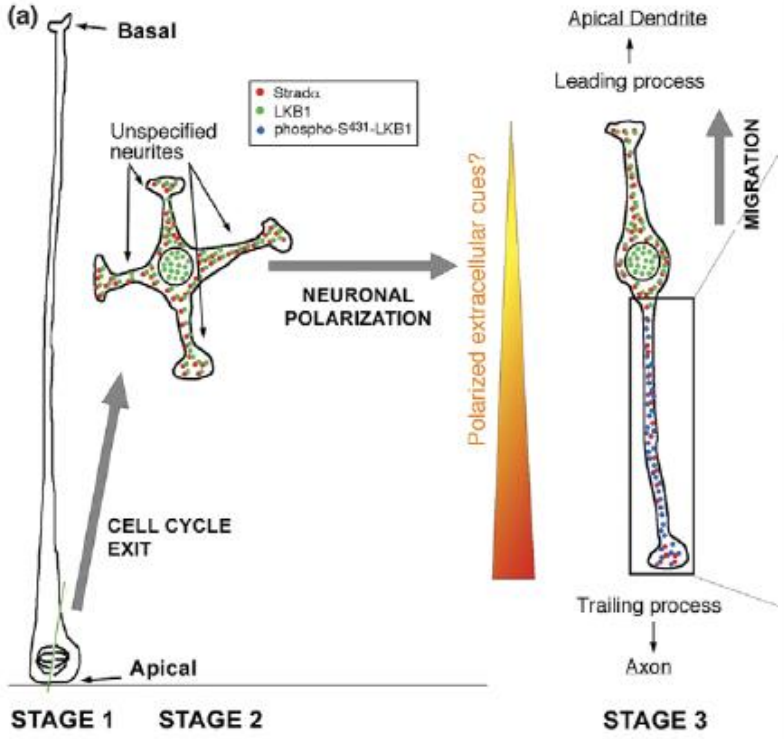
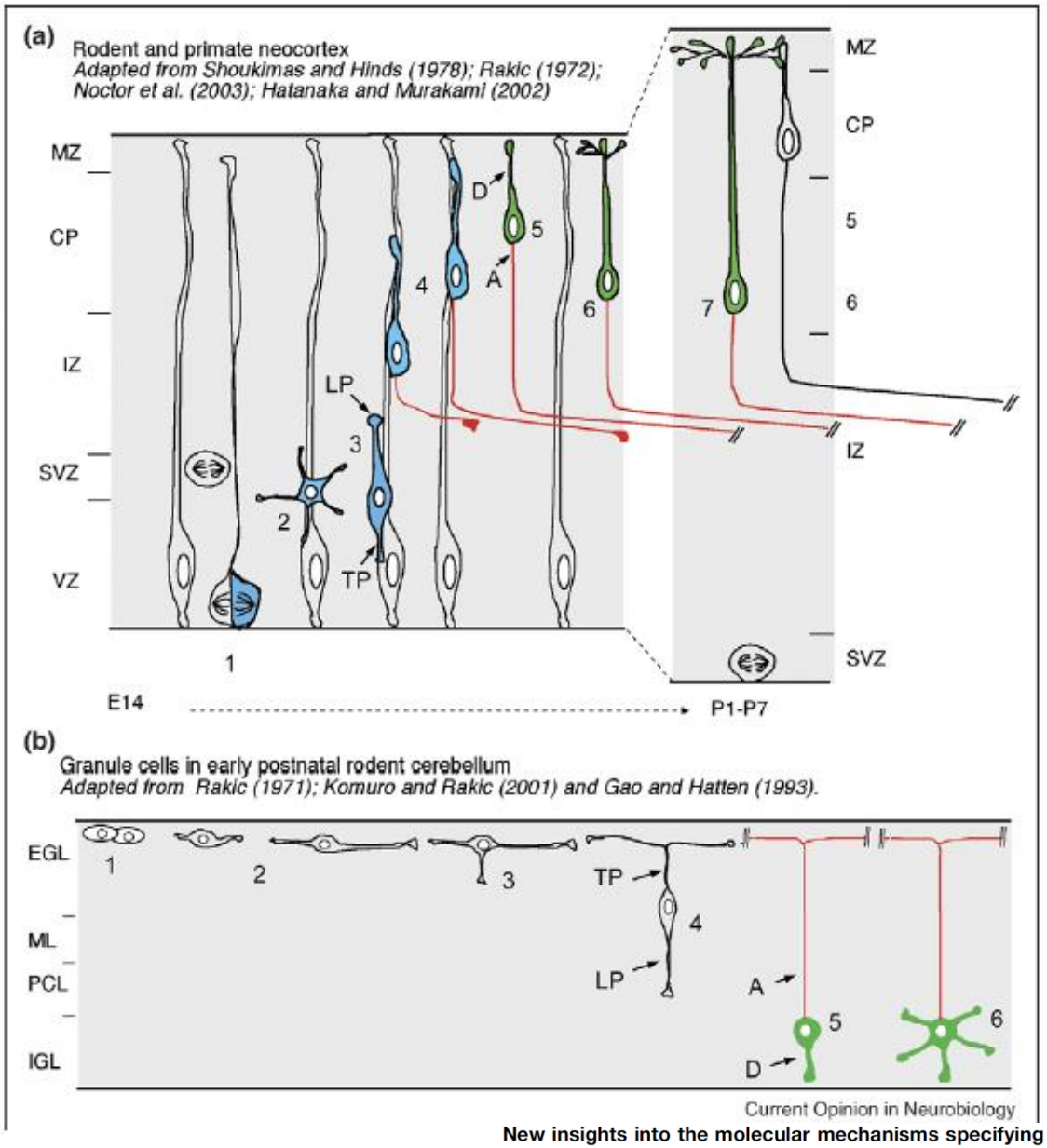
Neuronal polarity – axon / dendrite specification

Axon / dendrite specification in vivo



- postmitotic neurons leaving the germinative zone have uniform multiple processes (SVZ)
- radial / tangential migration requires polarized processes (leading / trailing process)



Current Opinion in Neurobiology

New insights into the molecular mechanisms specifying neuronal polarity *in vivo*

Anthony P Barnes[1], David Solecki[2] and Franck Polleux[1]

Axon / dendrite specification in vivo

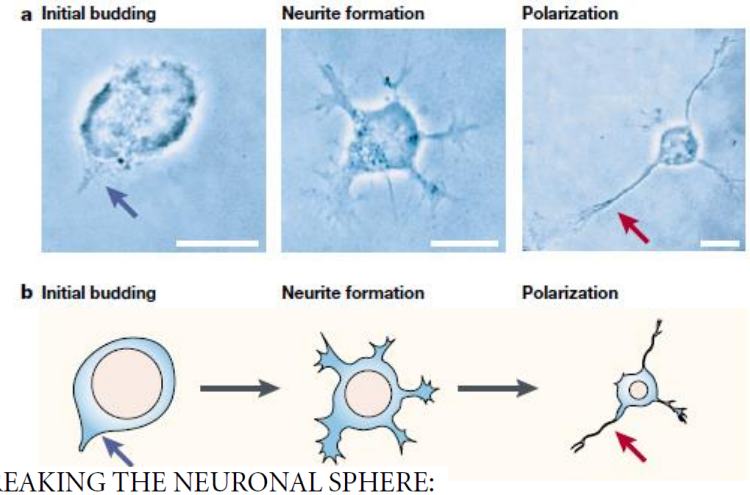
Molecular mechanisms underlying LKB1 function in neuronal polarization *in vivo*. **(a)** Upon asymmetric cell division of radial glial progenitors (Stage 1), early unpolarized post-mitotic neurons show a transient phase of non-directed neurite outgrowth in the subventricular zone (Stage 2) before adopting a bipolar morphology in the intermediate zone where they engage radial migration with a leading process directed toward the pial surface and a trailing process directed toward the ventricle. **(b)** On the basis of recent reports [21••,55••], we propose that *in vivo*, the trailing process is specified to become the axon in response to putative extracellular cues that preferentially induce phosphorylation of LKB1 on Serine431. This event might be mediated partly by cues providing chemotactic attraction of radially migrating neurons toward the cortical plate such as Sema3A [70] or any other extracellular cues neurotrophins (NTs) such as BDNF/NT4/NT3 [55••], Wnt [66•,67•], FGFs or other cues that can activate cAMP-dependent protein kinase (PKA) or p90 RSK (RSK1-3). One cannot exclude the possibility that another uncharacterized serine/threonine protein kinase can phosphorylate Serine 431 *in vivo* and play a role in neuronal polarization. Once LKB1 is activated by binding to its necessary co-activator Strad (α or β) and S431-phosphorylation (which occurs only in the neurite becoming the axon), LKB1 phosphorylates SAD-A/B kinases (and probably microtubule affinity-regulated kinases, MARK1-4) that are required for axon specification partly by phosphorylating microtubule-associated proteins such as Tau. On the basis of the function of SAD-kinases in presynaptic vesicular clustering in *C. elegans* [77], we can hypothesize that SAD-A/B kinases might also specify axon identity by directing vesicular trafficking in the neurite becoming the axon. On the basis of evidence obtained in *D. melanogaster*, Par1 can phosphorylate Par3 on two serine residues that constitute binding sites for the 14-3-3 protein Par5, an event that controls its localization during *D. melanogaster* oocyte polarity. At present this is the only potential link between the Par3/Par6/aPKC complex and Par4/Par1 dyad during cell polarization. See text for details. Modified from reference [21••].

