Shikimic Acid Pathway and Aromatic Amino Acid Biosynthesis

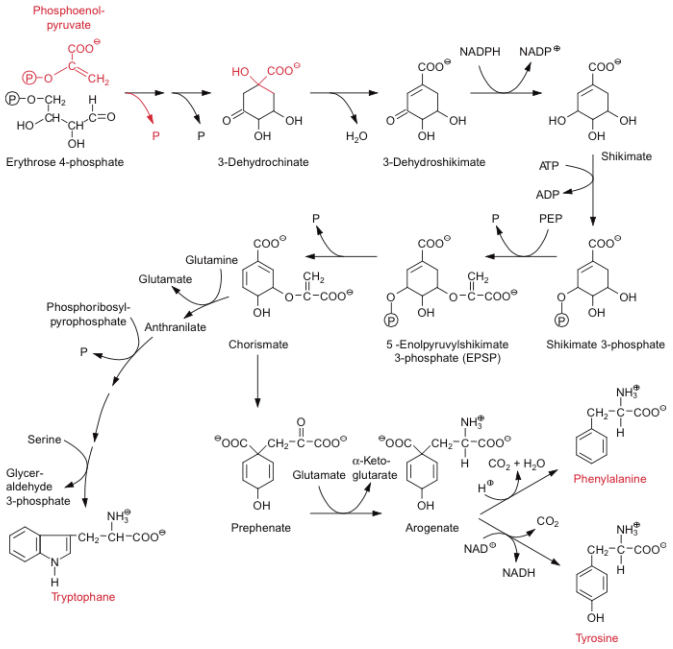
1. Define: *Pathway to **shikimic acid**, from which the **aromatic** amino acids **Trp, Tyr, Phe** are derived.*

2. Why is it important? Why study this pathway?

- among the **essential** amino acids we derive from our diet. Plants, microbes can synthesize them
- biosynthetic precursors for bioactive secondary plant metabolites (**phenylpropanoids** such as lignin, flavonoids, some alkaloids)
- example amino acid pathway for synthesis and regulation via feedbacks
- case study of agricultural biotechnology and herbicide resistant plants: **Glyphosate** (Roundup), a very popular and controversial herbicide acts on one enzyme of this pathway

3. Biosynthetic Precursors:

- **phospho-enol-pyruvat**e (derived from glycolysis)
- **erythrose-4-phosphate** (a 4C sugar, from **oxidative pentose phosphate** pathway)
- nitrogen is derived from several other amino acids: **glu**, **gln**, **ser**



4. Key enzymes and intermediates:

i) ***DAHP synthase*** (3-deoxy-arabino-heptulosonate-7-phosphate = DAHP) (= entry point)
ii) ***EPSP synthase*** (enolpyruvyl shikimate-3-phospate)
iii) chorismate synthase (chorismate))("fork" - branch point in the pathway)
iv) anthranilate synthase (branch specific for Trp)
*v) chorismate mutase (*makes prephenate*)*
iv) glutamate prephenate aminotransferase (adds amino group, to make arogenate)

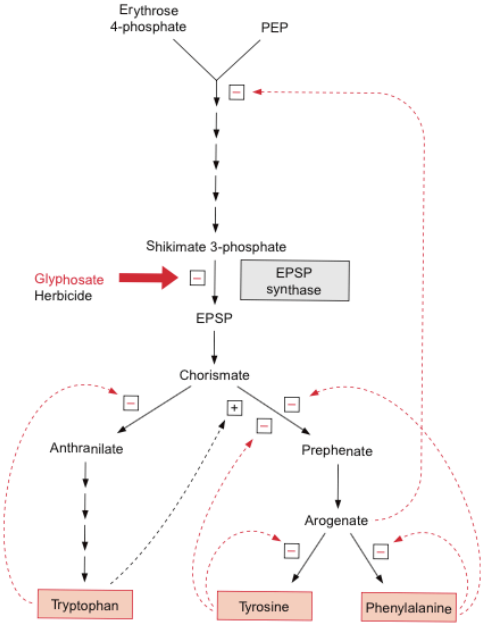
5. Localization: two locations for parallel pathways (although this is somewhat disputed)

- chloroplast, functions to provide amino acids for protein synthesis
- cytoplasmic, where Phe is required in large quantities for phenylpropanoid synthesis (lignin and secondary metabolites)

6. Regulation depends on the downstream products

i) **feedback control** (allosteric) by end products:

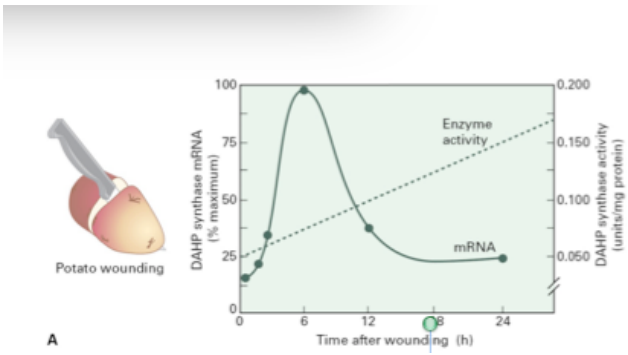
Trp - inhibits *anthranilate synthase*, ups *chorismate mutase* Phe and Tyr downregulate *chorismate mutase*
Phe also downregulates *arogenate dehydratase*
Arogenate (intermediate) inibits *DAHP synthase*

