

# **AraCyc and the OMICS Viewer: Making sense of metabolism in your favorite biological process**



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# Everyone is studying metabolism

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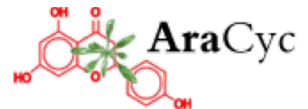
## □ Many biological processes connect to metabolism

- Drought tolerance – changes in osmolyte concentrations
- Hormone signaling – biosynthesis and degradation of hormones
- Photosynthesis – chlorophyll production and ROS scavenging
- Translation – amino acid biosynthesis and riboswitching
- Plant defense – phytoalexin synthesis

- Your favorite process . . .

- AraCyc can help you find these connections!

- Arabidopsis Metabolic EnCyclopedia
- Database of metabolic pathways found in Arabidopsis
  - [www.arabidopsis.org/biocyc/](http://www.arabidopsis.org/biocyc/) (TAIR)
  - [www.plantcyc.org/ARA](http://www.plantcyc.org/ARA) (Plant Metabolic Network)



# AraCyc 4.5 (released June 2008)

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Pathways	288
Compounds	1956
Reactions	1723
Genes (in pathways)	1914
Citations	2279

- How can you connect these to your research efforts?

# OMICS Viewer to the rescue . . .

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- Overlay experimental data on a metabolic map
  - **Transcript data (for enzymes)**
  - **Proteomic data (for enzymes)**
  - **Metabolomic data**
  
- Use multiple data sources
  - Your data
  - Publicly available data
    - Gene Expression Omnibus
    - NASC Proteomics Database
    - NSF2010 Metabolomics
    - Many more . . .
  
- Generate new testable hypotheses about your favorite
  - gene
  - metabolite
  - biological process
  - etc.

# Addressing common research conundrums . . .

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- ❑ My (single/double/triple/quadruple) mutant has “no phenotype”!!!!
  - **Compare transcript levels / protein levels / metabolite levels in wild-type and mutant plants using the OMICS viewer**
  - **Look for “hidden” perturbations in metabolism**
  
- ❑ I have a mutant with a defect in     (biological process)      
    . . . but I don’t know the mechanism
  - **Use the OMICS viewer to compare data from WT and mutant plants**
  - **Download publicly available data sets related to the biological process**
  - **Scan for affected areas of metabolism**
  
- ❑ I just did a microarray experiment and a bunch of metabolic enzymes popped up . . .
  - **Use the OMICS Viewer to quickly identify metabolic pathways that are up- or down-regulated**
  
- ❑ And many more . . .

# OMICS Viewer Overview

related pathways are grouped together

- △ Amino Acids
- Carbohydrates
- ◇ Proteins
- Purines
- ◇ Pyrimidines
- ▽ Cofactors
- ✦ tRNAs
- Other

