Microglia cytoskeleton rearrangement in microglia physiology

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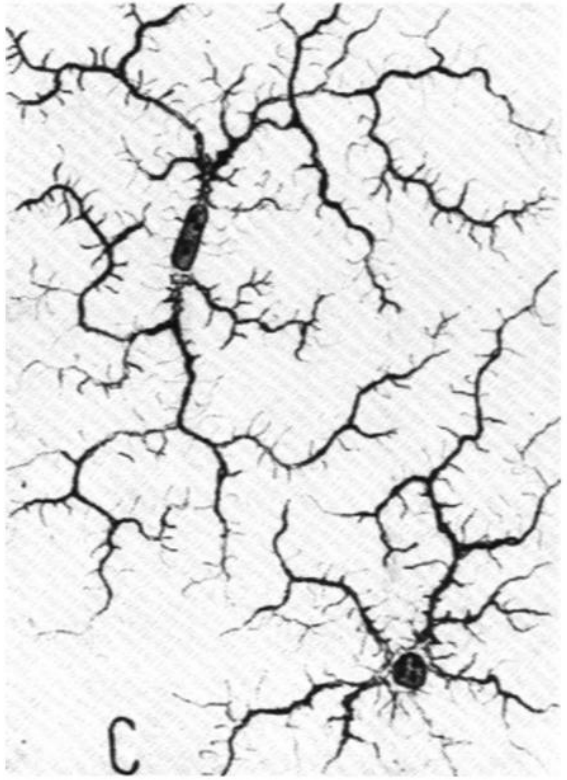
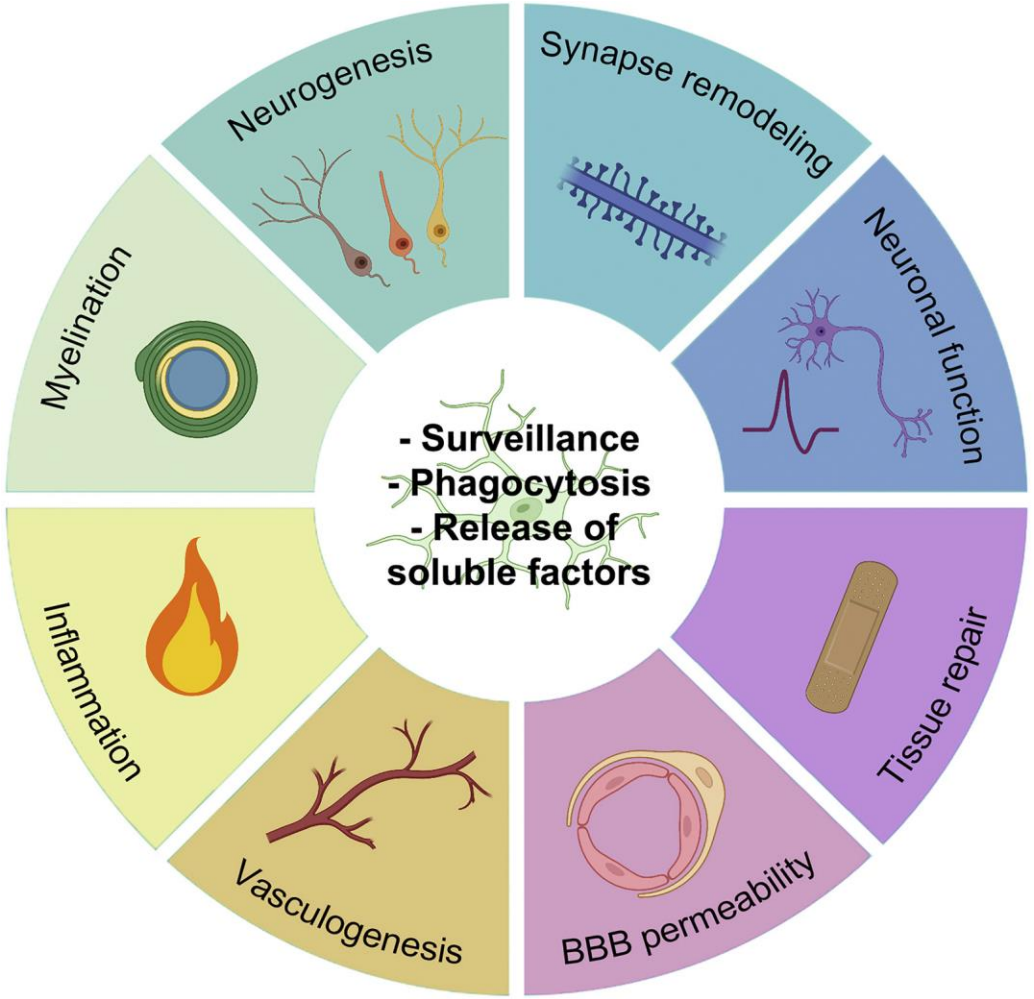
Microglia origin and physiology

- Microglia are the primary **immune cells** of the brain
- Microglia originate from **myeloid** precursors and migrate early during development in the brain parenchyma
- Microglia cells in the healthy mature brain display a **ramified morphology**, with a small soma and fine cellular processes
- When subjected to external damaging stimuli, microglia undergo a process called «activation», characterized by profound changes in the microglial cell shape, gene expression and functional behavior

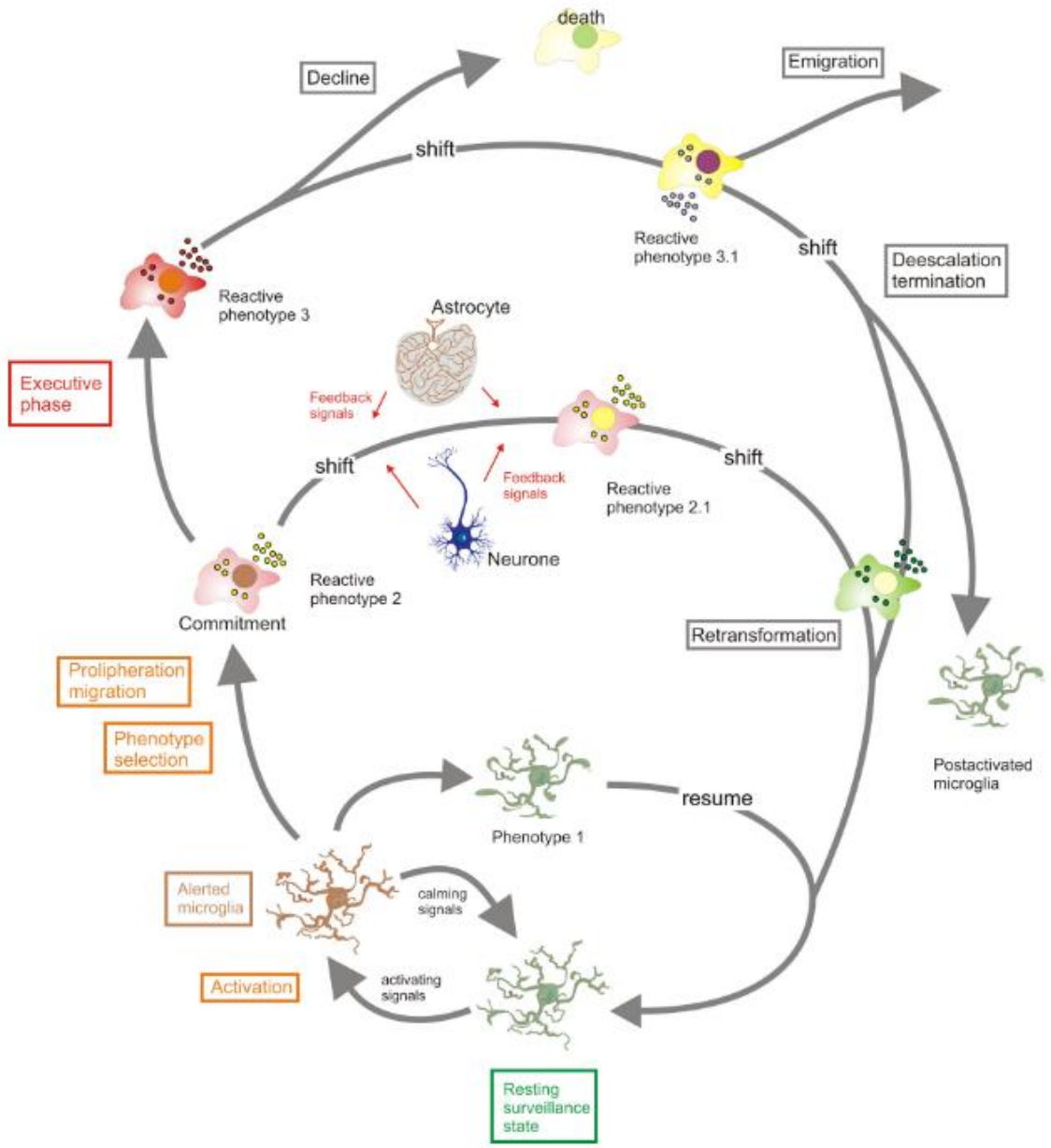


Microglial core properties and functions



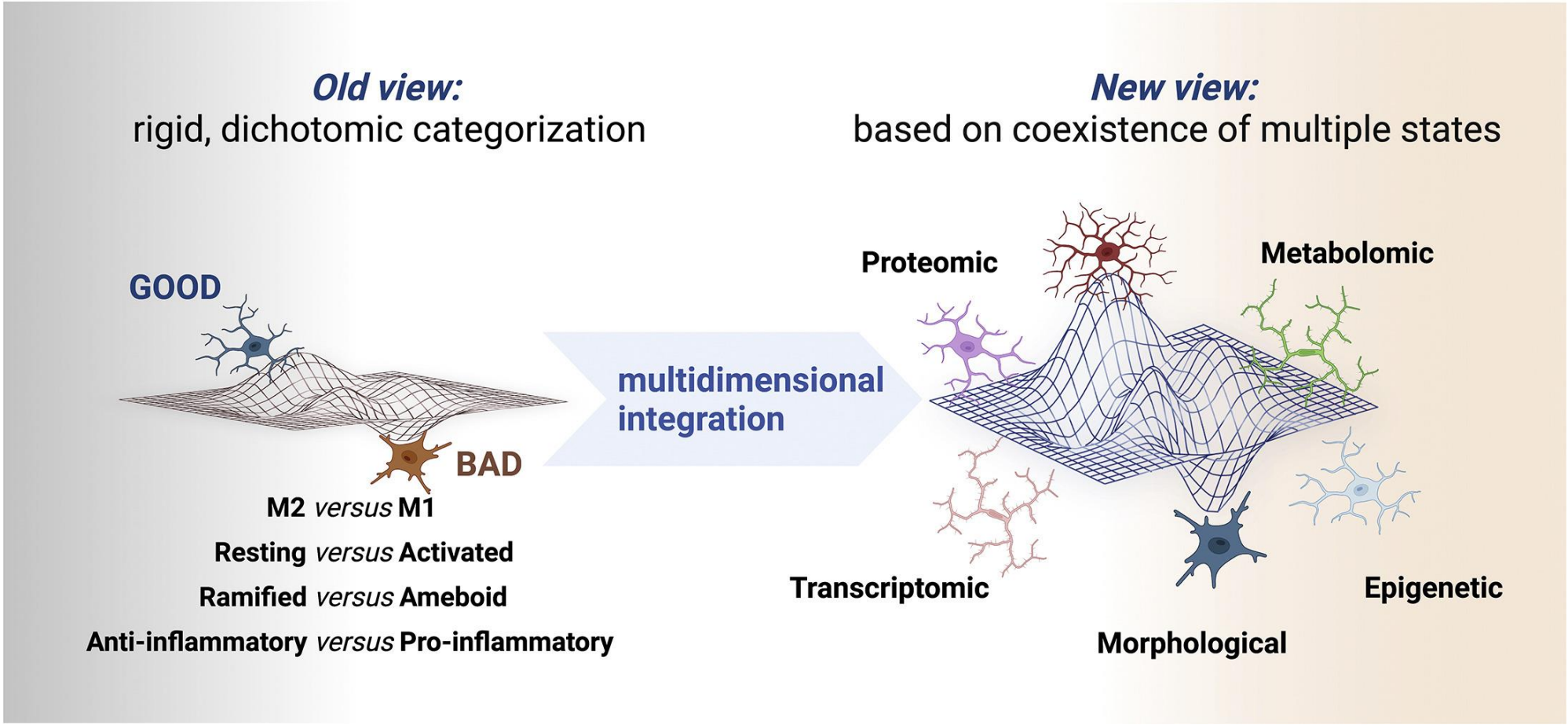
Phagocytosis, surveillance, and capacity for releasing soluble factors are core properties through which microglia contribute to key biological functions.

Microglia activation

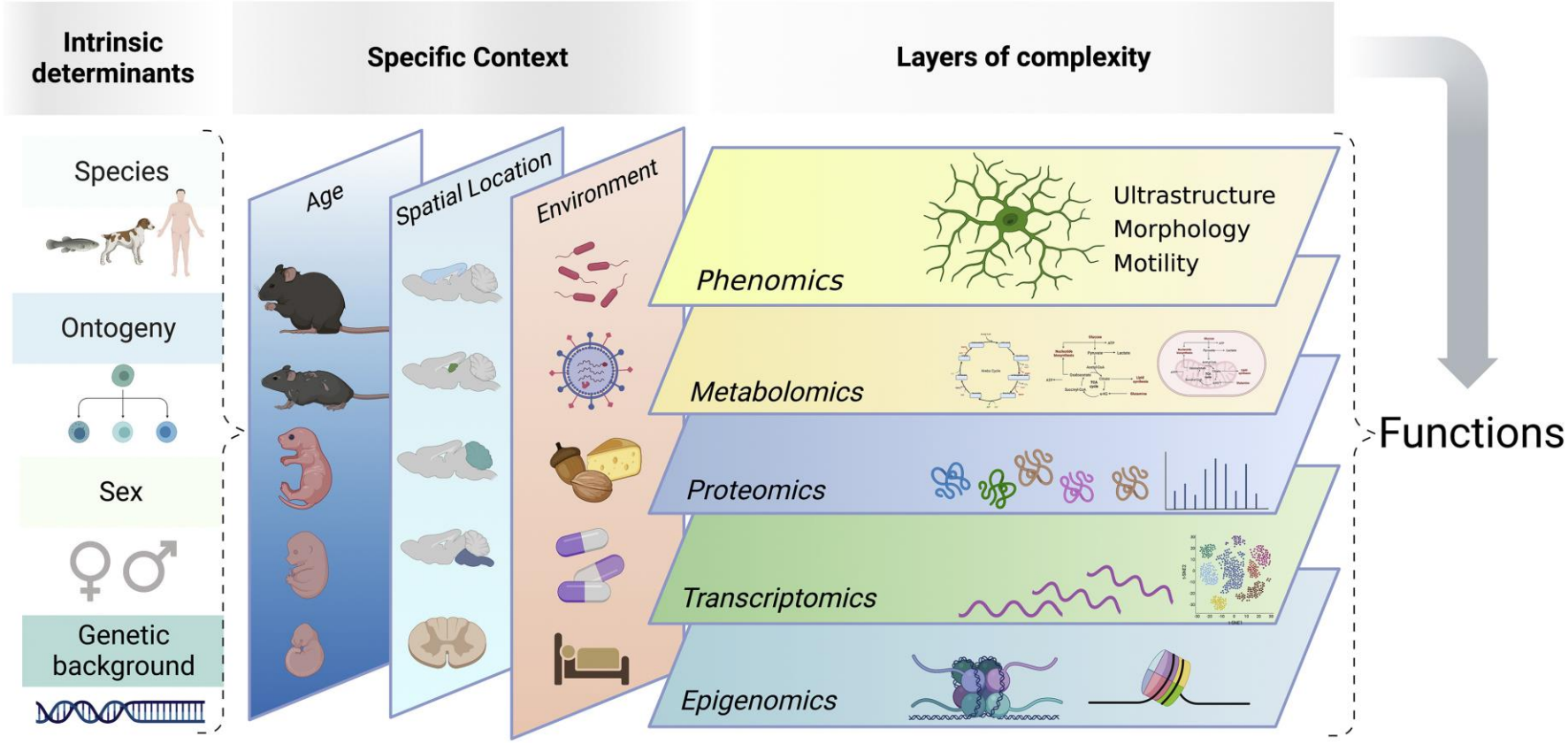


- Homeostatic microglia constantly and actively scan their environment for exogenous or endogenous signals indicating a threat to the homeostasis.
- Appearance of “activating” signals can then trigger transitions to alerted and activated states. Cells can commit to **distinct reactive phenotypes** depending on the challenging stimuli.
- Initial **reactive phenotypes** with **defense** orientation may **convert** to **repair-orientated** activity profiles.

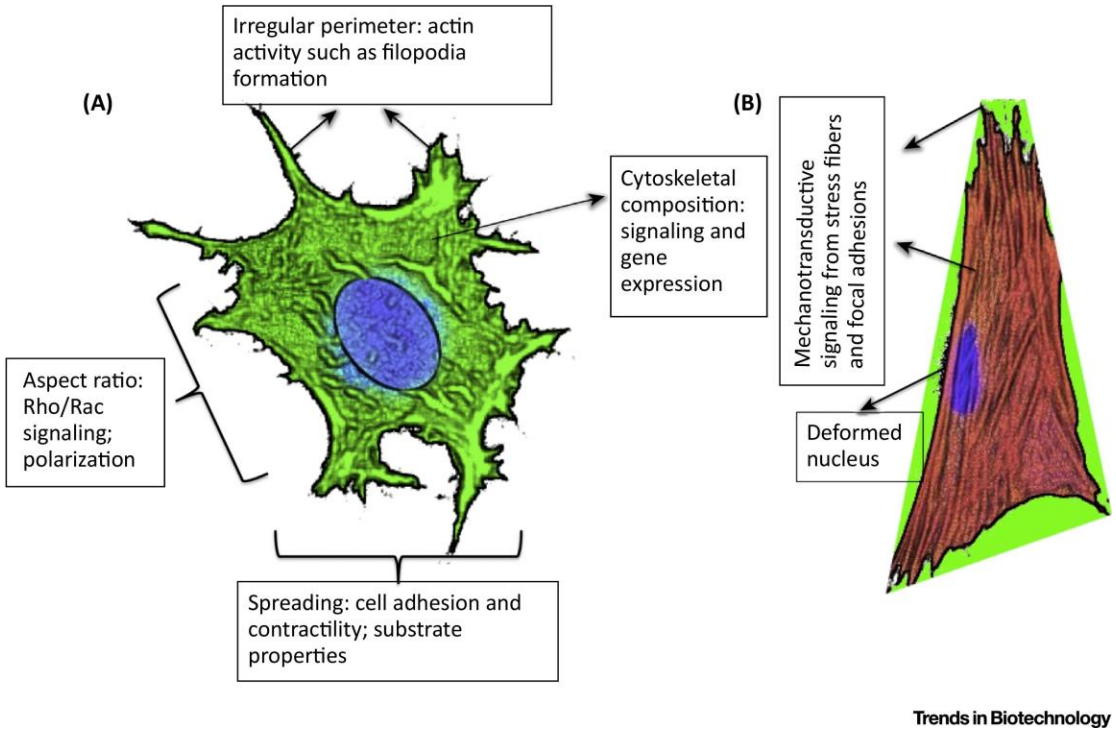
Microglia have been traditionally framed into dichotomic categories, but the current integration of epigenetic, transcriptomic, metabolomic, and proteomic data favors a **multidimensional integration** of **coexisting states**.

