

Pyrimidine Metabolism in Plants

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In presenting pyrimidine metabolism, most modern biochemistry texts contain a figure describing metabolism in bacterial cells and the alternative in animal cells. For plant biochemists, this discrepancy is disconcerting. Admittedly, pyrimidine metabolism has been largely overlooked in plants in favor of more glamorous endeavors. However, the importance of pyrimidines to almost all aspects of cellular biochemistry has led us to investigate pyrimidine metabolism in plants with the goal of elucidating these pathways.

Plants are true eukaryotes, so their genes are not organized in polycistrons, but rather are individually encoded and scattered throughout the genome like animal genes. The transcriptional machinery in plants differs from bacterial systems but is similar to the transcriptional machinery found in animal cells. Most of the work on pyrimidine metabolism in plants has been done in a few species, notably tobacco (*Nicotiana tabacum*) and pea (*Pisum sativum*); however, within the past decade, a small weed known as field pennycress or Arabidopsis (*Arabidopsis thaliana*) has become widely accepted as a "model" plant. The Arabidopsis genome project has completely sequenced about 40% of the Arabidopsis genome, so pyrimidine metabolism is being best characterized in Arabidopsis. Most of what we will present in this article, unless otherwise noted, is work based upon this plant.

Pyrimidine biosynthesis

In animals, the first three enzymes of pyrimidine

biosynthesis, Carbamoylphosphate synthase (CPSase), Aspartate transcarbamoylase (ATCase) and Dihydroorotase (DHOase) have been fused during evolution to form a single polypeptide called CAD¹. In contrast, the genomic organization of pyrimidine biosynthesis in plants is more like that of prokaryotes. Plants have individually encoded CPSases, ATCases and DHOases.

Carbamoylphosphate synthase

Carbamoylphosphate Synthase (CPSase) is encoded within the nucleus, but the mature protein is targeted to and functions within the chloroplast. This enzyme responsible for the production of carbamoylphosphate for both pyrimidine and arginine biosynthesis. Ornithine stimulates incorporation of NaHCO₃ into UMP and arginine. However, in the presence of exogenously added uridine, incorporation of NaHCO₃ into UMP was reduced while incorporation into arginine was unaffected².

Two cDNAs have recently been cloned which encode the small and large subunit (*carA* and *carB*) of this enzyme^{3, 4}. Like other enzymes of the *de novo* pathway, CPSase is more similar to a prokaryotic enzyme than the eukaryotic homologues. The small subunit shows 46% identity to the *E. coli* enzyme and 51% identity to the *Synechocystis* enzyme. The large subunit shares 66% identity to the *Synechocystis* enzyme. The C-terminus of the Arabidopsis large subunit contains a unique domain not found in other CPSases that may be important in subunit interaction³.

Aspartate transcarbamoylase

Aspartate Transcarbamoylase (ATCase) catalyzes the first committed step in pyrimidine biosynthesis. As in other organisms, this step is likely the major regulatory site. Indeed, studies in squash (*Cucurbita pepo*) tissue cultured cells have shown a 90% decrease in ATCase activity upon the addition of UMP to the growth media⁵. Further, addition of exogenous uridine to the growth media was able to inhibit incorporation of radiolabeled NaHCO₃ into UMP but had no effect on the incorporation into arginine. This also lends support to the claim that CPSase is not the major regulatory step in pyrimidine biosynthesis^{2, 6}.

Several cDNAs encoding ATCase (*pyrB1*, *pyrB2*, *pyrB3*) have been cloned from *Pisum sativum*^{7, 8} along with a single cDNA from Arabidopsis⁹. A motif has been identified as a putative pyrimidine binding site, based upon its homology to the known allosteric pyrimidine binding site of the *E. coli* regulatory ATCase subunit. This supports the argument that ATCase is the major regulated enzyme of pyrimidine biosynthesis. This binding site is also conserved in the Arabidopsis protein. The pea cDNAs contain N-terminal chloroplast targeting sequences which is in agreement with previous work reporting localization of ATCase activity to the chloroplast. No genes encoding ATCase from any plant have been deposited in the GenBank.

