



Neuronal Mitochondrion Trafficking

BCH441 Project: Defining a System

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The source code, notebook, and data pipeline can be found at github.com/thejmazz/biologicalsystem.
Cover image (mitochondrion in Purkinje neuron) by *Atlas of Ultrastructural Neurocytology*¹

¹synapses.clm.utexas.edu/atlas/1_1_2_8.stm

Contents

1	Introduction	2
2	The System	3
2.1	Systems Role Ontology	4
3	Gene Collection	5
4	Documentation	5
4.1	Signal Integration	5
4.1.1	RHOT1, RHOT2 <i>Mitochondrial Rho GTPase 1, 2</i>	5
4.1.2	TIAM2 <i>T-lymphoma invasion and metastasis-inducing protein 2</i>	6
4.2	Set	6
4.2.1	CLUH <i>Clustered mitochondria protein homolog</i>	6
4.2.2	MAP1B <i>Microtubule-associated protein 1B</i>	6
4.2.3	MAPT <i>Microtubule-associated protein tau</i>	6
4.2.4	MTM1 <i>Myotubularin</i>	6
4.2.5	SYBU <i>Syntabulin</i>	7
4.3	Transmit	8
4.3.1	BHLHA15 <i>Class A basic helix-loop-helix protein 15</i>	8
4.3.2	GABA <i>Gamma-amino-N-butyrate transaminase</i>	8
4.3.3	TRAK1, TRAK2 <i>Trafficking kinesin-binding protein 1, 2</i>	8
4.4	Transform	8
4.4.1	GAN <i>Gigaxonin</i>	8
4.4.2	TTL <i>Tubulin-tyrosine ligase</i>	9
4.5	Regulate	9
4.5.1	ATCAY <i>Caytaxin</i>	9
4.5.2	MGARP <i>Mitochondria-localized glutamic acid-rich protein</i>	10
4.5.3	MSTO1 <i>Protein misato homolog 1</i>	10
4.5.4	SNPH <i>Syntaphilin</i>	10
4.6	Output	11
4.6.1	KIF1B, KIF5B, KLC1 <i>Kinesin-like protein KIF1B, heavy chain, light chain</i>	11
5	Summary	11
6	Data Pipeline	11

1 Introduction

The “powerhouse of the cell” as it is so commonly called, the mitochondria is one of the most vital organelles in eukaryotes. This structure is thought to have developed through a symbiotic relationship among engulfed prokaryotic cells and their hosts. As such, it is rooted quite deeply evolutionarily, and one might expect its proper functioning to be absolutely vital, that is, knock-out mutants will not survive. This is true - but as we will see, it is not just the performance of this organelle which is centrally important, but where it is localized within the cell as well.

Images of isolated mitochondria were first observed in 1979 by Johnson et al.:
The variety of mitochondrion shape and size is clear, ranging from globular to filamentous to networked structures. As well, the authors observed movement during 15-30 sec intervals, between fluorescent and phase-contrast photographs.

The primary role of a mitochondrion is to supply energy to the cell in the form of ATP units, through the electron transport chain among the cristae. Where is that energy needed? Consider highly polar and elongated cells such as neurons. The cell body of a neuron is distant from its synaptic endings, where as it happens, large amounts of energy are required for neurotransmitter release and absorption. Following,