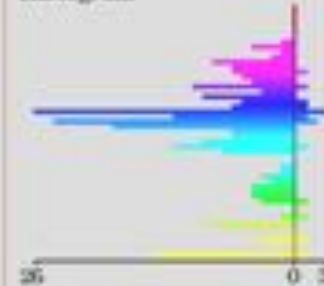
• (Filled): Phosphorylated

Expression Ratio



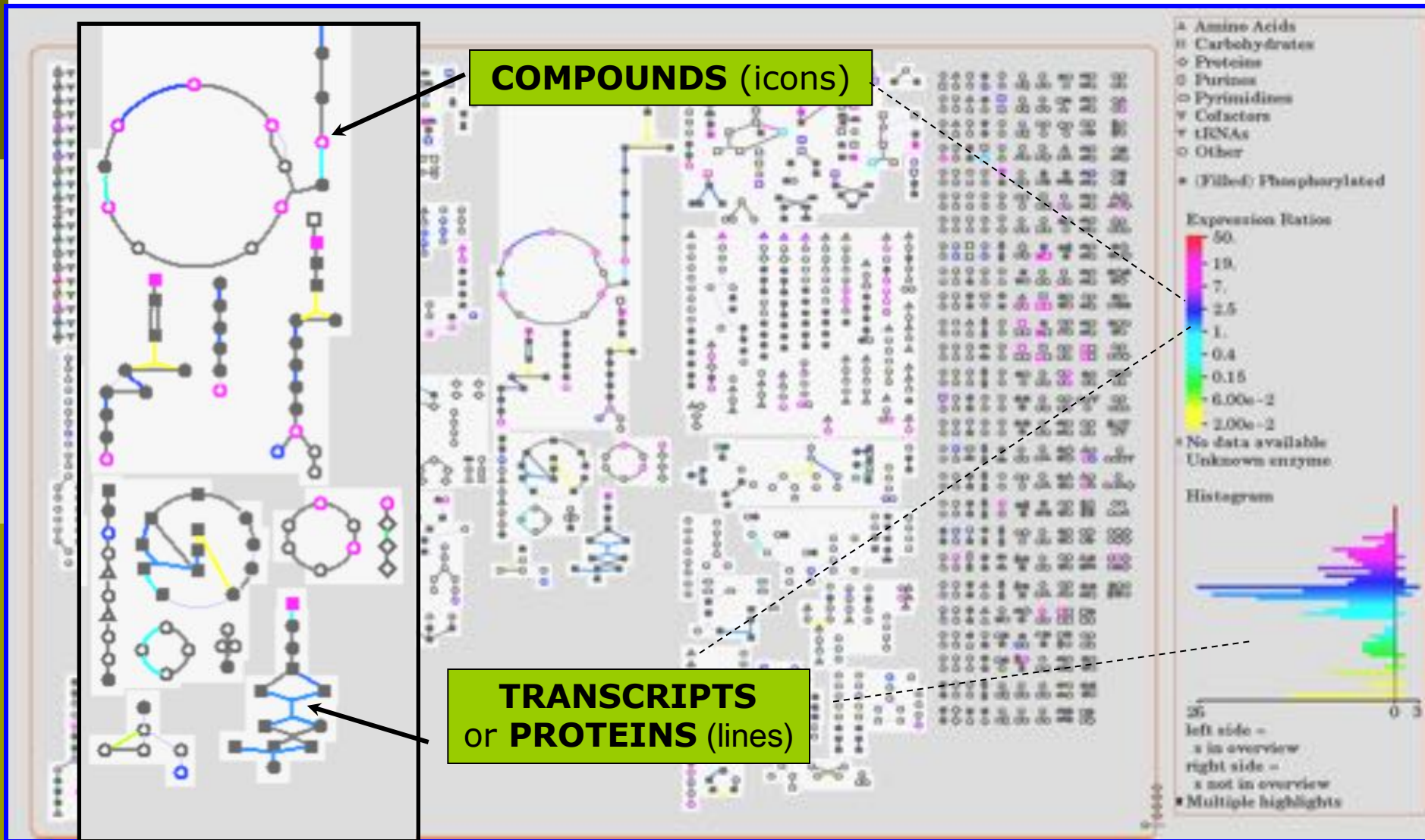
• No data available  
Unknown enzyme

Histogram



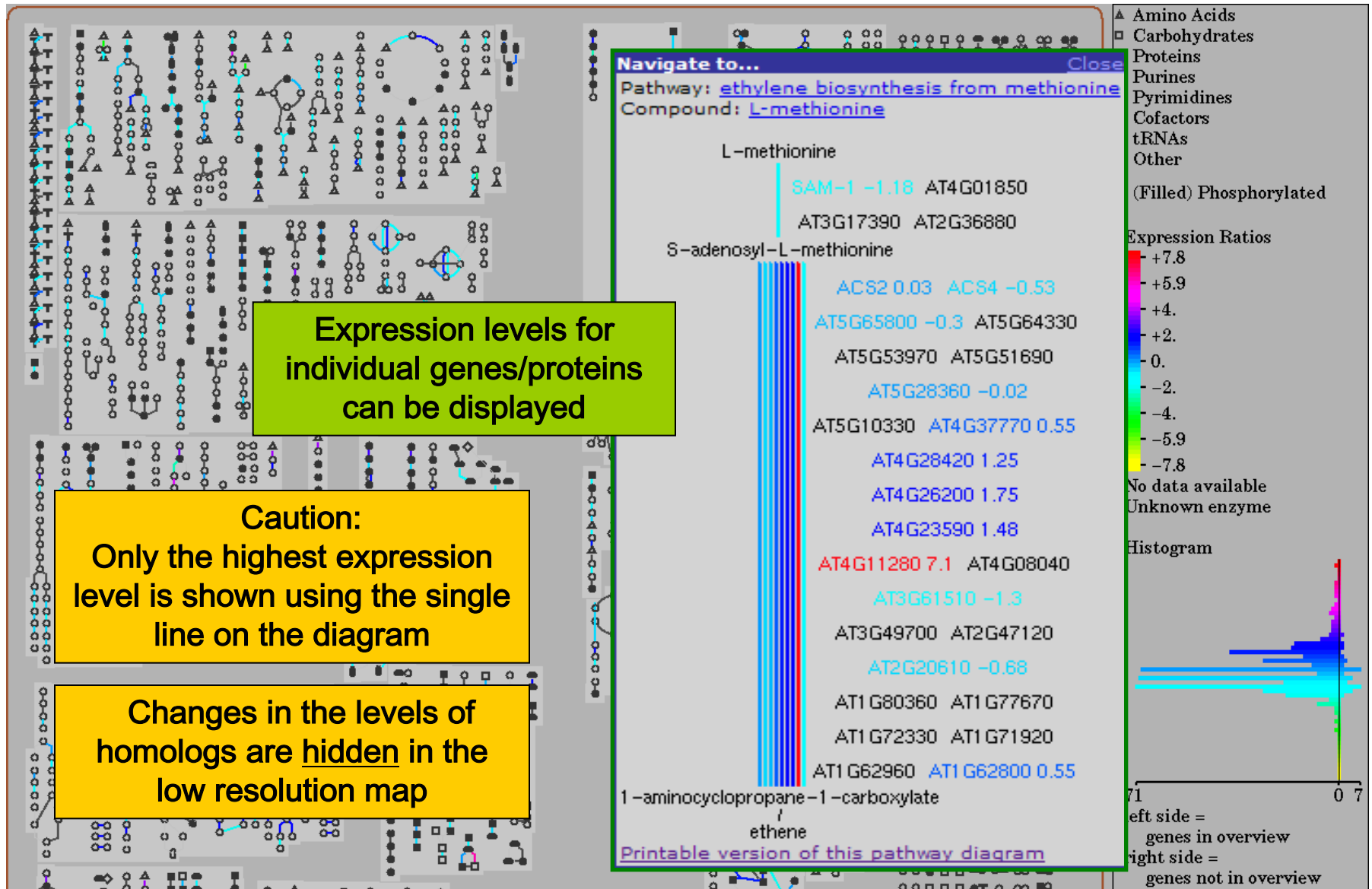
left side =  
x in overview  
right side =  
x not in overview  
• Multiple highlights

# OMICS Viewer Overview





# Viewing gene/protein families





## Pathway: choline biosynthesis III

Name: **chole biosynthesis III**  
 Pathway: **chole biosynthesis III**  
 Organism: **Escherichia coli**  
 Compound: **s-phosphatidylcholine**