Dihydroorotase

Dihydroorotase (DHOase) catalyzes the formation of dihydroorotate by cyclization of carbamoylaspartate. Unlike higher Eukaryotes, DHOase is not found as part of

a large multifunctional CAD enzyme in plants. Rather, like other members of the pathway, it is a nuclear encoded chloroplastic enzyme. The cDNA encoding this enzyme has been cloned from Arabidopsis and shares 54.5% identity to the *E. coli* enzyme¹⁰. Expression of DHOase has been examined during wheat germination. It was found that DHOase drops 50% during the first 30 min of imbibition and remains very low until approx. 12 hours after imbibition at which point, activity again rises. Two days after germination the activity is four fold higher than in the dry seeds¹¹ and therefore, this enzyme must be developmentally regulated.

Dihydroorotate dehydrogenase

To date the only Dihydroorotate dehydrogenase (DHO DH) homologue cloned from a higher plant was from Arabidopsis by complementation of a yeast auxotroph with plant cDNAs¹². In this case, the enzyme appears to be similar to its eukaryotic homologues. This protein also has N-terminal targeting sequences which direct the protein to the mitochondrial intermembrane space.

The Arabidopsis DHO DH gene was identified on chromosome V by Arabidopsis genome sequencing project¹³. The 2298 basepair gene is interrupted by 8 introns ranging in size from 87 to 193 base pairs. (Plant introns are typically much smaller than are introns from animal genes). There are several interesting features of the promoter region of this gene. It is relatively short (290 bp) and contains no TATA box. Furthermore the promoter is, most likely bifunctional, as an expressed superoxide dismutase gene is found immediately upstream and in

opposite orientation to the DHO DH gene.

UMP synthase

In plants, UMP synthase is a bifunctional protein catalyzing the final 2 steps of *de novo* pyrimidine biosynthesis. As in most higher eukaryotes, the plant UMP synthase has both orotate phosphoribosyl transferase (OPRTase) and orotidylate decarboxylase (ODCase) activities.

The Arabidopsis UMPS gene has an interesting physical structure. An unidentified open reading frame is found almost immediately upstream of the Arabidopsis gene resulting in an unusually short promoter region of only 400 bp (Kafer and Thornburg, manuscript in preparation). The promoter region of the Arabidopsis gene is currently being analyzed for its expression in transgenic plants. The coding region also has unusual features. The 5'-half of the gene encodes the OPRTase function while the 3'-half encodes the ODCase function. There are 5 introns found in the Arabidopsis gene ranging from 81 to 177 nucleotides. Interestingly, all five of these introns are found in the ODCase half and no introns are found in the OPRTase half. The *N. plum-baginifolia* gene contain introns in identical positions with the Arabidopsis gene; however there is an additional intron within the 5' half of the coding region of the tobacco gene (Kafer and Thornburg, unpublished). In genomic southern blots of both Arabidopsis and tobacco the gene appears to be single copy¹⁴.

Subcellular localization of pyrimidine biosynthesis in plants

Almost the entire pathway of pyrimidine biosynthesis

occurs within the chloroplast. There is one notable exception, DHO DH, which is localized in the mitochondria. The biosynthetic product of the first three *de novo* pathway enzymes, dihydroorotate accumulates in the chloroplast. To produce UMP, dihydroorotate must be transported from the chloroplast and be taken up by the mitochondria where it is converted into orotic acid. Subsequently, orotic acid must be transported from the mitochondria and reenter the chloroplast where it is converted into UMP by the enzyme UMP synthase. This remarkable shuttling of the pyrimidine metabolites between subcellular organelles results in some very interesting biochemistry.

Monika Löffler has previously argued in this journal¹⁵ that the coupling of pyrimidine biosynthesis to quinone reduction in the mitochondria results in the effective coupling of nucleotide biosynthesis to respiration. Although her work has been primarily concerned with animal systems, her conclusions may make even more sense when applied to plants. Plants frequently undergo oxygen stress induced principally by flooding. In many parts of the globe, flooding is an annual or a semiannual occurrence. The coupling of nucleotide biosynthesis with oxygen tension therefore would reduce growth and development during times of oxygen deficiency.