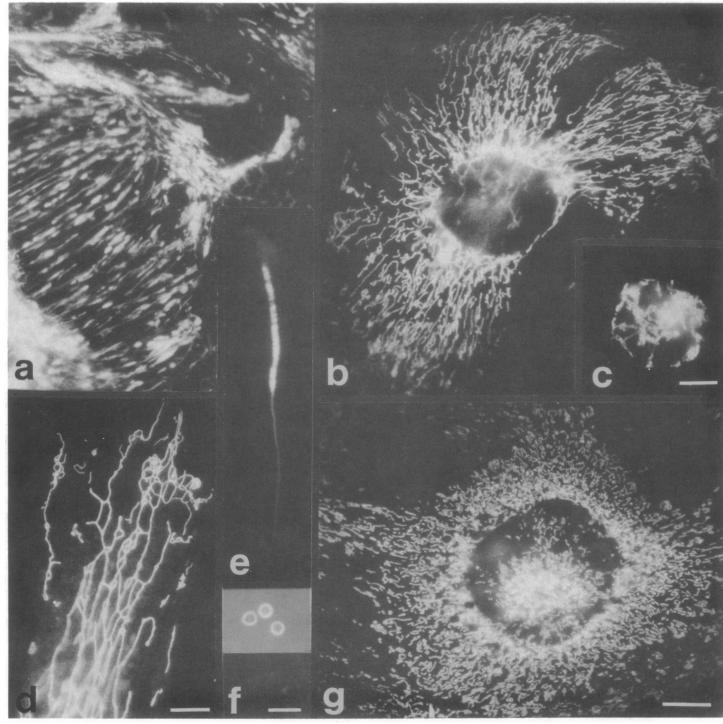


FIG. 5. Living cells stained with rhodamine 123: (a) rat cardiac muscle; (b) Pt K1 marsupial kidney; (c) mouse B lymphocyte; (d) mouse 3T6; (e) mouse sperm; (f) human erythrocytes (phase-contrast above and rhodamine 123-treated but unstained below); (g) rat embryo fibroblast. Bar represents: 15 μm in a, b, e, and g; 10 μm in c; 8 μm in d; 10 μm in f.

Figure 1: Mitochondria Morphology

we will investigate the **system** whose **functional role** is the **localization of mitochondrion within neurons**.

2 The System

<i>Name</i>	Localization/Trafficking of mitochondrion within neurons
<i>Description</i>	The collective of functional units represented by genes which process signals, transduce these events, initiate, and maintain the actions necessary to transport mitochondrion to distal points along the axon of a neuron.
<i>Associated GO Terms</i>	GO:0051646 (mitochondrion localization) <ul style="list-style-type: none"> • GO:0051659 (maintenance of mitochondrion localization) <ul style="list-style-type: none"> – GO:1990456 (mitochondrion-ER tethering) • GO:0034643 (establishment of mitochondrial localization, microtubule mediated) <ul style="list-style-type: none"> – GO:0034642 (mitochondrial migration along actin filament) – GO:0034643 (establishment of mitochondrial localization, microtubule mediated) <ul style="list-style-type: none"> * GO:0034640 (establishment of mitochondrion localization by microtubule attachment) * GO:0047497 (mitochondrion transport along microtubule) – GO:0090146 (establishment of mitochondrial localization involved in mitochondrial fission) <ul style="list-style-type: none"> * GO:0090147 (regulation of establishment of mitochondrion localization involved in mitochondrial fission) • GO:0048311 (mitochondrion distribution)

- GO:0048312 (intracellular distribution of mitochondria)
- GO:0000001 (mitochondrion inheritance)

Why this system? Originally I was looking into “mitochondrial localization.” Amongst the genes returned by the ontology, there appeared those related to mitochondrial localization during cellular reproduction, transport, microtubules, tethers, mRNA-binding, and various “popular” genes such as ubiquitins, serum albumin, leucine-rich repeat serine/threonine-protein kinase, basic helix-loop-helix protein. There was a fair amount of variety. In order to gather together a structured list of genes I would need to filter these out, and to filter these out I would need a functional goal. I decided to choose the neuronal process because it is one of the most extreme cases of mitochondrial movement in all cell types, there was a decent amount of related literature available, some elements of its processes had been recently elucidated, and it has important neurophysiological consequences. A review by Reis et al. (2009) explored the atypical Miro GTPases and their role in transporting mitochondria in neurons. The authors note that abnormal mitochondrial dynamics can contribute to Amyotrophic Lateral Sclerosis (ALS), Huntington’s, Parkinson’s, and Alzheimer’s diseases. A more recent experiment by Loss and Stephenson (2015) examines the role of TRAK1 and TRAK2 kinesin adaptor proteins which link mitochondria to kinesin motor proteins. Furthermore, Miro proteins are expressed in a large variety of cell types, potentially extending this current analysis to new domains (Reis et al., 2009).

