Homology-based search for microtubule associated proteins in *Caenorhabditis elegans*

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Abstract

Microtubule associated proteins (MAP) are essential for the regulation of microtubule dynamics involved in various cellular activities. MAP1 family proteins are widely known for their roles in development of axon and dendrites. Since MAPs in model organisms have shown to share noble similarities to that of their human homologs, it is hypothesized that *Caenorhabditis elegans* is a powerful tool to clarify functions of MAPs involved in neurite growth. Here we characterize worm homologs of MAPs collected from online databases based on regulatory and cellular functions to see whether *C. elegans* is a suitable model organism for explaining the aspects of MAPs on neurogenesis. From the conclusion that *C. elegans* proteins serve regulatory functions corresponding to its human homolog, this suggests maph-1 proteins in *C. elegans* can be used to determine functions of MAP1 family proteins in the growth of neurons.

Introduction

Microtubules are cytoskeletal structures that assemble from alpha- and beta- tubulin heterodimers at their two distinct ends, the fast growing plus ends and slow growing minus ends.[1] Many cellular activities depend on microtubules such as cell migration, budding, axonal growth, intracellular transport, intracellular cell signaling, and mitotic spindle assembly. Microtubule associated proteins (MAPs) play a crucial role in microtubule-dependent cellular events as they regulate microtubule dynamics by stabilizing and destabilizing microtubules, controlling their rate of growth, and linking them to various structures to facilitate these cellular activities.

Some human MAPs have evolutionarily conserved functions, showing similarities with homologs of model organisms [2] (ex. *Caenorhabditis elegans*, *Saccharomyces cerevisiae, Drosophila melanogaster*) By examining the *C. elegans* homologs, we will discuss whether this organism is a suitable tool to study the function of MAPs.

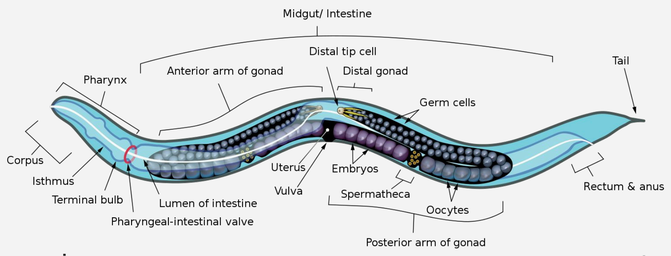

Figure 1: *Caenorhabditis elegans*
Schroeder, K. D. (2015, June 27). File:Caenorhabditis elegans hermaphrodite adult-en.svg. https://en.wikipedia.org/wiki/File:Caenorhabditis_elegans_hermaphrodite_adult-en.svg.

Methodology

Identifying Microtubule Associated Proteins

839 entries of MAPs were identified using an online database MAPanalyzer (http://systbio.cau.edu.cn/mappred/), and browsing all MAPs found in model organisms *(C. elegans, D. melanogaster, H. sapiens, M. musculus, R. norvegicus, S. cerevisiae, X. laevis)* updated in November of 2015. [3]
Additional 277 entries of MAPs were found through Uniprot using web parameters in July 2020.
[Uniprot web-parameters: *i) Interaction>Binary interaction, Subunit structure "Microtubule" ii) Function>Function[CC] "Microtubule Associated" iii) Function>Function[CC] "Microtubule binding" IV) Entry name [ID] "Microtubule Associated"]*
Excluding redundant genes and homologs, 843 MAPs and their expression and primary function were investigated.

Searching for Homology

Worm homologs of the MAPs were identified using BLAST in Wormbase (https://wormbase.org/tools/blast_blat)
Input in query sequences were FASTA-formatted peptide sequences obtained from Uniprot. In conclusion, 305 worm homologs of MAPs were identified.

Table 1: Primary and Secondary Function of MAPs

Primary Function	Secondary Function
Stabilizer	Polymerizes MT
	Reduce catastrophe
Destabilizer	Depolymerizes MT
	Increase dynamic instability
Linker Protein	MT and Actin
	MT and Organelle
	MT and Plasma Membrane
	Scaffolding Protein
Enzyme	Acetylase/Deacetylase
	Deacetylase
	Kinase (Phosphorylase)
	Polyglutamylase
	Ubiquitinase
	Others
Motor Protein	Stabilizer
	Destabilizer
End binding Protein (Plus/Minus)	
Others	

Table 2: MT-based cellular activities

MT-based cellular activities
MT Growth and Guidance
MT nucleation/Spindle assembly
Intracellular Transport
Cell signaling
Cell Division/Cell Cycle
Others

Results

305 worm homologs and their functions were characterized. **Figure 2** and **Figure 3** summarizes the number of MAPs for each category of MT-regulatory and cellular function.
Moreover, of all MAPs, **53** were present in touch receptor neurons, specifically **48** in Posterior lateral microtubule cells (PLM) and **27** in Anterior lateral microtubule cells (ALM).
Figure 4 is a visual representation of the expression of MAPs in different model organisms.

