The cell and developmental biology of alkaloid biosynthesis

Vincenzo De Luca and Benoit St Pierre

Plants produce unique natural products as a result of gene mutation and subsequent adaptation of metabolic pathways to create new secondary metabolites. However, their biosynthesis and accumulation remains remarkably under the control of the biotic and abiotic environments. Alkaloid biosynthesis, which requires the adaptation of cellular activities to perform specialized metabolism without compromising general homeostasis, is accomplished by restricting product biosynthesis and accumulation to particular cells and to defined times of plant development. The cell and developmental biology of alkaloid biosynthesis, which is remarkably complex, evolved in part by recruiting pre-existing enzymes to perform new functions.

Alkaloids are low molecular weight nitrogen-containing substances with characteristic toxicity and pharmacological activity. These properties, which have traditionally been exploited by humans for hunting, execution and warfare, have also been used for the treatment of disease. About 20% of plant species accumulate alkaloids, which are mostly derived from the amino acids, Phe, Tyr, Trp, Lys and Orn. In addition, the monoterpene indole alkaloids, which form a large class of complex compounds, are derived from Trp and terpenoid precursors. Over 12 000 different alkaloids have been described, indicating their structural and biosynthetic diversity compared to that of other secondary metabolites (Table 1).

Our extensive knowledge about the chemistry and pharmacology of alkaloids has led to their use in a range of medical applications. However, little is known about their biosynthesis or about the factors that regulate alkaloid production. Recently, intensive efforts have elucidated the basic biochemistry and molecular biology of some alkaloid pathways and, more generally, of secondary metabolism. These discoveries are now enabling more rapid consensus cloning and identification of highly specific biochemical reactions involved in the production of interesting alkaloids.

Root-specific scopolamine and nicotine biosynthesis

Roots have been shown to elaborate a remarkable variety of secondary metabolites, including alkaloids, flavonoids and terpenoids. Nicotine and tropane alkaloids, which are mainly produced in roots as suggested by reciprocal grafting experiments, accumulate mostly within vacuoles of plant roots and leaves. The early pathway leading to biosynthesis of both nicotine (Fig. 1) and tropane (Fig. 2) alkaloids involves the formation of a pyrrolidine ring that is derived from putrescine via the sequential action of an S-adenosyl-L-methionine-dependent putrescine-N-methyltransferase (PMT), a diamine oxidase and a spontaneous chemical rearrangement. The presence of PMT is unique to alkaloid-producing plants and it appears to have evolved from spermidine synthase (SPDS), which catalyzes the transfer of the aminopropyl moiety of decarboxylated S-adenosyl-L-methionine to form spermine.

PMT enzyme activity has primarily been detected within roots of tropane alkaloid-producing Atropa belladonna, Hyoscyamus niger and Datura stramonium, and this has been confirmed by RNA gel blot analyses for A. belladonna. Histochemical analyses of transgenic A. belladonna expressing β-glucuronidase behind

<table>
<thead>
<tr>
<th>Secondary metabolites</th>
<th>Number of structures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>12 000</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>30 000</td>
</tr>
<tr>
<td>Phenylpropanoids</td>
<td>2500</td>
</tr>
<tr>
<td>Others</td>
<td>2500</td>
</tr>
</tbody>
</table>

Table 1. Plant secondary metabolites with known structures
the PMT promoter shows that GUS is expressed specifically in root pericycle cells\(^3\) (Fig. 2). The pericycle-specific expression of hyoscyamine-β-hydroxylase, which catalyzes the last step in scopolamine biosynthesis, has also been demonstrated by in situ hybridization studies\(^1\) (Fig. 2). Localization of the first and last steps in scopolamine biosynthesis in the pericycle, suggested that the rest of the pathway could also be localized there. In addition this suggests that if the root pericycle is the manufacturing centre for tropane alkaloids, its proximity to the adjacent xylem cells probably facilitates the transport of alkaloids to leaves, where they are known to accumulate.

\(\text{N}\)-methylputrescine is converted to tropine, through an incompletely understood pathway (Fig. 2) that involves the participation of phenylalanine-derived phenylactic acid\(^4\). Tropine is then converted to tropine or to pseudotropine, respectively, by tropine reductase (TR) I or II, which catalyze opposite stereospecific reductions\(^5\). These enzymes are part of a short chain dehydrogenase or reductase gene family, with numerous homologous genes occurring in several plant species that do not make tropane alkaloids. This suggests that some plant homologs might function in other primary metabolic pathways, from which the TR enzymes were possibly recruited.