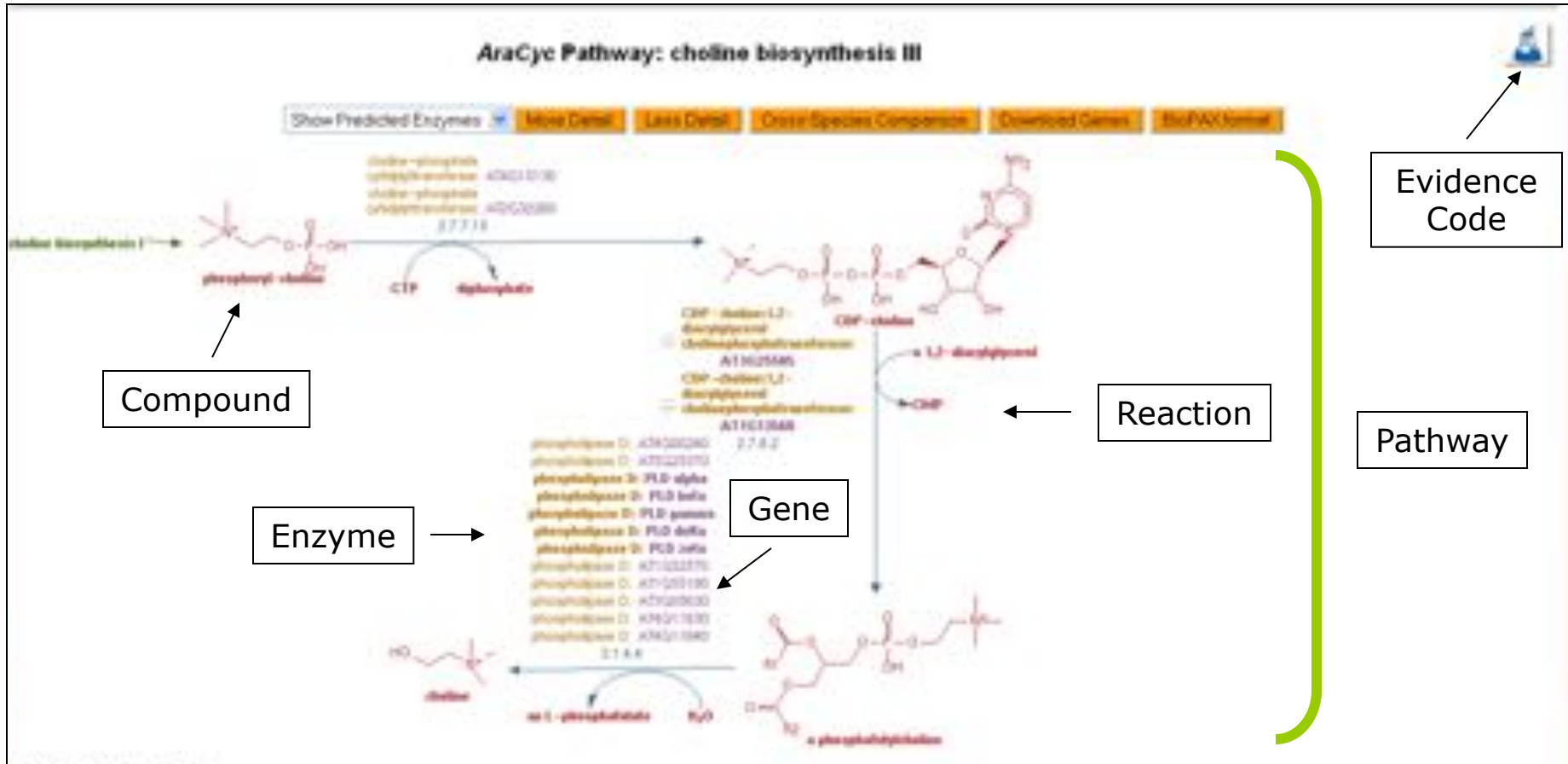
```

graph TD
    L-serine -- AIT042713 --> ethanolamine
    ethanolamine --> phosphoryl-ethanolamine[AFT018000]
    ethanolamine --> N-monomethylethanolamine
    phosphoryl-ethanolamine -- AIT027600, AIT048600 --> N-methylethanolamine_phosphate[AFT018000]
    N-methylethanolamine_phosphate -- AIT027600, AIT048600 --> N-dimethylethanolamine_phosphate[AFT018000]
    N-dimethylethanolamine_phosphate -- AIT027600, AIT048600 --> phosphoryl-chole[AFT018000]
    phosphoryl-chole -- AFA015130, AIT032200 --> CDP-chole
    phosphoryl-chole -- AFT025580, AIT013360-04 --> s-phosphatidylcholine
    CDP-chole --> choline
    s-phosphatidylcholine -- AFA000240, AFT025170 --> choline
    choline --> PLD-alpha[PLD-alpha, PLD-beta]
    choline --> PLD-gamma[PLD-gamma, PLD-delta]
    choline --> PLD-eta[PLD-eta, AIT052570]
    choline --> AIT052580
    choline --> AFT005630
    choline --> AFA011030
    choline --> AFA011040
    
```

A metabolic pathway diagram for choline biosynthesis in *Escherichia coli*. The pathway starts with L-serine, which is converted to ethanolamine by the enzyme AIT042713. Ethanolamine then branches into two main routes. The left route involves phosphorylation to phosphoryl-ethanolamine (AFT018000), followed by N-methylation to N-methylethanolamine phosphate (AFT018000, AIT027600, AIT048600), then N-dimethylation to N-dimethylethanolamine phosphate (AFT018000, AIT027600, AIT048600), and finally conversion to phosphoryl-chole (AFT018000, AIT027600, AIT048600). The right route involves N-monomethylethanolamine and N-dimethylethanolamine. Phosphoryl-chole then branches into CDP-chole (AFA015130, AIT032200) and s-phosphatidylcholine (AFT025580, AIT013360-04). s-phosphatidylcholine is further converted to choline (AFA000240, AFT025170) and then to various phospholipids (PLD-alpha, PLD-beta, PLD-gamma, PLD-delta, PLD-eta, AIT052570, AIT052580, AFT005630, AFA011030, AFA011040).