Cellular mechanisms underlying axon–dendrite polarization *in vivo*. **(a)** In the cerebral cortex, axon/dendrite polarization occurs when neurons engage in radial migration in the intermediate zone [19,20,21••]. Neurons are produced either from asymmetric ventricular division of radial glial progenitors in the ventricular zone (VZ, blue, step 1) and/or neurogenic symmetric abventricular division of transient amplifying cells in the subventricular zone (SVZ, step 1). Upon their last division, neurons often transiently display a multipolar morphology (step 2) before switching to a unipolar morphology where the leading process (LP) is attached to a radial glial process and initiates radial translocation (step 3). During radial translocation through the IZ, the trailing process (TP) elongates rapidly and becomes the axon while the cell body of the migrating neuron is still translocating toward the cortical plate (CP; steps 3-4). At this point, the leading process is actively engaged in neuronal translocation and will become the apical dendrite upon reaching the cortical plate where it will express dendrite-specific markers such as MAP2 (D; green), whereas the trailing process elongates at very rapid rate and already expresses axon-specific markers (a; red). Upon reaching the top of the CP, pyramidal neurons detach from the radial glial process (step 5), and the leading process/apical dendrite starts elongating again since subsequently generated neurons will bypass the first-generated neurons and accumulate in an 'inside-out' manner (steps 6–7). **(b)** This cellular mechanism is conserved in migrating neurons such as granule cells in the cerebellum: upon their terminal division in the external granular layer (EGL), young post-mitotic granule cell neurons (GCN) extend two processes (step 2) that become the trailing process, and ultimately the classically defined bifurcating axon of GCN (step 4) once the leading process emerges in the radial plane and GCN initiates radial migration toward its final position in the internal granular layer (IGL). The leading process defines the dendritic domain of GCN, whereas the trailing process defines the axon of GCN. Modified from references [74–76].

New insights into the molecular mechanisms specifying neuronal polarity *in vivo*

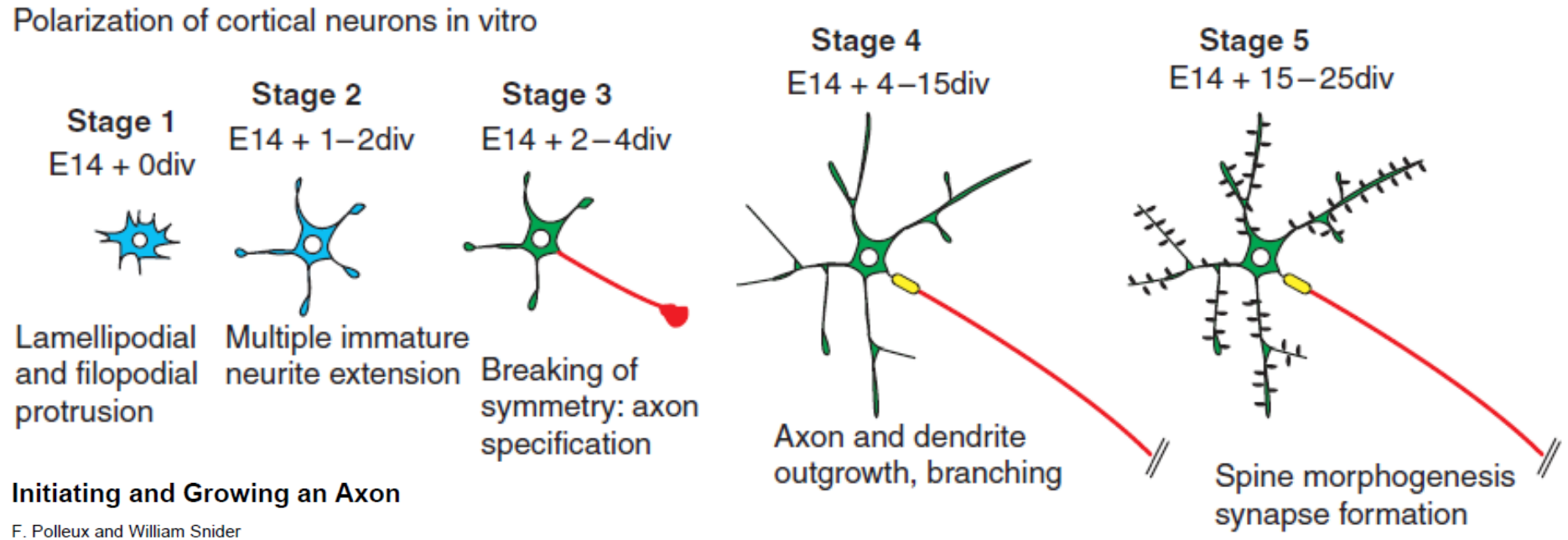
Anthony P Barnes[1], David Solecki[2] and Franck Polleux[1]

Axon / dendrite specification in vitro



BREAKING THE NEURONAL SPHERE:
REGULATION OF THE ACTIN
CYTOSKELETON IN NEURITOGENESIS

- studies mostly in dissociated embryonic hippocampal cell cultures
- characteristic morphology – characteristic stages in development and maturation



Initiating and Growing an Axon

F. Polleux and William Snider

Cold Spring Harb Perspect Biol 2010;2:a001925

Axon / dendrite specification in vitro

Figure 1. Parallels between neuronal polarization in vitro and in vivo. Comparison of the sequence of events leading to the polarization of cortical pyramidal neurons in vivo and in vitro. (*A*) In dissociated cultures, postmitotic cortical neurons display specific transitions as classically described for hippocampal neurons by Dotti and Banker (1988). At stage 1, immature postmitotic neurons display intense lamellipodial and filopodial protrusive activity, which leads to the emergence of multiple immature neurites, stage 2. Stage 3 represents a critical step when neuronal symmetry breaks and a single neurite grows rapidly to become the axon (purple), whereas other neurites acquire dendritic identity. Stage 4 is characterized by rapid axon and dendritic outgrowth. Finally, stage 5 neurons are terminally differentiated pyramidal neurons harboring dendritic spines and the AIS. (*B*) The axon–dendrite polarity of pyramidal neurons is derived from the polarized emergence of the trailing (TP) and leading processes (LP), respectively. In vivo, pyramidal neurons acquire other key features of their terminal polarity, such as the axon initiation segment (AIS; yellow cartridge) and dendritic spines (gray protrusions) during the first postnatal weeks of development.