In contrast with the pericycle-specific expression observed for the first and last steps in scopolamine biosynthesis, TR-1 protein is expressed strongly only in the endodermis and outer cortex\(^6\) (Fig. 2). The different cellular compartmentation of PMT, TR-1 and HH suggests that biosynthetic intermediates must be shuttling between the pericycle and the endodermis to complete the biosynthetic cycle leading to the production of scopolamine. Further studies to determine the sites of PMT, TR I and HH expression that only roots express these enzyme activities and contain the proteins that react to antibodies raised against each enzyme\(^7\). Dissection of young roots into 5-mm segments has revealed that the expression of each protein is restricted to a region 1–3 cm from the root apex, whereas they are absent in more differentiated root sections. In more mature roots, reactive PMT, TR I and HH proteins are only detected in the region 1–3 cm from the root apex of the developmentally younger lateral roots. These results confirm that tropane alkaloid biosynthesis is developmentally-regulated, in addition to being cell-type specific.

The localization of PMT to the pericycle\(^8\), where amino acids transported through the vascular tissue are unloaded, would allow ready access to ornithine or arginine, the precursors of putrescine\(^9\). \(\text{N}\)-methylputrescine or some biosynthetic intermediate could then be transported to the endodermis for further elaboration. It is unclear if tropine is then further elaborated into hyoscyamine before being transported back to the pericycle for conversion into scopolamine. In this context, it would be relevant to identify the location of other enzymes involved in the biosynthesis of tropane alkaloids, such as those that convert phenylalanine into phenylactic acid, and those involved in the formation of the tropine ester precursor of hyoscyamine\(^10\). This information could be useful to complete our understanding of the cell-specific compartmentation of tropane alkaloid biosynthesis.

**Indole alkaloid biosynthesis in the Catharanthus roseus model system**

*Catharanthus roseus* (Madagascar periwinkle) produces monoterpenoid indole alkaloids that are derived from the shikimate and the deoxyxylulose pathways. The biosynthesis of the indole moiety requires tryptamine, which is derived from tryptophan by the action of tryptophan decarboxylase (TDC). The biosynthesis of the terpenoid moiety requires secoligandin, which is derived from geraniol via a series of enzymatic conversions\(^11,12\). The committed step in monoterpenoid indole alkaloid biosynthesis involves a vacuole-specific\(^13\) strictosidine synthase (Str1), which catalyzes the stereospecific condensation of tryptamine and secoligandin to yield \(3\alpha\)-(5)-strictosidine (Fig. 3). Several thousand strictosidine-derived indole alkaloids have been characterized, a few of which are used for the treatment of hypertension and...
The regeneration of shoots and in shoot tissue cultures, the ability to synthesize and accumulate vindoline reappeared with sis was further corroborated by studies that suggested that the biosynthesis of catharanthine and tabersonine, but not vindoline is made in the intact plant and in dissecting the unique morphogenetic- and environment-specific events involved in activating the vindoline pathway. If Catharanthus seeds are germinated and grown in the absence of light, they accumulate high levels of tabersonine, as well as small amounts of four other intermediates between tabersonine and vindoline. The growth of etiolated seedlings in the presence of light, stimulated the quantitative, large-scale turnover of tabersonine and the intermediates of vindoline by sequential aromatic hydroxylation, O-methylation, hydration, N-methylation, hydroxylation and O-acetylation (Fig. 1). These results suggest that light treatment activates the late stages of vindoline biosynthesis, and that three hydroxylases, O-methyltransferase, N-methyltransferase and O-acetyltransferase are involved in the conversion of tabersonine into vindoline.

Further biochemical studies have resolved that:

- Tabersonine-16-hydroxylase belongs to the class of cytochrome P450-dependent mono-oxygenases, which are usually associated with the external face of the endoplasmic reticulum.
- N-methyltransferase is associated with chloroplast thylakoids.
- Terminal desacetoxyvindoline-4-hydroxylase (D4H) and deacetylvindoline-4-O-acetyltransferase (DAT) are cytosolic enzymes (Fig. 1).