# AraCyc Pathway pages

All items can be clicked on to obtain more information



+ Additional curated information

# AraCyc Pathway pages

Superclasses: [Biosynthesis](#) → [Fatty Acids and Lipids](#) → [Choline Biosynthesis](#)

## Classification

### Summary:

**General information:** Choline is a fundamental metabolite in plants because of its contribution to the synthesis of the membrane phospholipid phosphatidylcholine, which accounts for 40 to 60% of lipids in non-plasmid plant membranes [ [Mio02](#) ]. Choline is also a precursor for the formation of glycine betaine ( [glycine betaine biosynthesis II \(plants\)](#) ) in certain plants such as spinach, where this osmoprotectant is accumulated and confers also tolerance to salinity, drought, and other environmental stresses. In addition choline has been recognized as an essential nutrient for humans [ [McNeil01](#) ].

The choline biosynthetic pathway enables plants to decouple choline synthesis from lipid metabolism (Kennedy pathway - [triacylglycerol biosynthesis](#) ) and provides them with the metabolic flexibility to adapt to environmental conditions where large and variable amounts of choline are beneficial for survival [ [Boutant03](#) ].

**Pathway information:** The first step in choline biosynthesis is the direct decarboxylation of serine to ethanolamine [ [Boutant04](#) ], which is catalyzed by a serine decarboxylase unique to plants [ [Boutant03](#) ]. Ethanolamine is widely recognized as the entrance compound to choline biosynthesis.

The pathway variant displayed (nucleotide pathway) represents the biosynthetic route as found in diverse plant families. The synthesis of choline from ethanolamine may take place at three parallel pathways, where three consecutive N-methylation steps are carried out either on free-bases [ [Phytohorm02](#) ], phospho-bases [ [Nuccio00](#) ], phosphatidyl-bases [ [McNeil01](#) ] or a mixture of the latter [ [Duckett03a](#) ] [ [Duckett03b](#) ] [ [Hick01](#) ].

The synthesis of intermediates on both the phospho-base and phosphatidyl-base level includes the nucleotide pathway via CDP-phosphoaminoalcohol and the methylation pathway. However, it has been pointed out that the synthesis of phosphatidylethanolamine and phosphatidylcholine is characterized by a high degree of interaction and ligation on the various levels of arising intermediates. Consequently, it has been assumed that the reactions embedded in the nucleotide and methylation pathways may be two characteristics of one overall phosphoaminoalcohol pathway for the formation of phosphatidylcholine in plants [ [Kinney03](#) ].

The release of choline from the different pathway levels is also species-specific. Phosphocholine can either be directly dephosphorylated to release choline as observed in spinach [ [Summons93](#) ] or incorporated into phosphatidylcholine with the subsequent release of choline, as in tobacco [ [McNeil00](#) ]. The latter reaction has been shown to be specifically catalyzed by phospholipase D ( [phospholipases](#) ) in cyster bean [ [Wang04](#) ]. Although a well-defined physiological role of phospholipase D (PLD) still await further research, progress has been made to assign some members of the heterogeneous family of PLDs to distinct cellular functions [ [Hick00](#) ]. The remaining enzymes involved in this pathway, phosphoaminoalcohol cytidyltransferase and CDP-aminoalcohol phosphotransferase, cover a broader spectrum of substrates. This may be beneficial to process the heterogeneous mixture of possible substrates but it also indicates that the pathway flux is probably controlled more upstream [ [Kinney03](#) ].

Superpathways: [superpathway of choline biosynthesis](#)

## Superpathways

Variants: [choline biosynthesis I - choline biosynthesis](#)

## Summary

Unification Link: [MetaCyc:PWY3681](#)

## Pathway variants

### Legend for Pathway Diagram

If an enzyme name is shown in bold, there is experimental evidence for this enzymatic activity.