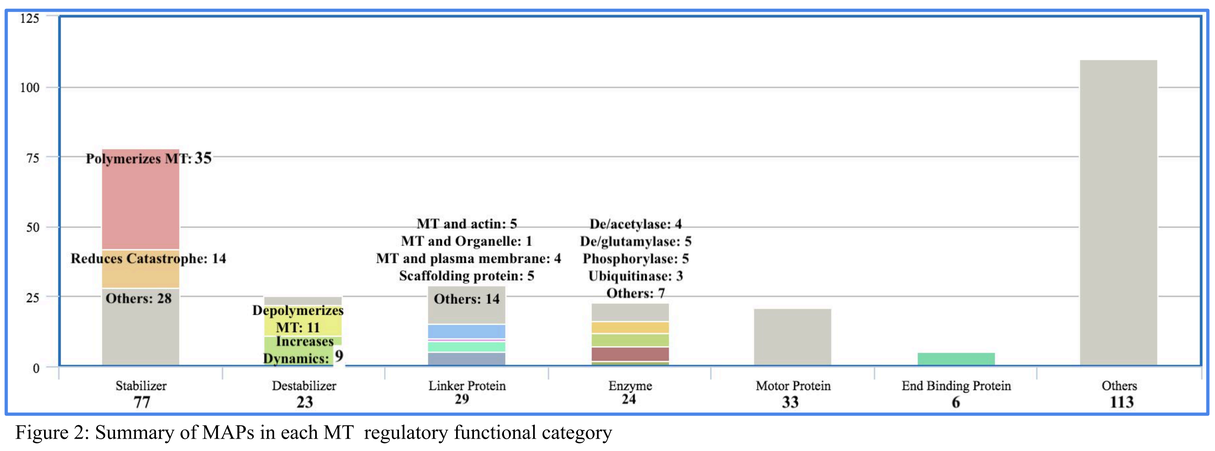

Figure 2: Summary of MAPs in each MT regulatory functional category

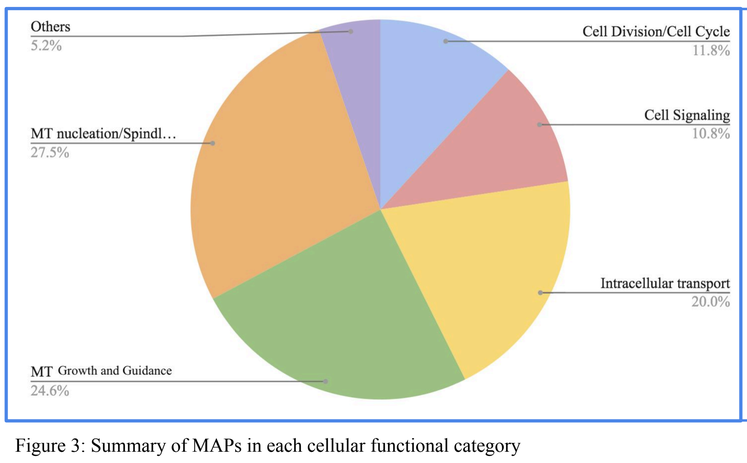

Figure 3: Summary of MAPs in each cellular functional category

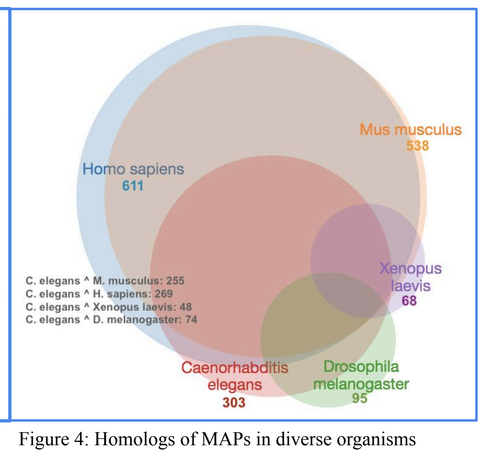

Figure 4: Homologs of MAPs in diverse organisms

Discussion

MAPs are broadly categorized into their functions determined by the mechanism in which they regulate microtubules dynamics

Microtubule Stabilizers

MAPs interact with tubulins to either stabilize or destabilize microtubules by regulating rate of polymerization and dynamic instability. [4] **Figure 5** shows a visual representation of how GTP/GDP binding on tubulin promotes polymerization/depolymerization.

1. Stabilize microtubules by *promoting microtubule polymerization*[4,5]: **XMAP215/zyg-9**, **Bora/spat-1**, **CAV1/CAV3/cav-1**, **Dystrophin/dys-1** and **33** more.
2. Stabilize microtubules by *suppressing microtubule dynamicity*[4,6]:
3. **CLASP family/cls-1,2,3**[4,6] **FHDC1/exc-6**, **PPP1CA/gsp-2**, **FNTA/fnta-1** and **11** more.