Initiating and Growing an Axon

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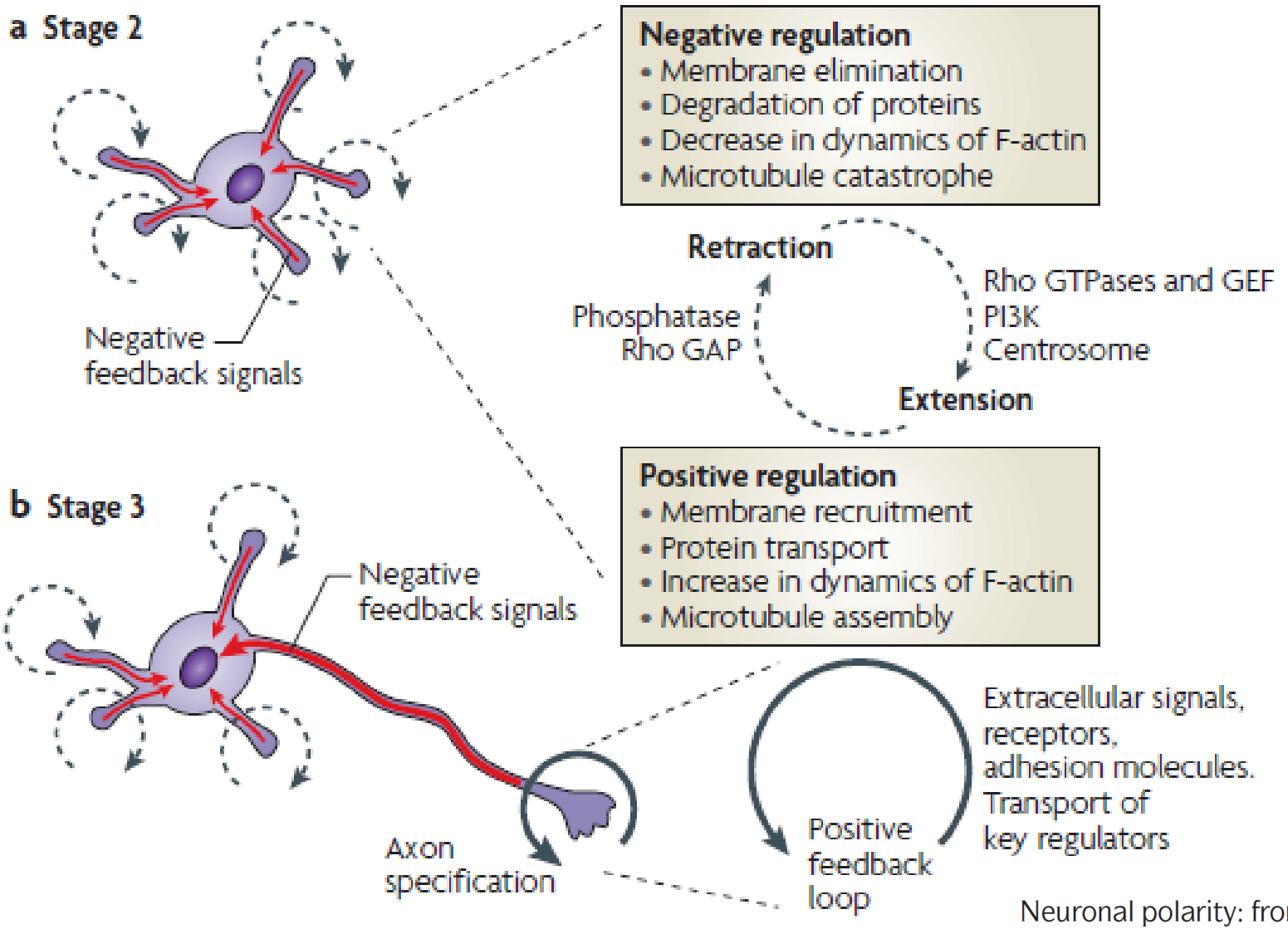
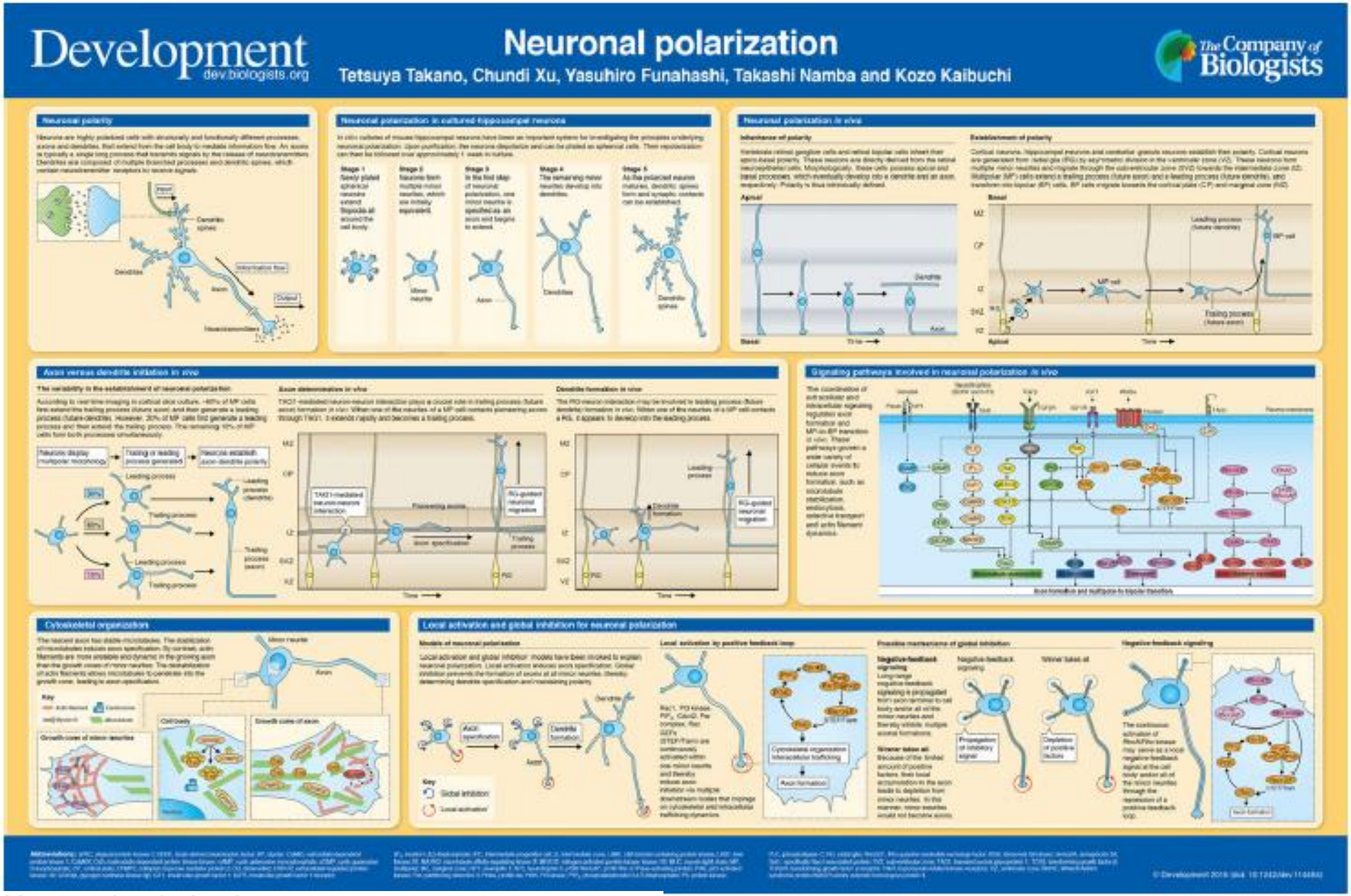
Neuronal polarization



Neuronal polarity: from extracellular signals to intracellular mechanisms

Nariko Arimura and Kozo Kaibuchi
NATURE REVIEWS | NEUROSCIENCE
VOLUME 8 | MARCH 2007 |

Neuronal polarization



Neuronal polarization

Tetsuya Takano, Chundi Xu, Yasuhiro Funahashi, Takashi Namba and Kozo Kaibuchi*

Development (2015) 142, 2088-2093 doi:10.1242/dev.114454

Initial axon specification (in vitro)

Figure 2 | **A tentative model for axon specification in neuronal polarization.** **a** | During stage 2, immature neurites extend and retract randomly to maintain their overall length. Neurite extension is driven by four main steps: an increase in the amount of plasma membrane by vesicle recruitment and fusion; the local concentration and activation of signalling molecules (such as Rho GTPase and phosphatidylinositol 3-kinase (PI3K)), an increase in actin dynamics; and an increase in microtubule formation. After extension, some signalling molecules (such as GTPase-activating proteins and phosphatases) counteract the positive regulation, induce microtubule catastrophe, decrease actin dynamics and decrease the amount of plasma membrane by endocytosis and by preventing vesicle fusion. **b** | When the balance between positive and negative signals is upset (in the transition from stage 2 to stage 3) by extracellular signals, (auto-) activation of receptors or adhesion molecules and by the recruitment of signalling molecules, one neurite elongates rapidly. Continuous elongation is supported by a positive feedback loop and sustains the activation cycle. The inhibitory signals that mutually antagonize neurite extension (negative feedback signals; red arrows in **a** and **b**) are progressively generated at the growing axon more than at the other neurites (a thicker red arrow in **b**), and interfere with their specification into axons. F-actin, filamentous actin; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor.

