Ecological Arsenal and Developmental Dispatcher. The Paradigm of Secondary Metabolism

Toni M. Kutchan*

Leibniz-Institut für Pflanzenbiochemie, Weinberg 3, 06120 Halle, Germany

PLANTS PRODUCE A LARGE NUMBER OF CHEMICALS OF DIVERSE STRUCTURE AND CLASS

Mankind has been exploiting plant chemicals in the form of potions and poisons for thousands of years. The attitude toward the physiological significance of this plethora of small molecules is reflected in the terminology that was assigned to them: secondary metabolites. Less flattering terms were also assigned: waste products, metabolic leftovers, and excrement. Two schools of thought concerning the function of secondary metabolites had developed into the 1970s. Prominent personalities such as Miriam Rothschild, an amateur naturalist in England, were proponents of a critical ecological function for secondary metabolites such as cardiac glycosides, cannabinoids, anthocyanins, and pyrrolizidine alkaloids. On the other side were followers of Kurt Mothes, a charismatic professor of plant biochemistry in East Germany who held the opinion that substances such as alkaloids have "no special physiological meaning" and that "many people apparently cannot live without the idea that everything in Nature has a purpose." That being said, how far have we come since 1975?

SECONDARY METABOLITES CAN BE KEY PLAYERS IN THE INTERACTION BETWEEN PLANTS AND THEIR ENVIRONMENT

Pioneering work published by the likes of Miriam Rothschild (13), Jeffrey Harborne (The University of Reading), Tom Eisner, and Jerry Meinwald (Cornell University) established a new field of study and coined its name, "chemical ecology." It is now accepted that there is an integral interaction between plants and their environment and that species-specific secondary metabolites are key players in this interaction. For example, the pigments that are produced in flower petals, "signatures" of mixtures of anthocyanins or betalains, determine in part how effectively a flower will be pollinated. In another form of interaction, juvenile forms of insects have become specialized to feed on poisonous plants. In fact, some insects can have an out right predilection for poisonous food plants. The protection proffered is 2-fold: Poisonous plants are avoided by large herbivores and the insects accumulate toxic plant secondary metabolites such as cardiac glycosides and pyrrolizidine alkaloids that serve to protect them in later stages of development. To further demonstrate function, the research group of Hans Grisebach (Freiburg University) showed that secondary metabolites have a role in plant defense. Phenylpropanoids were found to accumulate in soybean in response to treatment with pathogens (19). This then linked the field of secondary metabolism to phytopathology.

In recent years a discovery of the function of secondary metabolites in the interaction with symbionts, rather than pathogens, was made that was biochemically clearly verifiable. A groundbreaking observation by the group of Sharon Long (Stanford University) demonstrated that the flavone luteolin exuded from roots of alfalfa serves as a signal that activates rhizobial nodulation genes and therefore plays an integral role in root colonization (12).

SECONDARY METABOLITES CAN ALSO BE SIGNAL COMPOUNDS INVOLVED IN PLANT DEVELOPMENT

One of the classical secondary metabolites, methyl jasmonate (familiar as the scent of jasmine), is now recognized as part of a signal transduction chain that begins with the membrane associated fatty acid α-linolenic acid and results in pleiotropic effects ranging from defense gene activation to mechanotransduction, tuber formation, and plant senescence, among others (18). Using Arabidopsis mutants demonstrating dwarfism and defects in light-regulated development, another class of secondary metabolites, the brassinosteroids, has been found to have a role in the regulation of plant development (9, 16). A role for flavonols in functional pollen development was demonstrated using antisense chalcone synthase petunia plants and chalcone synthase mutant maize plants (10, 17). Plants that could not produce flavonols were male sterile, defective in pollen germination and tube growth, but the sterility could be overcome by addition of the flavonol aglycones kaempferol or quercetin to mature pollen at pollination. With the discovery of physiological roles for secondary metabolites such as jasmonates, brassinosteroids, and flavonols in critical processes in plant growth and development, the chapter in scientific history that relegated

* E-mail kutch@ipb-halle.de; fax 49–345–5582173.

secondary metabolites to being flukes of nature effectively came to an end.