2.1 Systems Role Ontology

To define this system in a structured manner, I considered its functionality in the context of the Systems Roles Ontology, which can be found at github.com/hyginn/SyRO:

SyRO – Systems Role Ontology (2015-10-27)

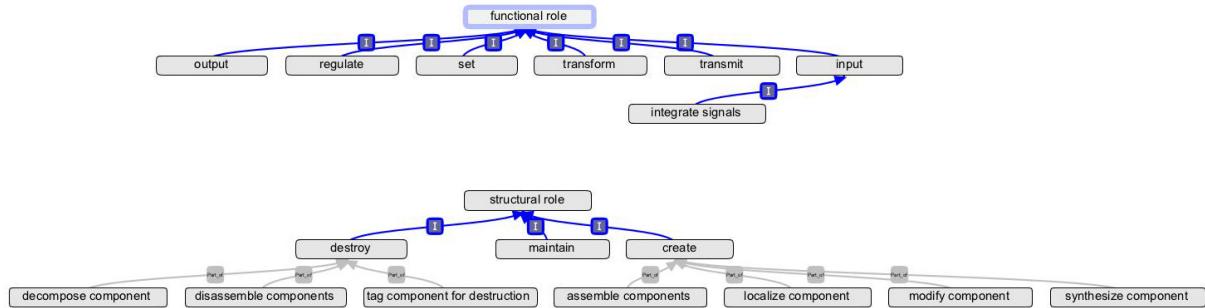


Figure 2: Systems Roles Ontology

Name	ID	Description
input	16	compounds or signals prompting the directed movement of mitochondrion
integrate signals	22	machinery which directs input signals/compounds to the system
output	21	mitochondrion transport towards synaptic endings
regulate	20	components which ensure regular mitochondrial motility
set	19	preparing the functional units, “setting the stage” as it were
transform	18	altering the system status so that it may be reversed, stopped, reinstated
transmit	17	the physical machinery to move mitochondria

These functions collaborate to produce the **functional role** of localizing mitochondria **for the proper functioning of neural communication**. I will define the bounds of this system as any genes which can be annotated with the functional role annotations above. Structural role annotations will be considered second to function, and will be used largely to describe how that component physically determines its function. With this goal in mind, I fetched and filtered data from the Gene Ontology Consortium, QuickGO, UNIPROT, IntAct, and STRING. Those genes which did not make the cut can be seen through the *Summary of first pool* in my [notebook](#).

3 Gene Collection

Gene	Accession	Name	SyRO
ATCAY	Q86WG3	Caytaxin	regulate
BHLHA15	Q7RTS1	Class A basic helix-loop-helix protein 15	transmit
CLUH	I3L2B0	Clustered mitochondria protein homolog	set
GABA	P80404	Gamma-amino-N-butyrate transaminase	transmit
GAN	Q9H2C0	Gigaxonin	transform
KIF1B	O60333	Kinesin-like protein KIF1B	output
KIF5B	P33176	Kinesin-1 heavy chain	output
KLCA1	Q07866	Kinesin light chain 1	output
MAPT	P10636	Microtubule-associated protein tau	set
MAP1B	P46821	Microtubule-associated protein 1B	set
MGARP	Q8TDB4	Mitochondria-localized glutamic acid-rich protein	regulate
MTM1	Q13496	Myotubularin	set
MSTO1	Q9BUK6	Protein misato homolog 1	regulate
RHOT1	Q8IXI2	Mitochondrial Rho GTPase 1	integrate signals
RHOT2	Q8IXI1	Mitochondrial Rho GTPase 2	integrate signals
SNPH	O15079	Syntaphilin	regulate
SYBU	Q9NX95	Syntabulin	set
TIAM2	Q8IVF5	T-lymphoma invasion and metastasis-inducing protein 2	integrate signals
TRAK1	Q9UPV9	Trafficking kinesin-binding protein 1	transmit
TRAK2	Q8IU62	Trafficking kinesin-binding protein 2	transmit
TTL	Q8NG68	Tubulin–tyrosine ligase	transform

4 Documentation

4.1 Signal Integration

4.1.1 RHOT1, RHOT2 *Mitochondrial Rho GTPase 1, 2*

RHOT1 and RHOT2, which are also known as MIRO1 and MIRO2, were first reported as a new family of Rho GTPases with in 2003 by Fransson et al.. An NCBI CDD search presents us with two GTPase domains at each terminus, with two EF hands in between. The C terminal TM domain targets this protein to the mitochondria.