These results indicate that the cytosolic face of the endoplasmic reticulum, the vacuole, the chloroplast thylakoid and the cytosol of each cell are all involved in vindoline biosynthesis.

**Cell-specific distribution of TDC, STR1, DAT and D4H**

The genes for cytochrome P450 tabersonine-16-hydroxylase (Ref. 26), D4H (Ref. 25) and DAT (Ref. 8) have been cloned recently. Detailed immunological and molecular studies strongly suggest that D4H and DAT are only expressed in the above-ground plant parts, whereas TDC and STR1 occur throughout the plant.

C. roseus has simple, elliptical mesomorphic leaves (Box 1) composed of several cell types. Indole alkaloid biosynthesis is under cell-, tissue-, development- and environment-specific control (Fig. 2).

**Fig. 2.** Histochemical localization of tropane alkaloid biosynthesis in root cross-sections. (a) Putrescine-N-methyltransferase (PMT) promoter GUS fusion expression has been detected by GUS staining in roots of *Atropa belladonna*. Reproduced, with permission, from Ref. 13. Immunological localization of (b) tropine reductase (TR-1) and (c) hyoscyamine 4b-hydroxylase (H6H) in roots of *Hyoscyamus niger*. (b) and (c) reproduced, with permission, from Ref. 17. The three biochemical steps leading to scopalamine biosynthesis and their cellular location are depicted, respectively, in red (PMT), green (TR-1) and blue (H6H). The Phenylacetic acid component of hyoscyamine is derived from Phe. Abbreviations: Ep, epidermis; OC, outer cortex; IC, inner cortex; En, endodermis; P, pericycle.

**Box 1.** The mesophyll corresponds to the mesophyll of stems, leaves (Fig. 3) and flower buds, whereas they appear in most protoderm and cortical cells around the apical meristem of root tips (Fig. 3). D4H and DAT are associated with laticifers and

for several antineoplastic ailments. In particular, vinblastine and vincristine from *C. roseus* are valuable chemotherapeutic agents currently used in the treatment of several cancerous diseases (Fig. 1).
idioblast cells of leaves, stems and flower buds (Fig. 3). These results provide significant new evidence that, in addition to intracellular specialization involving multiple cellular compartments, at least two cell types requiring intercellular translocation of a pathway intermediate are needed to allow the biosynthesis of vindoline in *C. roseus*.

Additional studies27 have shown that alkaloid biosynthesis only occurs transiently during early leaf, stem and root development. *In situ* hybridization and immunocytochemical studies have revealed that alkaloid biosynthesis follows a basipetal distribution in young expanding leaves. This expression pattern, together with the results obtained in young roots (Fig. 3), suggests that alkaloid biosynthesis is activated in young rapidly dividing cells, whereas it is down-regulated in more mature tissues.

The results raise interesting questions about:

1. The number of pathway steps occurring in the epidermis or in the root apex.
2. Whether alkaloid biosynthesis in roots and in epidermal tissues serves different biological and ecological functions by producing different indole alkaloid end-products.
3. Which of these tissues produces specific intermediates for vindoline biosynthesis.

4. How intermediates are mobilized into laticifers and idioblasts.
5. Why this elaborate cell-specific expression is required.
6. Which alkaloids accumulate in laticifers, idioblasts, epidermis and roots.

Although it is not known which alkaloids are made in the epidermis apart from strictosidine, it is well known that roots

---

**Box 1. Glossary**

**Idioblast**—specialized plant cell that contains a distinctive chemical composition compared with surrounding cells.

**Laticifer**—there are two main types, both are long and narrow and can be branched or unbranched.

**Nonarticulated (non-branched) laticifer**—specialized cell that contains a milky, chemically complex fluid called latex. Non-articulated laticifers initiate as single cells and grow to a large size by intrusive growth (pushing their way between other cells). They continue to grow as the plant grows and become giant coenocytic cells.

