nearly every major plant group has laticifers suggests that there must be a central module of genes providing the potential to repeatedly evolve laticifers. The question then becomes what is this central module of genes enabling repeated laticifer evolution?

A potential clue comes from the identification of S-cells within *A. thaliana* [28,29]. S-cells are inflorescence phloem associated elongated cells with altered cytoplasm and high levels of the glucosinolate secondary metabolites. As such, S-cells have been proposed to be identical developmental structures to laticifers. Removing the latex requirement for laticifer definition could allow for more plant species to have laticifer-like structures than has originally been described. While Arabidopsis leaves do not have classical S-cells they do have unique single cells scattered along the vascular bundle that can be seen when using glucosinolate-specific markers [30]. This positioning is identical to where an S-cell/laticifer should be located but these glucosinolate idioblasts do not connect to form a contiguous network. The repeated positioning near the vasculature for glucosinolate idioblasts, S-cells and laticifers suggests a hypothesis that laticifers/S-cells are evolutionarily derived from a vascular developmental module. This specialized cell type is often phloem associated suggesting that it may represent a novelization of the phloem developmental program. Additionally, laticifers can show tip development which is also true of xylem during secondary growth [31]. Thus, if this novel vascular hypothesis is true, it would require the combination of components from the xylem and phloem modules. Unfortunately, the absolute requirement for an intact vasculature to create a viable plant has inhibited our understanding of the underlying genetic modules. However, if this novelization of a vascular module hypothesis is true it suggests that identifying the genetic module controlling laticifer/S-cell development could be an easier avenue to understanding the genetics of vascular development. An interesting test of this model would be to analyze all known Arabidopsis
vascular or auxin mutants for alterations in S-cell development.

Another potential novel modification of a plant vascular developmental program for the specific purpose of secondary metabolism is present within the cucurbits. The cucurbits have two phloems, one within the vascular bundle, the fascicular phloem, and an extrafascicular phloem that is scattered through the stem. Recent work has shown that the extrafascicular phloem is largely transporting defense-related compounds like secondary metabolites while the fascicular phloem is facilitating the traditional phloem transport functions [32]. Interestingly, extrafascicular phloem proteins have a completely different proteome and metabolome suggesting that this is the evolution of a complete new transport system derived from the phloem, almost as if the phloem duplicated and then subfunctionalized. Very little is understood about the evolution of complicated new systems, networks or tissues and this system could provide a key system to investigate how a complex structure like the phloem can be retooled to create a completely new vascular component.

Asymmetric cell division and glandular trichomes

Another specialized developmental structure that is critical to the function of many plant defense compounds is glandular trichomes. These structures arise via a complex arrangement of precise cell divisions. For instance, the mint glandular trichomes first arise as protruding epidermal cells that then undergo a precise series of cell divisions including symmetric and asymmetric periclinal and anticlinal divisions to produce the precise glandular trichome structure [33] (Figure 1). Interestingly, glandular trichome development in Artemisia which also begins as an epidermal protrudance proceeds by a completely different timing and patterning of the cell divisions leading to a different glandular trichome structure [34] (Figure 1). Thus, both species have developed fundamentally the same structure using precise coordinated cell divisions but each species utilizing different patterns. The question then becomes have these two species utilized the same cellular regulatory genes to evolve these different sequence of cell division patterns. Further, is the final structure the optimal glandular trichome for each species or is the final structure a consequence of the stochastic nature of evolution and how and when the two species co-opted cellular division genes to the process of trichome formation? Intriguingly, some species have multiple types of glandular trichomes raising the possibility that each type has a different cellular program [35,36,37].

In spite of this fascinating developmental program with specific and differential cell division, almost nothing is known about the genetics underlying the development of glandular trichomes. The main trichome model is the Arabidopsis hair trichome which is a single cell with endoreduplication in contrast to the multi-cellular glandular trichome [38,39]. While it is tempting to postulate that hair trichomes and glandular trichomes utilize the same genetic program with hair trichomes eliminating the cellularization involved in forming glandular trichomes this may not be the case. In tomato that has both hair and glandular trichomes it is possible to genetically abolish glandular trichome formation without concomitant effects on hair trichomes suggesting that in tomato the hair and glandular trichomes use different genetic machinery [40]. Thus glandular trichomes and the specific cell division patterning are an underutilized study system that should provide enticing insights with significant implications both for plant defense and the control of cell division within plants.