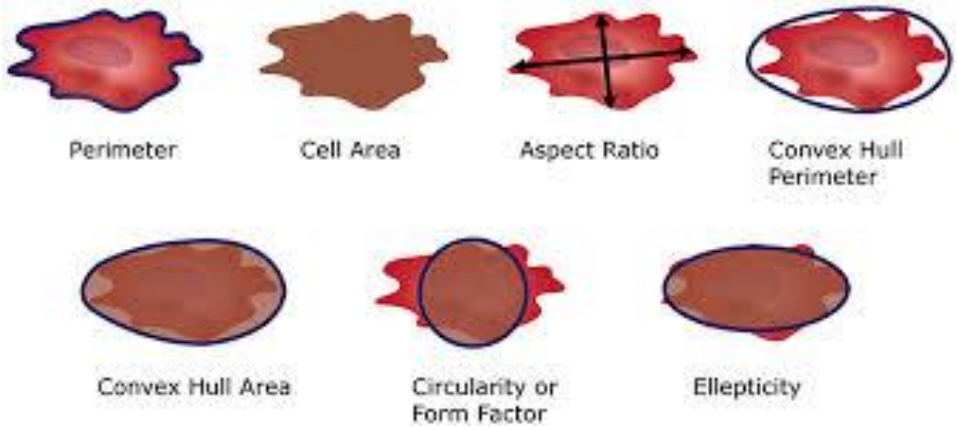


Microglial states depend on **intrinsic determinants** (such as species, ontogeny, sex, or genetic background) as well as the **specific context** they inhabit, including age, spatial location, and environmental factors (such as nutrition, microbiota, pathogens, drugs, etc.). All together, these factors impinge on microglia at **multiple levels** (i.e., epigenomic, transcriptomic, proteomic, metabolomics, ultrastructural, and phenomic), which ultimately determine microglial functions



Cell morphology



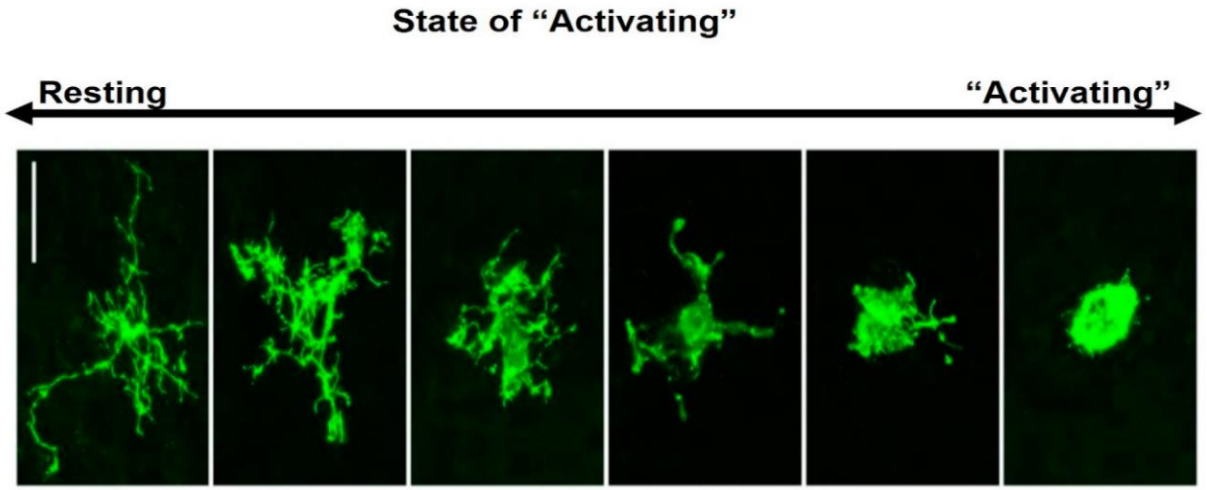


Morphology ⇄ Function

It’s something that can be **measured**

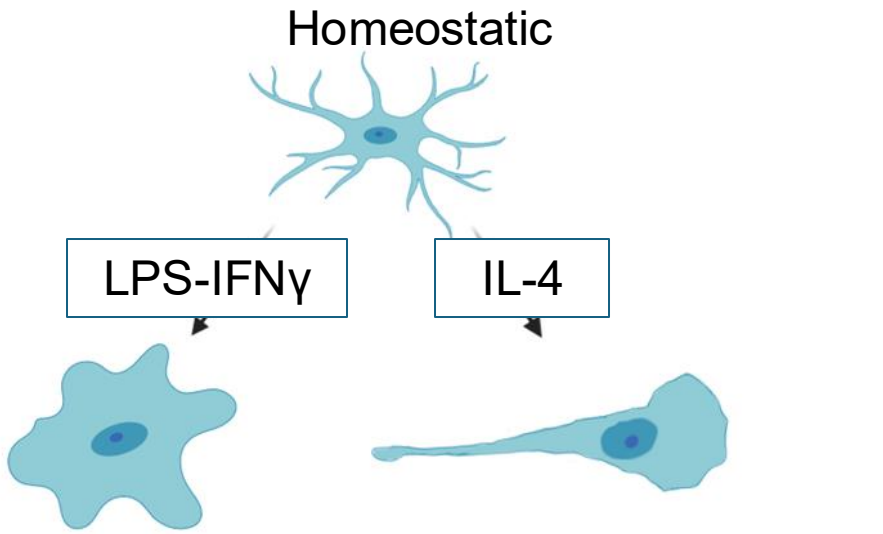
Microglia morphologies

In vivo



Rawlinson et al., 2020

In vitro



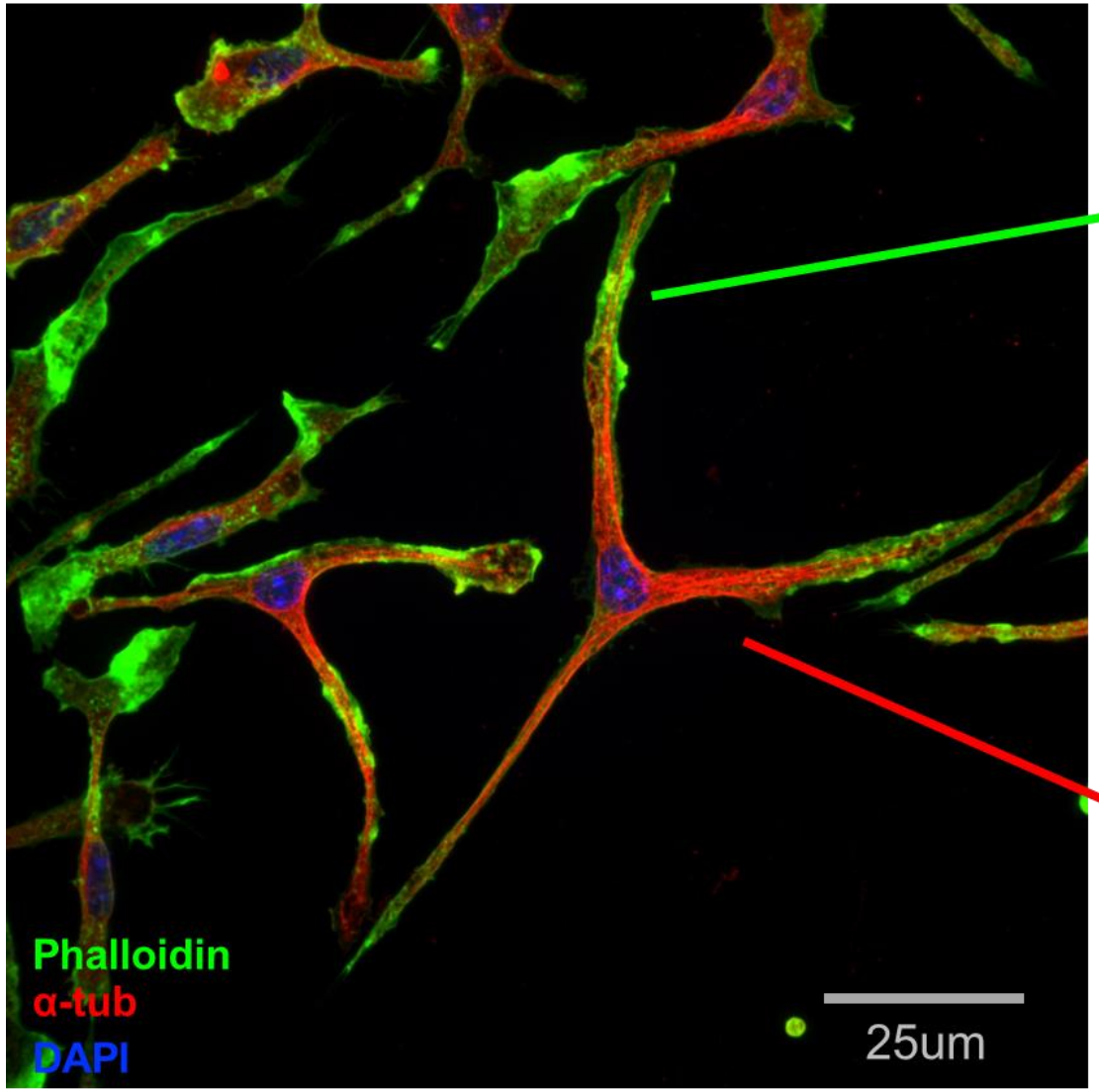
Morphology ⇄ Function

It's something that can be **measured**

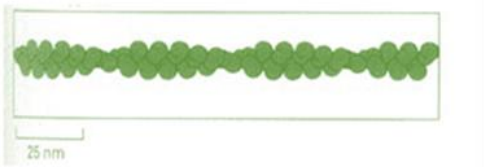
Although insufficient to define microglia state, morphology remains one of the first aspects to consider when approaching microglia functionality changes.

Microglia cytoskeleton

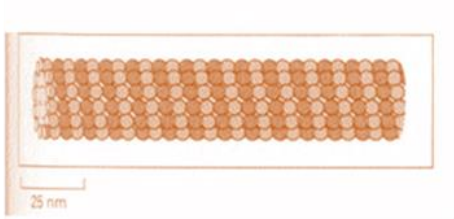
Primary murine microglia cells in culture



Actin



Microtubules



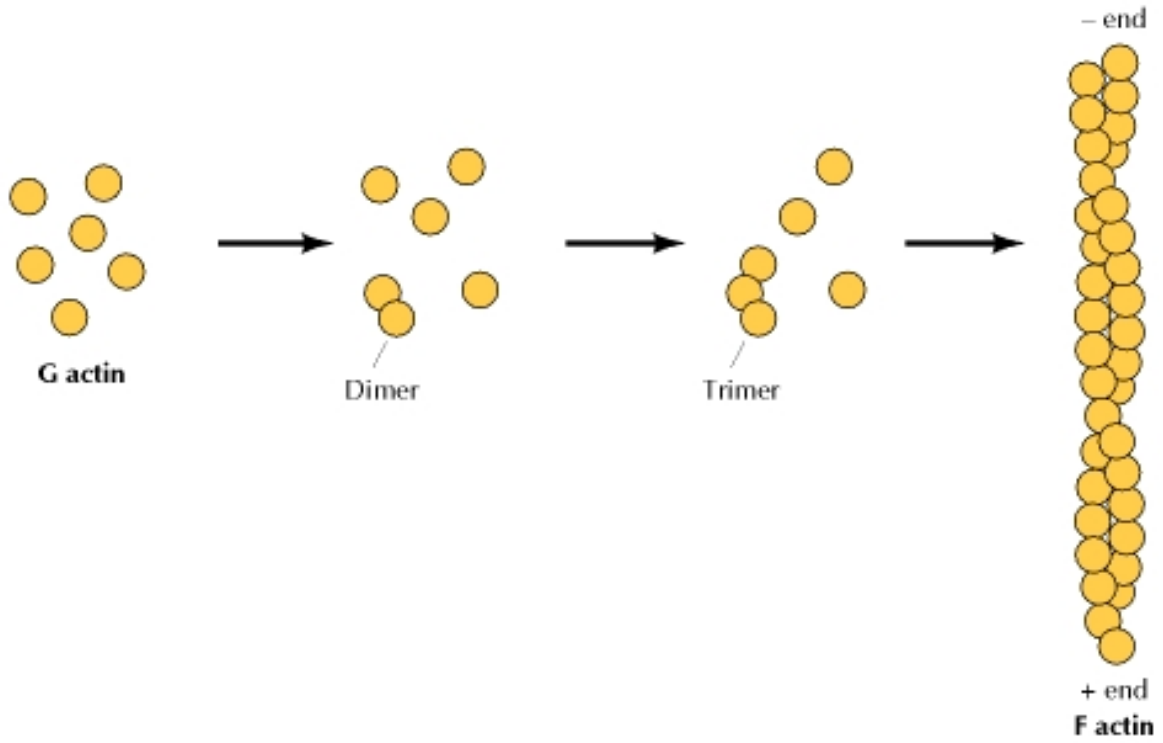
(…and intermediate filaments)

The actin cytoskeleton

Actin, a highly conserved protein found in all eukaryotic cells, plays a fundamental role in various cellular processes due to its dynamic structure and ability to form different types of filaments.

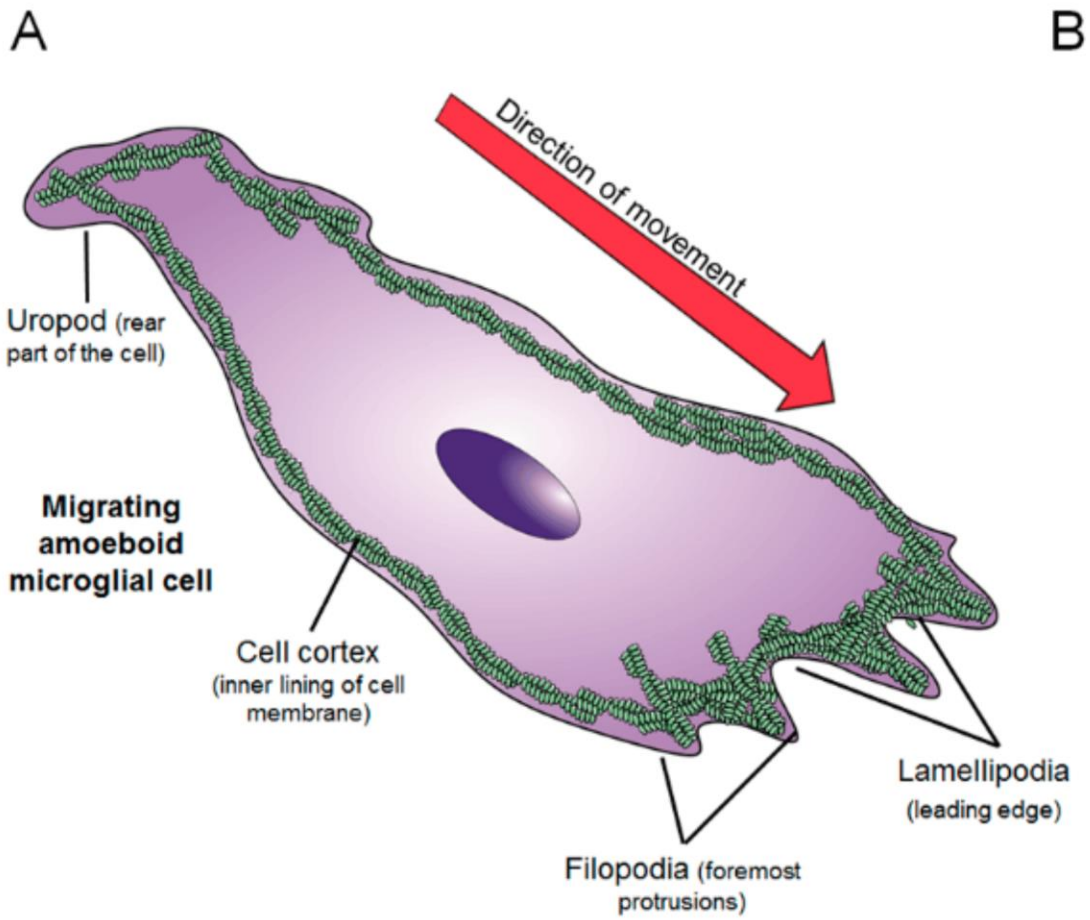
Actin monomeric form is known as **G-actin** (globular actin), and it has the potential to polymerize into **F-actin** (filamentous actin). The two forms of actin are maintained in a dynamic equilibrium.

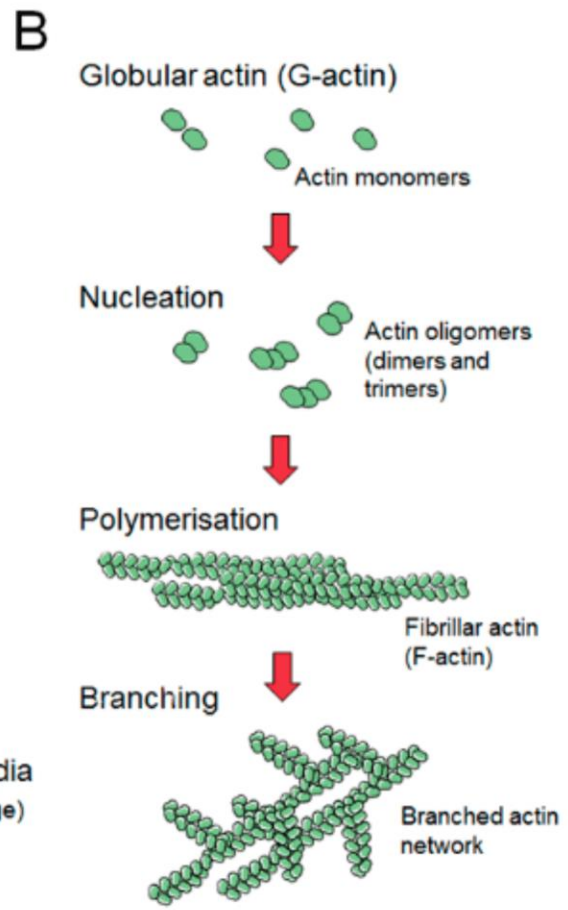
This process is crucial for **cell motility**, **shape** and **division** and it is regulated by various actin-binding proteins.