**Mesomorphic leaf**—leaves adapted to environments where there is adequate soil moisture.

---

![Cell-specific localization of idc, str1, d4h and dat mRNAs in developing Catharanthus leaves and roots27. Leaf or root cross-sections were reacted with antisense digoxigenin-labeled transcripts. Hybridized transcripts were localized with antidigoxigenin-alkaline phosphatase conjugate followed by BCP/Nitro tetrazolium color development. (a) Both idc and str1 (data not shown) are expressed in the upper and lower leaf epidermis. (b) Both d4h (data not shown) and dat are expressed in leaf idioblasts and laticifers. (c) Both idc and str1 (data not shown) are expressed in root protoderm and cortical cells around the apical meristem of root tips. Abbreviations: C, root cortex; CL, cross-connecting laticifer cells; LE, lower epidermis; PI, palisade mesophyll-associated idioblast cells; SI, spongy mesophyll-associated idioblast cell; T, tracheid; UE, upper epidermis. Reproduced, with permission, from Ref 27.](image-url)
accumulate many alkaloids, including catharanthine, tabersonine and derivatives of tabersonine. Therefore it is possible that root-generated catharanthine and tabersonine are transported via xylem and laticifer-associated tracheids to supply these precursors to laticifers. This is plausible because there are several examples of phloem and xylem-based transport of alkaloids2,5. The laticifer could convert tabersonine into vindoline, which would then be coupled by a non-specific peroxidase-mediated process36 with catharanthine to yield the antineoplastic agents vinblastine and vindicine. Therefore the localization of the late stages of vindoline catharanthine to yield the antineoplastic agents vinblastine and vindicine can be explained by the fact that the acyl donor, tigloyloxyacetate ester, is localized to a membrane fraction with a different specific gravity from those of the endoplasmic reticulum and plant vacuoles. The biochemical localization results were in part corroborated when the berberine bridge enzyme was cloned22 and it was shown to contain a 22 amino acid signal peptide directing it to the endoplasmic reticulum. Additional studies in the Catharanthus model system for indole alkaloid biosynthesis have also shown that Str1 contains a putative signal peptide that is cleaved from the mature protein25, which appears to be located within plant vacuoles23. These results suggest that unique specialized cellular compartments in plants serve as scaffolds for alkaloid biosynthesis.

Several vascular homologs of BBE (Accession no. A080254) and Str1 (Accession no. P09276) have been identified in Arabidopsis recently, which is not known to make alkaloids. The presence of these homologs could indicate that the BBE and Str1 reactions occur in the vacuole because these genes evolved from common ancestors that had other vacuolar functions. Therefore, the complex intracellular compartmentation of alkaloid biosynthesis might have occurred as a consequence of adapting compartmented reactions of primary metabolism to participate in alkaloid biosynthesis. The recent cloning of homospermidine synthase34, the first pathway-specific enzyme of pyrroloizidine alkaloid biosynthesis, clearly showed that it had evolved from deoxyhypusine synthase (DHS). DHS is highly conserved among eukaryotes and archaeabacteria where it catalyzes the first step in the activation of translation initiation factor 5A, which is essential for eukaryotic cell proliferation. This is a further example of recruiting a gene involved in a primary process to perform a pathway-specific function in alkaloid biosynthesis.

Conclusions

Recently, rapid progress has been made in our understanding of the biochemistry, molecular biology and cell biology of alkaloid biosynthesis in plants. This fundamental understanding is expected to lead to rapid progress in the isolation of almost any desired gene. The availability of these tools has been used to study the cell and developmental processes leading to the synthesize and accumulation of alkaloids that are often toxic to the host as well as to potential predators. The data from several different alkaloid-producing plants suggests that their biosynthesis and accumulation involve a highly regulated process that includes cell-, tissue-, development- and environment-specific controls. The evolution of alkaloid pathways together with their cellular compartmentation appears to be closely associated with the primary reactions from which they have evolved. With the availability of an increasing number of genes involved in alkaloid biosynthesis, increasing efforts will be made to identify the regulators35 that are associated with the development of specialized cell-types that accommodate alkaloid biosynthesis and accumulation. Continuing studies promise to yield exciting new discoveries about the biological origins of alkaloid biosynthesis in plants.

Table 2. Xylem and phloem transport of alkaloids

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Xylem</th>
<th>Phloem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinolizidine alkaloids</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Pyrrolizidine alkaloids</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Polyhydroxy alkaloids</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>Nicotian alkaloids</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>Tropane alkaloids</td>
<td>×</td>
<td>×</td>
</tr>
</tbody>
</table>

*Reproduced, with permission, from Ref. 5.

