

Identification of mitochondrial transfer sequences in a folic acid metabolism gene

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Neural Tube Defects and Folic Acid

- ▶ Second leading cause of congenital birth defects
- ▶ Around 1 in 1,000 births experience a neural tube defect (open spina bifida and anencephaly)
- ▶ Neural tube structure is a structure that gives rise to brain and spinal cord
- ▶ Nutritional levels of folic acid are linked to risks of neural tube defects
 - ▶ Increased levels of folic acid decrease risk of neural tube defects
- ▶ A possible mechanism suggests folic acid levels affect neural tube development
- ▶ Altered folic acid levels increase the risk of cancers such as colorectal and breast cancer



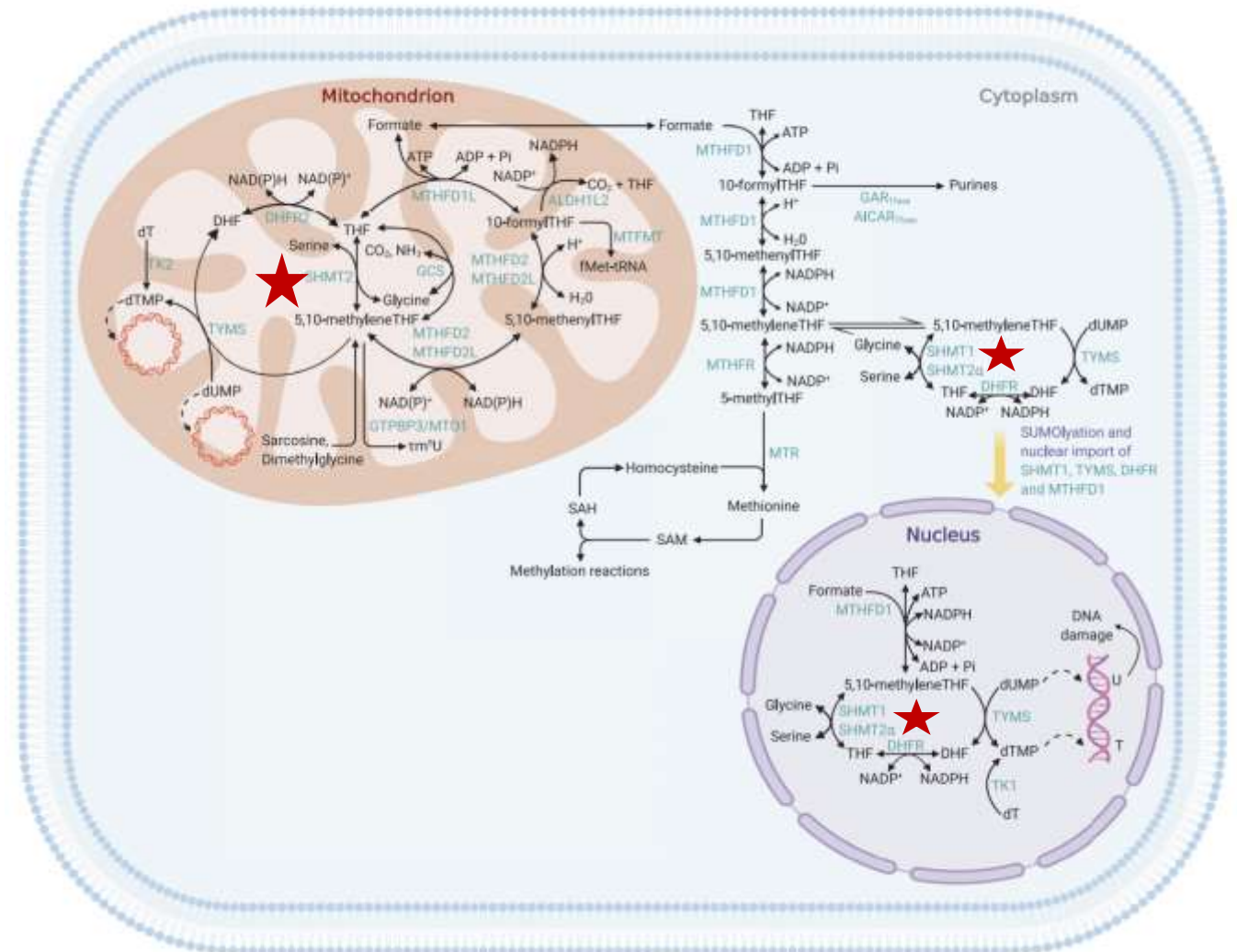
Open spina bifida



Anencephaly

Serine Hydroxymethyltransferase (SHMT)

- ▶ One of the many proteins in folic acid metabolism
- ▶ Metabolizes glycine to serine in folic acid metabolism
- ▶ Assists to create one-carbon groups
- ▶ Metabolism occurs in two areas:
 - ▶ cytosol and nucleus
 - ▶ mitochondria



Focus

- ❖ *C. elegans* have one *Shmt* gene while most Eukaryotes have two genes
- ❖ Q: Is the *C. elegans Shmt* homolog, *mel-32* localized to the cytosol, mitochondria, or both?
- ❖ Q: Do any other organisms have isoforms instead of genes?
- ❖ Q: Can these isoforms be used to determine any potential mitochondrial transfer sequences?