Actin's versatility allows microglia to **rapidly remodel their cytoskeleton**, facilitating processes such as **migration**, **phagocytosis**, and the formation of cellular protrusions necessary for **nanoscale surveillance** of the brain environment

Actin and microglia migration





Different actin structures are present in microglia:

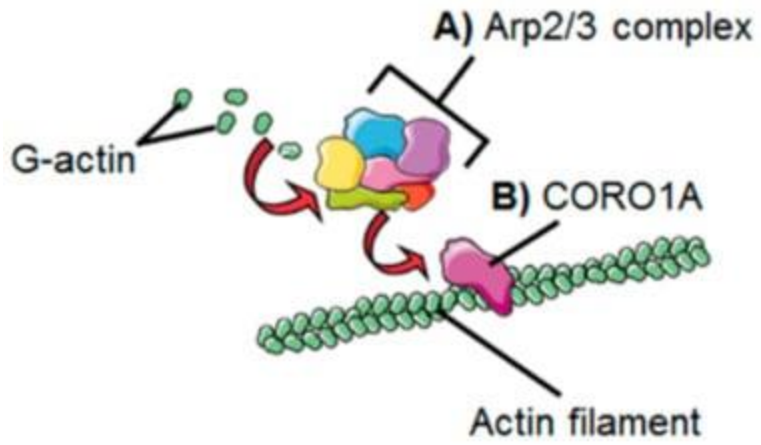
- **Cell cortex** (covering all the inner surface of the cell)
- **Filopodia** and **lamellipodia** (at the leading edge)
- **Uropod** (at the rear of the cell)

Mechanism of formation of the actin network includes:

- Oligomerization of G-actin (nucleation)
- G-actin polymerization into F-actin
- Recruitment of additional globular actin to form **branches**

Actin filaments need to be branched to form the lamellipodia → branching is essential for **directed motility**

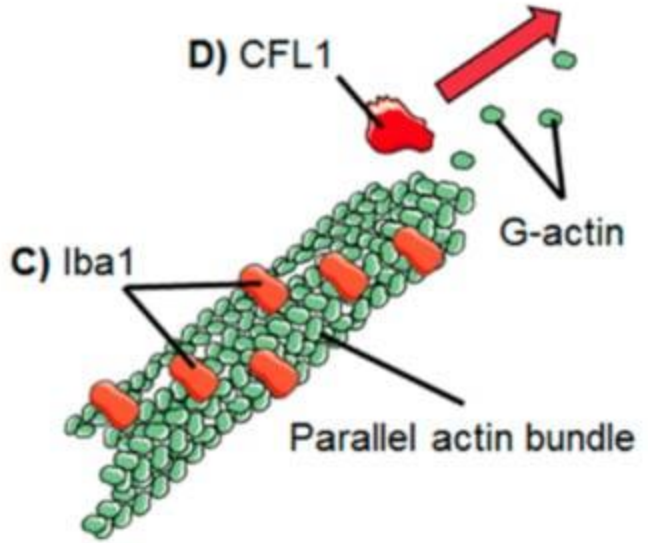
Branching



The process of **branching** at the lamellipodia and filopodia at the leading edge of moving cells is mainly controlled by the **Arp2/3** complex (actin-related proteins 2 and 3)**.**

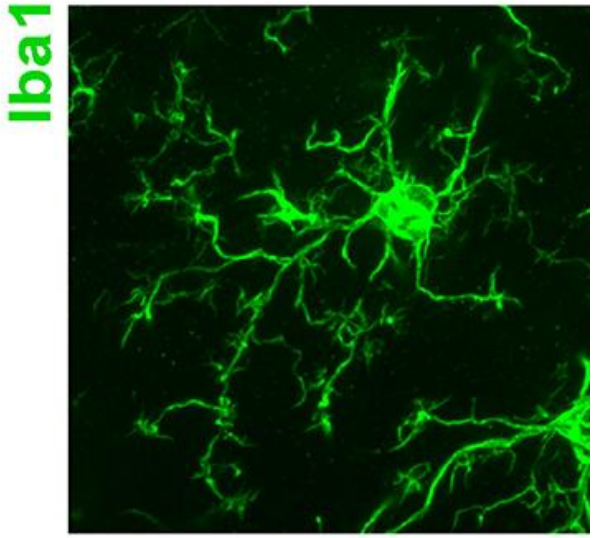
In the presence of ATP, the complex binds to the side of a filament and initiates a “subfilament” which stems from the “mother filament” at a characteristic angle of 70°. In microglia, this mechanism is regulated by **coronins**, such as coronin-1a (CORO1A).

Cross-linking



To control the cell shape and movement, actin polymers are connected with each other by a process called **cross-linking**. Cross-linking allows the network to shape into more complex structures.

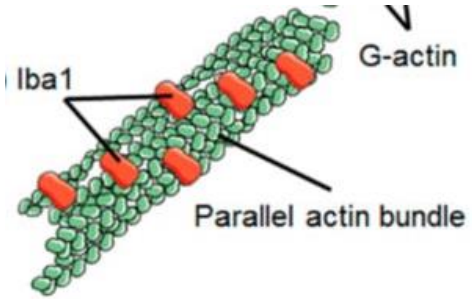
Iba1 promotes the formation of **parallel actin bundles**, scaffold-like structures that give shape to lamellipodia and filopodia.

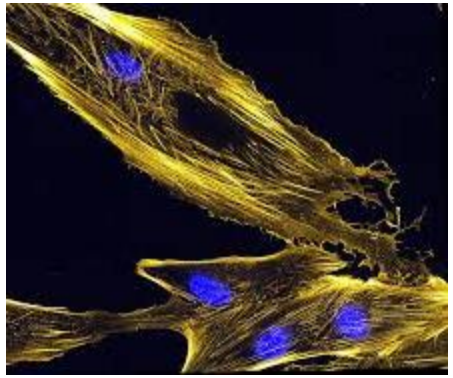


The ionized calcium-binding adapter molecule (Iba1) is also known as allograft inflammatory factor 1 (AIF1) is a widely used **microglia marker**

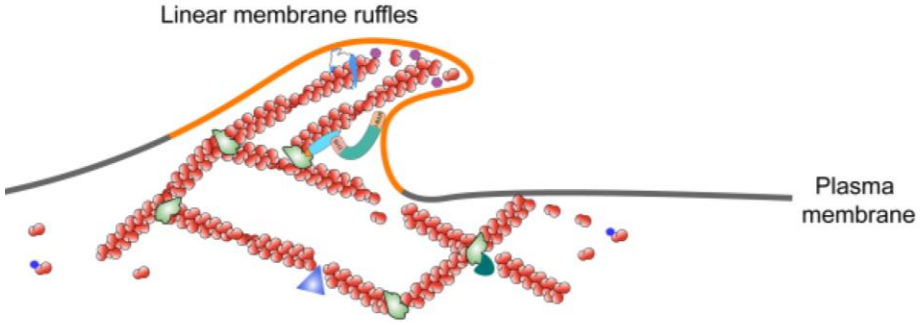
It is involved in **actin bundling** and **membrane ruffling**

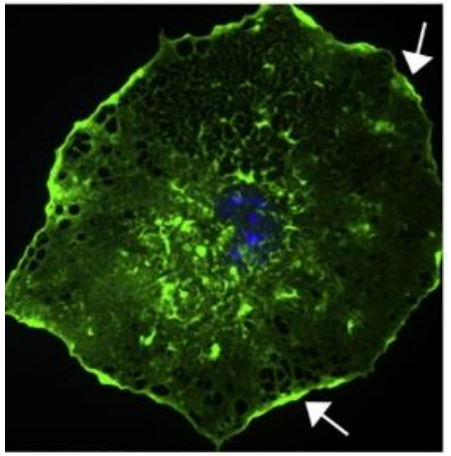
Actin bundles
Act like scaffolds, for lamellipodia and filopodia support



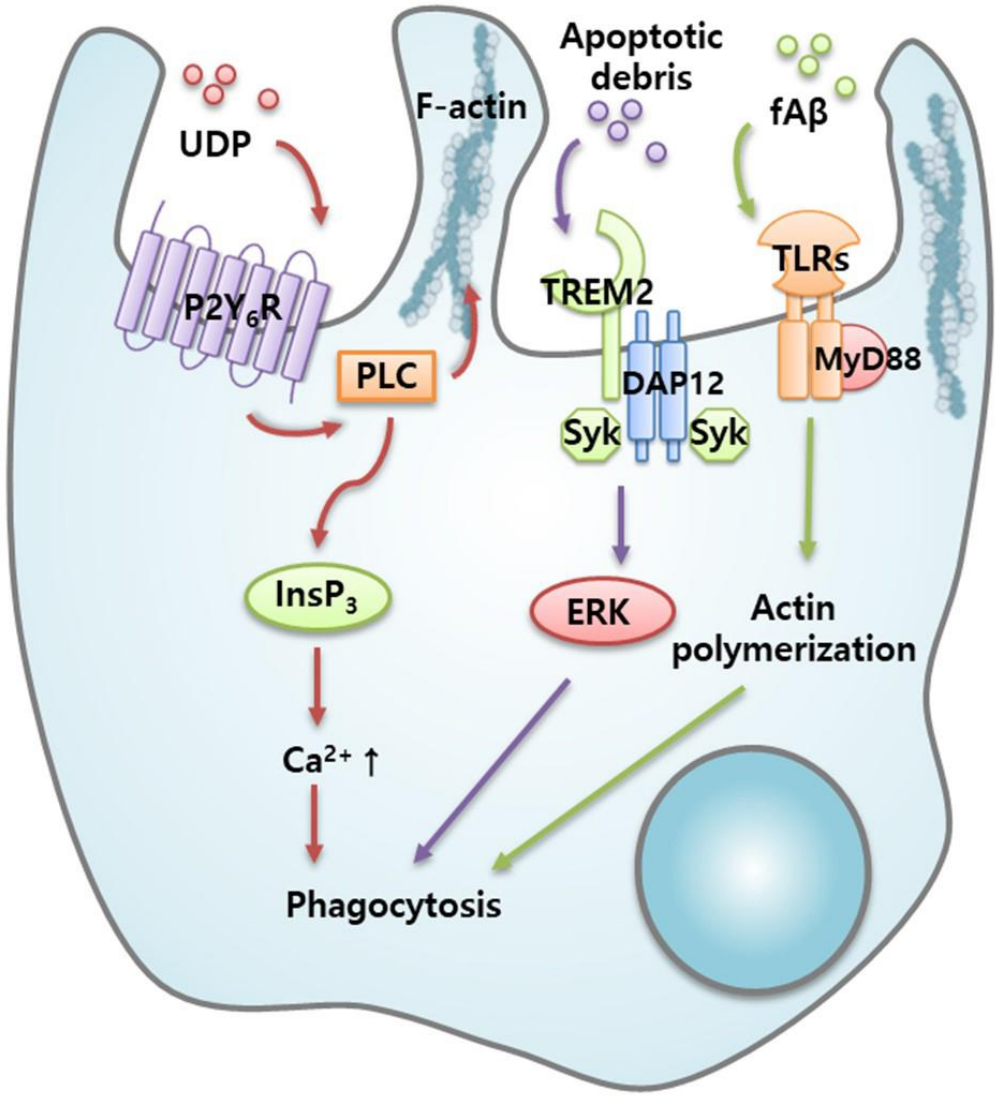


Membrane ruffling
Important for microglia migration and phagocytosis





Actin and microglia phagocytosis



Specific **receptors** and signal transduction pathways that contribute to the reconstitution of actin proteins are utilized for microglial phagocytosis, such as:

- Toll like receptors (**TLRs**), high affinity receptors that bind to external microbial pathogens

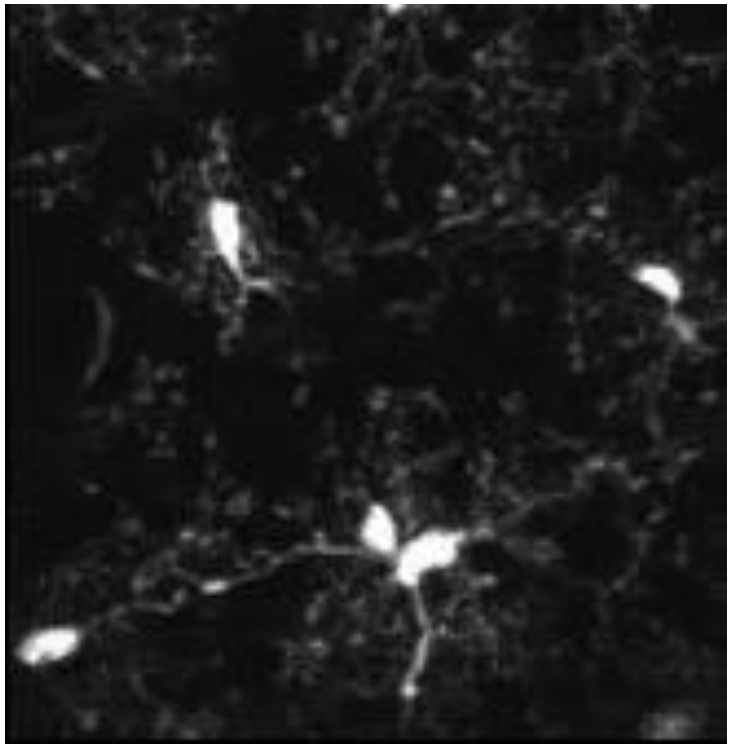
- Trigger Receptors Expressed on Myeloid cells 2 (**TREM-2**), that recognize apoptotic cell substances, leading to the reconstitution of F-actin mediating the removal of apoptotic neurons

- Purinergic P2Y G-protein binding 6 receptor (**$P2Y_6R$**, that actively respond to uridine diphosphate UDP and activate phospholipase C to induce the synthesis of inositol 1,4,5-triphosphate and to release Ca2+ and promotes actin, mediating cytoskeletal polarization to form filopodia-like protrusions, thereby promoting cell phagocytosis

Actin and microglia surveillance

Bernier et al., 2019

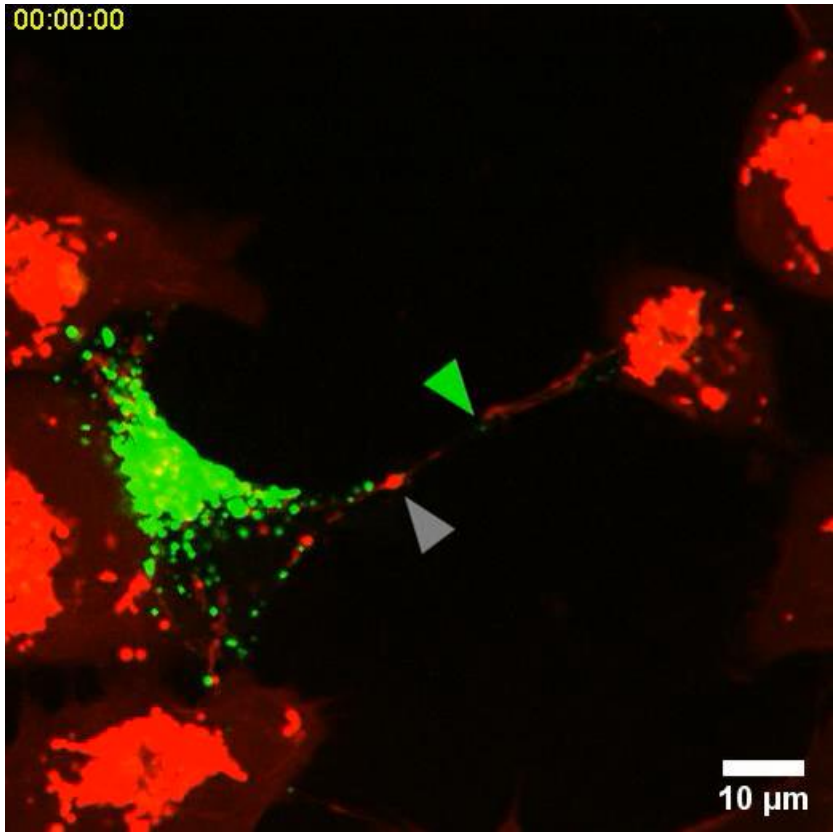
Microglia actively and continuously sense molecular cues within their local environment by using highly motile processes and ramified morphology



Nimmerjahn et al.,2005

Microglia use **actin-dependent filopodia** to efficiently sample the brain parenchyma

Actin and microglia tunnelling nanotubes



Bi-directional movement of α-Syn (green and yellow arrowheads) from neuronal cell to microglia and mitochondria (gray) in the opposite direction

Tunnelling Nanotubes (TNTs) facilitate **contact-mediated intercellular communication** over long distances.

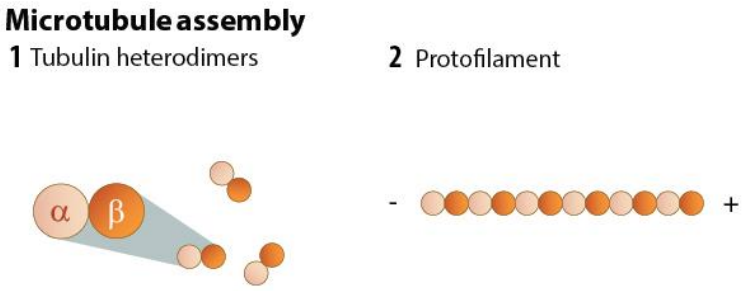
TNTs are thin, membrane-enclosed, **F-Actin-rich protrusions** able to transfer cargoes of different kinds between the connected cells, like Ca^{2+} signals, messenger- and micro-RNAs, organelles such as lysosomes and mitochondria, pathogens, apoptotic signals, and protein aggregates

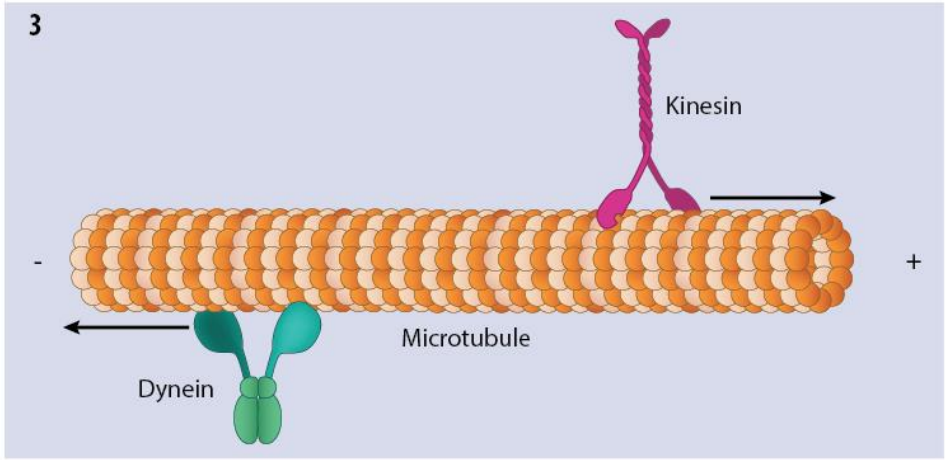
Microglia actin cytoskeleton

- Actin is fundamental to drive **rapid** rearrangements of microglia cytoskeleton
- It is important for microglia **migration**, as it forms microglia lamellipodia and filopodia at the leading edge of migrating amoeboid microglia. The main involved processes are actin polymerization, branching and cross-linking that allow the formation of actin bundles and membrane ruffles.
- Many receptors on microglia cell surface trigger actin polymerization that finally allow **phagocytosis**
- Specialized microglia protrusions are formed mainly by actin, such as the filopodia that allow microglia **nanoscale surveillance**, and **tunnelling nanotubes** to drive contact-mediated cell communication.