In maize, the shuttling of nucleotides between the chloroplast and the mitochondria appears to be mediated by a protein with similarity to microbial pyrimidine/purine transport proteins. This protein is encoded by the *lpe1* gene¹⁶. Indeed, normal plant development appears to be dependent upon such pyrimidine transport. Mutations in the *lpe1*

gene in maize result in abnormal chloroplasts and altered leaf development. Several homologues have been identified in the Arabidopsis EST databases. We are currently characterizing these cDNAs to identify the subcellular components of this shuttle system. Because the control of pyrimidine biosynthesis in plants involves this unusual subcellular compartment shuttling, these proteins may be an important and previously overlooked site of control for nucleotide metabolism.

Pyrimidine modification

Based upon current knowledge, it appears that formation of TMP and CMP is essentially identical to animal cell systems. Because fluorouracil induces thymine starvation in plant cells¹⁷ as it does in animal cells, the biochemistry must be similar.

UMP/CMP kinase

An Arabidopsis cDNA has been cloned and shows 50% sequence homology to the mammalian enzyme¹⁸. A conserved nucleotide binding region (GGPGS/AGK) is located near the N-terminus. This motif is found in all eukaryotic monophosphokinases and anchors the gamma phosphate of the nucleotide^{19, 20}. Like its eukaryotic counterparts, UMP and CMP are equally acceptable as substrates for the plant protein.

NDP kinase

Nucleoside diphosphokinase catalyzes the conversion of the diphosphonucleosides to the triphosphonucleosides. Most of the work done on these kinases has centered around their involvement in signal transduction pathways. These important kinases have been shown to be autophosphorylating^{21, 22}, involved in

phytochrome mediated light perception^{22, 23}, heat shock response²¹ and wound response²⁴. Most of the work has been centered around purine nucleoside diphosphokinase esp. GDP to GTP synthesis. A family of three NDP kinases have been identified in *Arabidopsis thaliana*.

CTP synthase, NDP reductase, dUTPase

CTP synthases have not been studied in plants. Our searches have identified at least three genes encoding CTP synthases from the Arabidopsis genome sequencing project. Likewise, the NDP reductase subunits have not been studied, nor have their genes been identified. In a search for meristem specific mRNAs, Pri-Hadash et al.²⁵ isolated a small cDNA from tomato that encoded a dUTPase. This mRNA was preferentially expressed in the growing meristems, but its expression dramatically declined in tissues further from the meristems. This enzyme is similarly expressed in root meristems²⁶ but is almost undetectable in mature root tissues.

Thymidylate synthase/ Dihydrofolate reductase

Thymidylate Synthase (TS) and Dihydrofolate reductase (DHFR) are responsible for the production of dTMP and regeneration of tetrahydrofolate, respectively. Like UMP synthase, the enzyme in Arabidopsis and also carrots²⁷, is a bifunctional polypeptide²⁸ which apparently has arisen from a gene fusion. The N-terminus has identity with DHFR while the C-terminus has identity with TS. The bifunctional nature of this enzyme is unusual. In bacteria and in most higher eukaryotes, including yeast, TS and DHFR proteins are separate enzymes encoded by

distinct genes. Other bifunctional TS/DHFR enzymes occur in protozoans such as *Leishmania amazonensis*²⁹, *Plasmodium falciparum*³⁰, and *Paramecium tetraurelia*³¹.

Sequence analysis by Lazar et al.²⁷ provides conflicting arguments on the two competing hypotheses for the origin of the plant and protozoan TS/DHFR enzyme. These enzymes have identical fusion sites between the functional domains. Based upon this, it is possible that the protozoan and plant enzymes have a common ancestor. Alternatively, these authors indicate that pairwise amino acid sequence analysis shows that these bifunctional enzymes are each more similar to the monofunctional enzymes than they are to each other. This raises the possibility that these enzymes may have arisen by convergent evolution. There are two copies of the gene in Arabidopsis that appear to have arisen by gene duplication²⁷.