Figure 3: RHOT1 Domains, CDD

NTPases are a large superfamily and operate in a large variety of systems. The on/off state of these proteins confers their ability to integrate signals into a pathway. In this way we can observe that whichever is activating Rhot, Rhot is integrating that signal downwards to its effector. Fransson et al. also observed an interesting property: overexpression of Miro1/Val-13 led to an aggregation of the mitochondrial network. The same authors separated this response into two distinct phenotypes later in ?. They observed that Miro-1 induced aggregation and thread-like mitochondria, whereas Miro-2 only induced aggregation. ? also

demonstrated interactions of Miro with GRIF-1 (TRAK2) and OIP106 (TRAK1), trafficking kinesin binding proteins. The EF hands can bind calcium, disrupting interactions with TRAK (Reis et al., 2009).

4.1.2 TIAM2 *T-lymphoma invasion and metastasis-inducing protein 2*

TIAM2 regulates the activity of RHO-like proteins (UNIPROT). This is confirmed with the existence of a GEF domain from the CDD.

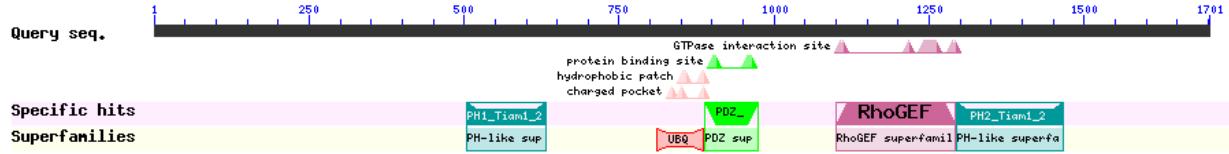


Figure 4: TIAM2 Domains, CDD

In this way TIAM2 has the ability to activate Rhot. TIAM2 has also been shown to promote the migration of neurons in the cerebral cortex (UNIPROT).

4.2 Set

4.2.1 CLUH *Clustered mitochondria protein homolog*

A CDD search with CLU1 from yeast presents the CLU domain (CLUstered mitochondria). This domain is required for mitochondrial positioning and transport; improper function can lead to mitochondrion clustering at the microtubule plus ends (CDD). Another uncharacterized CLU domain is present.

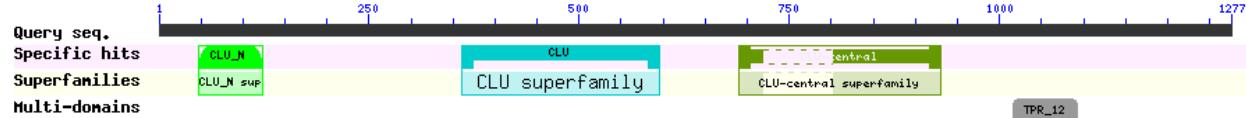


Figure 5: CLU1 Domains (Yeast), CDD

4.2.2 MAP1B *Microtubule-associated protein 1B*

By similarity, MAP1B facilitates tyrosination of α -tubulin in neuronal microtubules. Interacts with TIAM2 and TTL (UNIPROT). IntAct suggests an interaction with GAN.

4.2.3 MAPT *Microtubule-associated protein tau*

CDD for MAPT presents tubulin binding repeat domains. MAPT is expected to stabilize microtubules and potentially establish and maintain neuronal polarity (UNIPROT).

4.2.4 MTM1 *Myotubularin*

Hnia et al. (2011) observed that decreased MTM1 expression and mutations induced abnormal mitochondrial positioning, shape, dynamics, and function.