The microtubule cytoskeleton

Microtubules (MTs) are dynamic structures composed of α/β-tubulin heterodimers, playing diverse roles in cell shape maintenance, intracellular transport and cell division





- α/β-tubulin heterodimers string together to form the **protofilaments**.
- **Thirteen protofilaments** form the microtubule
- The diameter of a microtubule is 25 nm

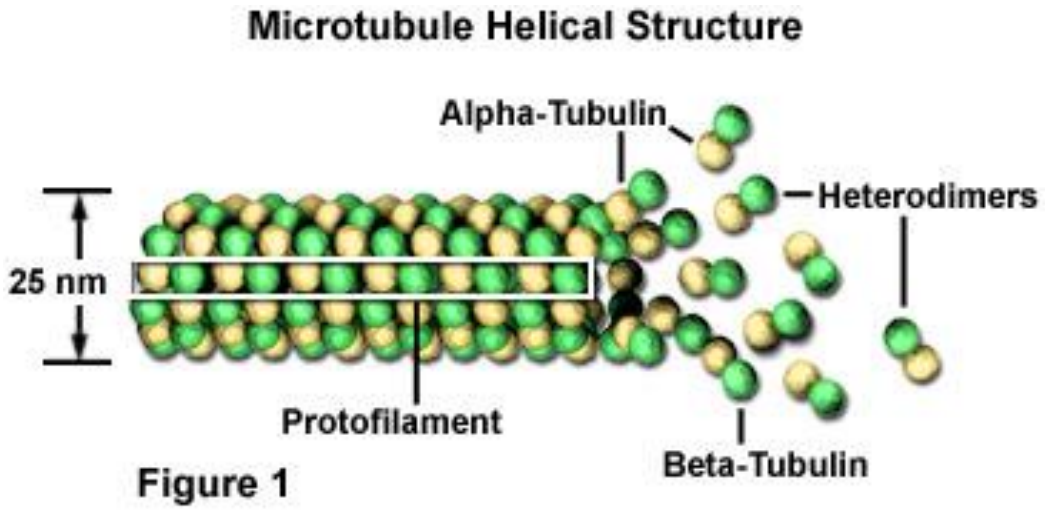
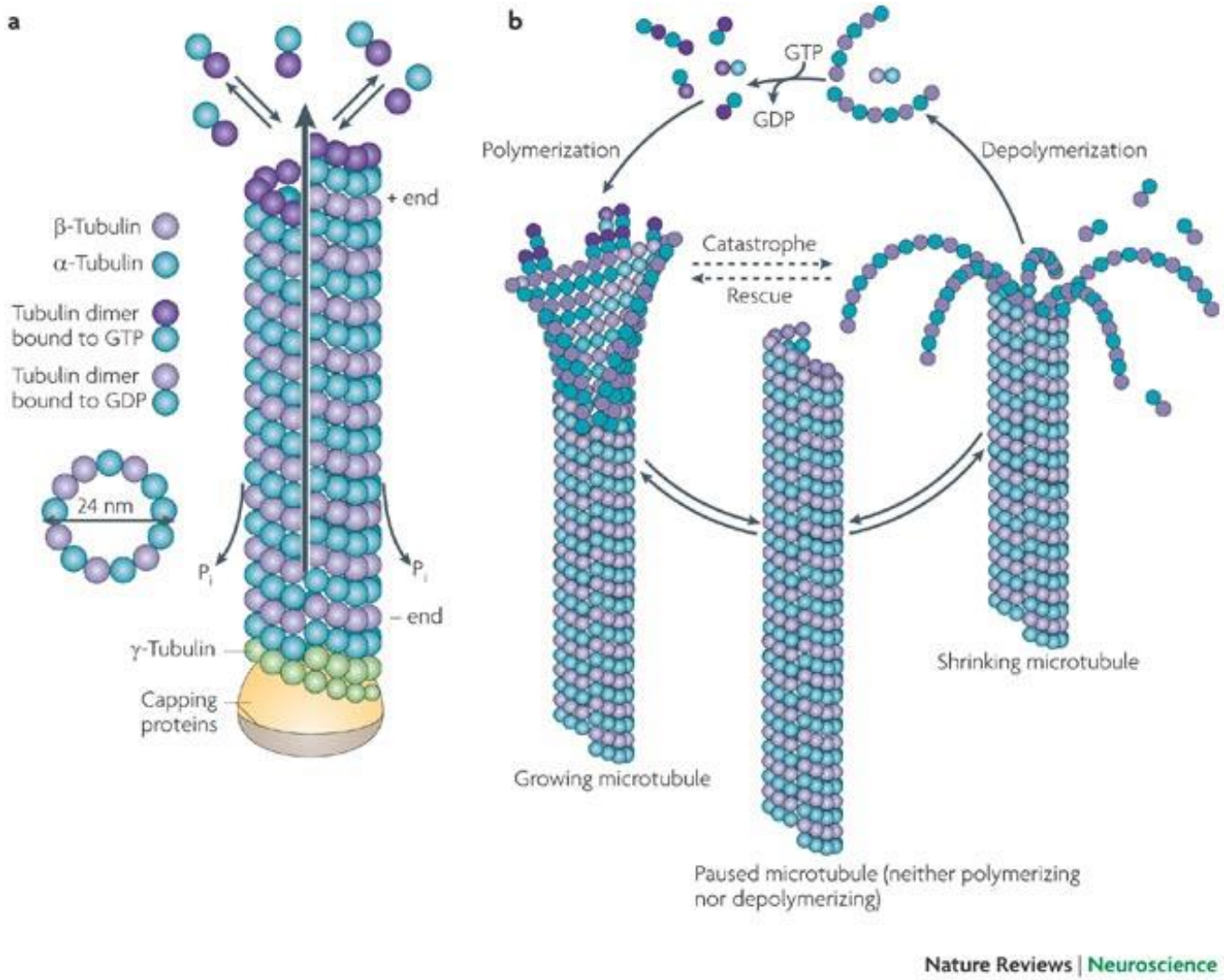


Figure 1

MTs are dynamic, with GTP-dependent reactions constantly adding and subtracting tubulin dimers at both ends of the filament.

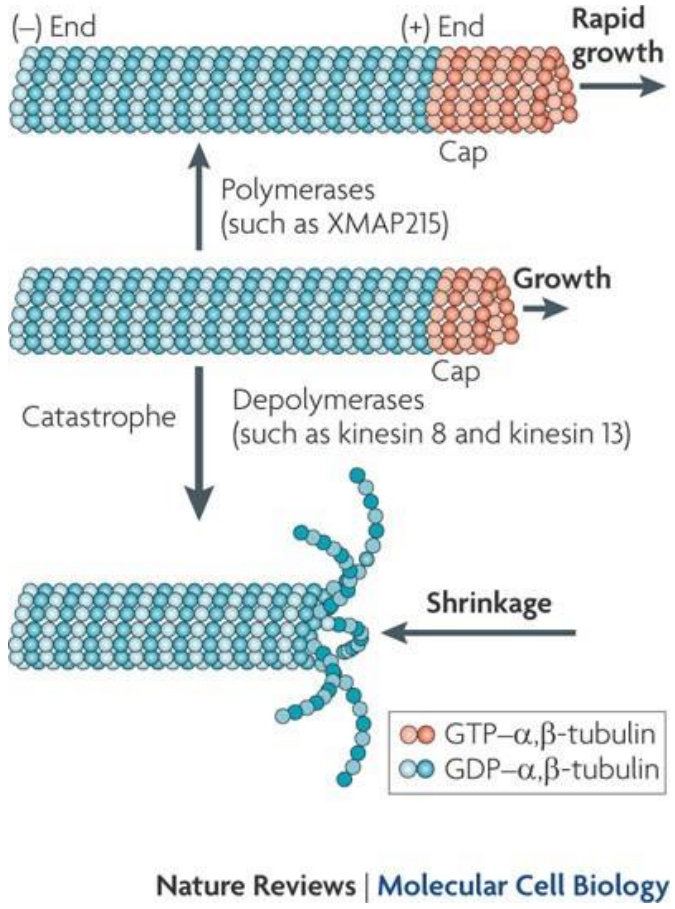


Dynamic instability

Microtubules in vitro coexist in growing and shrinking populations which interconvert.

This dynamic instability is a general property of microtubules

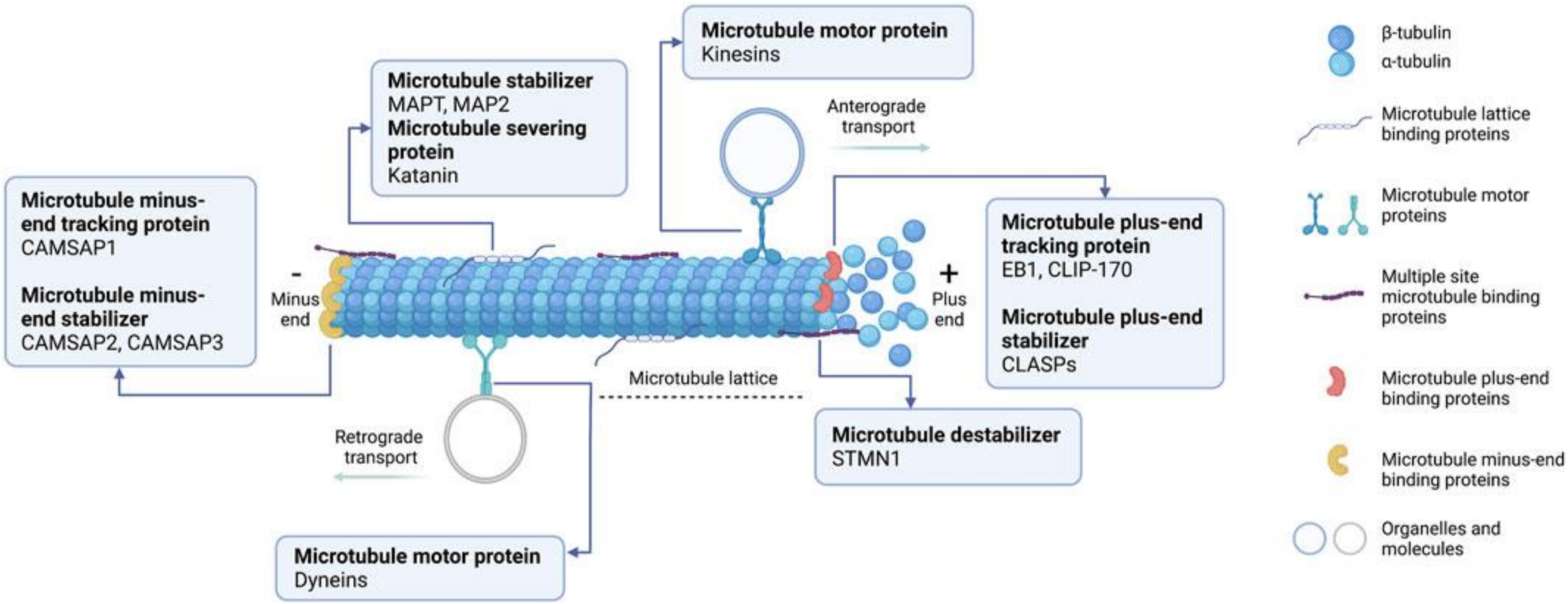
This process is dependent on GTP hydrolysis rate



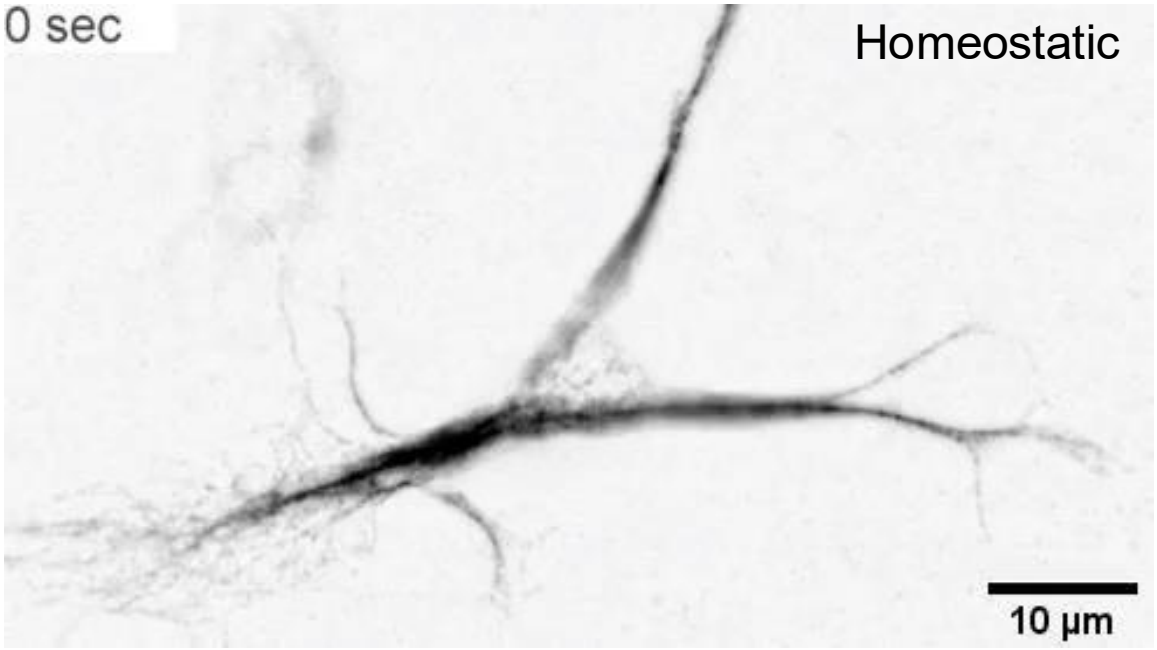
Nature Reviews | Molecular Cell Biology

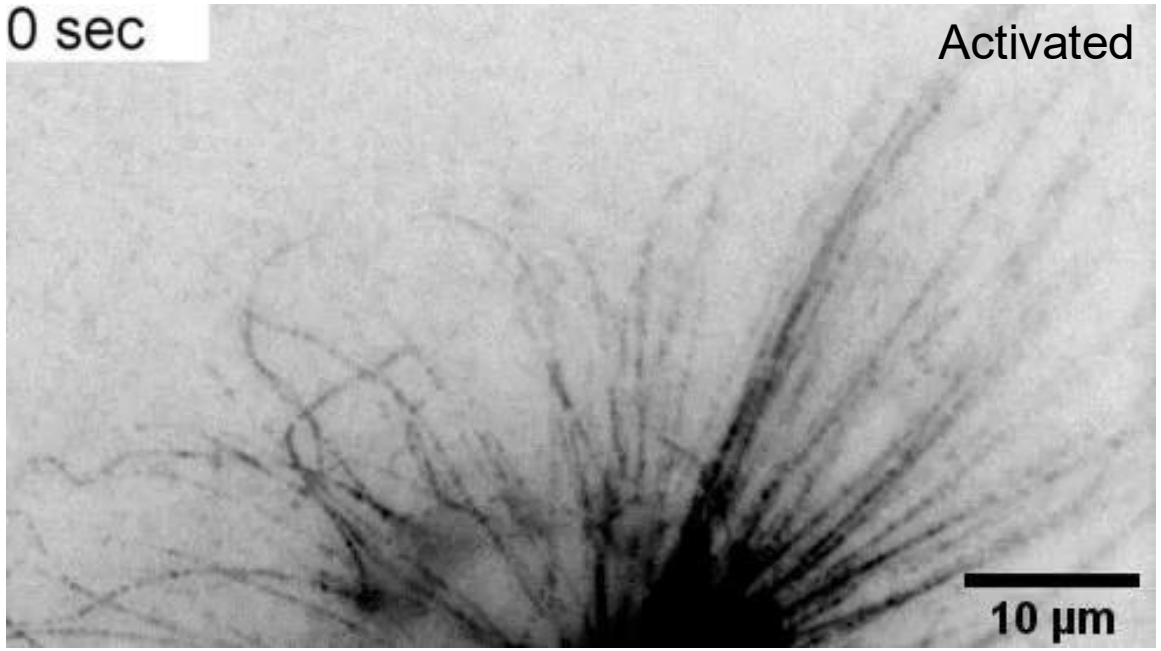
https://youtu.be/1Aid3fC6L94?si=19pCQ7T5Y2ilvg1Q

Many proteins interact with microtubules as stabilizers or destabilizers



Microtubule dynamics and microglia functions

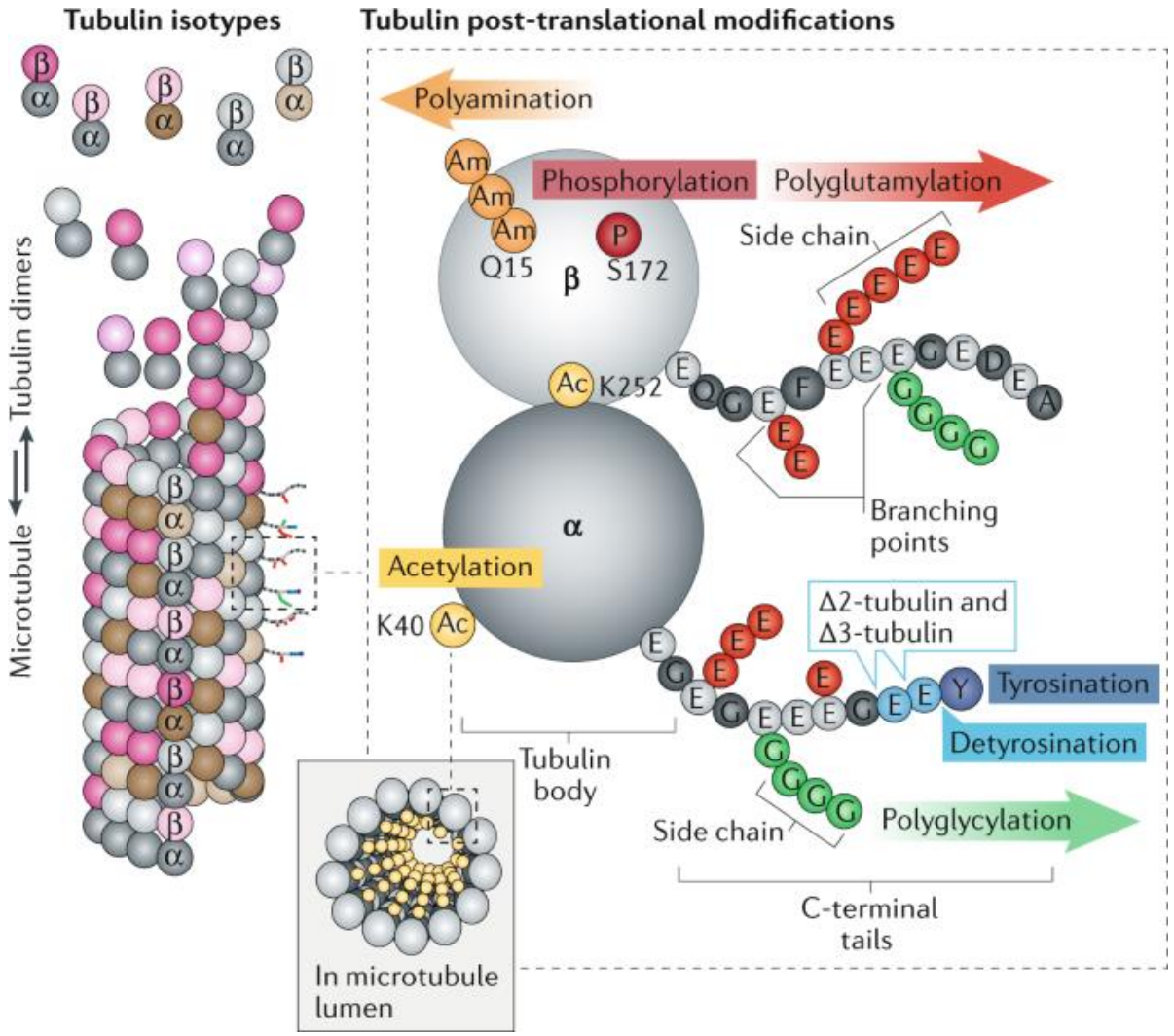




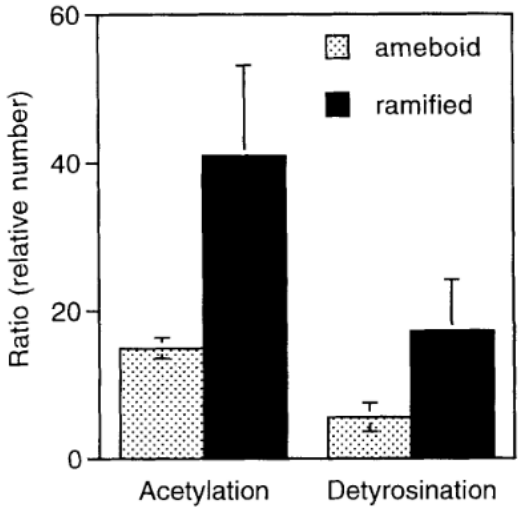
- Microglia activation increases MT dynamicity
- Highly ramified microglia have less dynamic microtubules
- Ameboid microglia have highly dynamic microtubules