Pyrimidine Salvage

Pyrimidine nucleosides are principally taken up into plant cells via one of two routes, Wu et al.,³² have identified 5-fluoro-2'-deoxyuridine FUdR-resistant mutants of Arabidopsis that are deficient in uptake of FUdR from tissue culture media. This protein also transports thymidine and may be similar to thymidine transporters from yeast and other eukaryotes. Uracil transport was unaffected in these mutants. In addition, our own work has demonstrated that the pyrimidine free bases orotic acid, uracil and cytosine and each of their fluorinated analogs are all taken up into plant cells. The proteins responsible for this transport have not been identified, but are apparently different from the transporter of Wu et al.,³².

Cytosine deaminase

It has been previously demonstrated that plants, like animals and unlike microorganisms, lack a cytosine deaminase activity³³. This lack of cytosine deaminase points to the inability of plants to salvage the cytosine free base. Indeed, because of this inability to salvage the free base, fluorinated cytosine cannot be metabolized in plants and animals and is therefore an effective antimicrobial compound. This has been very effectively utilized in animal systems, but has been widely overlooked in plant systems, especially plant tissue cultures.

Cytidine deaminase

Recently, we have identified and characterized the cDNA encoding the Arabidopsis *CDA1* (Kafer and Thornburg, submitted). Arabidopsis contains a gene family of seven genes encoding cytidine deaminase. Six of these genes *CDA2* through *CDA7* are present as a tandem repeat on a 15.7 kb stretch of DNA^{33a}.

Undoubtedly, some of the proteins encoded by these cytidine deaminase genes function in RNA editing. While RNA editing occurs in animal systems and the best studied example to date is the cytidine-to-uridine editing of apolipoprotein B (apo-B) RNA in mammalian intestine³⁴, plants have elevated RNA editing to new heights. RNA editing occurs in both the mitochondria and chloroplast of a wide variety of plants from bryophytes³⁵ to gymnosperms³⁶ to angiosperms including both monocots³⁷ and dicots³⁸. In most cases, the editing is simple conversion of cytidine into uridine by the action of cytidine deaminase thereby modifying codon occurrence. However, RNA editing also has been shown to

modify the coding region of genes including the creation of new stop and start codons^{39, 40} and creation of entirely new open reading frames³⁶. The editing is frequently identical across species, however it was recently shown that the patterns of RNA editing are different in angiosperms and gymnosperms³⁶.

RNA editing is also regulated both developmentally and in a tissue-specific manner in plants⁴¹. This change in mRNA editing with development or growth conditions results in expression and accumulation of proteins with altered polypeptide sequences within organelles. This alteration of protein sequence during development may result in modification of organelle function as the plant develops⁴².

Interestingly, all of the plant cytidine deaminases are more similar to the *E. coli* enzyme than they are to other eukaryotic enzymes. The bacterial enzymes are 2 dimers of 31 kDa subunits. The eukaryotic enzymes from humans, yeast, *Cenorhabditis*, and insects are tetramers of 15 kDa subunits. The plant enzyme is translated as a 32.5 kDa monomer that shows conserved identity with the *E. coli* enzyme throughout its length. Indeed, so closely related is the major Arabidopsis cytidine deaminase that we have modeled the active site of the plant enzyme using the active site of the bacterial enzyme (Kafer and Thornburg, submitted). All active site residues of the *E. coli* enzyme have identical structural counterparts in the plant enzyme implying that the catalytic mechanism must be the same.

UPRTase

Plants appear to have several different UPRTase

genes. We have recently identified one gene from *Arabidopsis thaliana* which is most similar to the *Toxoplasma gondii* and yeast enzymes rather than the bacterial enzymes (Weers and Thornburg, in press). However the *N. tabacum* enzyme has greater identity with the bacterial enzymes than to eukaryotic enzymes.