Microtubule Destabilizers

1. Destabilize microtubules by *promoting microtubule depolymerization*: Kinesin, Serine-Threonine Protein family (Refer to Figure 6) (ex.**KIF2/klp-7)**[7], **TTBK1,3/C39H7.1**, **MIP-T3/dyf-11,** and **8** more.
2. Destabilize microtubules by *increasing dynamic instability and catastrophe rates*:[8,9] **Spastin/spas-1** (MT severing protein), **ARL3/arl-3,** and **10** more

Linker Protein

Some MAPs, can connect microtubule ends to different organelles (Golgi, mitochondria), cytoskeletal elements, plasma membrane, or kinetochores.

1. *Actin and MT Linkers:* Connect growing ends to stable actin bundles to steer direction of polymerization. **GAS2-like Protein/D2096.11** and **4** more. [10]
2. *Kinetochore and MT linkers:* Facilitates attachment of MT to kinetochore for accurate chromosome segregation.[11]
3. *Cell cortex and MT linkers:* Forms stable routes for vesicle transport, regulates MT growth.[11] **CLASP family/cls-1,2,3** and **7** more
4. *Organelle and MT linkers:* Facilitates movement of vesicles and organelles.[11] ie. **Dynein/dlc-1,dyhi-1,dhc-1**
5. *Plasma membrane and MT linkers:* For force generation required in MT network positioning. [11] **GEPH/lin-46/moc-1,2** and **1** more

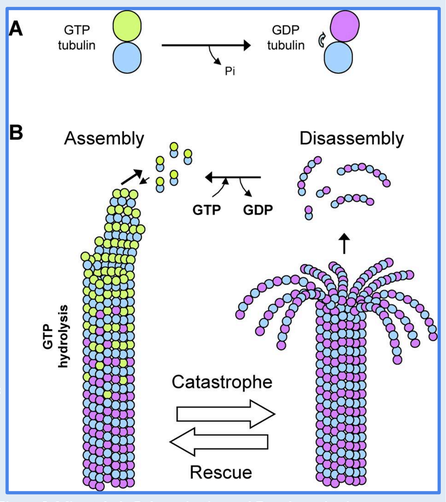

Figure 5: Microtubule Polymerization and Depolymerization
Al-Bassam, J., & Chang, F. (2011). Regulation of microtubule dynamics by TOG-domain proteins XMAP215/Dis1 and CLASP. *Trends in cell biology*, *21*(10), 604–614. https://doi.org/10.1016/j.tcb.2011.06.007

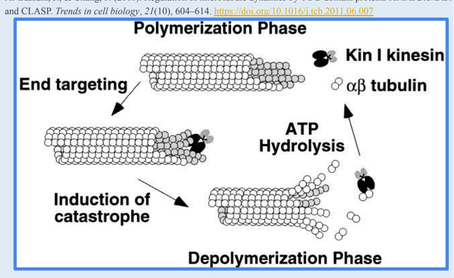

Figure 6: Kin I Kinesin Inducing Catastrophe
Desai, A., Verma, S., Mitchison, T. J., & Walczak, C. E. (1999). Kin I Kinesins Are Microtubule-Destabilizing Enzymes. *Cell*, *96*(1), 69–78. https://doi.org/10.1016/s0092-8674(00)80960-5

Enzymatic Modifiers

Some MAPs are enzymes that post-translationally modify tubulins to regulate microtubule dynamics.[12] ie. *acetylation*, *tyrosination*, *phosphorylation*, *polyglutamylation*, *ubiquitination*
Ex. **Tubulin acetylation** influences microtubule stability, assembly, and ability to interact with other proteins (ie. Luminal and motor proteins in **mec-17, hda-6**).[12] Acetylation also induce restriction of motion of tubulin αK40 loop, stabilizing microtubules and decreasing lateral interaction with tubulin monomers. (31072936)

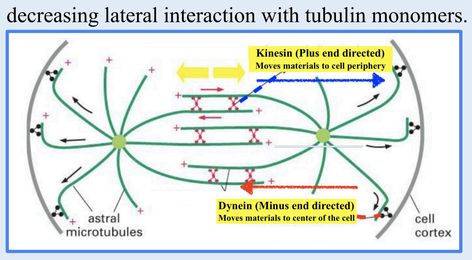

Figure 7: Motor Proteins
Desai, A., Verma, S., Mitchison, T. J., & Walczak, C. E. (1999). Kin I Kinesins Are Microtubule-Destabilizing Enzymes. *Cell*, *96*(1), 69–78. https://doi.org/10.1016/s0092-8674(00)80960-5