Microtubule “stability”

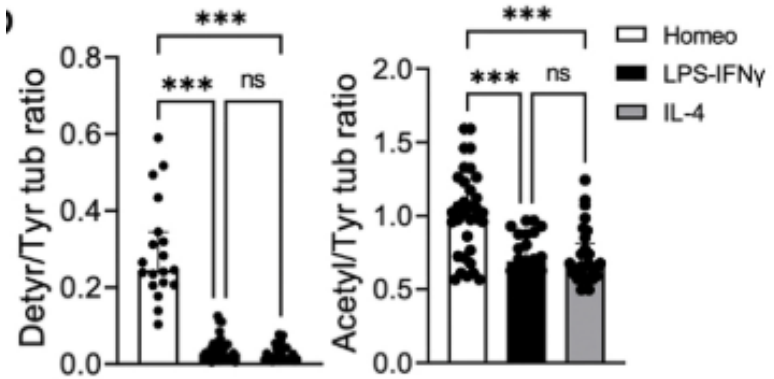
- Microtubules are considered “stable” if they **live long enough** to become substrates for microtubule-modifying enzymes
- Certain **tubulin modifications** are therefore **indirect signs of microtubule “stability”**
- Tubulin modifications decode a **tubulin “code”** to determine the state of the microtubule



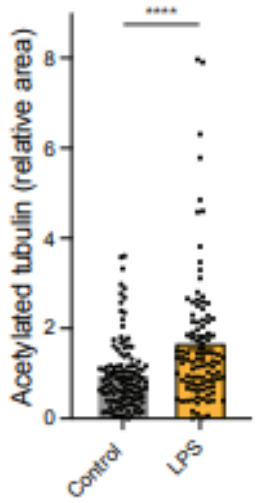
There is no consensus on which posttranslational modifications of microglial microtubules can reliably indicate their state across different microglial functional states



Ilschner and Brandt, 1996



Rosito et al, 2023



Adrian et al, 2023

Ramified, homeostatic → “stable” modifications
Amoeboid, activated → less “stable” modifications

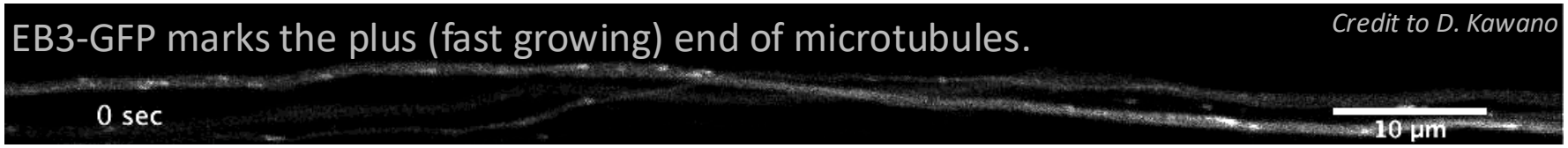
Ramified, homeostatic → less “stable” modifications
Amoeboid, activated → “stable” modifications

The importance of the in vitro model: it is challenging to replicate microglial functional states and their corresponding morphological changes that reflect cytoskeletal rearrangements

MT polarity and molecular transport

One MT end grows more rapidly and is called the **plus end**, whereas the other end is known as the **minus end** → the two ends are not symmetrical → MTs are **polar** structures

The plus end can be visualized with end-binding proteins (EB) → **EB comets**

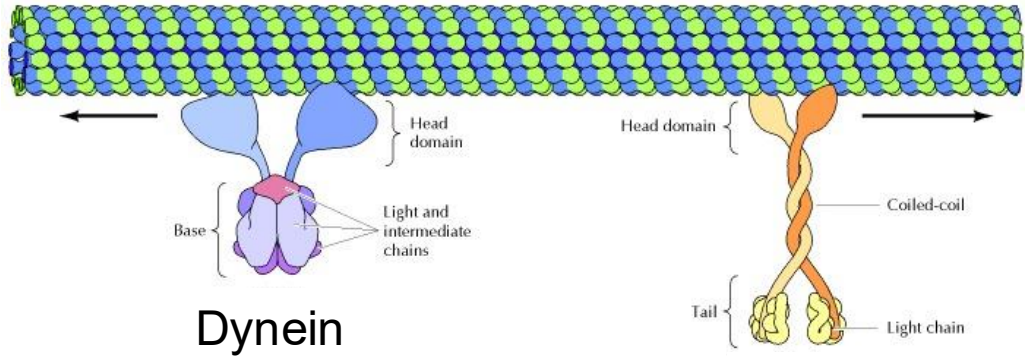


Movement along microtubules is based on the action of motor proteins that utilize energy derived from ATP hydrolysis to produce force and movement. Members of two large families of motor proteins, the **kinesins** and the **dyneins**, are responsible for powering the variety of movements in which microtubules participate.

Kinesin and dynein move in opposite directions along MTs, toward the plus and minus ends, respectively

Minus end

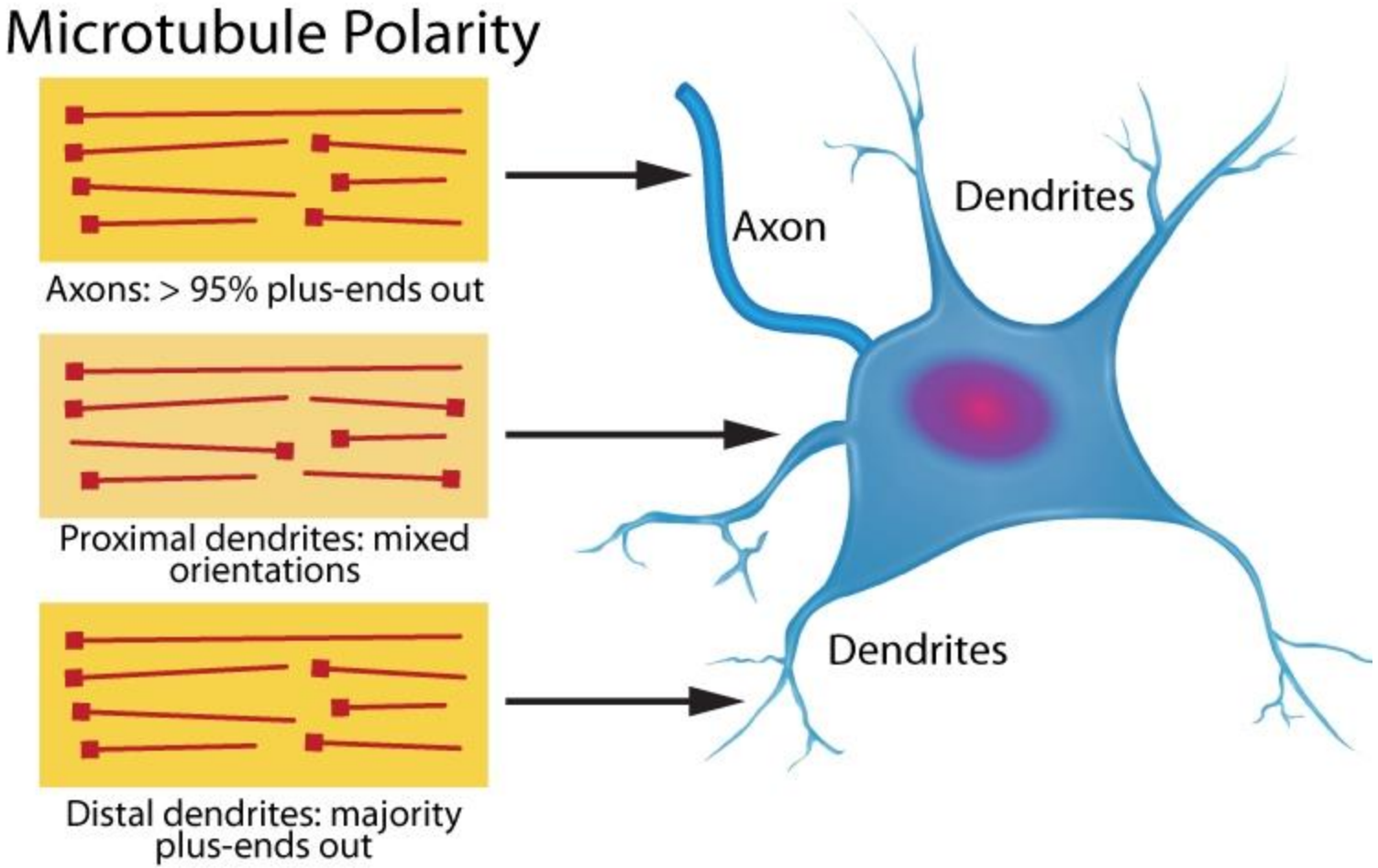
Plus end



Dynein

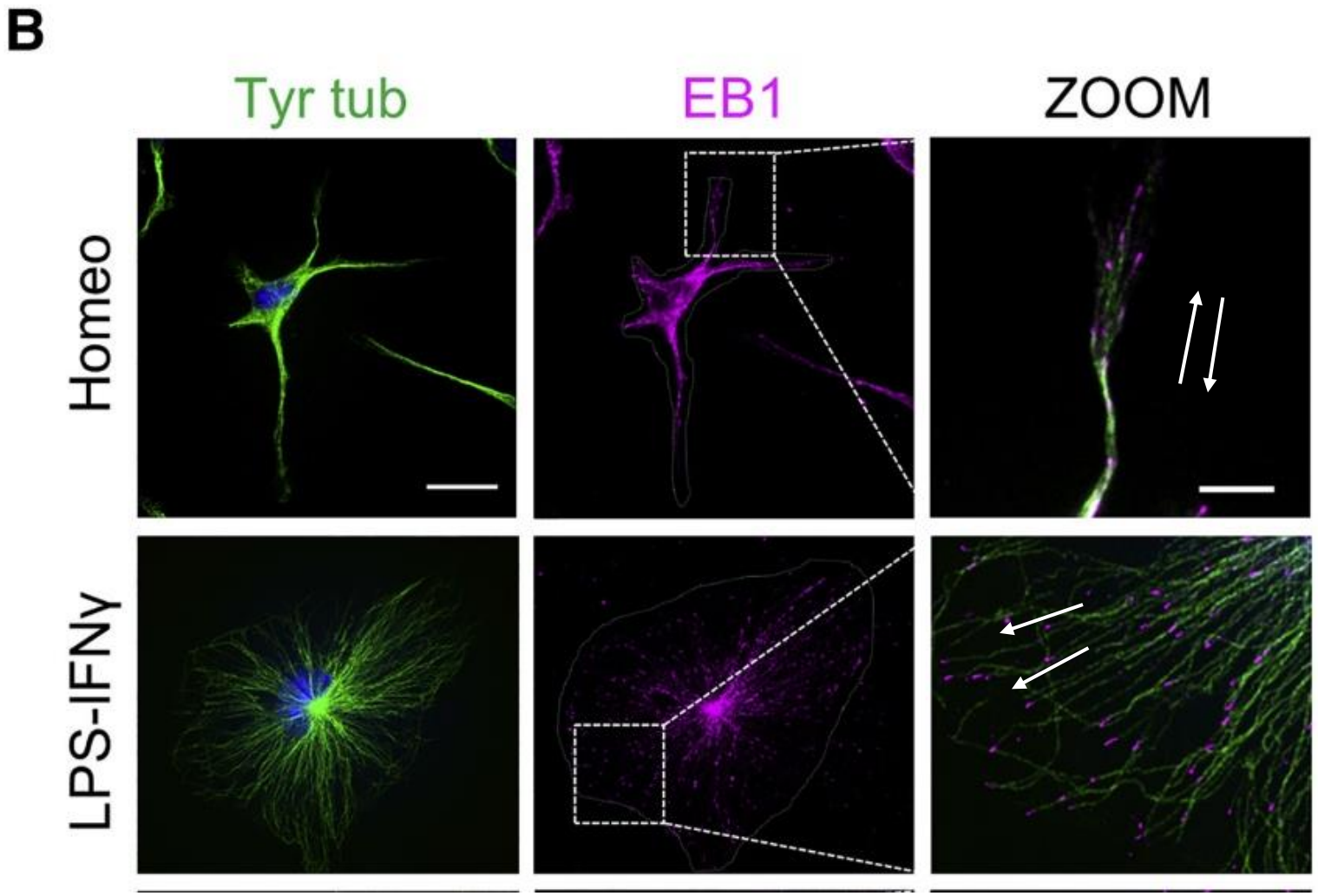
Kinesin

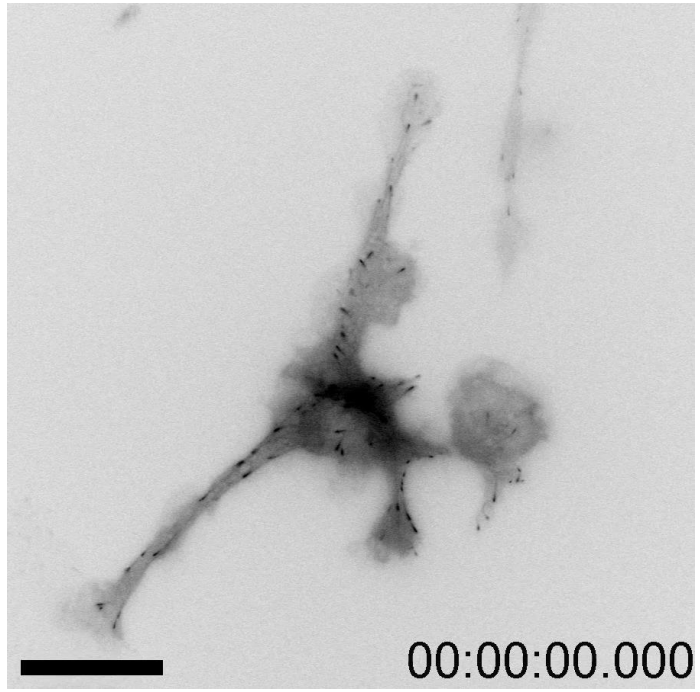
Microtubule polarity in neurons



MT polarity is **uniform in the axon**, **mixed in the dendrites**. This allows for the control of the direction of the molecular cargos along the MTs.

This peculiar pattern allows for homogeneous transport along the axon (to send signals), and more flexible and heterogeneous transport in the dendrites (to receive signals)

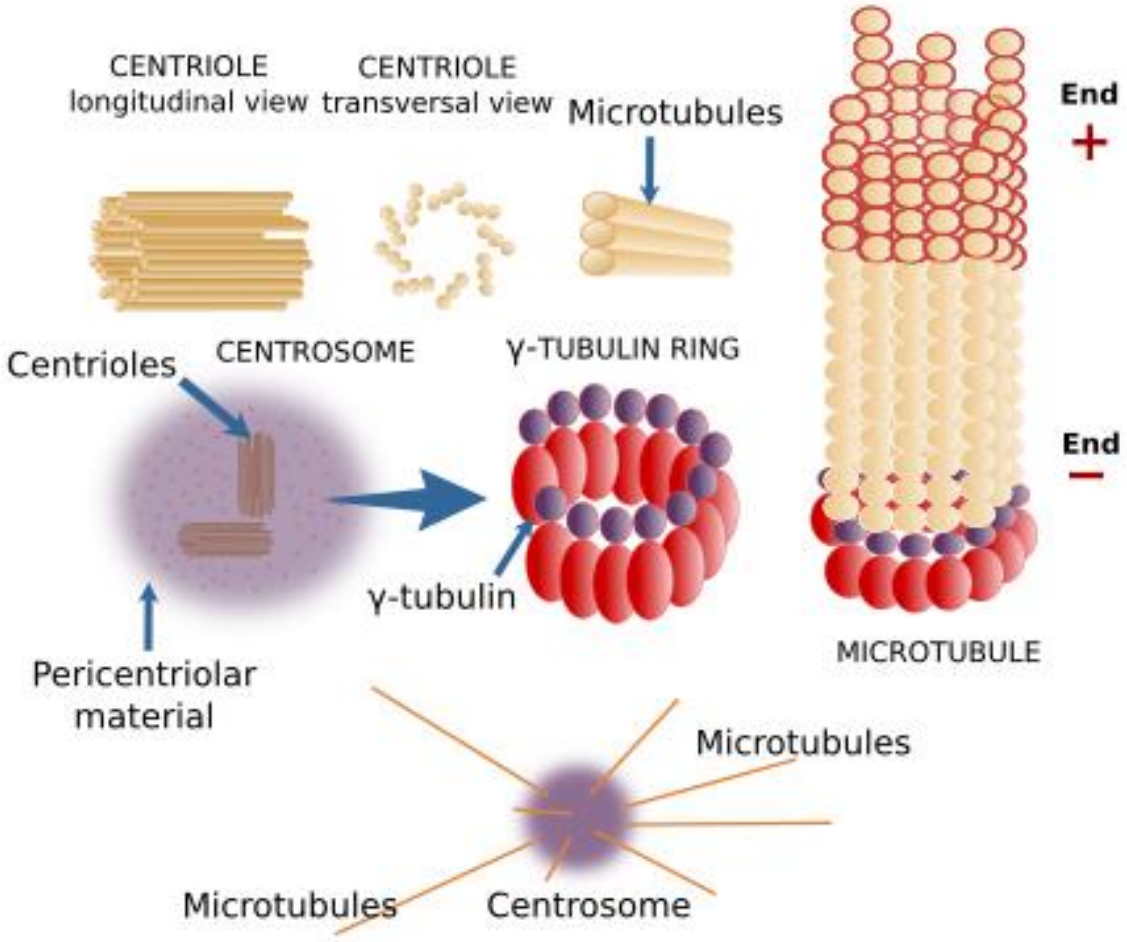


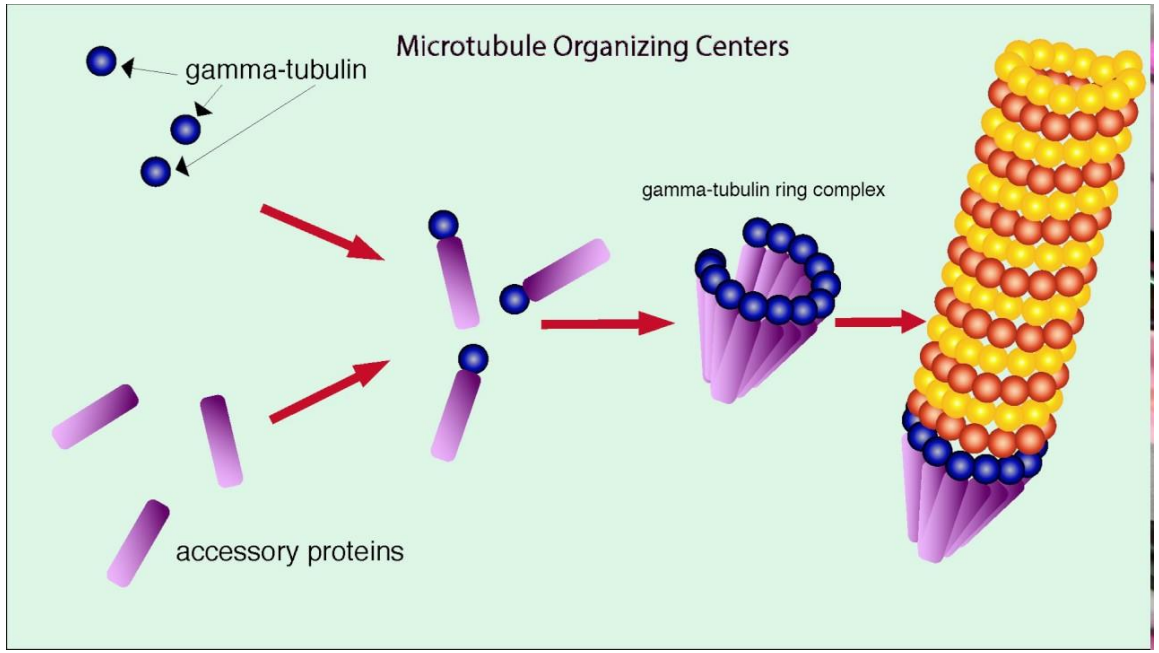


Microglia MTs display mixed polarity when ramified, uniform polarity when amoeboid

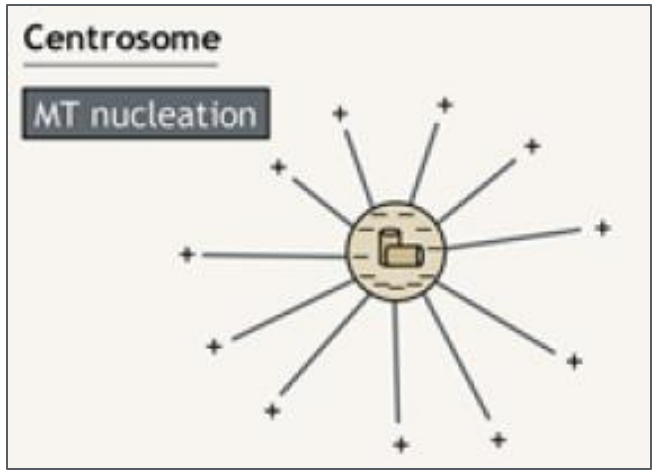
The MT minus ends are anchored in structures called **microtubule organizing centers** (MTOCs).