Abbreviations: ×, transported; −, not transported.

The chloroplast as the site of quinolizidine alkaloid biosynthesis

Biosynthesis of lysine-derived quinolizidine alkaloids, which are found in many legumes, such as lupins (Lupinus), appears to occur within the mesophyll chloroplasts of green leaves1. A biochemical-localization study has been performed with two acyltransferases that catalyze terminal acylations leading to the production, respectively, of (+) p-coumaryloxyphenylethylamine and (+) -13x-tigloyloxy-methylfuranone (Fig. 1). This study in Lopinus albus demonstrated that one acylation occurred within the cytoplasm and the other in mitochonrdia, but notwithin chloroplasts. These findings suggest that although chloroplasts might be responsible for the formation of the quinolizidine skeleton, further modifications must be occurring after intracellular transport to the cytosol and to the mitochondria. The need for tigloylation to occur within the mitochondria can be explained by the fact that the acyl donor, tigloyloxyacyl-CoA, which is derived via a three-step process from isoleucine, is produced within mitochondria in animal systems. Therefore an analogous pathway might be present in plant mitochondria. The products of biosynthesis are thought to accumulate particularly within the vacuoles of the epidermis of lupin plants, where their defensive roles can be most effectively exploited by the plant33. This adds the mitochondria to the growing list of cellular organelles that might participate in alkaloid biosynthesis. These studies also illustrate how plants have been opportunistic to adapt primary metabolic processes, as well as the organelles in which they evolved, to permit the diversification of secondary metabolism.

Isoquinoline alkaloid biosynthesis

The biosynthesis of isoquinoline alkaloids13,18 proceeds via the decarboxylation of tyrosine or DOPA to yield the respective amine precursors. Dopamine and p-hydroxyphenylacetaldelyde are then combined by the action of (-) norcoclaurine synthase to yield (S)-norcoclaurine, which is a central precursor to several thousand isoquinoline alkaloids. In situ-hybridization studies in opium poppy (Papaver) with tyrosine/DOPA decarboxylase (TYDC) have revealed that the expression of two subgroups of differentially expressed genes are restricted to the metaphloem and to the protoxylem of vascular tissues in mature stems and roots31. Opium poppy alkaloids accumulate within laticifers, which are derived from the metaphloem. The restriction of TYDC expression to these particular cells illustrates the strict control to which this gene family is subject.

Further biochemical localization studies36 have been performed using Berberis wilsoniae cell cultures. Berberine bridge enzyme (BBE) and (S)-tetrahydroberberine oxidase, which catalyze the third to last and final steps of berberine (Fig. 1) biosynthesis, are localized to a membrane fraction with a different specific gravity from those of the endoplasmic reticulum and plant vacuoles. The biochemical localization results were in part corroborated when the berberine bridge enzyme was cloned22 and it was shown to contain a 22 amino acid signal peptide directing it to the endoplasmic reticulum. Additional studies in the Catharanthus model system for indole alkaloid biosynthesis have also shown that Str1 contains a putative signal peptide that is cleaved from the mature protein25, which appears to be located within plant vacuoles23. These results suggest that unique specialized cellular compartments in plants serve as scaffolds for alkaloid biosynthesis.
Acknowledgements

We thank Takashi Hashimoto for the photographs used in Figure 2. We also thank Michael Wink for sharing unpublished reviews. This work was supported by a grant to V.D.L. from the Natural Sciences and Engineering Council of Canada.

References


Vincenzo De Luca is at Novartis Agribusiness Biotechnology Research Inc., 3054 Cornwallis Road, Research Triangle Park, NC 27709-2527, USA. Benoit St Pierre is at Laboratoire de Physiologie Vegetale, EA 2106, UFR des Sciences et Techniques, Universite de Tours, Parc de Grandmont, 37200 TOURS, France (tel +33 247 367101; fax +33 247 367042; e-mail saint.pierre@uv-tours.fr).

*Author for correspondence (fax +1 919 541 8575; e-mail vince.decoru@nabr.novartis.com).

Students

Did you know that you are entitled to a 50% discount on a subscription to Trends in Plant Science?
See the subscription order card in this issue for details.

April 2008, Vol. 5, No. 4 173