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Making iridoids/secoiridoids and monoterpenoid indole alkaloids: progress on pathway elucidation

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Members of the Acanthaceae, Apocynaceae, Bignoniaceae, Caprifoliaceae, Gentianaceae, Labiatae, Lamiaceae, Loasaceae, Loganiaceae, Oleaceae, Plantaginaceae, Rubiaceae, Saxifragaceae, Scrophulariaceae, Valerianaceae, and Verbenaceae plant families are well known to accumulate thousands of bioactive iridoids/secoiridoids while the Apocynaceae, Loganiaceae and Rubiaceae families also accumulate thousands of bioactive monoterpenoid indole alkaloids (MIAs), mostly derived from the secologanin and tryptamine precursors. Several large-scale RNA-sequencing projects have greatly advanced the tools available for identifying candidate genes whose gene products are involved in the biosynthesis of iridoids/MIAs. This has led to the rapid comparative bioinformatics guided elucidation of several key remaining steps in secologanin biosynthesis as well as other steps in MIA biosynthesis. The availability of these tools will permit broad scale biochemical and molecular description of the reactions required for making thousands of iridoid/MIAs. This information will advance our understanding of the evolutionary and ecological roles played by these metabolites in Nature and the genes will be used for biotechnological production of useful iridoids/MIAs.

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Introduction

Plants are the source of many thousands of complex chemicals that include specialized metabolites such as phenols, terpenes and alkaloids. The alkaloids alone contain nitrogen in their basic structures that are derived mainly from the a few amino acid precursors and that confer unique biological activities to this class of molecules. In spite of their important pharmacological activities and medicinal uses, the pathways of alkaloid biosynthesis remain poorly understood. This lack of progress has been attributed to the unavailability of the substrate intermediates required for detecting the biochemical reactions in multistep pathways and to inherent difficulties associated with the slow traditional forward genetic approaches for identifying the genes involved.

The present review will focus on the recent use of large-scale transcriptome projects [Phytometasyn (http://www.phytometasyn.com/; [1•,2•]); Medicinal Plant Genomics Consortium (http://www.medicinalplantgenomics.msu.edu/; [2•,3•,4]); Medicinal Plant Transcriptome Project (http://www.uic.edu/pharmacy/MedPlTranscriptome/index.html)] and CATHACyc (http://www.cathacyc.org/; [5•]) that are speeding up the discovery of monoterpenoid indole alkaloid biosynthesis pathways.

Monoterpenoid indole alkaloids

The monoterpenoid indole alkaloids (MIAs) represent one of the largest and most biologically active classes of special metabolites found in thousands of species of plants of the Apocynaceae, Loganiaceae and Rubiaceae families. These MIAs include the *Catharanthus roseus* vinblastine/vincristine and *Camptotheca acuminata*/*Ophiorhiza pumila* camptothecin anticancer alkaloids, the antimalarial quinine from *Cinchona ledgeriana*/*C. succirubra* and the antihypertensive drug ajmalicine from *Catharanthus rosues*/*Rauvolfia serpentina*. The pathways involved require the condensation of secologanin with tryptamine to yield strictosidine that is then converted to several thousand MIAs found in Nature. While almost 200 MIAs have been described in *C. roseus*, catharanthine and vindoline are present in greatest abundance and have great commercial value since dimers of these two compounds are drugs used in cancer chemotherapy. The Catharanthus literature related to MIA biosynthesis, its organization in different leaf cell types [6–8] and its biotechnological applications [9•] has recently (been) reviewed and has been extensively reviewed over the past 10 years.

Missing genes for the supply of geraniol in iridoid biosynthesis

Higher plants have cytosolic mevalonic acid and plastidic methyl erythritol phosphate (MEP) pathways that provide isoprenoid precursors for a large range of monoterpene, sesquiterpene, diterpene, triterpene, tetraterpene and polyterpene. In "*range of monoterpene, sesquiterpene, diterpene, triterpene, tetraterpene and polyterpene products*" *roseus*, most of MEP pathway genes have been characterized and were shown to be preferentially expressed in specialized Internal Phloem Associated Parenchyma (IPAP) cells (Figure 1; [6,7]) in order to supply precursors for the assembly of iridoid precursors required for the biosynthesis of secologanin in *C. roseus*. These IPAP cells also preferentially express geraniol 10-hydroxylase (G10H) (Figure 1, reaction 2) and 10-hydroxygeraniol oxidoreductase (10HGO) (Figure 1, reaction 3), while the two terminal reactions in secologanin biosynthesis are known to be preferentially expressed in the leaf epidermis (Figure 1, reactions 8 and 9 [6]). However several genes including isopenenyl diphosphate synthase, geraniol synthase (Figure 1, reaction 1) and other gene products responsible the formation of loganic acid remained to be described (Figure 1, reactions 4–7; iridoid synthase/monoterpene cyclase, 7-deoxyloganetic acid synthase, 7-deoxyloganetic acid glucosyltransferase, and 7-deoxyloganic acid hydroxylase).