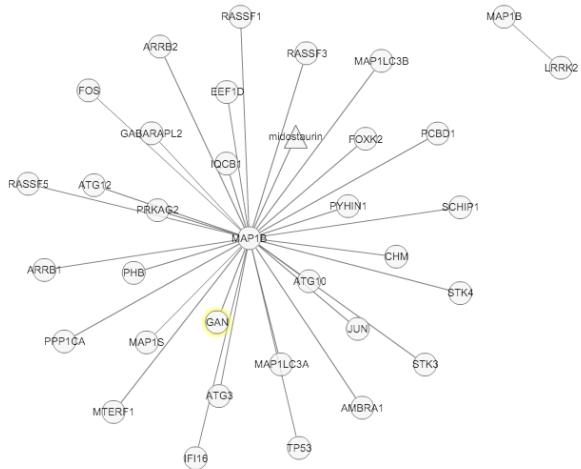


Figure 6: MAP1B Protein-Protein Interactions, IntAct

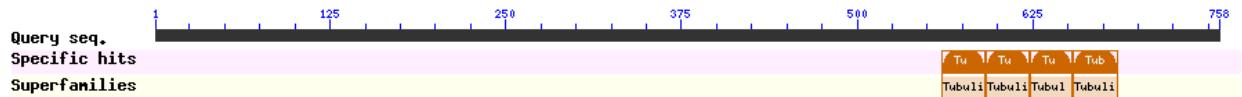


Figure 7: MAPT Domains, CDD

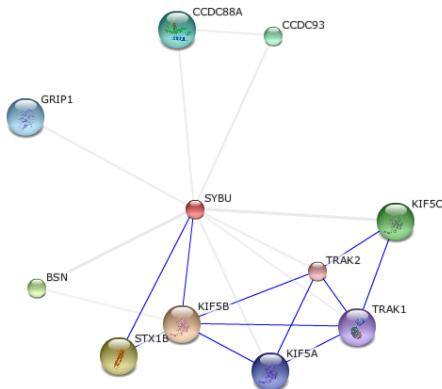


Figure 8: SYBU Protein-Protein Interactions, STRING

4.2.5 SYBU *Syntabulin*

SYBU belongs to a kinesin motor-adapter complex. It is critical for forward axonal transport (UNIPROT). STRING demonstrates binding interactions with KIF5B, which in turn binds with KIF5A and TRAK2, of which both bind TRAK1.

Reis et al. (2009) note that syntabulin has been found to interact with kinesin heavy chain in hippocampal neurons.

4.3 Transmit

4.3.1 BHLHA15 *Class A basic helix-loop-helix protein 15*

Required for mitochondrial calcium ion transport (UNIPROT). Ensembl electronic annotation has tagged BHLHA15 with the biological process [calcium-mediated signalling](#). This protein is expressed specifically in brain, liver, spleen and skeletal muscle (UNIPROT).

4.3.2 GABA *Gamma-amino-N-butyrate transaminase*

GABA is located within the mitochondrial matrix (UNIPROT). Does not appear particularly involved on its own, yet we will see it interacts with TRAK1 and TRAK2.

4.3.3 TRAK1, TRAK2 *Trafficking kinesin-binding protein 1, 2*

Reis et al. (2009) identify Milton as an adapter protein required for the transport of mitochondria in axons. The closest human equivalents are TRAK1 and TRAK2, which each contain a coiled-coiled domain. Below are the TRAK1 and TRAK2 CDD results.

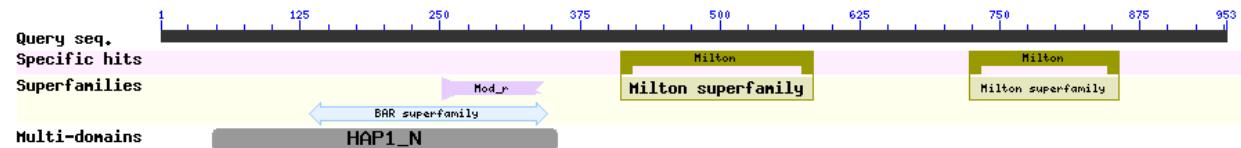


Figure 9: TRAK1 Domains, CDD

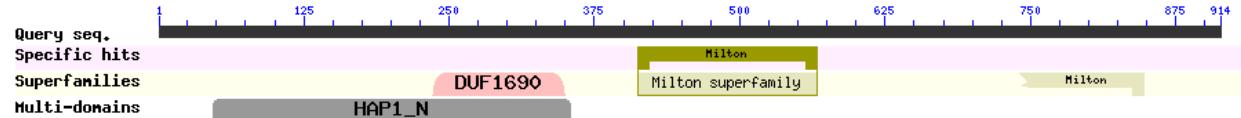


Figure 10: TRAK2 Domains, CDD

The Milton domain is credited for recruitment of kinesin heavy chain to the mitochondria (CDD). TRAK1 and TRAK2 have demonstrated interactions with GABA receptors (?). It was previously noted that GABA is located within the mitochondrion and mitochondrial matrix. UNIPROT mentions TRAK is involved in the regulation of trafficking GABA receptors.

STRING corroborates with interactions for SYBU above. As well make note of the identified binding between TRAK2, MGARP and RHOT1.