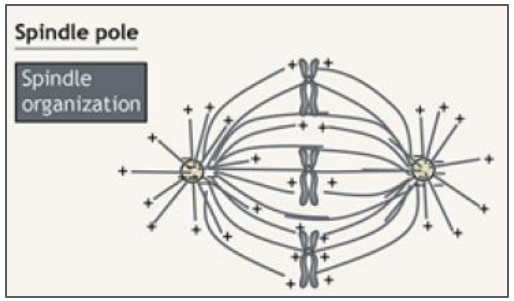
The primary MTOC in a cell is called the **centrosome**, and it is usually located adjacent to the nucleus.

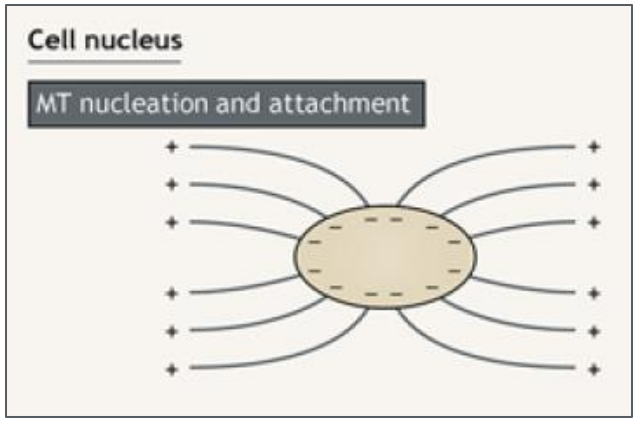


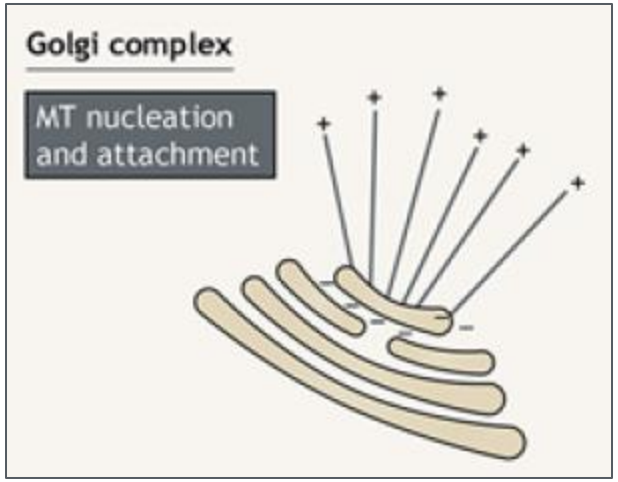


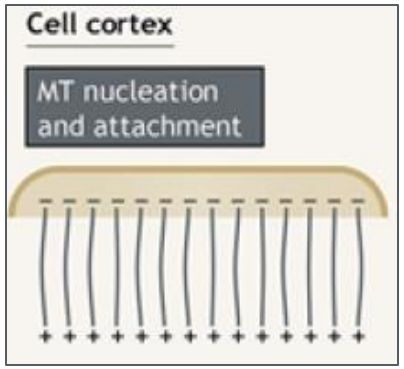
Microtubule organizing centres

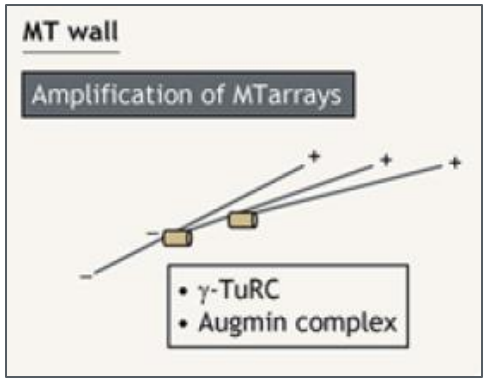








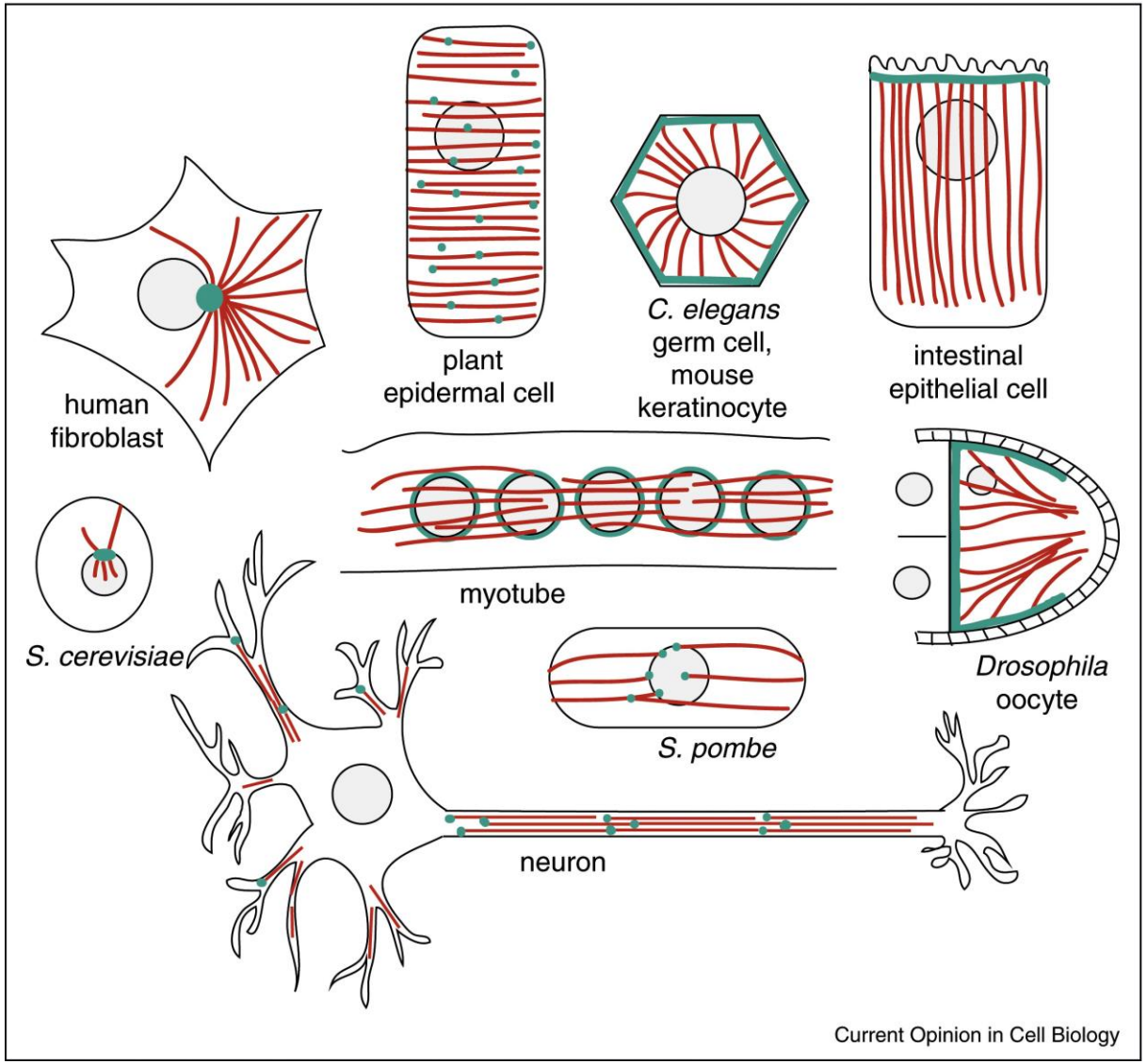


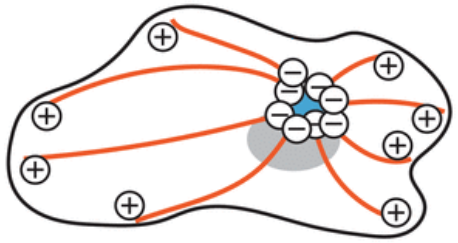


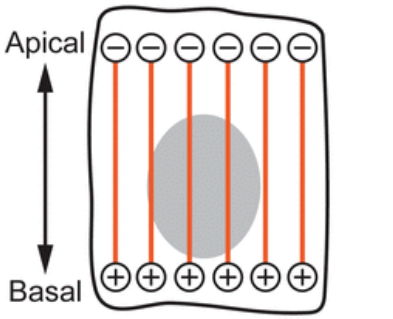
The main MTOC is the centrosome but there are many other structures that can anchor MTs and act as MTOCs

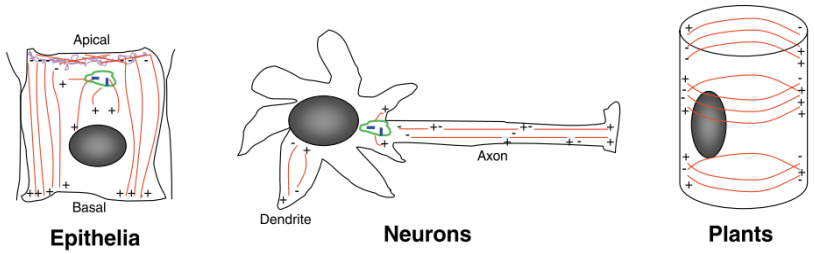
MTs are therefore distinguished beetween **centrosomal** and **non-centrosomal** MTs.

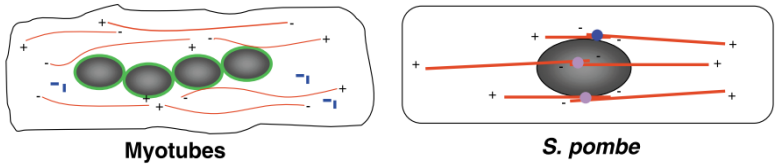
Differentiated animal cells often establish non-centrosomal MTOCs

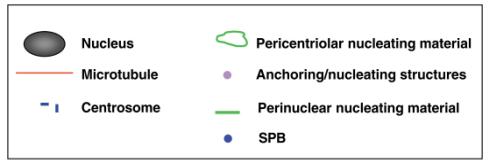




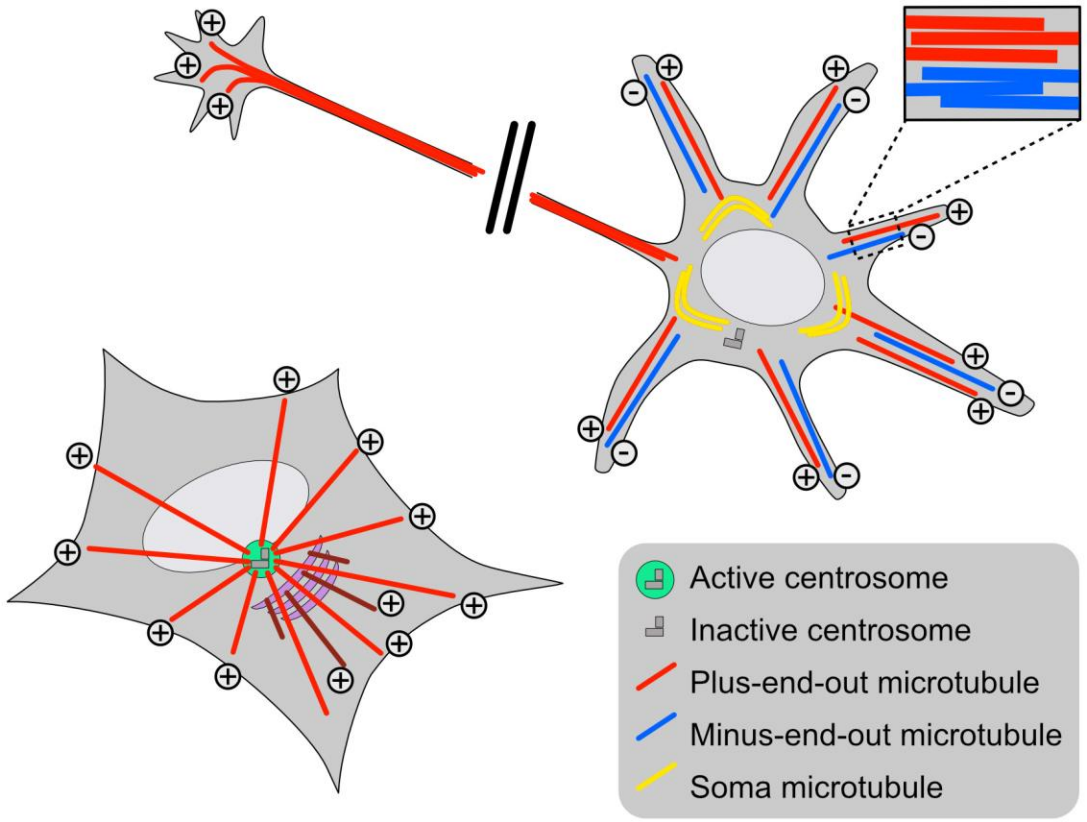








Centrosomal and non-centrosomal microtubules



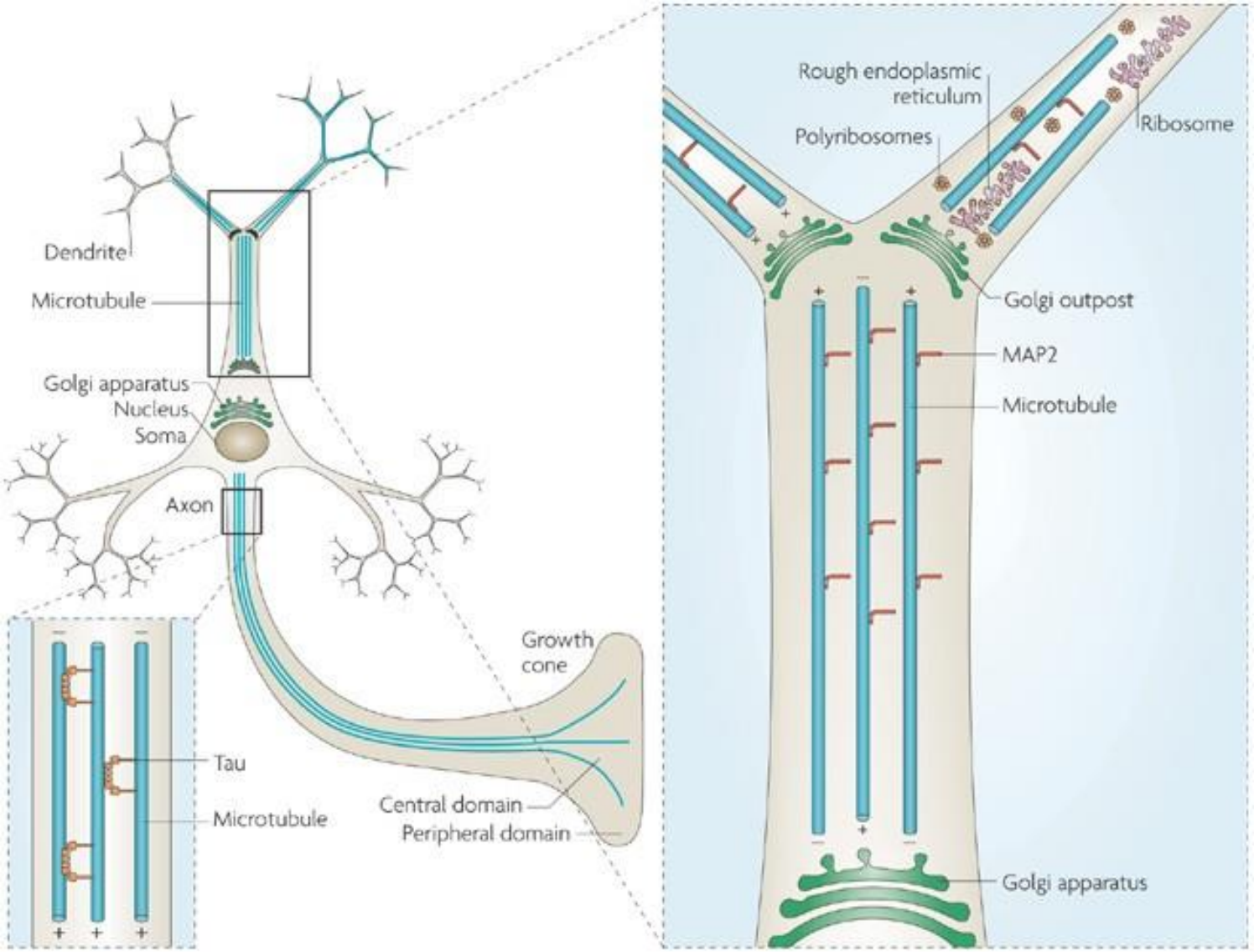
In differentiating, cycling cells, MTs are radially organized around the centrosome, the main MTOC. The centrosome is active.

In neurons, which are post-mitotic cells, centrosome is present but has lost MTOC activity.

Neurons rely on local MT nucleation and stabilization to accommodate their extended morphology, such as axons and dendrites. This organization allows:

1. **Support for Neurite Growth:** Non-centrosomal microtubules enable the elongation of axons and dendrites.
2. **Efficient Intracellular Transport:** They ensure proper transport of organelles and molecules along long distances.
3. **Structural Plasticity:** They adapt to changes in neuronal architecture during development and synaptic plasticity.