Methodology

Sampled 17 species from the 3 domains, 4 kingdoms within Eukaryotes, and 11 phyla within the animal kingdom

Identified *Shmt* homolog(s) from sampled species

Determined whether if the *Shmt* was mitochondrial or cytosolic

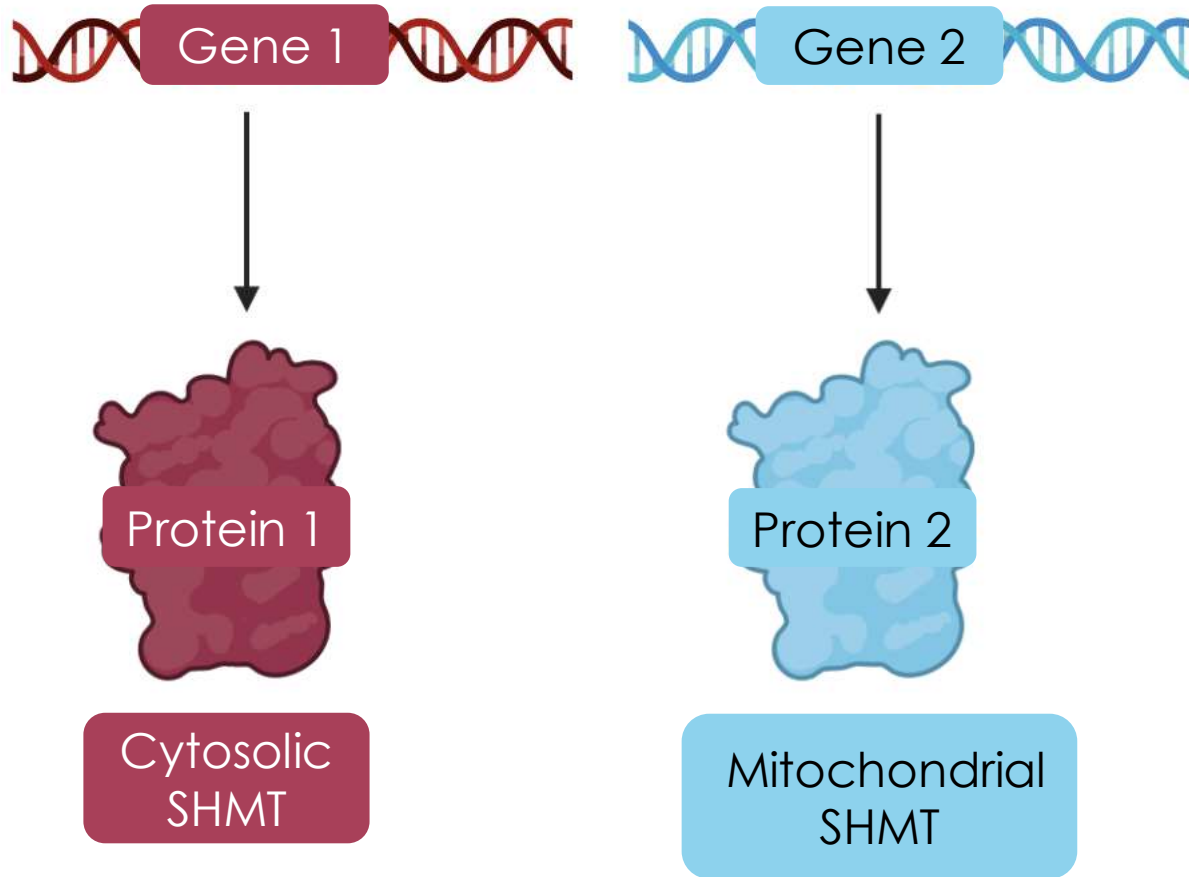
Compared *Shmt*(s) within species to determine if they were isoforms or independent genes