Neuronal polarity: from extracellular signals to intracellular mechanisms

Nariko Arimura and Kozo Kaibuchi

NATURE REVIEWS | NEUROSCIENCE

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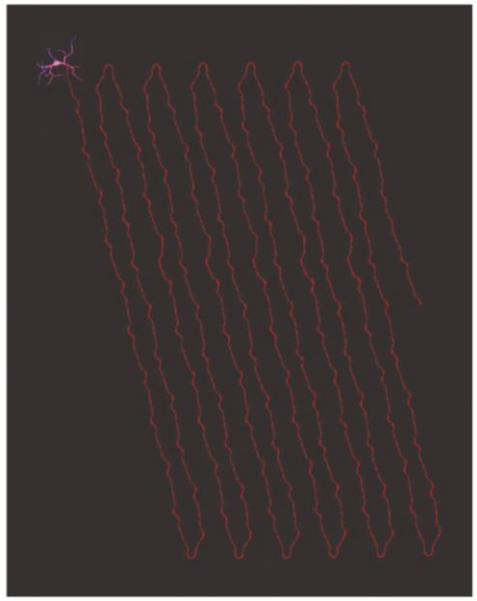
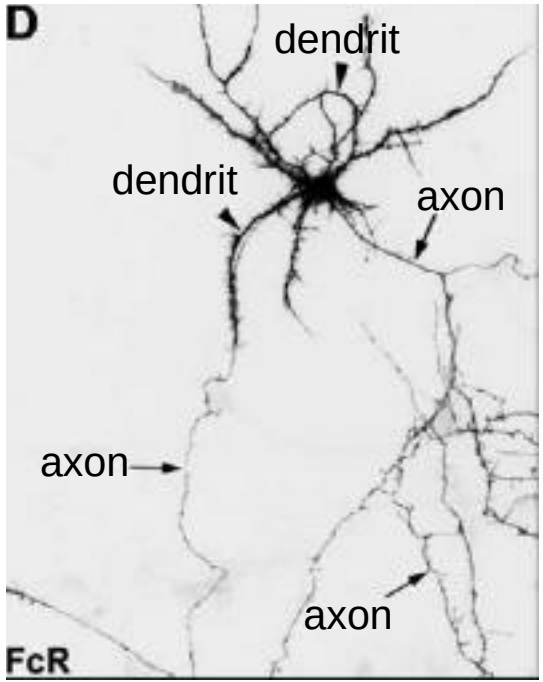
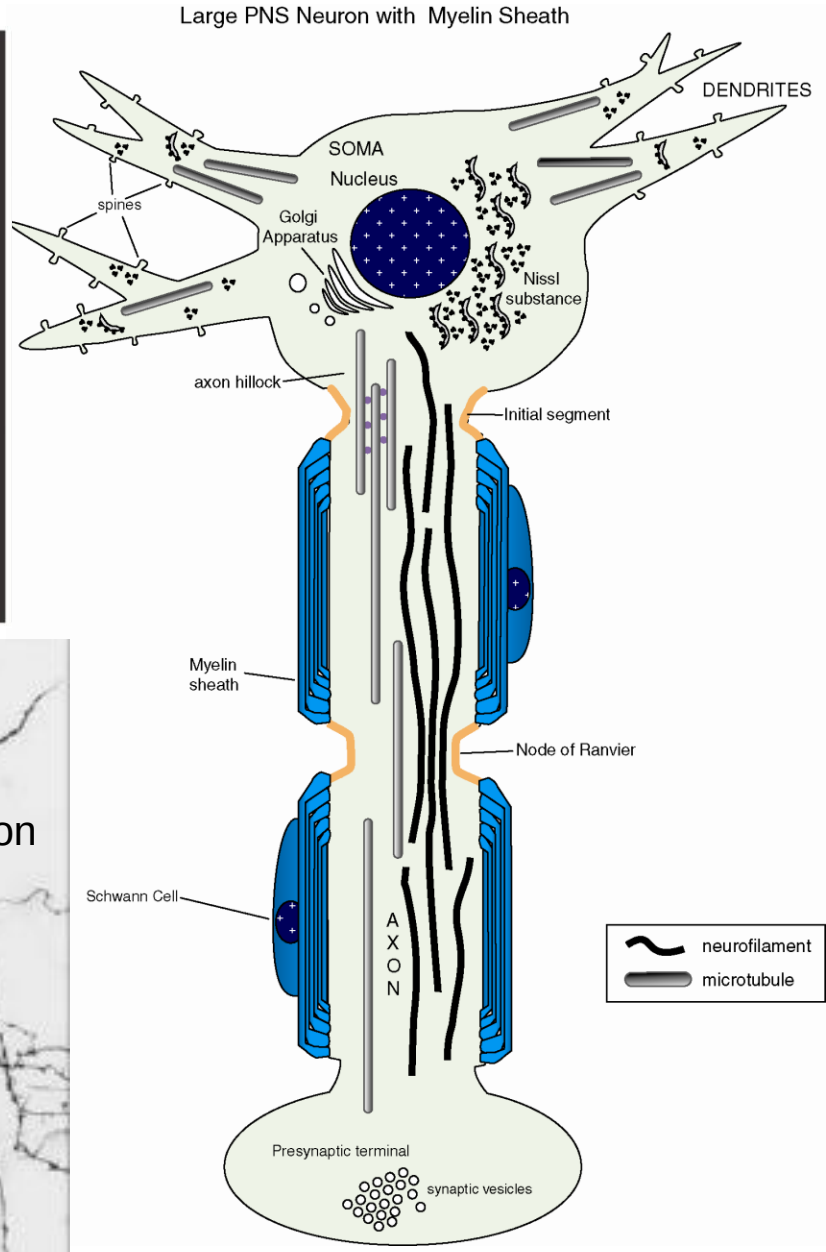
General features of axons and dendrites

- number, appearence
- membrane structure
- length, morphology, branching
- myelin
- protein synthesis, organelles
- endosomes
- cytoskeleton, MAPs
- synaptic features
- electrical activity