Motor Proteins

Motor proteins power a variety of cell movements including intracellular transport and organelle positioning.[13]
Kinesins: Plus-end tracking motor protein that transport materials towards cell periphery. **Dynein**: Minus-end tracking motor protein that transports materials towards center of cell

End binding proteins

Minus- or plus- end-tracking proteins (+TIPs,-TIPS)) that concentrate at growing microtubule ends.
Autonomous tip trackers: binds to MT ends purely through interaction with tubulin subunits[11, 14] **End-binding protein/ebp-1,2,3, NUF2/him-10, KIF2/klp-7** and **6** more.
Hitchhikers: concentrates at MT ends through interaction with autonomous tip trackers.[11] **BICD/bicd-1, CLIP170/clip-1** and **15** more.

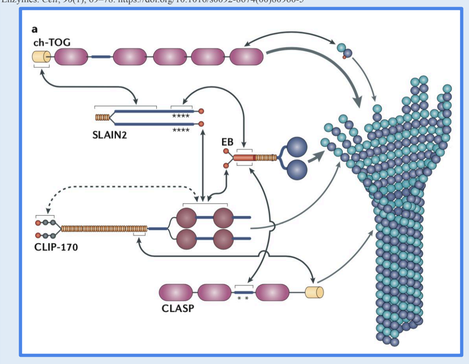

Figure 8: Recognition of TIP by Autonomous tip tracker (EB) and Hitchhikers (SLAIN2, CLIP-170, CLASP, ch-TOG)
Akhmanova, A., & Steinmetz, M. O. (2015). Control of microtubule organization and dynamics: two ends in the limelight. *Nature reviews. Molecular cell biology*, *16*(12), 711–726. https://doi.org/10.1038/nrm4084

Microtubule-Associated Protein in Neurite Growth

MAPs recruit at microtubules to control dynamics and reorganize cytoskeletal elements to drive morphological change for neurite growth in response to extracellular cues.
MAPs initiate neurite formation by extending microtubules into actin rich filopodia:[15] Distributes stable microtubules to actin rich protrusions or induces polymerization ie. **MAP2C, MAPT, MAP1B**

MAPs regulate neurite growth by linking microtubules to actin filaments:

1. Allows motor proteins to use actin as a scaffold to allow MT to generate force on cell membranes to push neuron forward.[16]
2. Allows growth cone actin filaments to capture and guide microtubule during early steps of neurite formation.[17]

MAP1 Family

MAP1A, MAP1B, MAP1S are MT-stabilizers that regulate brain development and neuronal plasticity. MAP1B is essential for regeneration of MT and neurite growth as a cytoskeletal stabilizer and actin and microtubule linker protein.[18,19] Figure 9 displays the delay in axon growth of MAP1B mutant mice B compared to the wild type A. Table 3 notes the mislocalization and reduction in size of axons in the growth cone area in MAP1B-deficient neurons.

	Growth Cone Area	Number of mislocalized growth cones	
	Axons	Minor neurites	
WT Neurons	180 ± 22	65 ± 10	0.20 ± 0.05
MAP1B-Deficient Neurons	88 ± 15a	72 ± 8	5.5 ± 0.5a

Table 3: Growth cone shape parameters

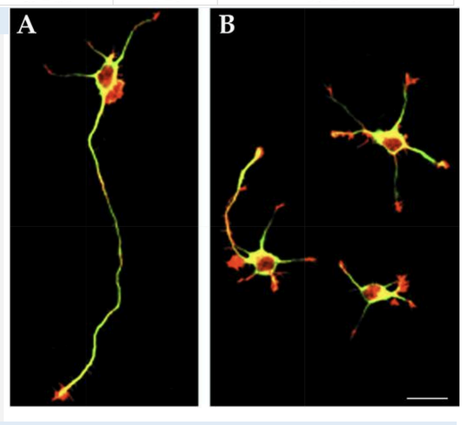

Figure 9: Confocal micrograph of a polarized hippocampal pyramidal neuron (A: WT, B: Mutant)
Gonzalez-Billault, C., Avila, J., & Cáceres, A. (2001). Evidence for the role of MAP1B in axon formation. Molecular biology of the cell, 12(7), 2087–2098. https://doi.org/10.1091/mbc.12.7.2087

Conclusion

Majority of the studied worm proteins have been shown to regulate microtubule dynamics correspondingly to their human homolog. This suggests that *C. elegans* is a powerful tool that can help recognize the underlying mechanism in MAPs to regulate microtubule dynamics.

MAP1 family of proteins are predicted to play an essential role in regulating growth of neurons. However, its underlying mechanisms are not fully understood, which is why future studies should focus on functional assays on the *C. elegans* homologs maph-1.1, 1.2 and 1.3 in *C. elegans* to elucidate some of these key points.[20,21]

Acknowledgement and References

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