Identified mitochondrial transfer sequences from isoforms

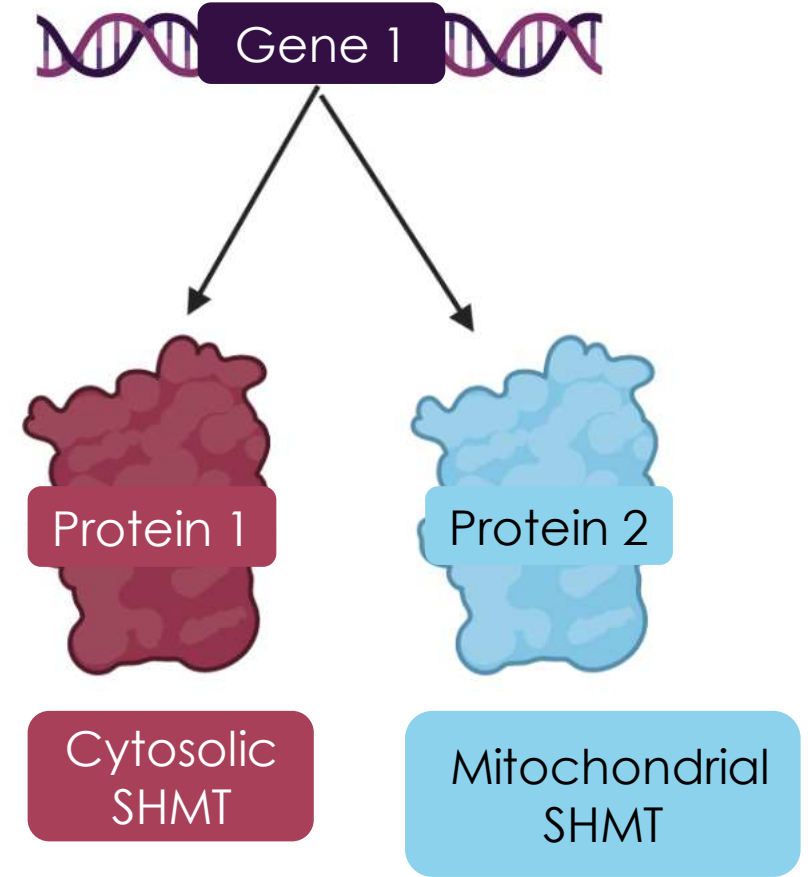
Analyze SHMTs for phylogenetic similarities (all, cytosolic, and mitochondrial)

What is an Isoform?

1 Gene = 1 Protein

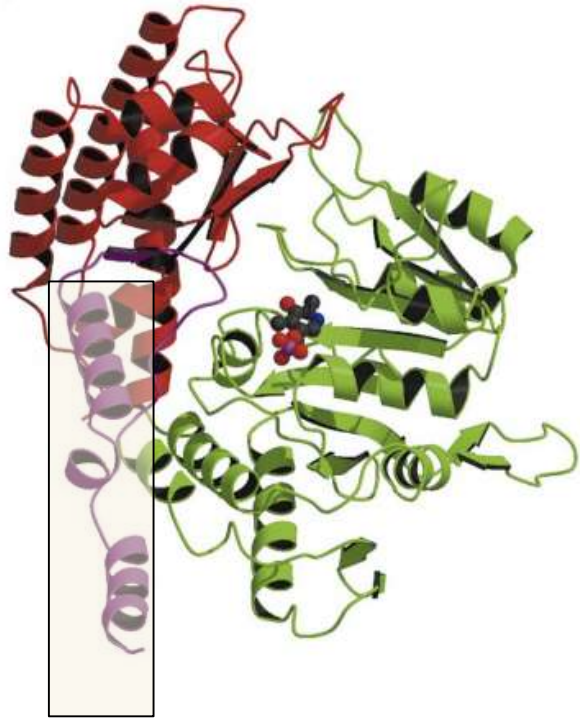


1 Gene = Multiple Proteins



Since isoforms are localized to different targets, the difference must be in the targeting sequence!

Proteins are Localized Through Target Sequences



- ▶ A target sequence is required for proteins to be localized to the mitochondria (like a zip code!)
- ▶ N-terminal encodes the target sequences
- ▶ Sequences have similar physiochemical properties
- ▶ Research shows a conserved pattern for mitochondrial target sequence: $\phi X X \phi \phi$
 - ▶ ϕ (phi) indicates a bulky hydrophobic residue (but can tolerate glycine, proline, and alanine)
 - ▶ X indicates any amino acid
- ▶ The N-terminal amino acid sequence interacts with the receptors, translocase of the outer and inner mitochondrial membranes