### References

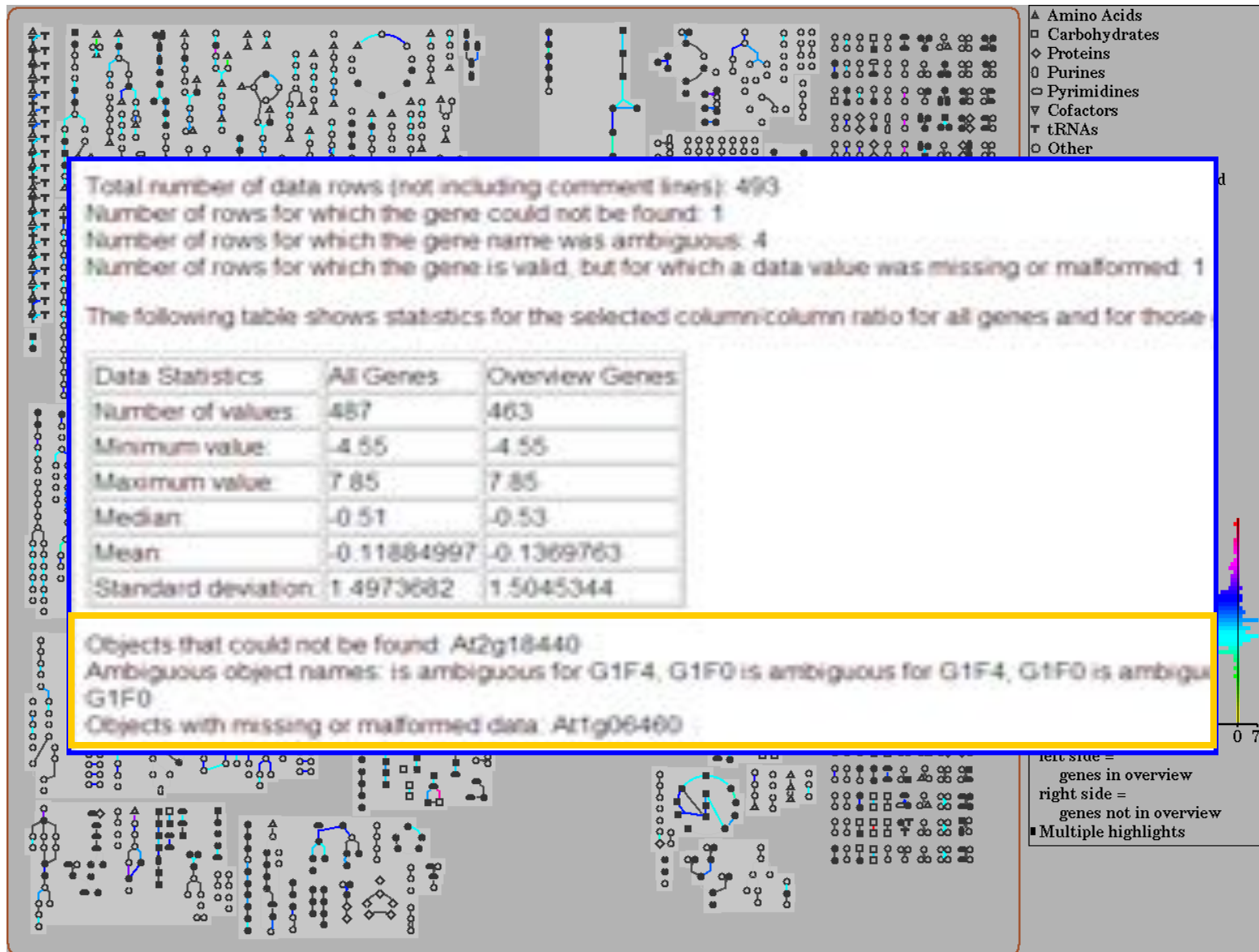
[Duckett03a](#) Duckett AH, Mudd SH. (2003) "Enzymes of phosphatidylcholine synthesis in *Lemna*, *Arabidopsis*, and *carrot*." *Plant Physiol.* (2003), 90, 1330-1340.

[Duckett03a](#) Duckett AH, Mudd SH. (1999) "Phosphatidylcholine synthesis: differing patterns in *Arabidopsis* and *carrot*." *Plant Physiol.* (1999), 90, 954-961.

[Hick01](#) Hick WD, Rhodes D, Hanson AD. (2001) "Radioisotope evidence implicating phosphoryl and phosphatidyl bases as intermediates in betaine synthesis by water-stressed barley leaves." *Plant Physiol.* (2001), 90, 914-922.

## References

# OMICS Viewer Statistics



# OMICS Viewer in action

- Multiple data sets can be entered using the same input file
- An animation can show changes in data sets:
  - wild type /mutant a / mutant b / mutant c / . . .
  - time points 0, 1, 2, 3, . . .
  - compound concentration x, y, z, . . .