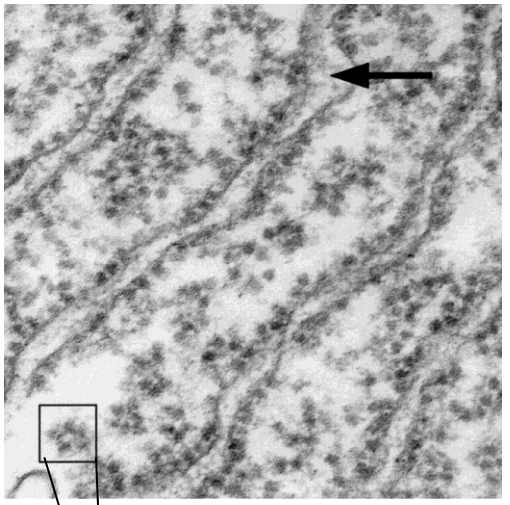






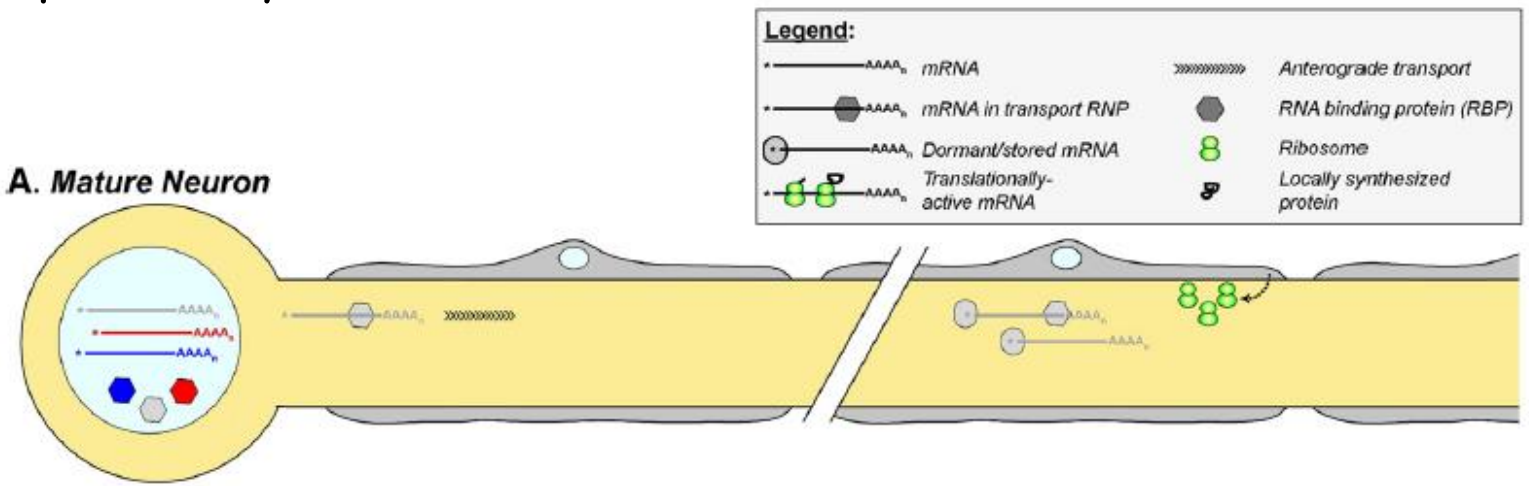
Neuronal protein synthesis and sorting

- Nissl granules: within the perikaryon and proximal dendrites



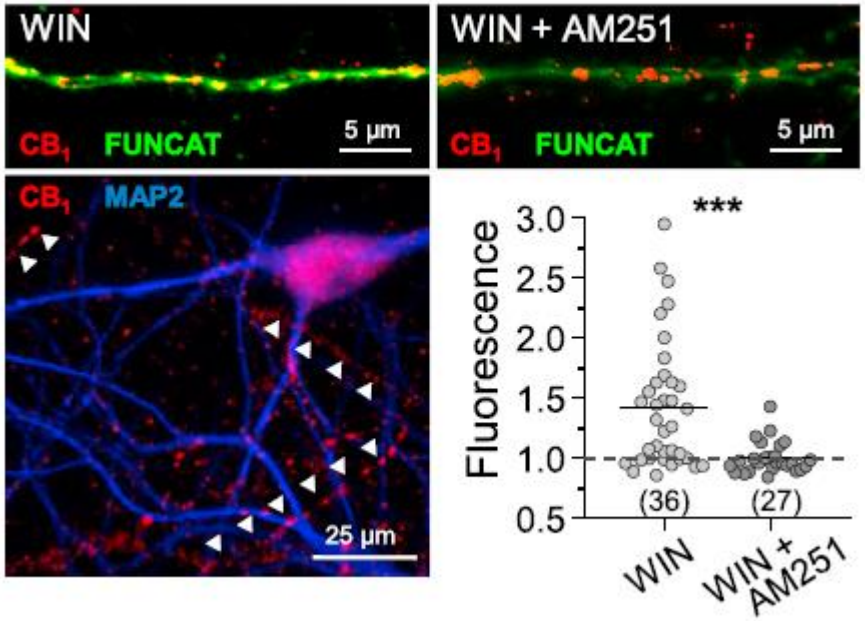
free ribosomes (rosetta)

- cytoplasmic, membrane-associated proteins:
  - synthesis on free ribosomes or bound polysomes
    - somatodendritic compartments, rarely in the axon (mainly during development)
  - directly / indirectly attached to the cytoskeleton
  - axon: mainly in resting state (RNA granulum) – activation upon injury -> local protein synthesis



Neuronal protein synthesis and sorting

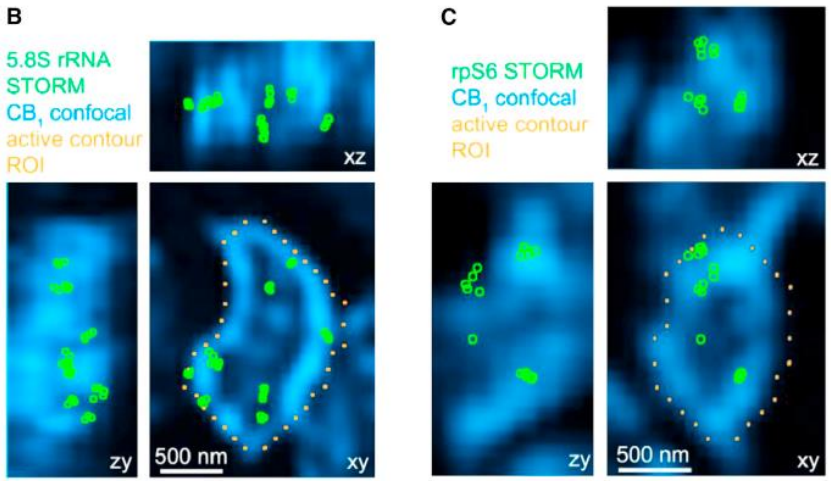
- new results: presynaptic protein synthesis is required for long-term presynaptic plasticity



FUNCAT: fluorescent non-canonical amino acid tagging

- no *de novo* protein synthesis during the fast presynaptic events, but it is required for long-term effects

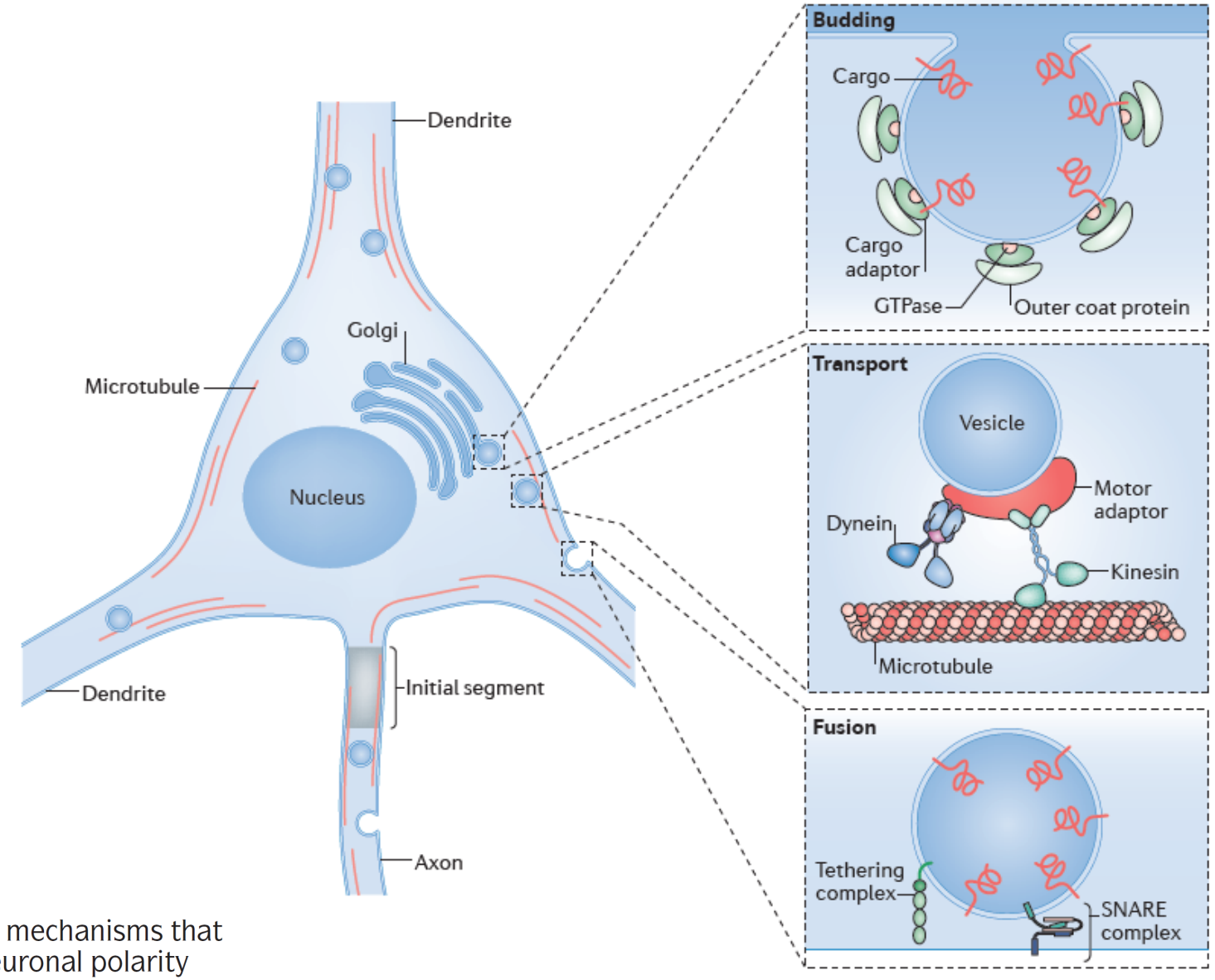
- ribosomes can be detected by STORM superresolution within the presynaptic terminals