IF SECONDARY METABOLITES HAVE A DEFINITE AND EVOLVED ROLE IN THE LIFE CYCLE OF A PLANT, THEN SPECIFIC ENZYMES SHOULD UNDERLIE THEIR FORMATION

This was another point of contention encompassing two opposing hypotheses: Enzymes of primary metabolism fortuitously catalyze the formation of secondary metabolites, or enzymes specific to the biosynthesis of secondary metabolites have arisen during the course of evolution. Pioneers of the enzymology of plant secondary metabolism, such as Eric E. Conn (University of California, Davis) and Hans Grisebach began to demonstrate that specific enzymes, such as Phe ammonia lyase of phenylpropanoid biosynthesis, did exist. However, higher plants have a relatively sluggish rate of expression of secondary metabolism, and steady-state concentrations of biosynthetic enzymes are low. In addition, large amounts of tannins and other phenolics that accumulate in plants interfere with the extraction of active enzymes. A breakthrough in the study of the enzymology of secondary metabolite formation came with the establishment of plant cell suspension cultures that produce quantities of secondary metabolites that match or surpass those levels found in plants. These experiments in the research group of Meinhart H. Zenk (Bochum University, University of Munich) went on to show with the discovery and purification of more than 80 new substrate-specific enzymes of phenylpropanoid and alkaloid anabolism that the dogma that assigned secondary metabolites as mistakes or a simple play of primary metabolism was incorrect (20).

DEDICATED, SPECIES-SPECIFIC BIOSYNTHETIC ENZYMES ARE PRESENT IN PLANTS, BUT FROM WHERE DO THEY COME?

The earliest identified enzymes of plant secondary metabolism were of general phenylpropanoid metabolism, Phe ammonia lyase, 4-coumarate:CoA ligase, and chalcone synthase. Since their discovery they have also been the most intensely studied enzymes. The entry of the area of plant secondary metabolism into the age of molecular genetics came in part with the isolation of the cDNAs encoding these three enzymes by the groups of Rick Dixon (University of London), Chris Lamb (Salk Institute), and Klaus Hahlbrock (Freiburg University) (3, 7, 15). This was followed within a few years by the isolation of the first cDNAs of alkaloid biosynthesis, strictosidine synthase, of terpenoid biosynthesis, 4*S*-limonene synthase, and of cyanogenic glucoside biosynthesis, $P\text{-}450_{Tyr}$ by the groups of Toni Kutchan (University of Munich), Rod Croteau (Institute of Biological Chemistry, Pullman), and Birger Lindberg Møller (Royal Veterinary and Agricultural University, Copenhagen), respectively (1, 6, 8). Technological breakthroughs such as molecular cloning advanced many research fields simultaneously and plant secondary metabolism was included in this surge forward. Given knowledge of the complete amino acid sequence of enzymes of secondary metabolism, insight can now been gained into their evolutionary origin. Orthologs of some plant secondary metabolite biosynthesis genes such as those encoding cytochromes P-450, aldo-keto reductases, and methyltransferases exist in organisms from widely unrelated kingdoms such as mammals and bacteria. A strictosidine synthase-like gene has recently been found to be strongly expressed in human brain (5). Inter-relatedness based on primary amino acid sequences has been demonstrated between enzymes of flavonoid and alkaloid anabolism. Plant polyketide synthases such as chalcone synthase, stilbene synthase, and acridone synthase have provided evidence for how small changes in gene evolution can lead to diverse chemical structures such as the phenylpropanoid-derived chalcone and resveratrol, and acridone alkaloids. Although the phylogeny of plant secondary metabolites remains to be resolved, great progress is being made.

SECONDARY METABOLISM IS A MUSE TO NEW CONCEPTS

In the 1990s, secondary metabolism clearly emerged as a field of research from which ideas were born that transcended plant sciences into areas such as human health and materials engineering. In a study of the enzymes involved in lignan/lignin biosynthesis in Forsythia, the research group of Norman G. Lewis (Institute of Biological Chemistry, Pullman) discovered a fundamentally new type of biomolecule, the dirigent protein, which functions as a scaffold that determines the chirality of a molecule after chemical transformation by an enzyme (2). A tantalizing implication of these results is that one of our major building materials, lignin, may be chiral.

The 5′-deoxyadenosyl radical derived from coenzyme B_{12} is involved in an intramolecular migration in the conversion of L-methylmalonyl CoA into succinyl CoA. In mammals this step is part of the metabolism of certain amino acids and odd chain-length fatty acids into Glc. Plants do not produce vitamin B_{12} and it has been a long-standing question what molecule takes the role of this cofactor in intramolecular migration reactions. The answer has been provided very recently by the group of János Rétey (University of Karlsruhe) who discovered that during an intramolecular migration in the biosynthesis of the anticholinergic alkaloid scopolamine in Jimson weed, 5′-deoxyadenosyl radical is formed from *S*-adenosyl-Met (11). This work has defined a new role in plants for *S*-adenosyl-Met, known in plant metabolism mainly as a major methyl group donor. It also indicates how plants, similar to bacteria, have evolved to use a cofactor that is structurally simpler than cobalamin to affect intramolecular migration reactions.