Pyrimidine degradation

Pyrimidine degradation has not been examined in plants. Sequences putatively corresponding to Dihydrouracil dehydrogenase, Dihydropyrimidine dehydrogenase, and -ureidopropionase have all been identified either as ESTs or genomic sequences from the Arabidopsis genome project. None of these have been well characterized to date, but their identification leads us to conclude that overall pyrimidine degradation in plants is similar to that in other organisms.

Regulation of Nucleotide Metabolic Genes

Plants undergo developmental processes that theoretically require high levels of pyrimidine nucleotides. This includes processes such as germination, pollen tube growth, flowering, and seed set. In spite of these numerous processes that require large amounts of nucleotides, nucleotide metabolism has not been particularly well studied in plants.

Germination

Expression of the enzymes of *de novo* pyrimidine and pyrimidine salvage have been examined during germinating seedlings. Following imbibition, dry seeds typically have high concentrations of free bases that appear within a few hours⁴³. These appear to arise by salvage from nucleo-

sides stored within the seeds. Based upon incorporation of labeled precursors into RNA, salvage appears to be the preferred source during the first few hours following germination. After the first day, *de novo* synthesis becomes the predominant source of nucleotides⁴⁴. Similarly, the *de novo* and salvage enzymes have been examined in dry seeds and most enzymes are present; however, following a brief decline in activity, the activity of the *de novo* enzymes dramatically increases within only a few hours following imbibition¹¹. In contrast, the salvage enzymes UPRTase and Uridine kinase were high in dry seeds and showed little change during the first 24 hours of germination. The fact that relative high levels of salvage enzymes are maintained following germination along with the induction of the *de novo* pathway results in maximum funneling of all nucleotides towards pyrimidine accumulation.

Tissue Cultured Cells

In some tissue cultures the relative activities of the salvage and *de novo* pathways change markedly depending upon the stage of the culture. The key salvage pathway enzymes UPRTase and Uridine kinase, increase just after transfer of the cultures. Enzymatic reactions of the *de novo* pathway, namely CPSase and UMP synthase, increased after the initial post transfer lag phase with a maximum velocity during the active cell division phase⁴⁵. The results are quite similar to the picture seen in germinating seeds where the initial phase is largely one of pyrimidine salvage which then quickly turns biosynthetic with salvage playing less of a role.

The question of how the two pathways (*de novo* biosyn-

thesis and salvage) are coordinately regulated still remains. That the activities of CPSase and UMP synthase increased in *Vinca rosea* (Madagascar periwinkle) cultures during the cell division phase led Kanamori-Fukuda and coworkers to postulate that the *de novo* pathway may not be feedback regulation but may rather be controlled by enzyme synthesis⁴⁵. Likewise, these workers showed evidence that the salvage pathways were also controlled by the availability of the key salvage enzymes.

Studies with metabolic inhibitors

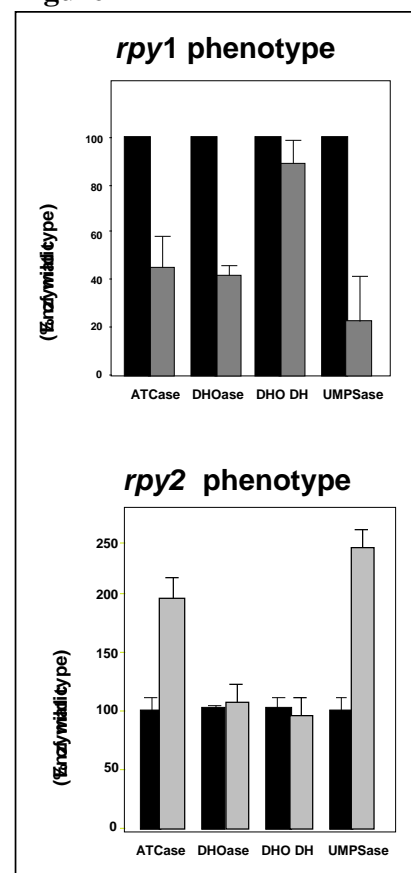
The pathways of arginine biosynthesis and pyrimidine biosynthesis share the carbamoylphosphate precursor pool and are tightly coordinately controlled. Metabolic inhibitors of each of these pathways should be useful in elucidating the regulatory mechanisms of each. The metabolite, 5-fluorouracil, is toxic to plant cells just as it is in animal cells^{46, 47}. Phaseolotoxin is a pathogenesis factor from *Pseudomonas syringae* pv *phaseolicola*, a pathogen of beans. It inhibits the condensation of carbamoylphosphate with ornithine and is capable of reversing the toxic effects of fluorouracil⁴⁸. The result of treating cells with phaseolotoxin is the increase in intracellular ornithine which subsequently stimulates CPSase activity. The resulting carbamoylphosphate enters the *de novo* pyrimidine biosynthetic pathway thereby increasing the levels of UMP which overcomes the toxicity of fluorouracil.