The alternative MTOCs and the Golgi outposts

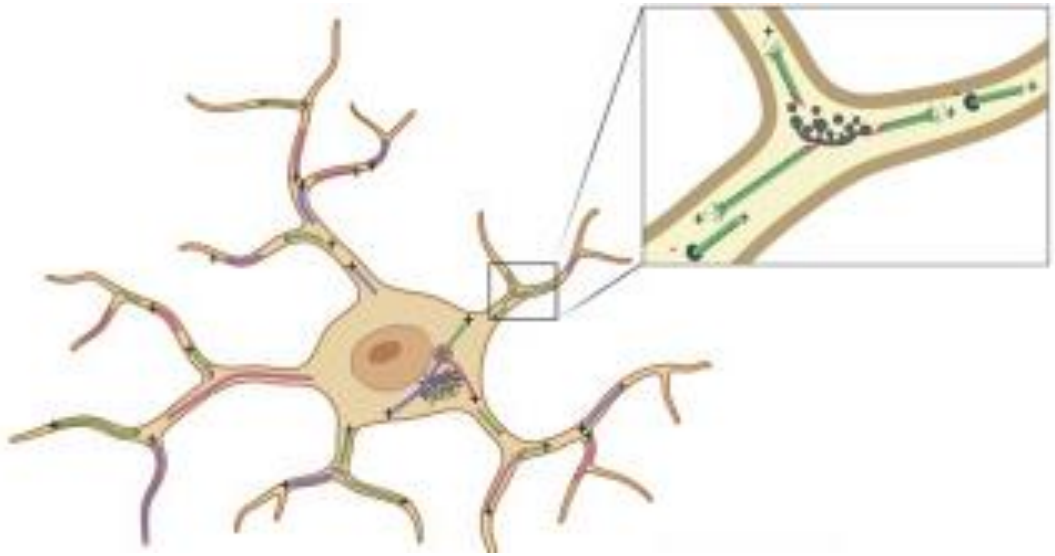


Non-centrosomal microtubules can be locally nucleated at alternative MTOCs, such as structures called **Golgi outposts**

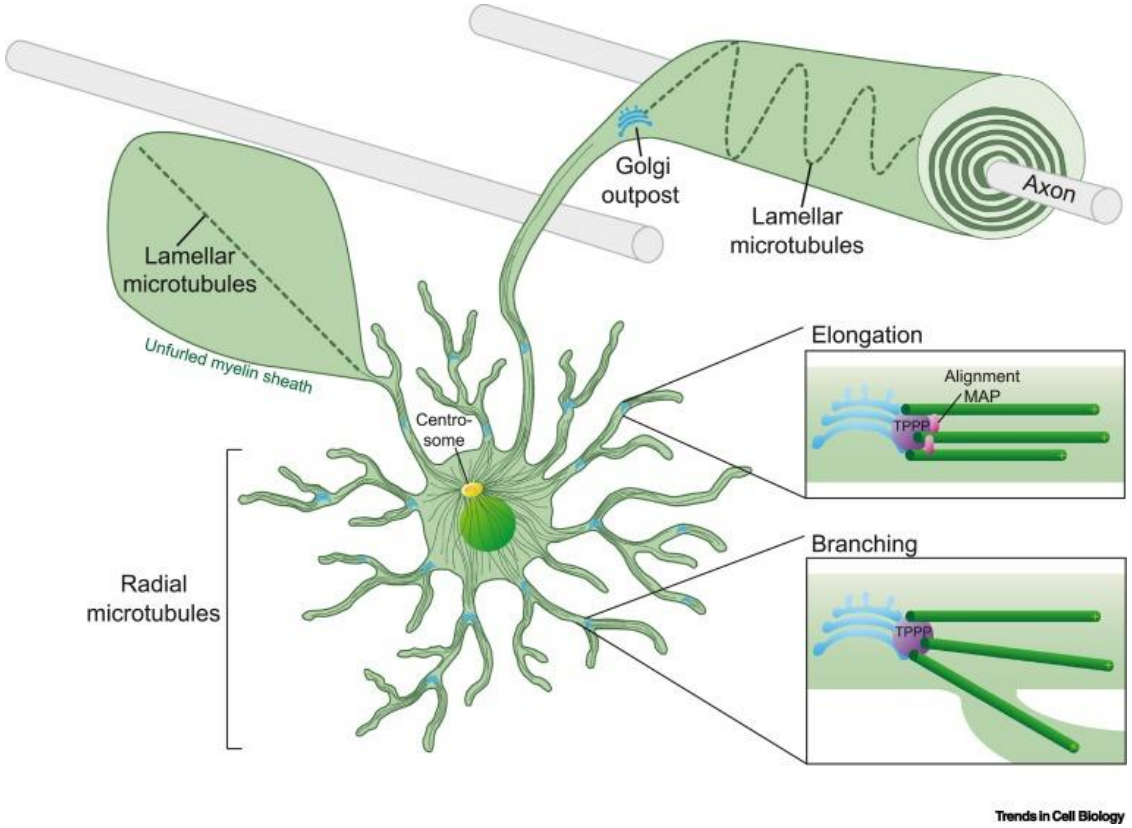
Glial Golgi outposts

Golgi outposts are present in glial cells such as oligodendrocytes and microglia

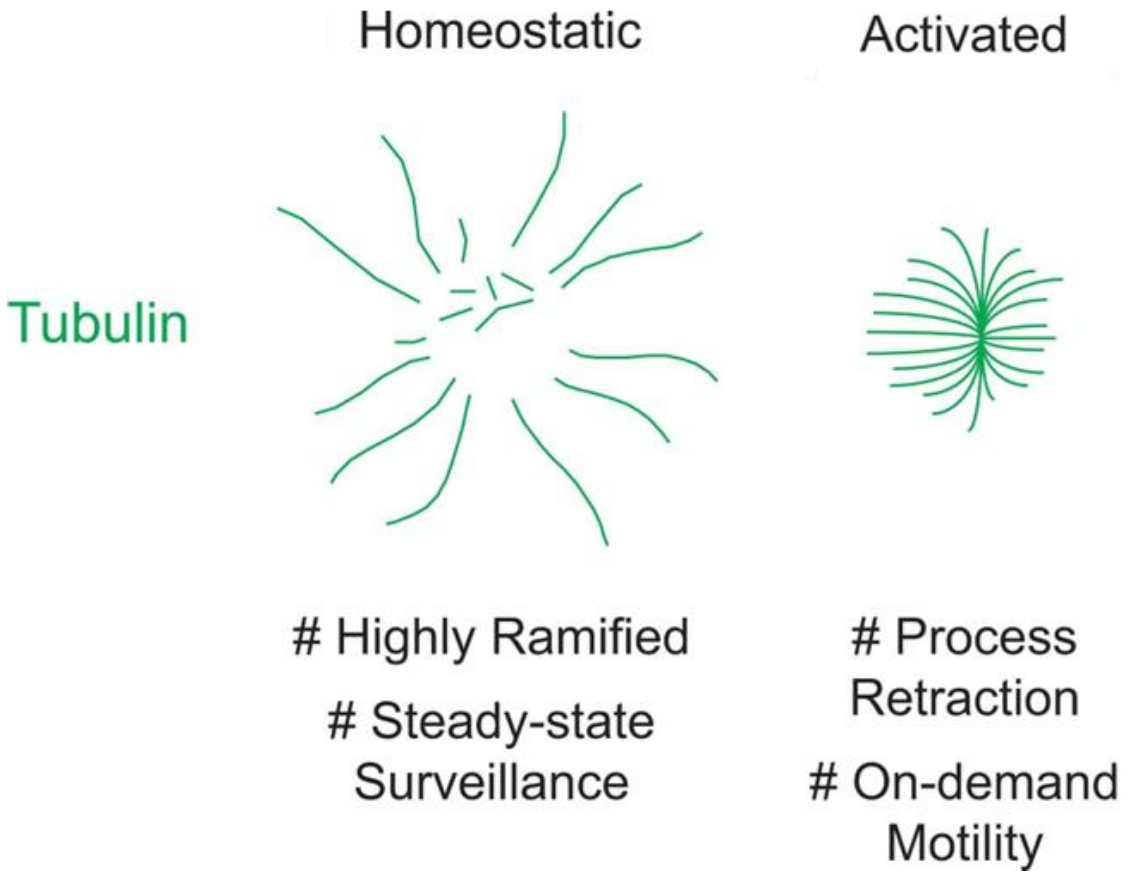
In microglia, they are important for MT elongation and branching in highly ramified morphologies

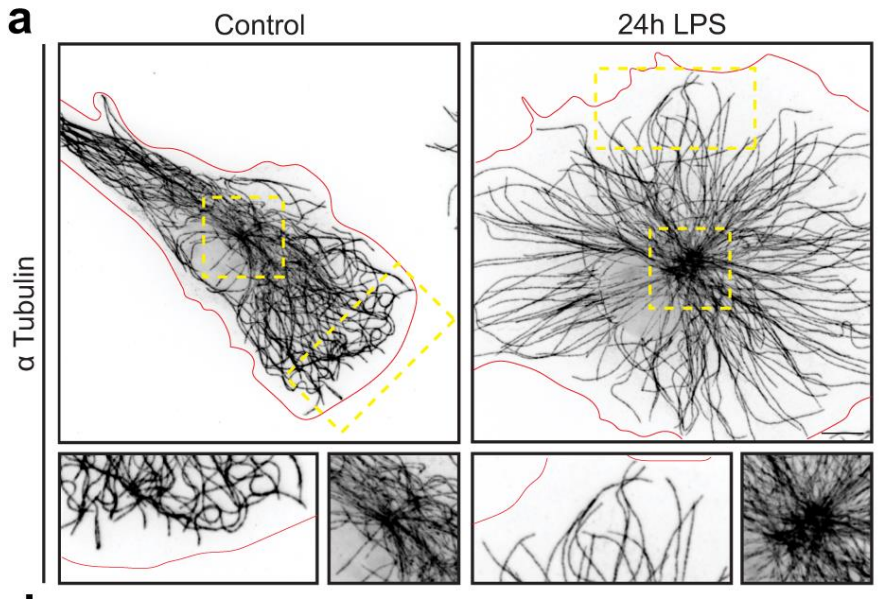


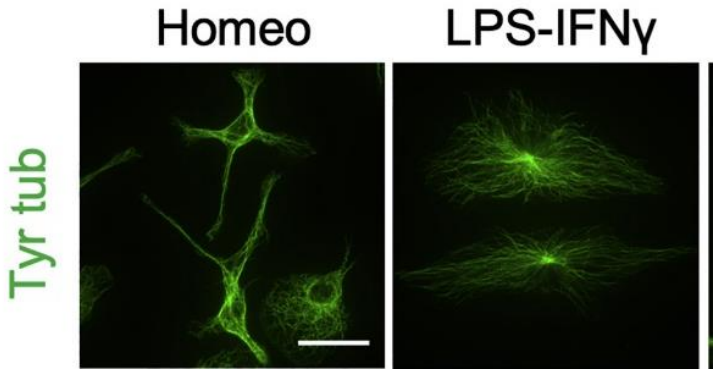
In oligodendrocytes, they are functional for MT elongation, branching and for the formation of myelin sheath



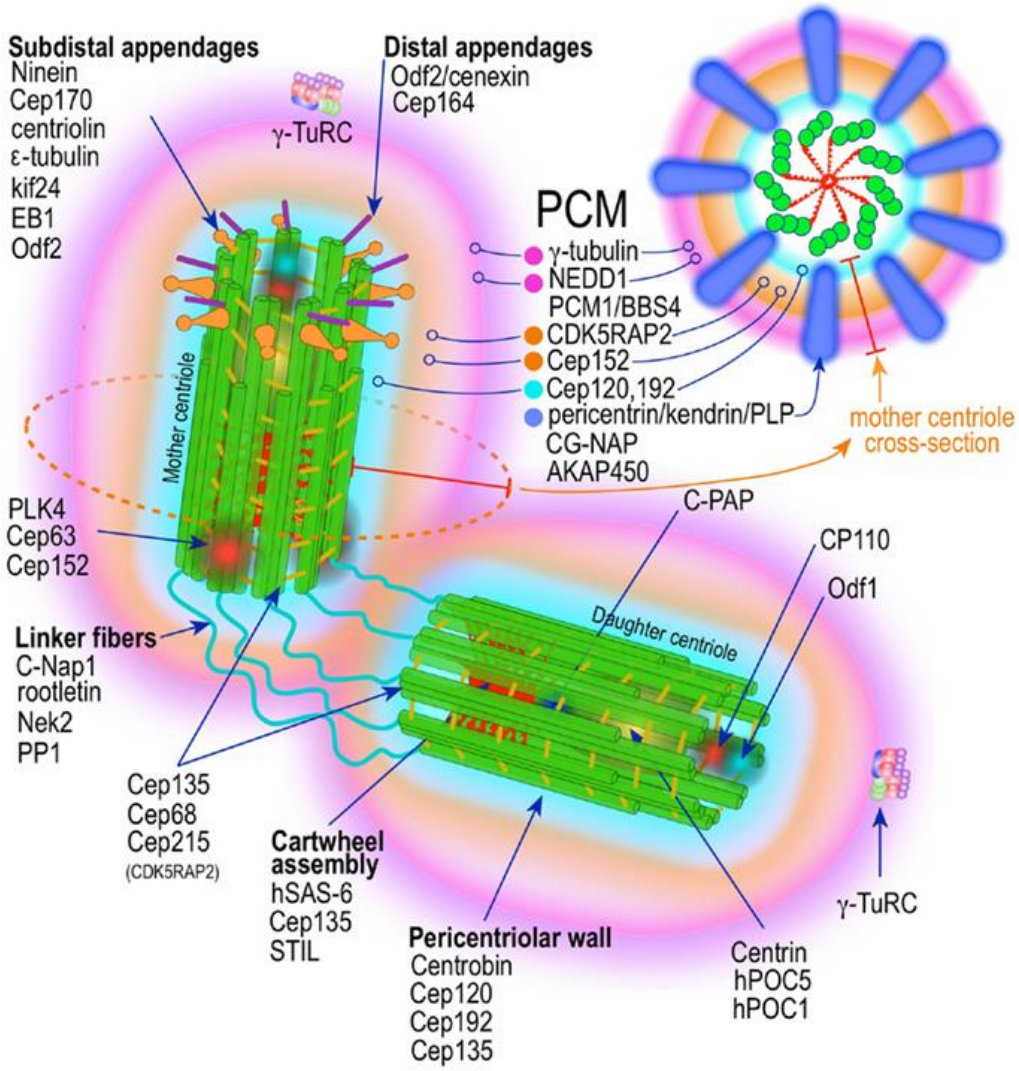
Microglia display different morphologies associated with different activation states, with **non-centrosomal** microtubules present in **ramified** morphologies, and **centrosomal** MT organization in **amoeboid** morphologies







The centrosome



The centrosome is the microtubule organizing center of animal cells. The centrosome is the best-characterized microtubule organizing centers.

The centrosome has three important activities:

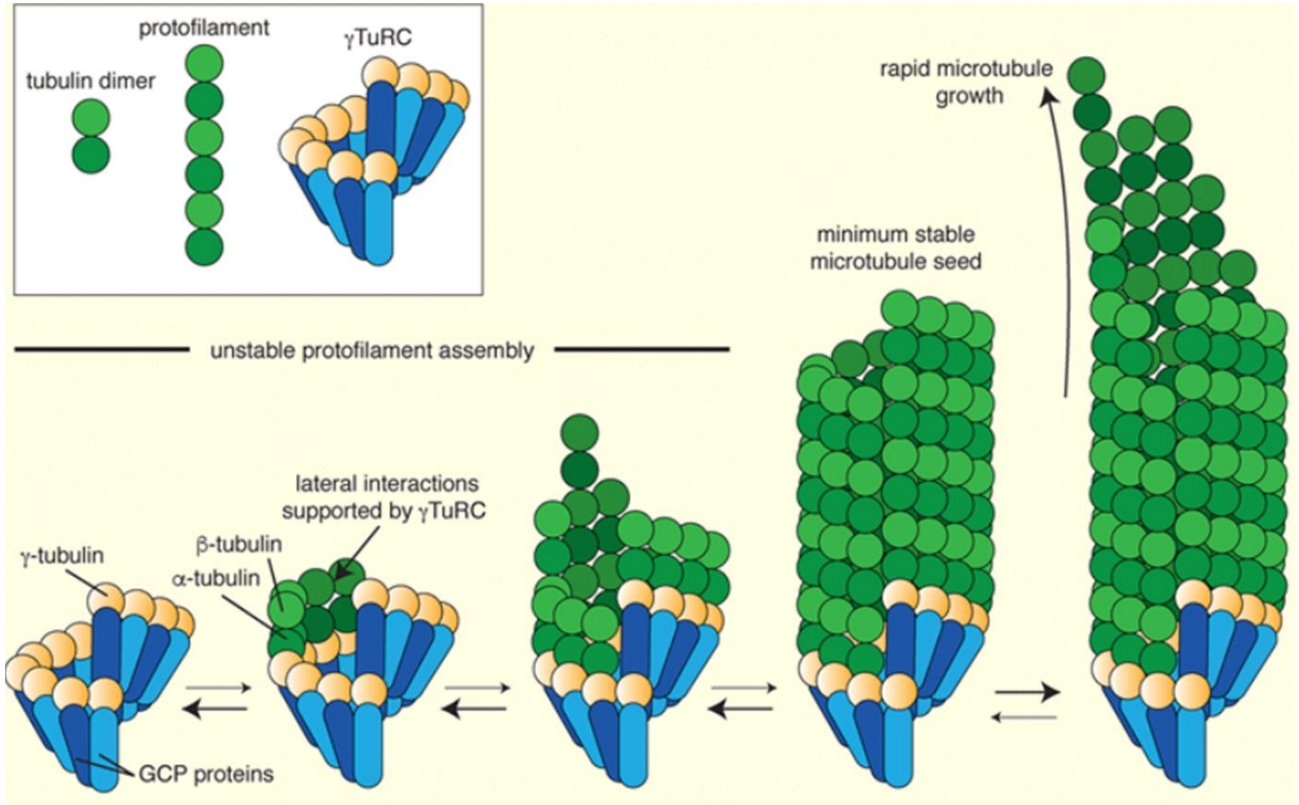
- it **nucleates** the polymerization of tubulin subunits into the long polymers that are **microtubules**;
- it **organizes** the nucleated **microtubules** into useful arrays;
- it **duplicates** once every cell cycle

The centrosome consists of two short cylindrical centrioles (each made of 9 triplets of MTs), usually oriented perpendicular to each other, surrounded by pericentriolar material (PCM)

The PCM contains elements that nucleate and organize microtubules, such as γ-tubulin