Discovery of Isoforms of *mel-32*

- ▶ *mel-32* is the *C. elegans* homolog of *Shmt*
- ▶ *mel-32* previously has not been published as producing isoforms because it is not widely researched
- ▶ *mel-32* isoforms were found on genetic database (Wormbase: C05D11.11)

mel-32: isoform A
Cytosolic: 0.0442
Signal: 0.0018
Mitochondrial: 0.9541



mel-32: isoform B
Cytosolic: 0.9999
Signal: 0
Mitochondrial: 0.0001



Table 1: Determined SHMT Localizations and Percent Identity within Species

Kingdom/ Phylum Representation	Organism	Mitochondrial SHMT	Cytosolic SHMT	Undeclared SHMT ²	Percent Identity
Stramenopiles (Protists)	<i>Thalassiosira</i>	XP_002295557	XP_002289669		54.58-
	<i>pseudonana</i>		XP_002293993		58.54
Plant	<i>Arabidopsis</i>	NP_195506	NP_193129	NP_001119098	47.59-
	<i>thaliana</i>	NP_001331385	NP_193125 NP_001323098 NP_564473		85.24
Fungi	<i>Saccharomyces</i> <i>cerevisiae</i>	AAA21024	AAA21023		59.10
★ Porifera	<i>Amphimedon</i> <i>queenslandica</i>	XP_019854079	XP_019854080	XP_003387864	99.20- 100.00
Cnidaria	<i>Actinia tenebrosa</i>	XP_031559133	XP_031558549		63.00
Platyhelminthes	<i>Opisthorchis</i>	OON24063	OON23958		54.60-
	<i>viverrini</i>	XP_009166916	XP_009166918		100.00
Annelida	<i>Capitella teleta</i>	ELU01860	ELU03449		64.39
Mollusca	<i>Crassostrea gigas</i>	XP_011420488	XP_011435353		59.79-
			XP_034311075		100.00
Rotifera	<i>Brachionus</i> <i>plicatilis</i>		RMZ93562 RNA14241		24.17
★ Nematoda	<i>Caenorhabditis</i> <i>elegans</i>	NP_741197	NP_001367440		100.00
★ Arthropoda	<i>Drosophila</i> <i>melanogaster</i>	NP_572278	NP_001138162		100.00
Echinodermata	<i>Strongylocentrotus</i>	XP_030829045	XP_798074		54.29-
	<i>purpuratus</i>		XP_011661053		100.00
Chordata	<i>Ciona intestinalis</i>	XP_002126094	XP_002127233		60.50
Chordata	<i>Homo sapiens</i>	NP_005403	NP_004160		63.45

Identification of Mitochondrial Transfer Sequences

- ▶ Mitochondrial transfer sequences are based off the motif, φ X X φ φ
- ▶ The three species sampled from Porifera, Nematoda, and Arthropoda had at least two isoforms with different localizations

A. *Amphimedon queenslandica* Isoforms

XP_019854079.1	-----MLITVFRKAAKVTFRALDRRFQQVMA	26
XP_003387864.2	MSCKLFWIMSITCMGATIVGVFLKKAESLRRTRERCITVITKAAKVTFRALDRRFQQVMA	60
XP_019854080.1	-----MA	2

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B. *Caenorhabditis elegans* Isoforms