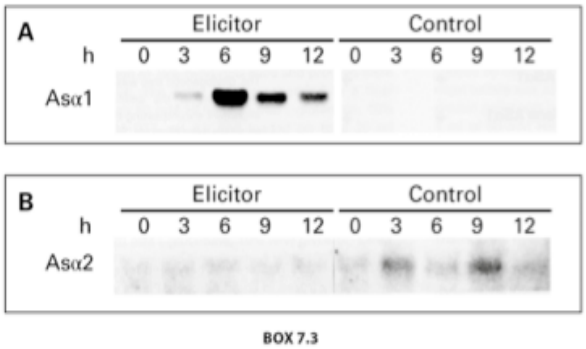


ii) **environmental control:**

- light induces the chloroplast enzymes via thioredoxin
- pest and pathogen attack triggers the cytoplasmic' shikimate pathway (specialized for phenylpropanoids)
  example 1: *DAHP synthase* and *shikimate dehydrogenase* are elicitor-induced
  example 2: *anthranilate synthases* (AnS) in *Ruta graveolens*, required for inducible defense chemicals

AnSa2 is **trp**-inhibited, but not elicitor-sensitive, **AnSa1** is very elicitor-sensitive, but not **trp**-inhibited.
Why might different regulatory mechanism be important?



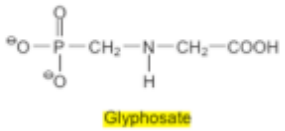


BOX 7.3

7. Glyphosate action and engineering of resistance into crop plants

Glyphosate ("RoundUp") is a herbicide that inhibits *EPSP synthase*
- very effective and relatively non-toxic
- originally, very low rates of resistance in weeds, but this has now changed with resistant weeds appearing

What are the possible strategies to generate glyphosate-resistant crop? What biochemical and molecular approaches might be available?



Glyphosate

The development of glyphosate-resistant plants took years of research and testing by Monsanto and others

i) overexpress a *Petunia* EPSP synthase gene, ie get high expression of the enzyme (failed)
ii) screen bacteria for (*E. coli)* glyphosate-resistant EPSP synthase mutants. This approach worked but the resistance is not sufficiently high for resistance
iii) screened and isolated a *Agrobacterium* EPSP gene that is more resistant than from other sources
-> then selected for mutations in this ESPS gene to enhance the resistance
-> overexpress the gene in plants with strong promoter. This worked and produces functionally glyphosate resistant plants

8. Overview of some herbicides in the context of nitrogen metabolism

Herbicides ideally are plant-specific toxins, and target pathways that animals don't have (photosynthesis, nitrogen assimilation and amino acid synthesis. A number of herbicides do target photosynthesis (i.e., Paraquat)

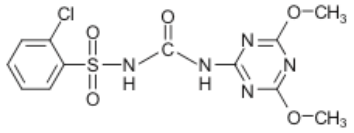
Nitrogen metabolism-specific herbicides

1. Chlorate Ion (ClO^{3-})
- reduced by nitrate reductase to chlorite (ClO^{2-})
- very non-specific, no longer used

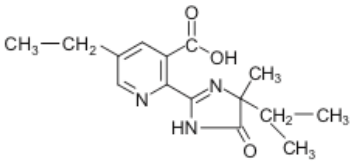
2. Glyphosate ("RoundUp")
- inhibits EPSP synthase (shikimate pathway)
- resistance engineered using a bacterial EPSP synthase ("Roundup Ready")

3. Chlorsulfurone ("Glean")
- inhibit acetolactate synthase (valine, leucine synth)
- natural resistance discovered in the field , and was crossed into crop cultivars via traditional breeding

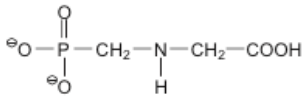
4. Glufosinate (Phosphinotricine - "Basta")
- inhibits glutamine synthetase
- resistance engineered with phosphinotricine acetyl transferase (bacterial) ("LibertyLink")



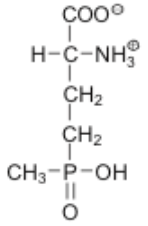
Chlorsulfurone



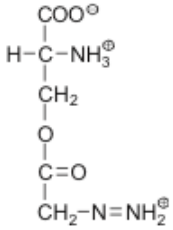
Imazethapyr



Glyphosate



Glufosinate



Azaserine

For Discussion:

Genetically engineered herbicide-resistant crops of various types are commonly used in modern 'industrial' agriculture. Roundup Ready Canola is very common on the Canadian prairies, and is very popular among growers. The benefits and costs or dangers have been discussed.

Do you think you have ingested any herbicide resistant plant products? Do you think or worry about it?

What are the benefits of herbicide resistant crops?

Do you see any obvious health risks?

What are the potential problems associated with herbicide resistant crops? What are the big environmental issues, if any. Any agronomic problems?