Presynaptic Protein Synthesis Is Required for Long-Term Plasticity of GABA Release

Younts et al., 2016, Neuron 92, 479–492
October 19, 2016 © 2016 Elsevier Inc.
http://dx.doi.org/10.1016/j.neuron.2016.09.040

Neuronal membrane trafficking



The cellular mechanisms that maintain neuronal polarity

Marvin Bentley and Gary Banker

NATURE REVIEWS | NEUROSCIENCE

Neuronal membrane trafficking

Figure 1 | **Neuronal membrane trafficking.** Neurons are composed of two regions: the somatodendritic domain and the axon. The axon initial segment (grey) marks the boundary between these domains. Axonal and dendritic membrane proteins are synthesized in the rough endoplasmic reticulum (not shown) and undergo post-translational modification in the Golgi complex. These organelles are restricted to the cell body and dendrites. The delivery of proteins from the Golgi complex to the plasma membrane involves budding, transport and fusion events; each of these processes is orchestrated by a complex set of trafficking proteins. Vesicle budding is initiated by the recruitment of a small GTPase from the cytosol to the membrane. In turn, this GTPase binds to and recruits cargo adaptors, which recognize transmembrane 'cargo' proteins containing sorting motifs and concentrate them in the forming bud. Cargo adaptors also recruit additional coat proteins from the cytosol that drive membrane curvature and fission. Long-range vesicle transport is mediated by kinesins and dyneins, which translocate along microtubules. Motors are bound to vesicles by adaptors that may also regulate their activity. Fusion is initiated by tethering factors that form a complex, which links the vesicle to its target membrane. Subsequent formation of a SNARE (*N*-ethylmaleimide-sensitive factor attachment protein receptor) complex drives fusion of the lipid bilayers.

The cellular mechanisms that maintain neuronal polarity

Marvin Bentley and Gary Banker

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Polarized membrane trafficking

kymographs

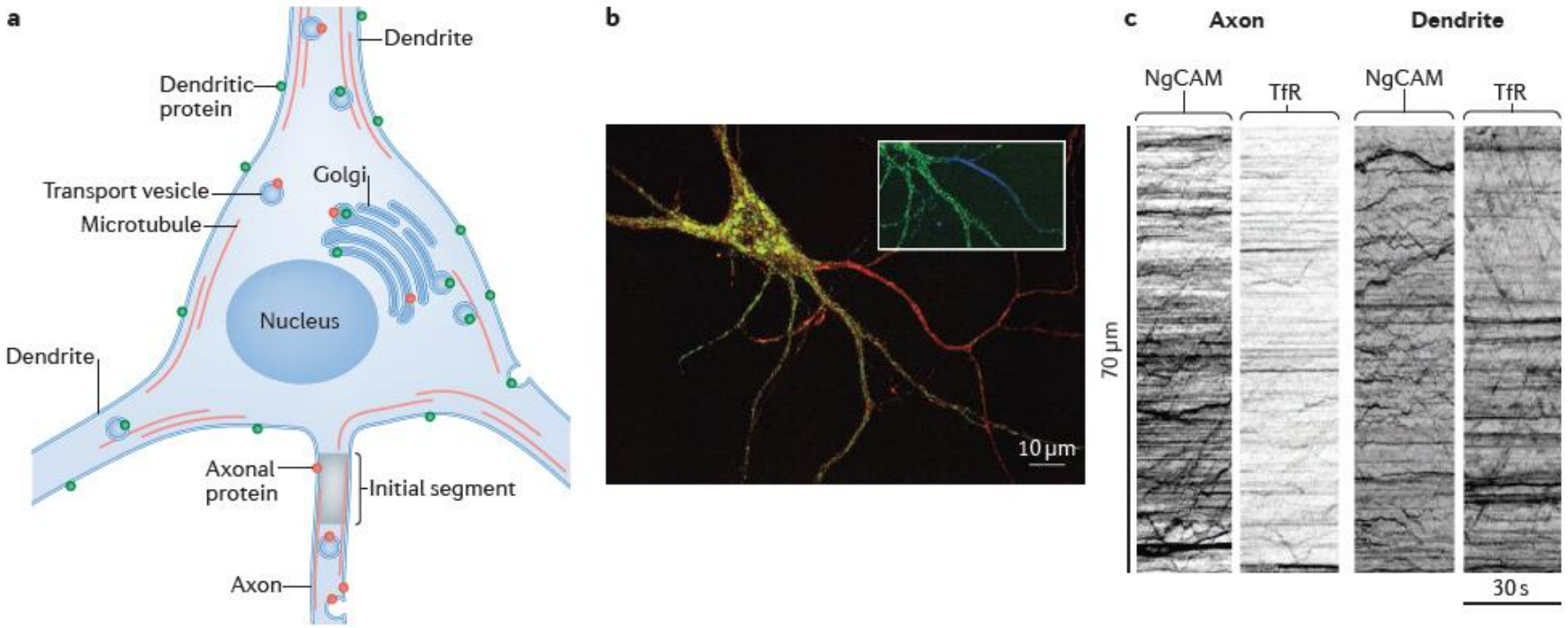
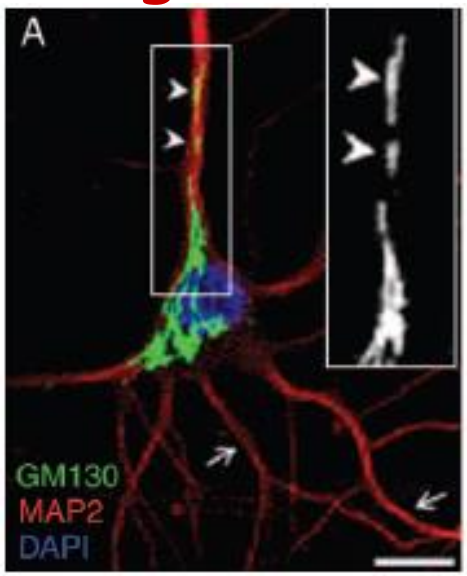