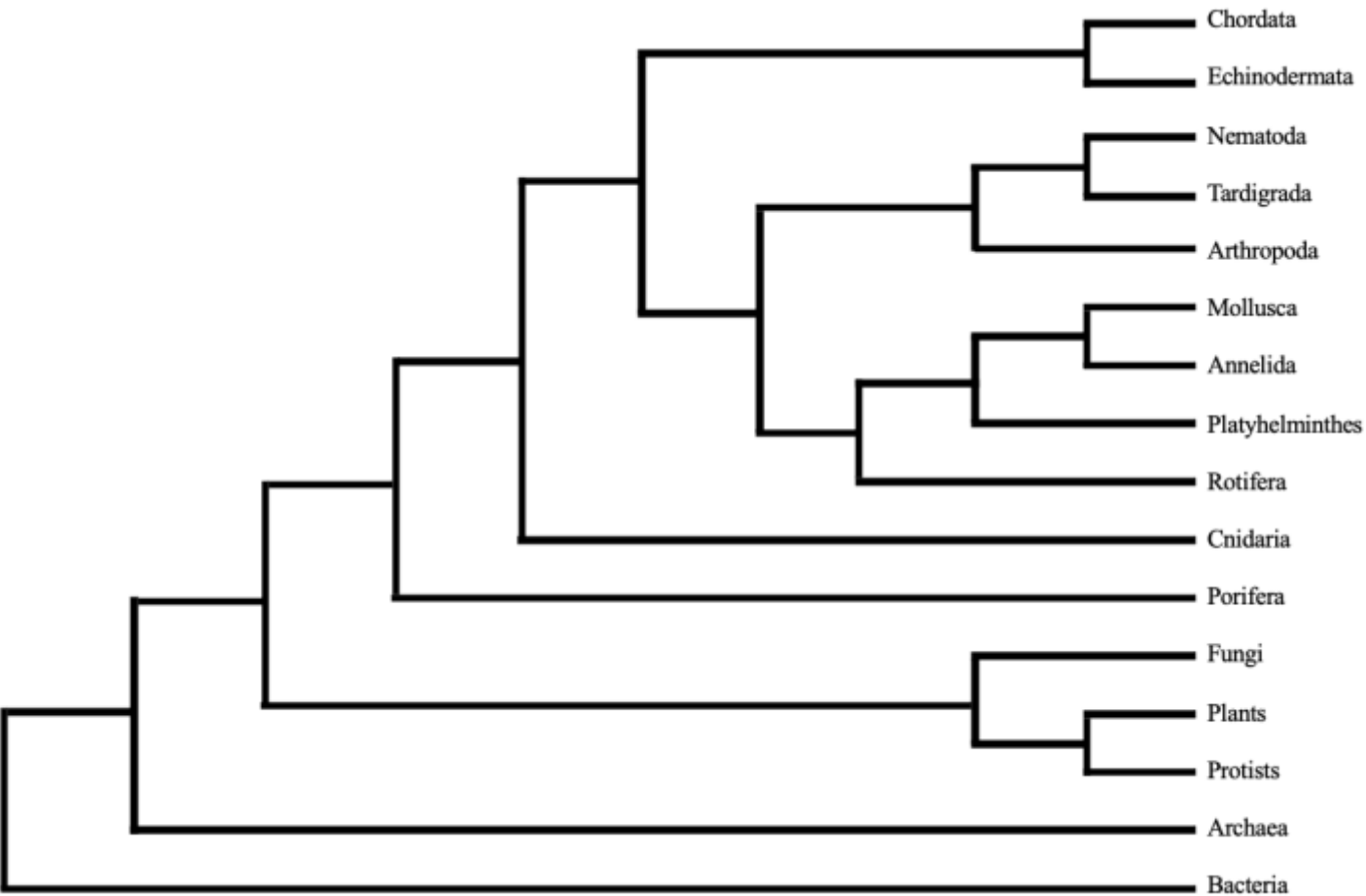
NP_741197.1	MFARIVSRRAATGLFAGASSQCKMADRQVHTPLAKVQRHKYTNNENILVDHVEKVDPEVF	60
NP_001367440.1	-----MADRQVHTPLAKVQRHKYTNNENILVDHVEKVDPEVF	37

C. *Drosophila melanogaster* Isoforms

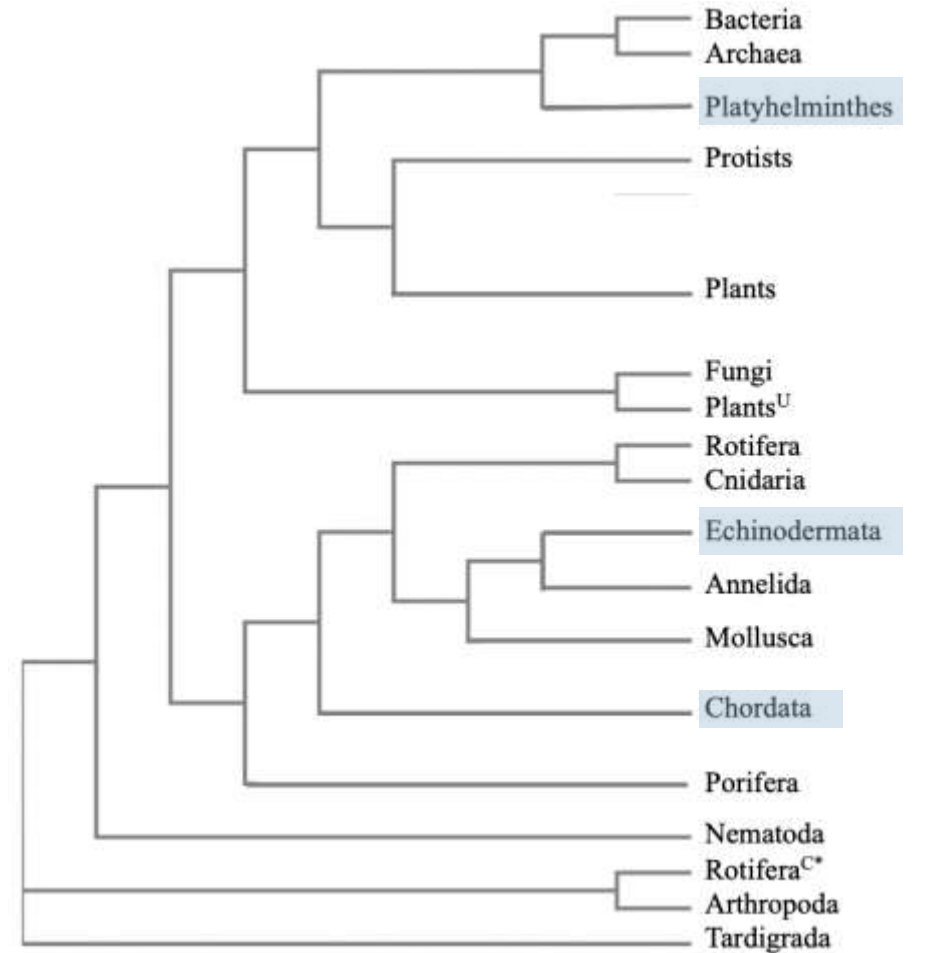
NP_572278.1	MQRARSTLTQKLRFLSRDLNTKVGPNVNFETGKLSGALTRIAAKKQPSPTPFLPAIRRY	60
NP_001138162.1	-----	0