4.4 Transform

4.4.1 GAN *Gigaxonin*

Allen et al. (2005) observed that GAN interacts with the light chain of MAP1B. IntAct also suggests this interaction.

When Allen et al. deleted sequences for binding to GAN from MAP1B, the N terminus of MAP1B was not ubiquitinated. This suggests that GAN plays a regulatory role with MAP1B, and since MAP1B facilitates forward movement of mitochondria along microtubules, GAN has the potential to arrest this process.

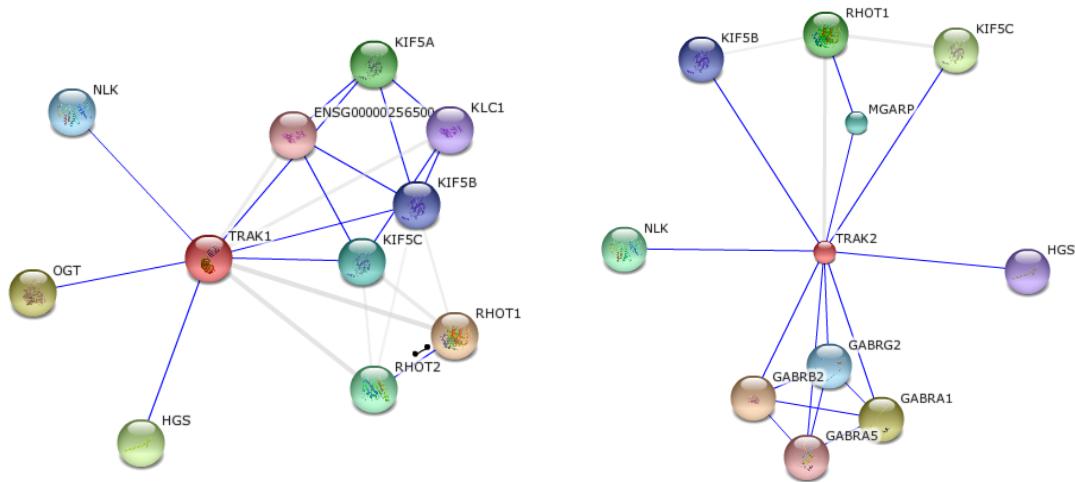


Figure 11: TRAK1 and TRAK2 Protein-Protein Interactions, STRING

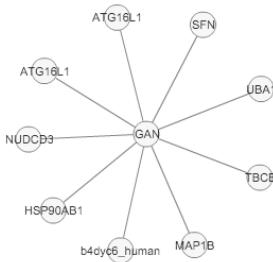


Figure 12: GAN Protein-Protein Interactions, IntAct

4.4.2 TTL *Tubulin–tyrosine ligase*

$\text{ATP} + \text{detyrosinated } \alpha\text{-tubulin} + \text{L-tyrosine} = \alpha\text{-tubulin} + \text{ADP} + \text{phosphate}$ (UNIPROT). TTL is involved in microtubule organization, given its modifications to α -tubulin, a primary component of microtubules.

4.5 Regulate

4.5.1 ATCAY *Caytaxin*

Has been annotated with [kinesin binding](#) GO molecular function (UniProtKB, inferred from physical interaction). UNIPROT suggests it may regulate the localization of mitochondria within axons and dendrites. A CDD search presents the SEC14p-like lipid binding domain, found in lipid regulated proteins such as RhoGAPs and RhoGEFs.

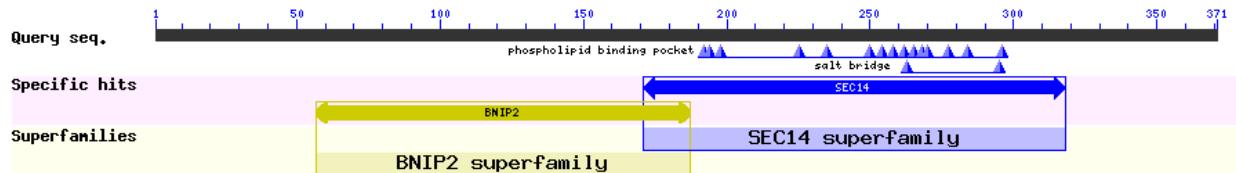


Figure 13: ATCAY Domains, CDD

In this way it is possible ATCAY may perform a similar function to TIAM2.