In order to understand the regulation and expression of pyrimidine biosynthesis in plants, we have examined the effect of the metabolic inhibitor, 5-fluoroorotic acid, on UMP

synthase expression in cell cultures of *Nicotiana plumbaginifolia*¹⁷. UMP synthase is the rate limiting step of pyrimidine biosynthesis in plants⁴⁷. Addition of fluoroorotic acid causes an up-regulation of UMP synthase enzyme activity, resulting from transcriptional induction of the UMP synthase gene. Exogenously added thymine reversed this up-regulation. Methotrexate and aminopterin, which affect thymine levels by inhibiting DHFR also up-regulate UMP synthase in *N. plumbaginifolia* cells.

We have previously selected a large number (>180) of plants and plant cell lines^{17, 47} that grow in the presence of fluoroorotic acid plus uracil. Our characterization of these plants and cell lines has demonstrated several unique classes of mutants. We call these cell lines *rpy* for regula-

Figure 1



tor of pyrimidine biosynthesis. The *rpy1* phenotype was identified by examining enzymes in the *de novo* pyrimidine biosynthetic pathway. These cells show down-regulation of three of the four enzymes in the pathway (Figure 1). Because CPSase funnels metabolites to both pyrimidine and arginine biosynthesis, we did not examine this enzyme. *rpy2* plant cell lines shows constitutively two to four-fold elevated levels of UMP synthase and ATCase. The *rpy2* mutants show normal levels of DHOase and DHO DH. In wildtype plants, UMP synthase is normally expressed at low levels, but it increases three to four-fold during thymine starvation⁴⁷. The *rpy2* mutants constitutively expresses UMP synthase and cannot be regulated by thymine levels (Santoso and Thornburg, manuscript in preparation). Apparently, the *rpy2* mutants have lost the ability either to detect thymine levels within cells or to transmit this information to the promoter of the ATCase and UMP synthase genes. Both *rpy1* and *rpy2* represent regulatory mutations that have not previously observed in either plants or animals. We are currently trying to isolate the genes for *rpy1* and *rpy2* by positional cloning methods.

Secondary metabolites

Plants produce an incredibly rich diversity of biochemical compounds. Of the 100,000 secondary metabolites that have been identified by organic chemists, 80% of these are produced uniquely by plants. Most of these compounds are produced by plants as defensive compounds to combat bacteria, fungi, insects, or even other plants. Chief among these secondary metabolites are the terpenes and