NP_572278.1	SDSKQSTLKNMADQKLLQTPLAQGDPELAELIKKEKERQREGLEMIASENFTSVAVLESL	120
NP_001138162.1	-----MADQKLLQTPLAQGDPELAELIKKEKERQREGLEMIASENFTSVAVLESL	50

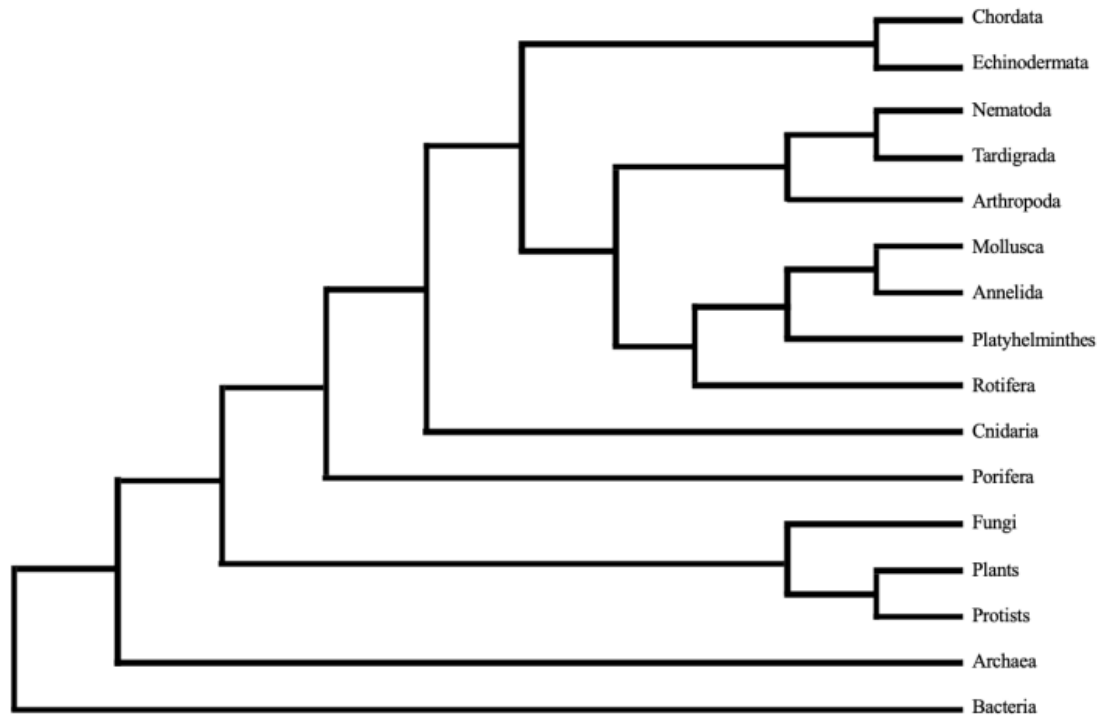
Accepted Phylogeny



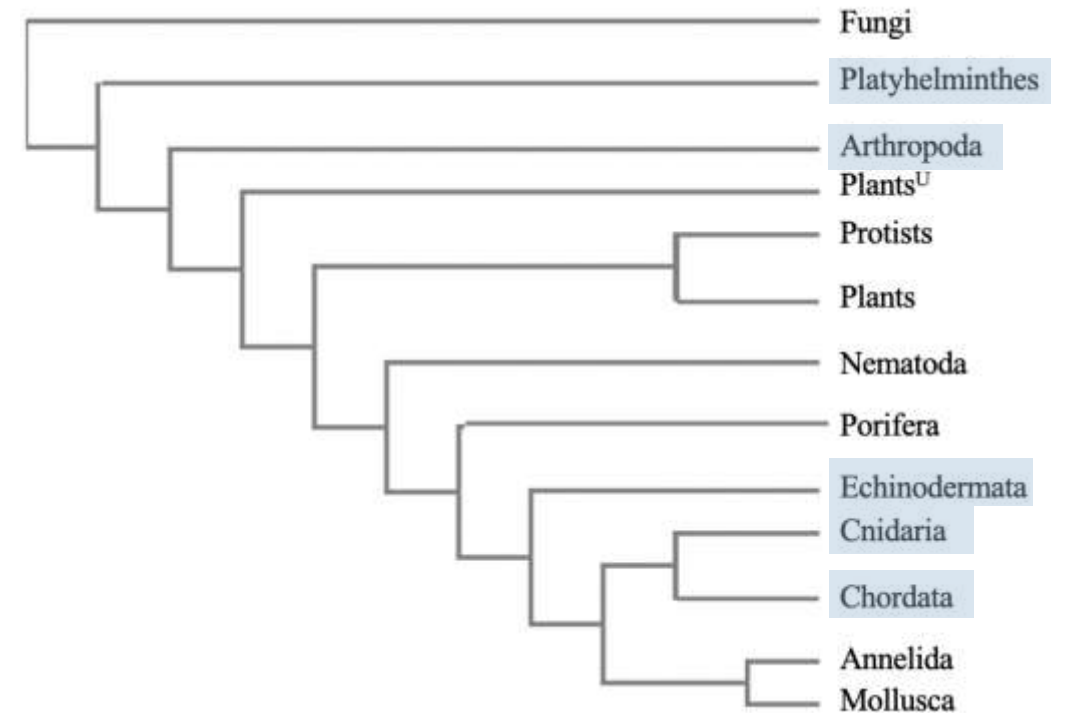
Tested Cytosolic SHMT Phylogeny



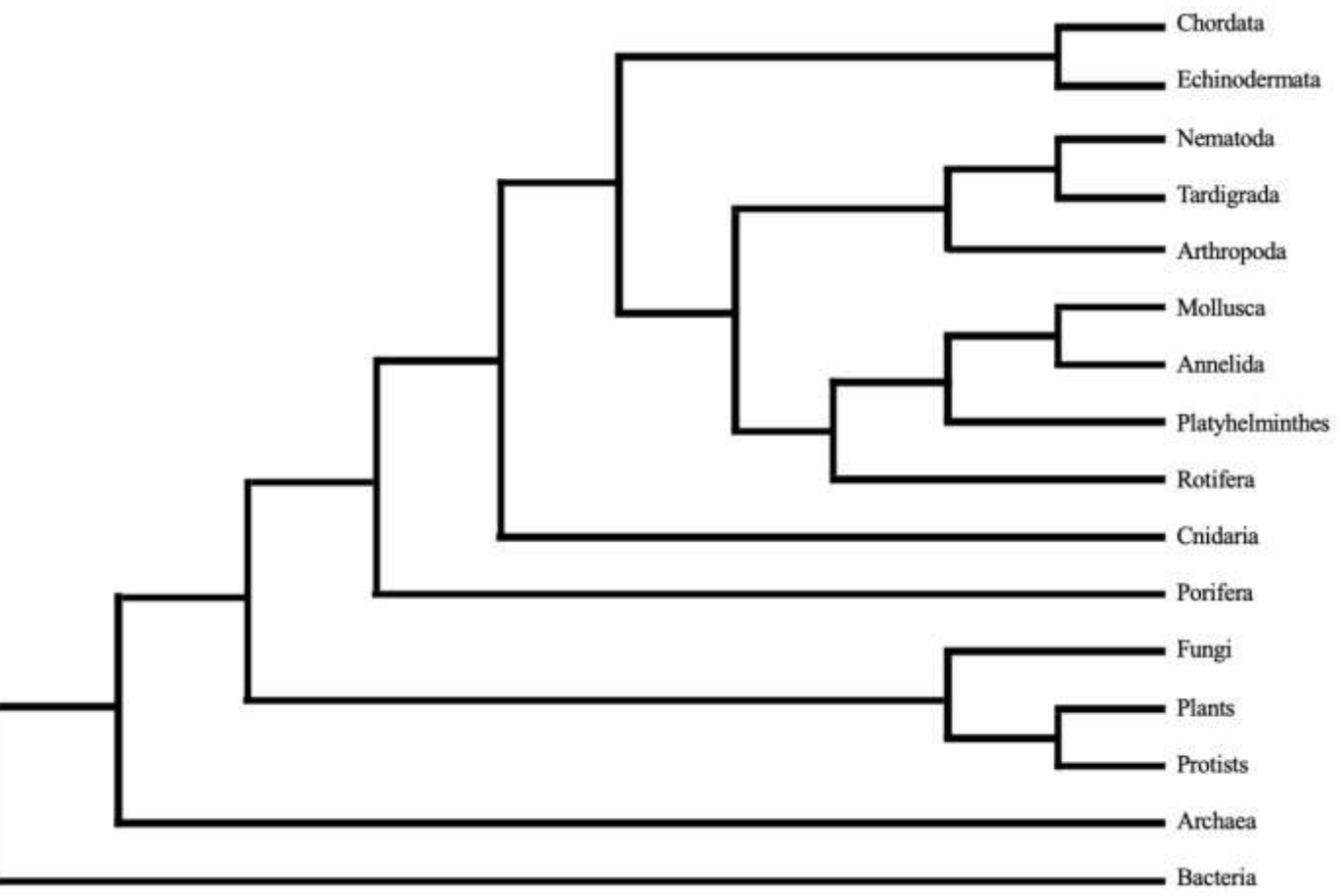
Accepted Phylogeny



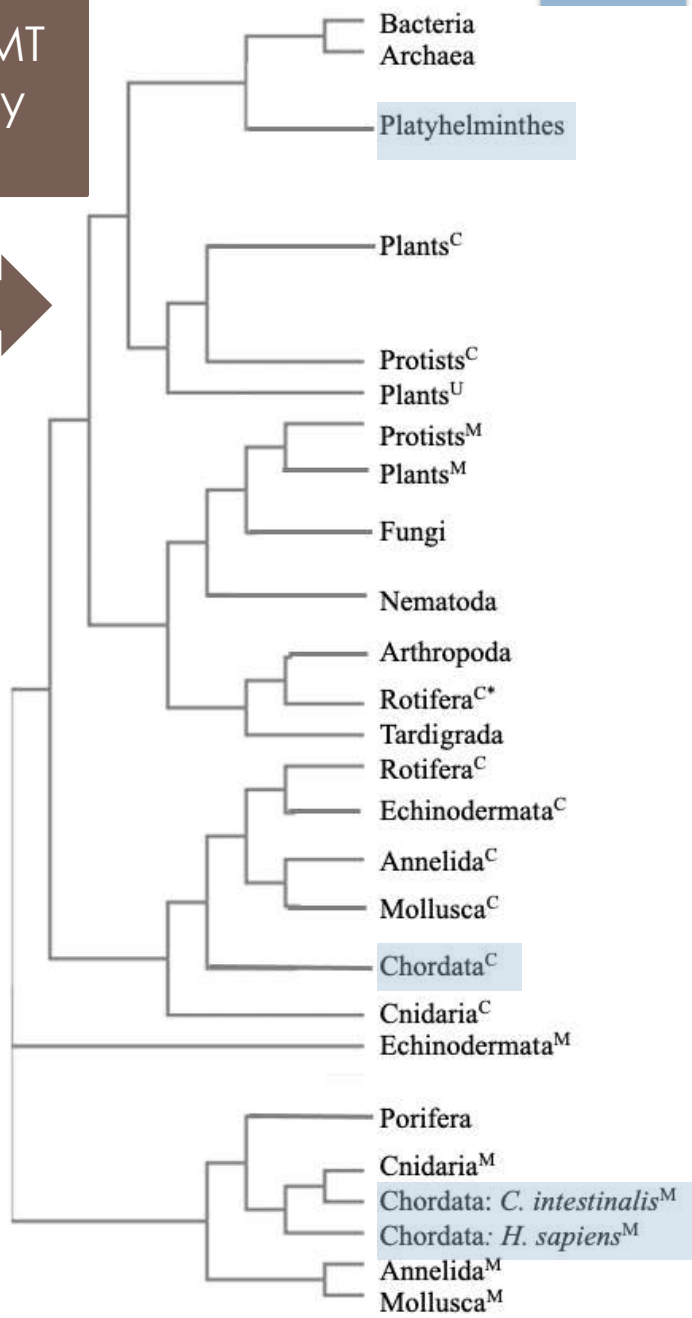
Tested Mitochondrial SHMT Phylogeny



Accepted Phylogeny



Tested SHMT Phylogeny



Results and Takeaways

Identified Mitochondrial Transfer Sequences

- ▶ Successful in identifying potential mitochondrial presequences
- ▶ Future experiments can be done to:
 - ▶ (1) confirm *C. elegans* isoform is localized to the mitochondrial,
 - ▶ (2) determine if both isoforms in *C. elegans* are essential
 - ▶ (3) determine which amino acid(s) are essential for mitochondrial targeting

Phylogenetic Comparisons

- ▶ Cytosolic SHMT and Mitochondrial SHMT were closer together unless the SHMTs originated from isoforms
- ▶ Key differences include (1) Platyhelminthes difference, and (2) similarity between Cnidaria and Chordata
- ▶ Due to Tardigrada's association with Nematoda and Arthropoda, it can be hypothesized that **Tardigrada has a mitochondrial SHMT isoform** (which has not been identified yet)

THANK YOU TO DR. SULLIVAN-BROWN FOR ALL YOUR HELP!

Questions?