4.5.2 MGARP *Mitochondria-localized glutamic acid-rich protein*

MGARP is annotated as an [integral component of mitochondrial outer membrane](#), inferred by sequence or structural similarity by UniProtKB. By STRING we can see that it binds with RHOT1 and TRAK2.

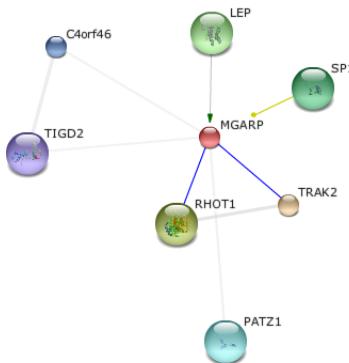


Figure 14: MGARP Protein-Protein Interactions, STRING

The CDD describes a “mitochondrion localization sequence” as well, supporting its description as a component of the mitochondrial outer membrane.

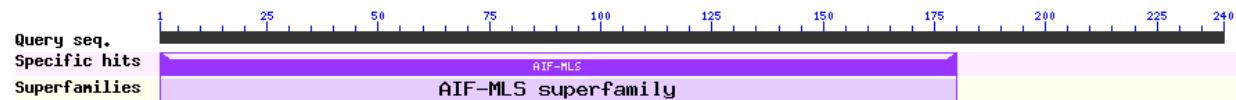


Figure 15: MGARP Domains, CDD

It is suggested, by similarity, to translocate TRAK2 from cytoplasm to mitochondrion (UNIPROT). In this way, we see that RHOT1, RHOT2, TRAK1, TRAK2 and MGARP all localize at the mitochondrion.

4.5.3 MSTO1 *Protein misato homolog 1*

Misato localizes to the outer membrane of mitochondria. It contains a tubulin like domain and is responsible for mitochondrial fission and localization (CDD).

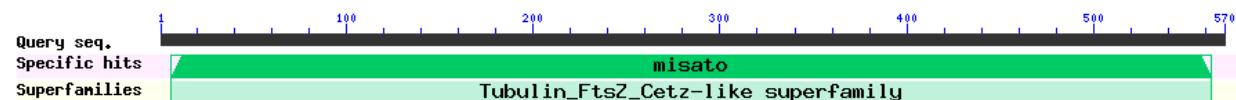


Figure 16: MSTO1 Domains, CDD

4.5.4 SNPH *Syntaphilin*

Syntaphilin is brain specific and found in synapses (UNIPROT). Syntaphilin has been found co-localized with mitochondria, where it has been proposed to act as receptor for docking for mitochondria in axons. Furthermore, deletion of the SNPH gene has resulted in increased mitochondrial motility and reduced mitochondrial density in axons (Reis et al., 2009).

4.6 Output

4.6.1 KIF1B, KIF5B, KLC1 *Kinesin-like protein KIF1B, heavy chain, light chain*

Kinesin activity is the final output of these system. We have seen KIF1B, KIF5B interact with TRAK1 through binding interactions. Kinesin-like protein KIF1B, kinesin heavy chain, and kinesin light chain will carry mitochondria towards the plus end of the microtubule. Due to microtubule arrangement in the cell handled by other processes, this will be towards the synapses, away from the cell body.

5 Summary

I have prepared a diagram summarizing the proteins I have found to be involved in the trafficking of mitochondrion in neurons. "MT" is "microtubule".