Using defense chemistry to find new cell types

Developmental structures are almost always defined by a visual characteristic either directly observable like trichomes or flowers or enhanced by chemical staining like the casparian strip. Thus, our understanding of plant development is limited by these very developmental assays and visualization approaches. This limitation in visualization raises the potential that the application of new techniques to developmental investigations may identify previously unknown cell types. One such technology being applied to developmental questions is mass-spectrometry to map the patterning of secondary growth patterns.
metabolites within specific tissues. Mapping flavonoid accumulation in Arabidopsis flowers showed that the petal actually has two different developmental zones that can only be separated by the differential accumulation of two flavonoids [41]. A similar mapping of glucosinolate accumulation within the Arabidopsis leaf identified three sites of accumulation, near the vasculature, at the leaf margin and distributed cells within the leaf [42]. While the vasculature sites are probably unrecognized vascular idioblasts based on gene-fusion studies [30,43,44] and the leaf margin is a relatively unstudied tissue, the potentially most interesting accumulation site are the distributed cells within the leaf. These appear to be single cells that are previously unrecognized mesophyll idioblasts and show a stochastic distribution within the mesophyll. These glucosinolate mesophyll idioblasts are solely recognizable by the accumulation of glucosinolates and the expression of specific promoters for glucosinolate pathway genes [45,46]. This glucosinolate mesophyll idioblast would only have been found using molecular approaches targeted specifically to the glucosinolate pathway. The finding of a new potential tissue with a single metabolic pathway raises the intriguing question of how many other unrecognized cell types may be found when researchers begin targeting each specific secondary metabolic pathway and its tissue patterning in intact tissues.

Defense chemistry modifying development

While development is usually considered to be the framework upon which secondary metabolism is organized, there is beginning to be intriguing evidence that plant secondary metabolites may be perceived by the plant to control developmental processes. In tomato, a tomatine derivative can lead to the induction of programmed cell death in what appears to be a direct recognition rather than a generic toxicity mechanism [47,48]. Similarly, glucosinolates have been linked to controlling developmental processes like flowering time in Arabidopsis via what also appears to be a specific recognition event [49]. This ability of plants to specifically utilize their secondary metabolites to regulate development is best illustrated in Raphanus sativa where a specific glucosinolate has taken over the role of auxin in controlling hypocotyl responses to light by interacting with the TIR1 receptor [50,51]. In this system, the plant has evolved a replacement for auxin in a key developmental process using a chemical that is specific to R. sativa. Thus, it is possible to rapidly evolve developmental programs both in terms of their consequence and their cause. The impact of plant secondary metabolites on plant development is typically not investigated so it remains to be seen how frequently secondary metabolites may influence development.

Conclusions and future perspectives

This review has focused on how secondary metabolites and their accumulation sites can provide unique opportunities for the study of plant development and cellular patterning. The very fact that secondary metabolites are not conserved may actually simplify these studies by allowing these developmental systems to be genetically manipulated. Further, the developmental structures while apparently independently evolved like laticifers are probably facilitated by the use of a common developmental genetic program possibly from a more essential tissue like the central vasculature. This will allow for rapid application of information from one laticifer system to another or one glandular trichome to another. Finally, the development of better metabolite visualization platforms should greatly help our understanding of how many cell types and tissues actually occur within a plant. Secondary metabolism and developmental studies have the potential for great future synergism that will hopefully be quickly tapped.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


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The authors look at the dual phloem system of the cucurbits and show that while the physical structures of the two are highly similar, the molecular details are nearly completely divergent. This suggests that plants have an amazing ability to adapt complex developmental structures to new purposes.


The authors provide evidence that the S-cells within Arabidopsis are likely equivalent structures to laticifers and thus provide a drive to remove the requirement for latex accumulation from the definition of a laticifer.


The site of synthesis and accumulation in plant secondary metabolism is frequently in different tissues. This requires a complex transport system with numerous specific transporters. The authors begin the process of finding some of the first secondary metabolite-specific transporters.


6 Growth and development


The authors provide evidence that the specific glucosinolates within Arabidopsis are likely perceived by the plant to alter key developmental and physiological processes like the circadian clock and flowering time. This continues to raise the likelihood that development and secondary metabolism are interlinked to optimize the resulting defense phenotype.