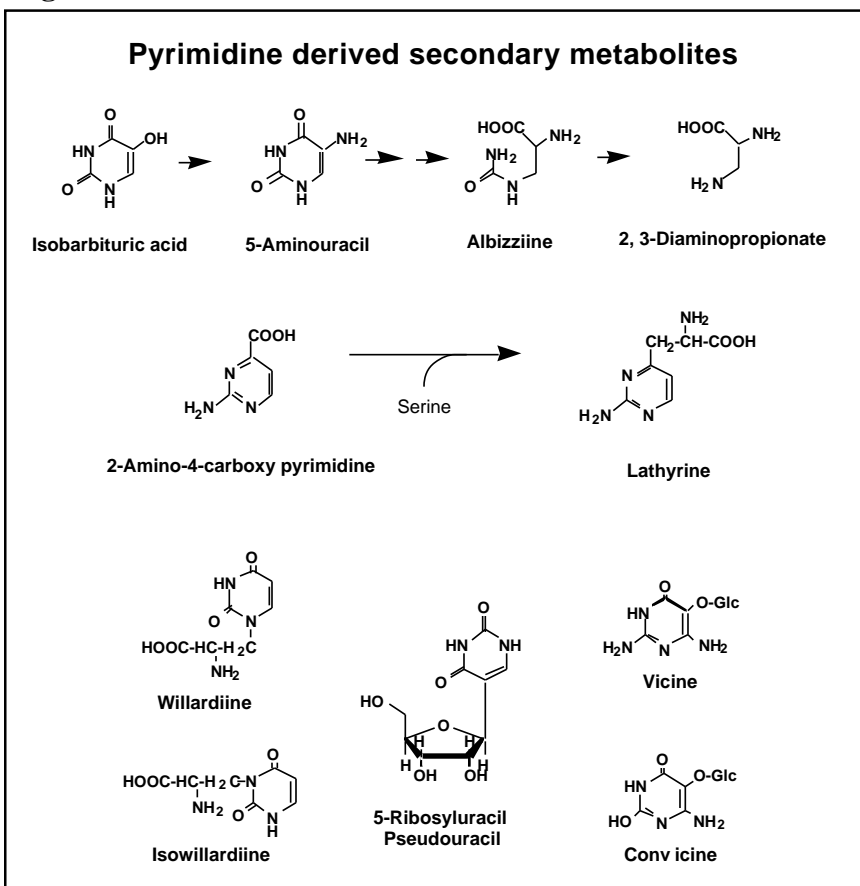
phenolic compounds; however, secondary metabolites derived from pyrimidines have also been described (see Figure 2).

One of the most important of these is the compound 5-aminouracil, a defensive compound produced by plants of the Mimosa family⁴⁹. 5-aminouracil blocks the mitotic cycle and inhibits incorporation of guanosine into nucleic acids⁵⁰. It is widely used as a cell cycle inhibitor⁵¹. Presumably it is synthesized from uracil by way of isobarbituric acid⁵². Plants can detoxify 5-aminouracil and these detoxification products also accumulate intracellularly. It is likely that these detoxification products also have defensive properties. Albizziine is a non-protein amino acid derived from 5-amino uracil by ring cleavage between N3 and C4. In secondary metabolite producing plants, the relative activities of

CPSase and ATCase were 4 to 6 times more active than non-metabolite producing plants⁵². In addition, the salvage enzymes 5'-nucleotidase and uridine hydrolase are also up-regulated in metabolite producing plants. Thus, both the *de novo* biosynthetic pathway and the salvage pathway provide pyrimidines for conversion into large amounts of albizziine. In addition, 2, 3-diaminopropionic acid, a product of 5-aminouracil catabolism also accumulates in these plants⁵².

Other uracil-derived secondary metabolites include lathyrine which is produced from 2-amino-4-carboxypyrimidine by addition of an alanine sidechain donated by serine⁵³. The source of 2-amino-4-carboxypyrimidine is uracil and it has been shown to accumulate in plants⁵⁴, but the biosynthetic route is unclear⁵⁵. Further metabolic modifica-

Figure 2



tions of lathyrine also occur yielding γ -glutamyl-lathyrine⁵⁶ or *cis*-5-hydroxy-L-pipecolic acid⁵⁷. Lathyrine synthase is an unusual biotin-stimulated pyridoxal phosphate containing enzyme that catalyzes the simultaneous decarboxylation of 2-amino-4-carboxypyrimidine and condensation of this product with the alanyl sidechain of serine⁵⁸. Lathyrine has antimicrobial activity, but surprisingly acts as a cytokinin in some plant cells, stimulating soybean cells to divide and maintaining chlorophyll retention in radish leaf discs⁵⁹. This activity, however, was not universal for all species tested.