One of the highest impact discoveries in secondary metabolism of the last 10 years is the discovery and elucidation of the non-mevalonate biosynthetic pathway to isoprenoids (4, 14). This breakthrough has changed textbook knowledge that had long been accepted and unchallenged. The pathway is currently being dissected by simultaneously using plant and bacterial model systems. Although closure on the pathway will still take some years of research effort, the impact of this knowledge will likely be far reaching to the field of human medicine in the form of new treatments for bacterial- and parasite-related diseases such as tuberculosis and malaria.

WHAT THEN HAS CHANGED IN 25 YEARS?

In the past 25 years we have transcended the view of plant secondary metabolites as one of nature's meaningless waste products to one in which secondary metabolites play critical roles in plant development and defense. They are no longer only fortuitously formed chemicals that serve mankind as pharmaceuticals and pesticides. We now understand that secondary metabolites can provide a local or a systemic defense response to pathogen and herbivore attack. They have an integral role in plant growth, development, symbiosis, and reproduction. This list is certain to grow as we discover additional important functions for secondary metabolites in the years to come.

LITERATURE CITED

1. **Colby SM, Alonso WR, Katahira EJ, McGarvey DJ, Croteau RJ** (1993) Biol Chem **268:** 23016–23024
2. **Davin LB, Wang HB, Crowell AL, Bedgar DL, Martin DM, Sarkanen S, Lewis NG** (1997) Science **275:** 362–366
3. **Edwards K, Cramer CL, Bolwell GP, Dixon RA, Schuch W, Lamb CJ** (1985) Proc Natl Acad Sci USA **82:** 6731–6735
4. **Eisenreich W, Schwarz M, Cartayrade A, Arigoni D, Zenk MH, Bacher A** (1998) Chem Biol **5:** 221–233
5. **Fabbri M, Delp G, Schmidt O, Theopold U** (2000) Biochem Biophys Res Commun **271:** 191–196
6. **Koch BM, Sibbesen O, Halkier BA, Svendsen I, Møller BL** (1995) Arch Biochem Biophys **323:** 177–186
7. **Kuhn DN, Chappell J, Boudet A, Hahlbrock K** (1984) Proc Natl Acad Sci USA **81:** 1102–1106
8. **Kutchan TM, Hampp N, Lottspeich F, Beyreuther K, Zenk MH** (1988) FEBS Lett **237:** 40–44
9. **Li J, Nagpal P, Vitart V, McMorris TC, Chory J** (1996) Science **272:** 398–401
10. **Mo Y, Nagel C, Taylor LP** (1992) Proc Natl Acad Sci USA **89:** 7213–7217
11. **Ollagnier S, Kervio E, Rétey J** (1998) FEBS Lett **437:** 309–312
12. **Peters NK, Frost JW, Long SR** (1986) Science **233:** 977–980
13. **Reichstein T, von Euw J, Parsons JA, Rothchild M** (1968) Science **161:** 861–866
14. **Rohdich F, Wungsintaweekul J, Eisenreich W, Richter G, Schuhr CA, Hecht S, Zenk MH, Bacher A** (2000) Proc Natl Acad Sci USA **97:** 6451–6456
15. **Ryder TB, Cramer CL, Bell JN, Robbins MP, Dixon RA, Lamb CJ** (1984) Proc Natl Acad Sci USA **81:** 5724–5728
16. **Szekeres M, Nemeth K, Koncz-Kalman Z, Mathur J, Kauschmann A, Altmann T, Redei GP, Nagy F, Schell J, Koncz C** (1996) Cell **85:** 171–182
17. **Van der Meer IM, Stam ME, van Tunen AJ, Mol JN, Stuitje AR** (1992) Plant Cell **4:** 253–262
18. **Weiler EW, Laudert D, Stelmach BA, Hennig P, Biesgen C, Kubigsteltig I** (1999) Novartis Found Symp **223:** 191–204
19. **Zahringer U, Ebel J, Grisebach H** (1978) Arch Biochem Biophys **188:** 450–455
20. **Zenk MH** (1991) Phytochemistry **30:** 3861–3863