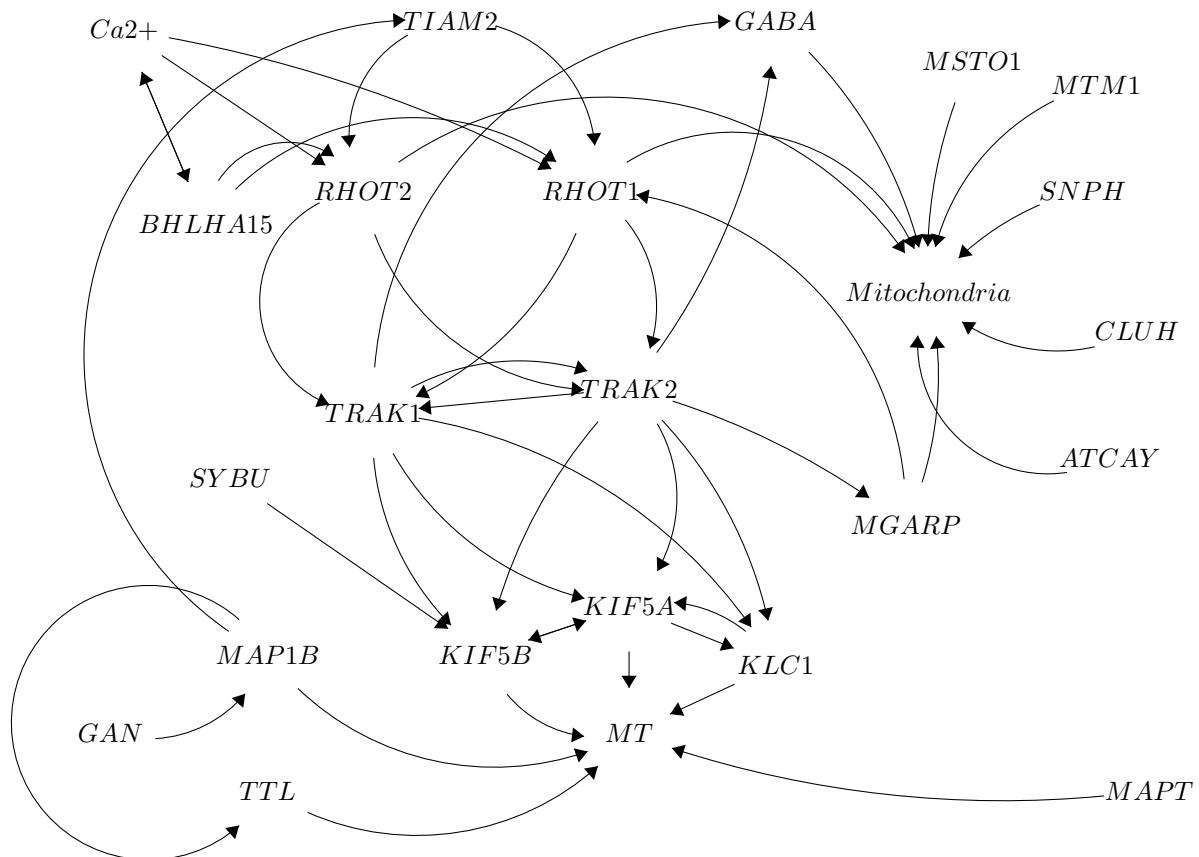


Figure 17: Overview of the Neuronal Mitochondrion Trafficking System

6 Data Pipeline

For this project, I implemented a data pipeline using `dat` and `gasket`. I wrote a small module which wraps the QuickGO REST API and provides responses in Promise and Stream format: github.com/thejmazz/bionode-quickgo. I plan on submitting this to the bionode project and publishing on npm. The CLI for bionode-quickgo was used in my gasket pipeline:

```
{  
  "get-annotations": [
```

```

    "bionode-quickgo --gannotation --goid GO:0019896 --format tsv",
    "node src/parse-tsv.js",
    "dat import -d GO0019896-annotations"
],
"get-uniprot": [
    "dat export -d GO0019896-annotations",
    "node src/get-uniprot.js > files/0019896-proteins.json"
],
"make-markdown": [
    "node src/gen-uniprot-md.js files/0019896-proteins.json > files/0019896-proteins.md"
]
}

```

This pipeline will take a GO ID, get its annotation from QuickGO in the specified format, get the Uniprot XML for each protein, bundle all of these into one object separated into arrays by gene name, sort by number of proteins per gene, and then output a neatly formatted markdown file complete with links to Uniprot. I found this essential in getting an idea of the proteins representative of each GO term. Data is intermediately stored in the version controlled file storage program dat. Currently, this pipeline requires manual editing to perform on a new GO term. With a modern text editor this can be done quickly for all occurrences, yet it is not satisfactory. I had begun writing github.com/thejmazz/bionode-ob, a streaming OBO 1.2 parser, but could not complete it in time. I would've liked to specify a GO term, and have the above pipeline run for each child term, recursively. It would be interesting to implement a web interface composed of “nodes” and “pipes” to interface with biological databases and file types, much like how complex shaders (see: [Procedural Material Monkeys](#)) are created in programs like Blender.

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