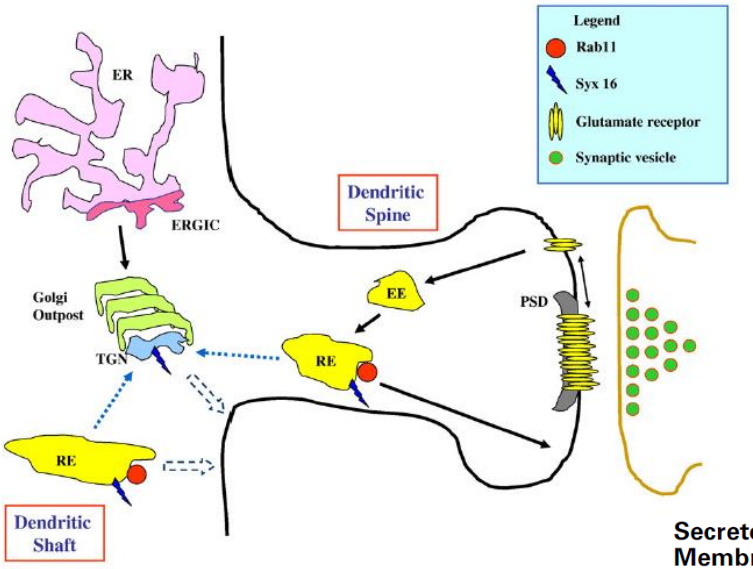
Figure 2 | **The trafficking of polarized proteins in neurons. a** | A drawing of a neuron illustrating the different trafficking patterns of axonal and dendritic proteins. As proteins exit the Golgi complex, dendritic proteins (green) and axonal proteins (red) are sorted into different vesicles, which undergo microtubule-based transport to reach the axon and dendrites. Vesicles containing dendritic proteins are transported bidirectionally in dendrites but do not enter the axon. Vesicles containing axonal proteins are also transported into dendrites, but their transport is biased towards the axon. **b** | Still images from a movie of a living cultured hippocampal neuron expressing an axonal membrane protein, NgCAM (red) and a dendritic membrane protein (green). The inset shows the initial segment, visualized with antibodies against neurofascin (blue). **c** | Kymographs from the cell shown in part **b** compare the movement of vesicles containing the axonal protein NgCAM and vesicles containing the dendritic protein TfR. NgCAM-containing vesicles move in the axon and the dendrite, whereas TfR-containing vesicles move in the dendrite and do not enter the axon. Kymographs plot the maximum intensity at each position along the axon (*y* axis) as a function of time (*x* axis). Moving vesicles are represented by the diagonal lines; lines with positive slope represent vesicle movement away from the cell body; lines with negative slope represent movement towards the cell body. Contrast was inverted in kymographs so that bright vesicles appear dark.

https://images.nature.com/full/nature-assets/nrn/journal/v17/n10/extref/nrn.2016.100-sv1.mov

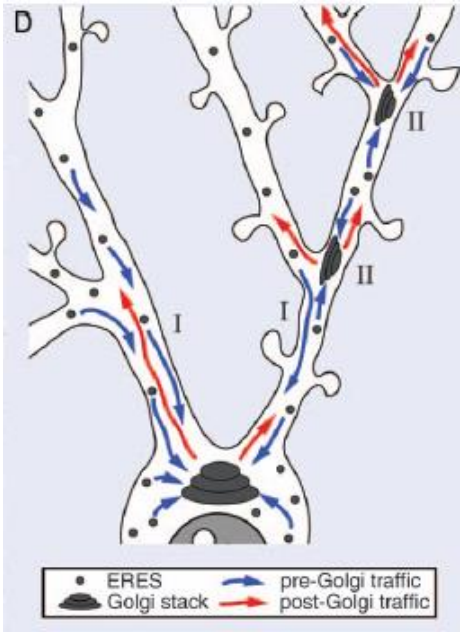
Neuronal protein synthesis and sorting

- transport of transmembrane proteins in the dendrites:
  - ER, Golgi: also within the dendrites
    - continuous ER-network
    - Golgi outposts
  - bidirectional pre-Golgi transport
  - „basic" secretion: somatic ER -> Golgi, long path towards the plasma membrane
  - local protein synthesis within the dendrites:
    - specific cargo (eg. BDNF)
    - dendritic secretion / release
    - spine and PSD development





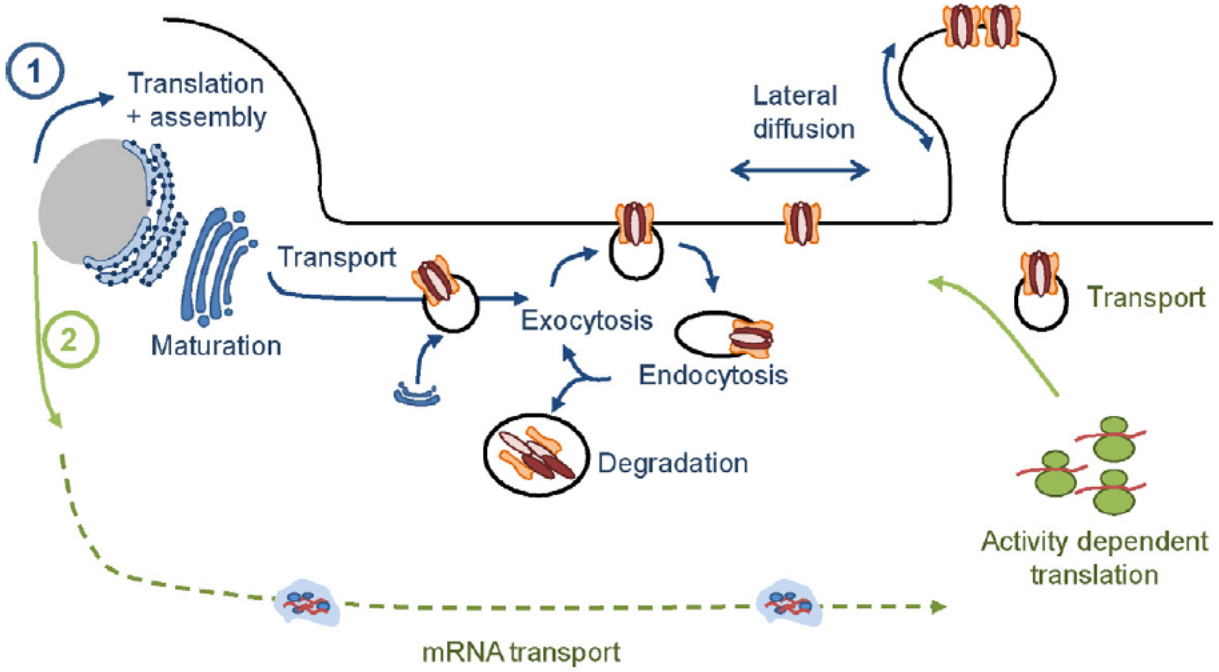
Emerging aspects of membrane traffic in neuronal dendrite growth; Tang 2008



Secretory Outposts for the Local Processing of Membrane Cargo in Neuronal Dendrites

Neuronal protein synthesis and sorting

- transport of transmembrane proteins in the dendrites:
  - AMPARs are transported in vesicles from the Golgi, but can be locally synthetised upon activation within the dendrites