Willardiine and isowillardiine are neuroexcitatory non-protein amino acids. They are related uracilyl amino acids in which O-acetyl serine donates the alanine side chain and are typical of a class of acetylation products of N-heterocyclic compounds⁶⁰. Enzymes capable of synthesizing willardiine and isowillardiine were isolated from pea etioplasts^{61, 62, 63, 64} and apparently separate enzymes are involved in the synthesis of these two compounds.

Vicine and convicine accumulate in the stems and roots of *Lupinus hispanicus* (Spanish Lupins), *Vicia faba* (Faba beans) and related species during seedling development and during seed development where they serve a protective purpose. In the guts of insects, vicine and convicine are hydrolyzed by insect gut α -glucanases into divicine and isouramil, respectively⁶⁵. These compounds inhibit the insect glucose-6-phosphate dehydrogenase⁶⁶ thereby leading to reduced insect growth.

Pseudouracil is another pyrimidine derivative that accumulates in plant tissues⁶⁷.

Feeding experiments indicate that its synthesis proceeds from orotic acid through uracil and UTP. Pseudouracil is most commonly found in tRNAs, so presumably the accumulation arises as a result of RNA turnover.

Unsolved Mysteries

Fate of nucleotides from DNA and RNA turnover

One remaining metabolic mystery in plants is the fate of dCMP arising from DNA turnover. While DNA degradation is probably not extensive in most eukaryotic organisms, even a low level of turnover could lead to unacceptable accumulation of nucleotide intermediates in plants if they were not recycled. Indeed, the oldest known living organism is a plant that is roughly 43,000 years old⁶⁸. Clearly, plants must have the ability to recycle these intermediates; however, the mechanisms are as yet unclear. We have tested whether dCMP can be deaminated to dUMP by cytidine deaminase. It cannot. Likewise we have also tested whether it can be converted to dCDP by UMP/CMP kinase. It cannot. Therefore, it is not clear how dCMP is recycled in plants.

Another accumulating compound that may pose a problem for long lived plants is pseudouracil. In animals, pseudouracil is not degraded, but rather is excreted in urine. Land plants do not have similar excretory mechanisms, therefore the degradation of pseudouracil in plants would be of more importance. It is not clear how such compounds are degraded. An alternative to degradation may lie in the deciduous nature of plants. If these compounds accumulate to toxic levels, this could trigger the senescence process that

leads to tissue degradation and loss of plant tissues. Alternatively, the deciduous nature of plants could circumvent the accumulation of toxic metabolites.

Pyrimidine biosynthesis vs salvage

Because most of the genes that encode the *de novo* and salvage pathways have been isolated from Arabidopsis, we have begun to ask more comprehensive questions about pyrimidine metabolism in plants. For example, it is clear that the supply of pyrimidines to plant tissues is the sum of *de novo* biosynthesis plus salvage. With these molecular tools in hand, can we examine the roles of each of these pathways in global pyrimidine metabolism in plants. Further, can we define mechanisms of gene regulation that control entire pathways for pyrimidine metabolism. Our results on *rpy1* and *rpy2* suggest that we can.

Pyrimidine catabolism

Pyrimidine catabolism is probably similar to other organisms based upon the identification of cDNAs and genes from the Arabidopsis genome project. However, these steps need to be examined in more detail to discern any potential differences.

Summary

A large portion of our work has been the general characterization of the plant cDNAs and genes that encode the pyrimidine metabolism in plants. A surprisingly large number of these plant genes are more similar to prokaryotic sequences than they are to other eukaryotic sequences. Perhaps this was the state of evolution when plants branched off from other eukaryotes approximately one billion years ago.

The pyrimidine *de novo* and salvage pathways also show a very interesting coordination in their regulation. The ease of production of mutants and transgenic plants may make plants the ideal eukaryotic organism to elucidate the details of such complex regulatory mechanisms.

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