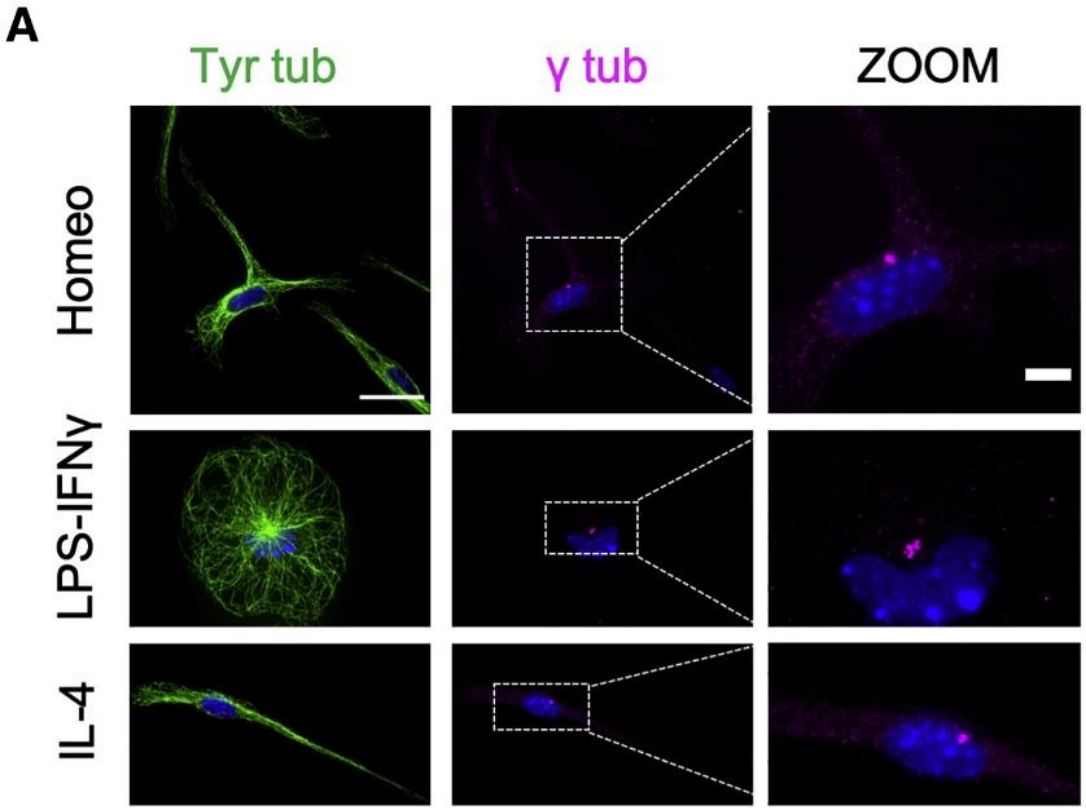
Microtubule nucleation

γ-tubulin is a conserved component of all microtubule organizing centers, localized to the pericentriolar material of the centrosome



Microtubule nucleation occurs in the pericentriolar material, and γ-tubulin is required for this nucleation activity. The γ-tubulin is part of a large protein complex that is organized into an open ring of roughly 25 nm diameter, the same as the diameter of a microtubule

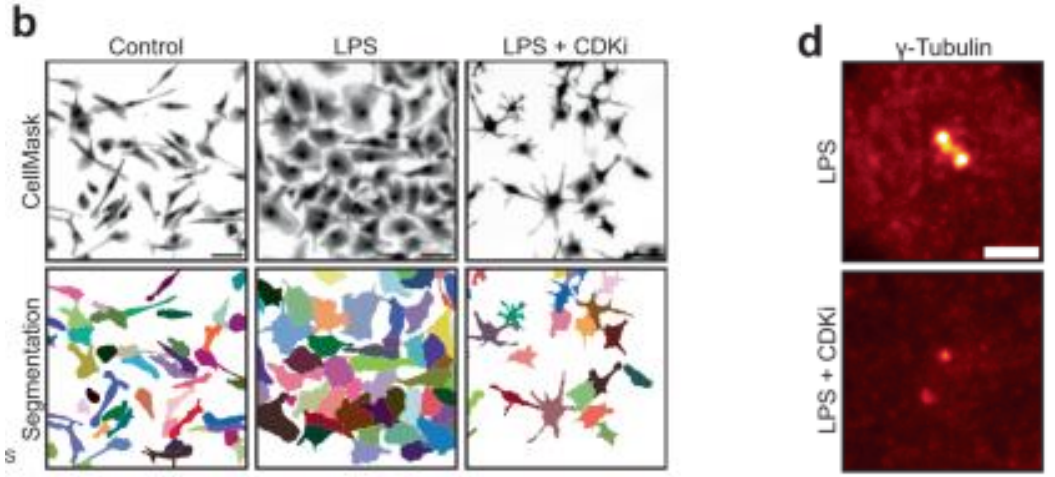
In microglia, γ-tubulin redistributes from a diffuse pattern in homeostatic cells to a concentrated pericentrosomal area in activated microglia

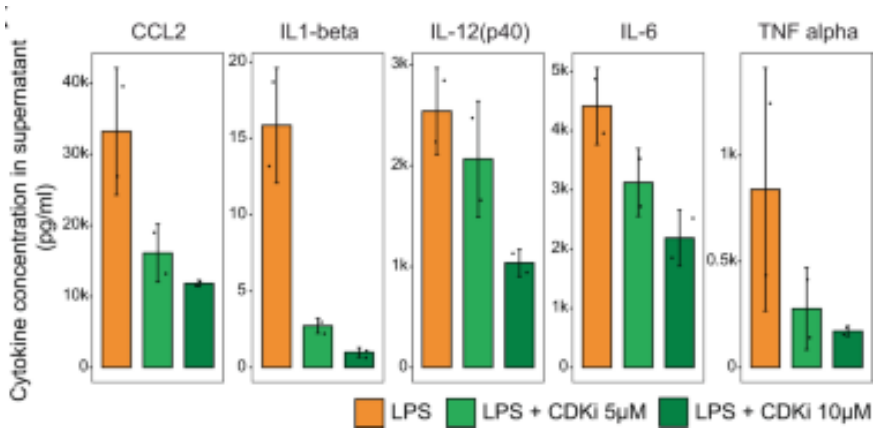


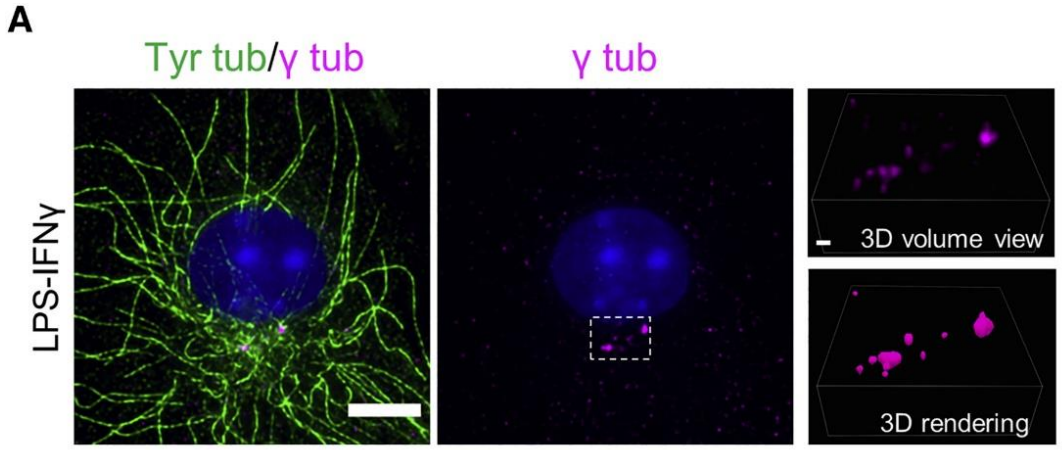
This redistribution is important for cytokine release

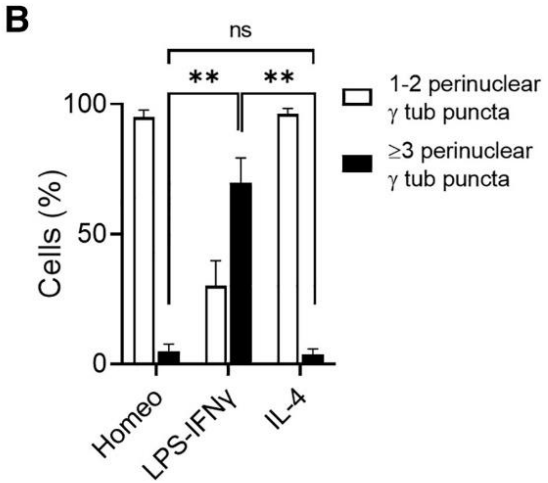
Cyclin-dependent kinase 1 (Cdk1) is an upstream regulator of microtubule remodeling and morphological changes

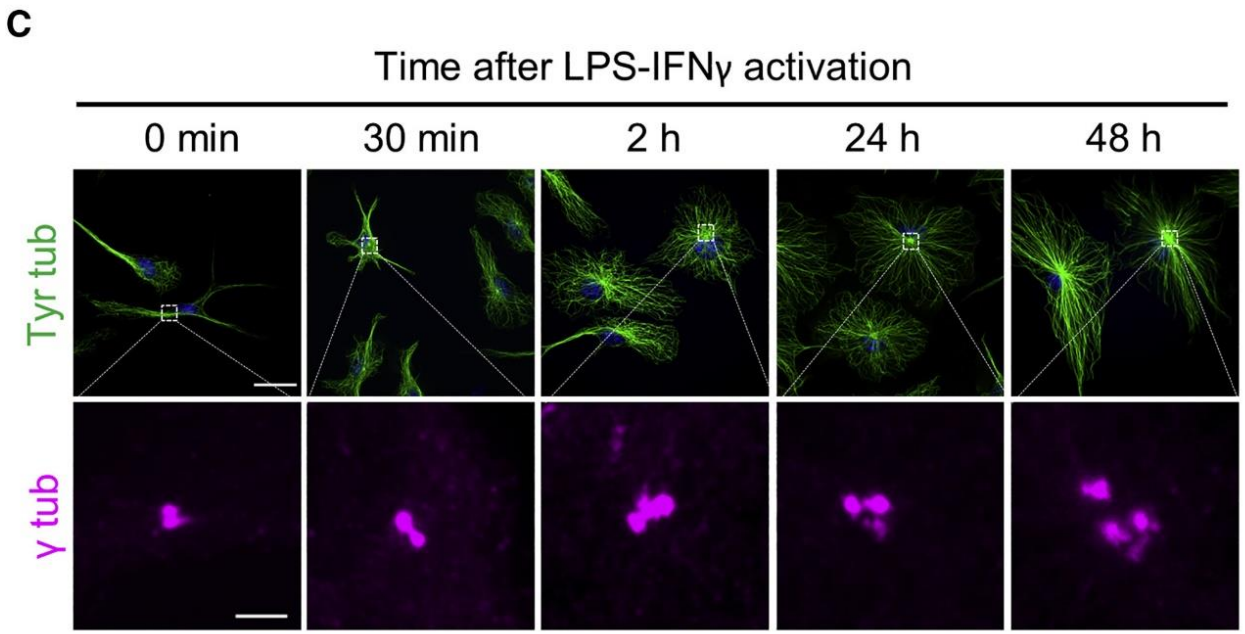
Cdk1 activation is essential for MT remodeling and functional cytokine release

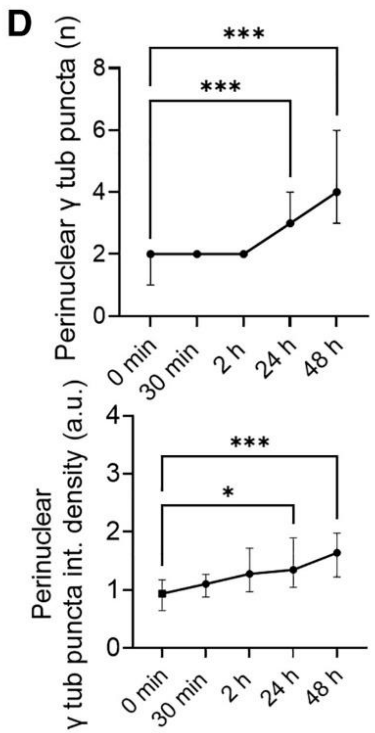












Microglia activation with LPS-IFNγ drives the formation of multiple pericentrosomal γ-tubulin positive structures

Microglia microtubule cytoskeleton

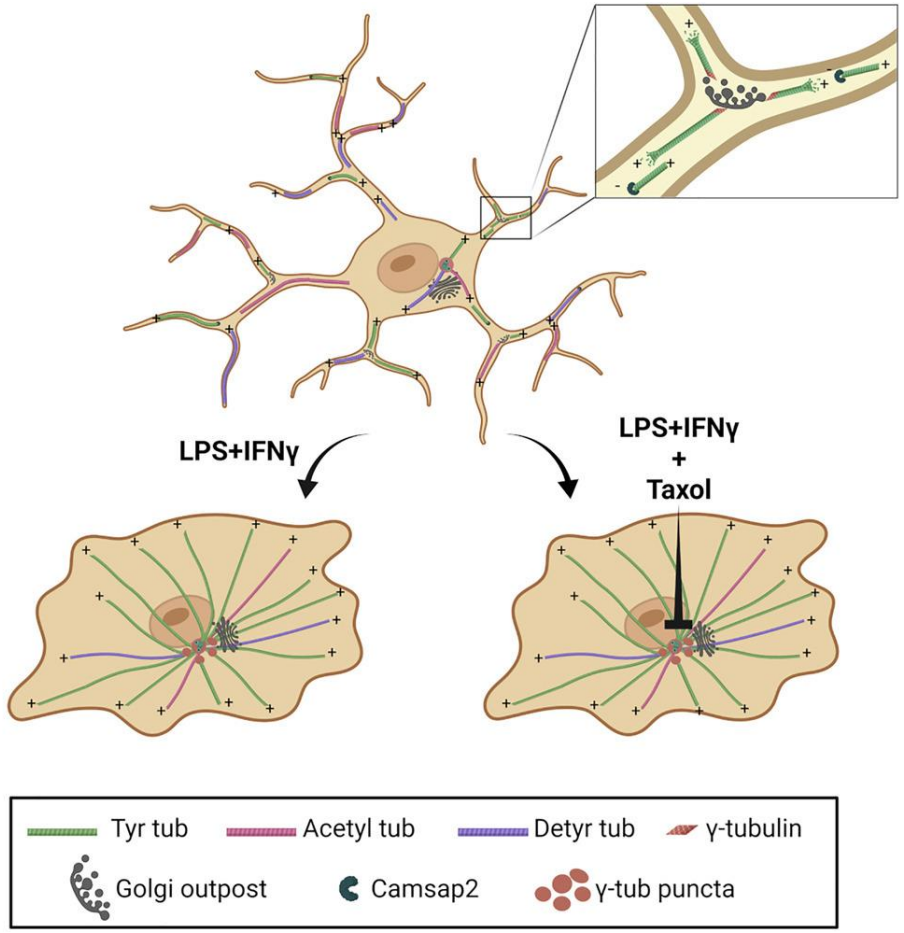
- In microglia, MTs dynamicity increases with activation
- MT polarity, fundamental to establish intracellular transport direction, is mixed in ramified microglia and uniform in amoeboid microglia
- Ramified microglia display non-centrosomal MTs, amoeboid microglia rearrange MTs in centrosomal organization
- Ramified microglia display Golgi outposts as alternative MTOCs
- Microglia reorganize γ-tubulin during activation, and this drives cytokine release

Why is microglia microtubule cytoskeleton interesting?

To find novel target to modulate neuroinflammation

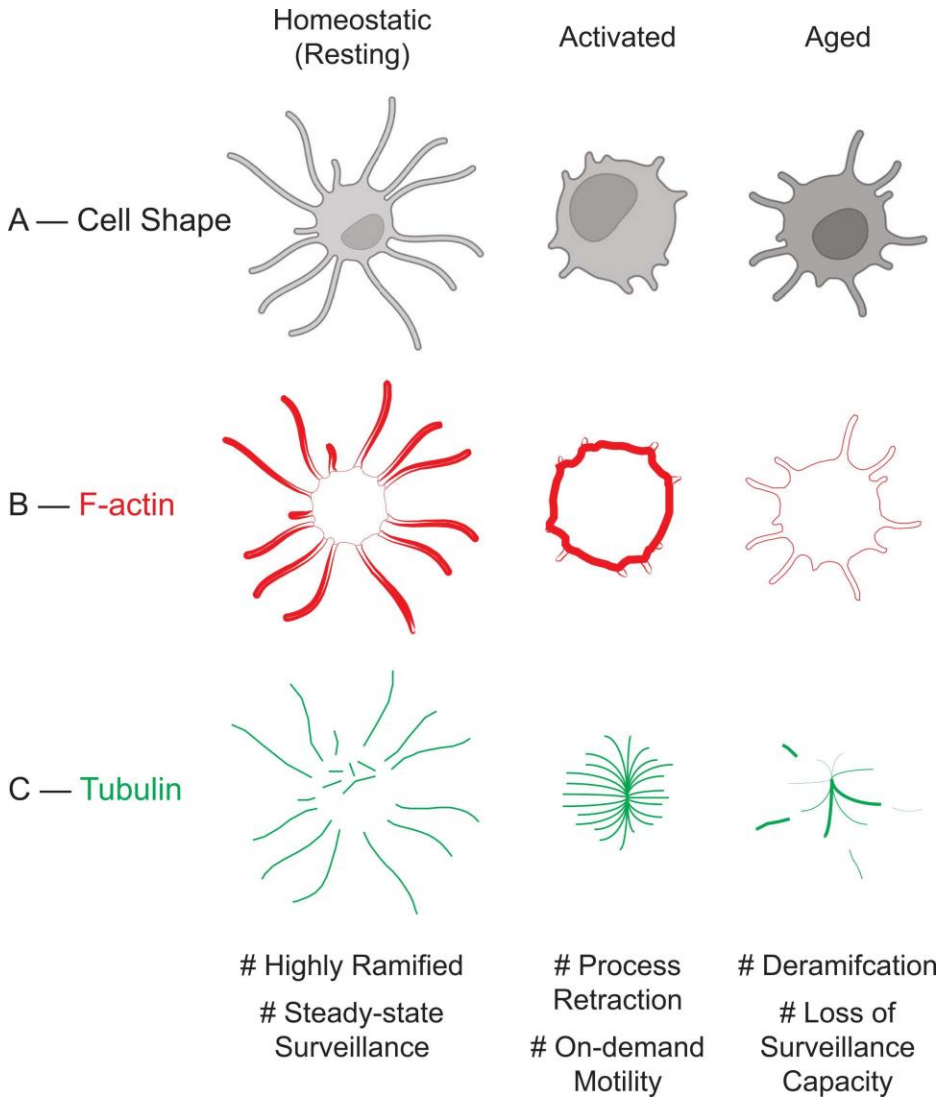
The huge morphological changes in microglia morphologies rely on the MT cytoskeleton rearrangements, therefore MT cytoskeleton components can become:

- Proxies for neuroinflammation and aging
- Target for neuroinflammation modulation



Rosito et al., 2023

A cytoskeleton symphony: Actin and microtubules in microglia dynamics and aging



Actin and microtubule cooperatively shape the microglial cytoskeleton behavior.

- Actin and microtubule dynamics underpin microglia homeostatic functions.

- Several MT reorganizing molecules can bridge microtubule dynamics to microglia reactivity.

- Actin polymerization coordinates microglial motility across diverse functional states.

- Disorganization of the actin-microtubule network is a hallmark of aged microglia.

- Aging in microglia is associated with notable changes in MT stability, leading to a disrupted MT network.

Useful links

10.1016/j.neuron.2022.10.020 microglia states and nomenclature
https://doi.org/10.1038/s41419-023-05835-8 tunneling nanotubes
https://www.cell.com/current-biology/fulltext/S0960-9822(99)80201-2 the centrosome
10.1016/j.pneurobio.2024.102586 actin and MTs in microglia
10.3390/cells8060639 microglia actin
https://doi.org/10.1016/j.celrep.2023.112104 microglia MTs
https://doi.org/10.1038/s41467-023-41891-6 microglia MTs
https://doi.org/10.1242/jcs.03227 generation of non-centrosomal arrays