Isopentenyl diphosphate isomerase (IDI) mediates the equilibrium and supply of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) required for the assembly of geranyl pyrophosphate (GPP). Recent studies have described a single gene in Catharanthus with alternative transcription sites to generate different isoforms of CrIDI that appear to be targeted to peroxisomes, mitochondria and plastids [10]. Presumably the plastid targeted isoform would also be expressed in IPAP cells where it would participate in providing IPP and DMAPP for biosynthesis of GPP.

While the MEP pathway is responsible for the formation of IPP and DMAPP, geranyl pyrophosphate synthase (GPPS) is required for the formation of GPP. Recently several *CrGPPS* genes identified from *C. roseus*, through database searches (www.ncbi.nlm.nih.gov/and http://medicinalplantgenomics.msu.edu) [11] were functionally characterized. Three separate types of CrGPPS were functionally characterized with two of them being expressed in chloroplasts and one of them more likely to be targeted to mitochondria. While the results complemented previous studies on the functional characterization and plastid localization of one of these genes [12] several other CrGPPS genes were also described to be present in Catharanthus [11] that make it difficult to establish how many of these genes provide precursor GPP for the biosynthesis of iridoids. Therefore, it is interesting and perhaps understandable that neither study reported if any of these CrGPPS genes might be preferentially expressed in IPAP cells.

The molecular cloning of *C. roseus* geraniol synthase (*CrGS*) was also recently described and recombinant protein was shown to convert geraniol pyrophosphate (GPP) to geraniol when expressed in *Escherichia coli* [13]. A significant finding of this study was that *CrGS* appears to be preferentially expressed in IPAP cells where it converts MEP-derived GPP to produce the geraniol required for the G10H that produces 10-hydroxygeraniol. IPAP cells are known to convert geraniol to 10-oxogeranial via G10H (Figure 1, reaction 2) and 10HGO (Figure 1, reaction 3). Together these characterizations of CrGPPS and CrGS complete the metabolic connection of the MEP pathway to G10H.

Assembly of secologanin

Over 2500–3000 iridoids/secoiridoids with a wide variety of biological properties [14,15] have been characterized after isolation from members of the Acanthaceae, Apocynaceae, Bignoniaceae, Caprifoliaceae, Gentianaceae, Labiatae, Lamiaceae, Loasaceae, Loganiaceae, Oleaceae, Plantaginaceae, Rubiaceae, Saxifragaceae, Scrophulariaceae, Valerianaceae, and Verbenaceae plant families. These unusual monoterpenoids contain a methylcyclopentan-[*c*]-pyran skeleton typically fused to a six-membered oxygen containing heterocycle. While iridoids participate in MIA biosynthesis within the Apocynaceae, Loganiaceae and Rubiaceae families this mostly involves a single iridoid, secologanin that provides a C_{10} or C_9 or ocassonally a C_8 fragment to the formation of MIAs. However the levels of MIAs produced may be small compared to the levels of iridoids that accumulate within MIA producing plant species. Recent studies in *C. roseus* have shown that the levels of secologanin in Catharanthus leaves were 10–15 times higher than those of the major MIAs, catharanthine and vindoline [16–18]. This raises important questions about the biological importance of maintaining high levels of secologanin in Catharanthus and perhaps in other MIA accumulating plant species where little documentation exists about the levels of these iridoids. The mobility of iridoids was recently documented when photosynthesis was measured in Snapdragon (*Antirrhinum majus*) leaves by feeding them with $^{14}CO_2$ [19]. Measurements showed that 47% of the phloem mobile ^{14}C-photoassimilate was the iridoid antirrhinoside with sucrose making up the rest. The study suggested that rapid conversion of the products of photosynthesis to toxic antirrhinoside could provide a selective advantage to iridoid producing species by deterring herbivory by phloem feeding insects and this was supported in other studies with *Alonsoa meridionalis* and *Asarina barclaiana* describing the phloem mobility of antirrhinoside [20]. This raises interesting questions about the mechanisms of iridoid biosynthesis that might involve biochemical cellular specialization events that may be common to iridoid and/or MIA producing plant species