## Suberin Biosynthesis

## Wild type



mutant A



mutant B





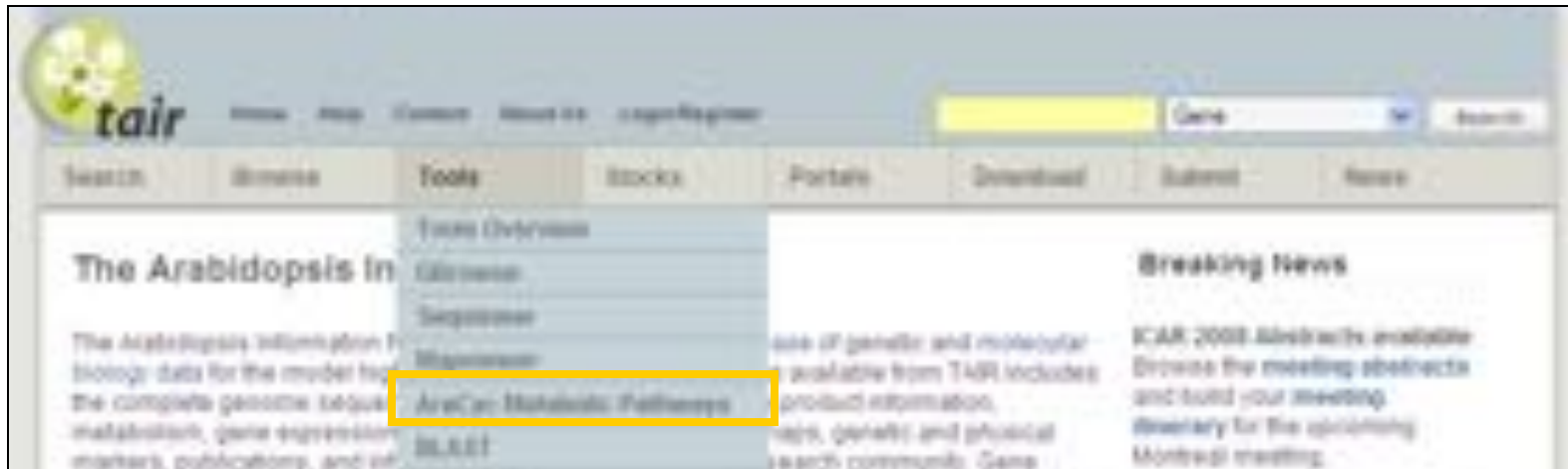
# OMICS Viewer data inputs

- Input file
  - tab-delimited
  - pre-analyzed
  - Identifiers
    - Genes
      - AGI
    - Enzyme
      - AGI
      - Enzyme
      - EC
    - Compound
      - Compound
  - Data in absolute
  - Sample file

	A	B	C	D	E	F	G
1	Existing Metabolic Detail						
2	Column 0	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
3							
4	Gene	WT	mutant1	mutant2	WT + salt	mutant1 + salt	mutant2 + salt
5	AT2G24770	1.20	2.40	1.60	4.00	9.60	6.72
6	AT1G042970	0.20	0.10	0.07	1.24	0.62	0.43
7	AT1G030440	0.30	0.39	0.27	0.54	0.70	0.49
8	AT3G022960	3.60	9.72	6.00	14.40	17.44	12.28
9	AT5G042920	1.26	0.51	0.36	7.94	3.17	2.22
10	AT3G022960	4.17	8.34	5.64	7.51	15.01	10.51
11	AT5G060680	0.24	0.12	0.08	0.96	0.48	0.36
12	AT5G060680	8.60	11.18	7.60	19.72	26.34	17.04
13	AT5G008570	0.77	2.08	1.46	1.39	3.74	2.62
14	AT4G28390	0.40	0.19	0.13	1.92	0.77	0.54
15	AT3G042980	0.67	1.34	0.94	4.15	8.31	5.82
16	AT3G048860	3.20	1.60	1.12	6.76	2.89	2.02
17	AT3G068910	4.10	5.33	3.73	12.17	15.62	11.07
18	AT3G049160	0.60	1.62	1.13	1.08	2.92	2.04
19	AT3G004050	0.38	0.16	0.11	1.52	0.61	0.43
20	AT3G025960	0.42	0.84	0.59	2.60	5.21	3.66
21	AT3G068880	0.08	0.05	0.03	0.16	0.08	0.06
22	AT2G38630	0.17	0.22	0.15	1.06	1.37	0.96
23	AT2G38630	1.40	4.00	2.00	2.66	7.19	6.03
24	AT2G329580	7.10	2.84	1.98	26.40	11.36	7.96

# OMICS Viewer data upload

**TAIR - [www.arabidopsis.org](http://www.arabidopsis.org)**



**AraCyc - [www.arabidopsis.org/biocyc](http://www.arabidopsis.org/biocyc)**





# OMICS Viewer data upload

PMN – [www.plantcyc.org](http://www.plantcyc.org)

The screenshot shows the PMN website with the logo 'PMN Plant Metabolic Network' at the top left. A search bar with a dropdown menu set to 'PlantCyc' and a 'search' button is located at the top right. Below the search bar is a navigation menu with tabs: 'About PMN', 'Databases', 'Downloads', 'Tools', 'Useful Sites', 'Submit Data', 'Help', and 'Feedback'. The 'Tools' tab is active, showing a dropdown menu with options: 'Tools Overview', 'Metabolic Maps', 'OMICS Viewer', 'Pathway Tools Biocyc Bundle', and 'Tutorials'. The 'OMICS Viewer' option is highlighted with a yellow box. To the right of the 'Tools' dropdown, the 'Aracyc (Arabidopsis)' option is also highlighted with a yellow box. The main content area on the left has a green header 'Introduction' and text describing the PMN as a collaboration between plant scientists and biochemists. On the right, there is a section titled 'Home of the PlantCyc' and a 'Tutorial 1.0' link. At the bottom right, a text block mentions 'On June 17, 2008, PlantCyc made its web debut, containing biochemical pathways with information from over'.

# OMICS Viewer data upload

## Pathway Tools Omics Viewer

The Pathway Tools Omics Viewer (formerly the Pathway Tools Expression Viewer) paints data values from the user's high-throughput and other experiments onto the Metabolic Overview diagram for an organism.

The Omics Viewer can be used for:

Select a dataset:

Arabidopsis thaliana COL ▼

File containing experimental data (NOT a URL):

C:\Documents and Settings\...

Browse...

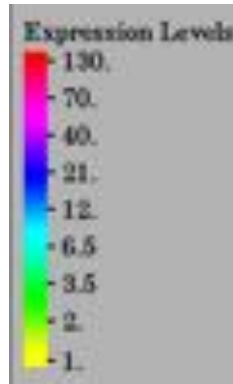
Do you want to display absolute or relative data values?

Relative ▼

- ❑ Select species
- ❑ Upload data file
- ❑ Enter appropriate parameters
- ❑ GO!

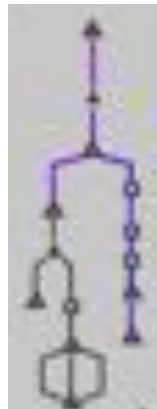
# New ideas to pursue . . .

Wild type



The transcript levels  
of several enzymes in the  
**lysine biosynthesis pathway**  
are reduced  $\sim 4$ -fold!

Mutant with  
“no phenotype”



Mutant with  
a metabolic  
phenotype



New hypotheses and experiments

# Acknowledgements

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## AraCyc, the PMN, and TAIR

Sue Rhee (*PI and Co-PI*)

Eva Huala (*Director and Co-PI*)

### Current Curators:

- Peifen Zhang (*Director and lead curator- metabolism*)
- Tanya Berardini (*lead curator – functional annotation*)
- David Swarbreck (*lead curator – structural annotation*)
- Debbie Alexander (*curator*)
- A. S. Karthikeyan (*curator*)
- Donghui Li (*curator*)

### Recent Past Contributors:

- Christophe Tissier (*curator*)
- Hartmut Foerster (*curator*)

### Tech Team Members:

- Bob Muller (*Manager*)
- Larry Ploetz (*Sys. Administrator*)
- Raymond Chetty
- Anjo Chi
- Vanessa Kirkup
- Cynthia Lee
- Tom Meyer
- Shanker Singh
- Chris Wilks

### Metabolic Pathway Software:

- Peter Karp and SRI group (**NIH**)

# Need more help?

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**If you have any questions or want a live demo . . .**

**Come to the Curation Booth – Booth #1  
Open throughout the conference!**



banner by Philippe Lamesch

Please stop by during a poster session

# Thank you . . .

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[www.arabidopsis.org](http://www.arabidopsis.org)

[curator@arabidopsis.org](mailto:curator@arabidopsis.org)



[www.arabidopsis.org/biocyc](http://www.arabidopsis.org/biocyc)

[curator@arabidopsis.org](mailto:curator@arabidopsis.org)



[www.plantcyc.org](http://www.plantcyc.org)

[curator@plantcyc.org](mailto:curator@plantcyc.org)

# More Help and Tutorials

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[http://www.arabidopsis.org/help/tutorials/aracyc\\_intro.jsp](http://www.arabidopsis.org/help/tutorials/aracyc_intro.jsp)

[curator@arabidopsis.org](mailto:curator@arabidopsis.org)



[http://www.plantcyc.org/tutorials/tutorials\\_index.faces](http://www.plantcyc.org/tutorials/tutorials_index.faces)

[curator@plantcyc.org](mailto:curator@plantcyc.org)