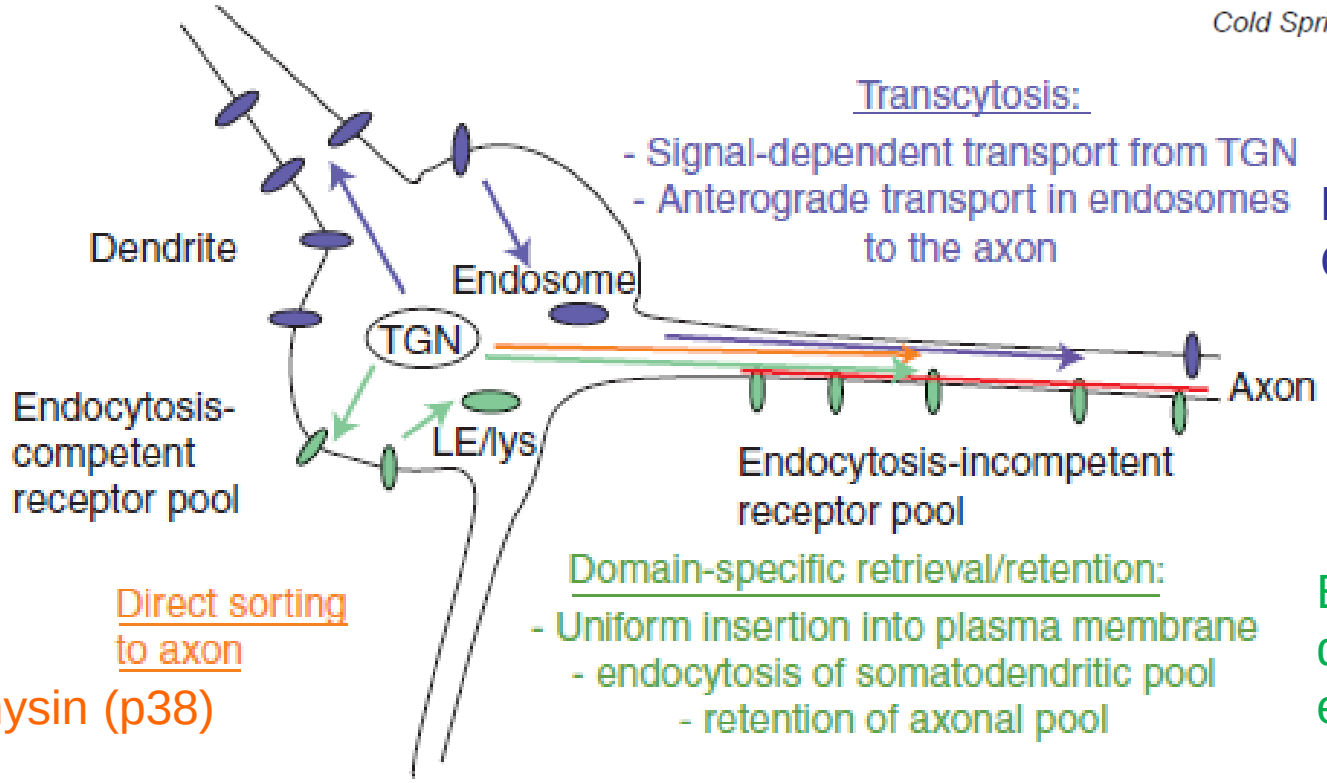
Brain Research Bulletin 93 (2013) 57–68

Fig. 2. General mechanisms of AMPAR sorting and trafficking.

Biophysical mechanisms regulating AMPA receptor accumulation at synapses
Katalin Czöndör[a,b], Olivier Thoumine[a,b,*]

Pathways of axonal transmembrane protein transport

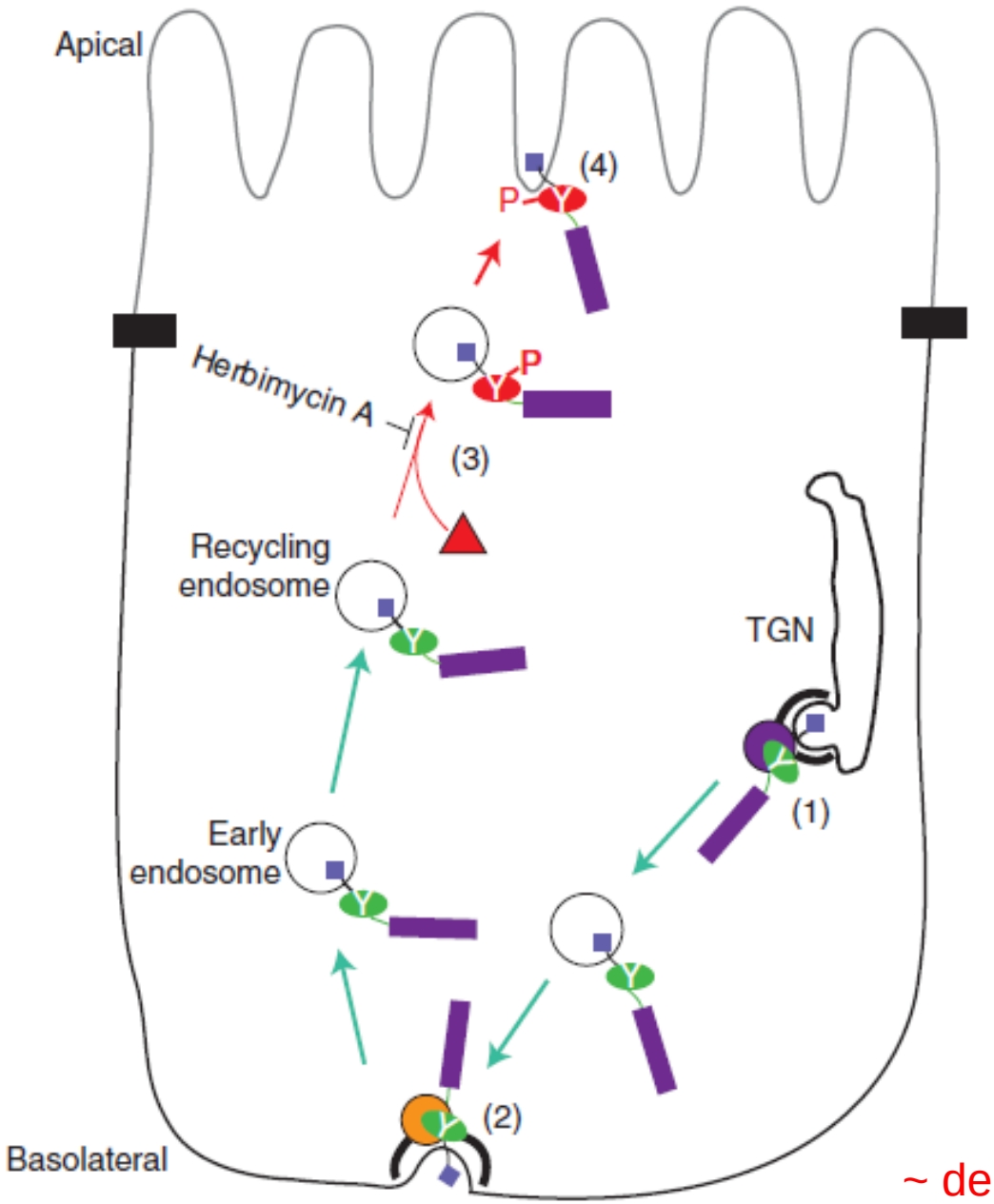
Trafficking Guidance Receptors
Bettina Winckler[1] and Ira Mellman[2]
Cold Spring Harb Perspect Biol 2010;



1. axonally directed, polarised sorting from the TGN + selective binding to axon-specific delivery motors
2. indirect, polarised transport (transcytosis): endocytosis in dendrites, followed by selective anterograde axon-directed transport
3. non-polarised transport + selective retrieval / retention

Transcytosis

~ axon



~ dendrite

Trafficking Guidance Receptors
Bettina Winckler[1] and Ira Mellman[2]
Cold Spring Harb Perspect Biol 2010;

L1/NgCAM

Lumenal domain

Tyrosine-based motiff (YRSLE)

active

Inactive

Transmembrane domain

Glycine-rich region

Ankyrin-binding domain

Clathrin

AP-1B bazolaterális adaptor

AP-2 klatrin adaptor

c-src kináz

Transcytosis

Trafficking Guidance Receptors

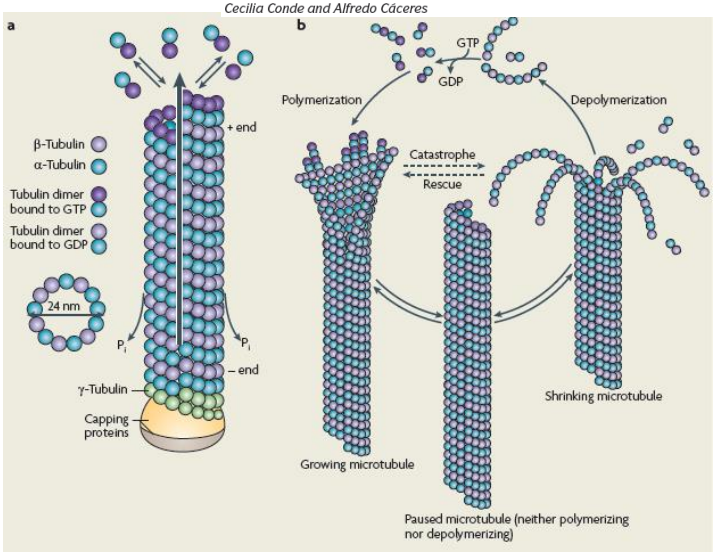
Bettina Winckler[1] and Ira Mellman[2]

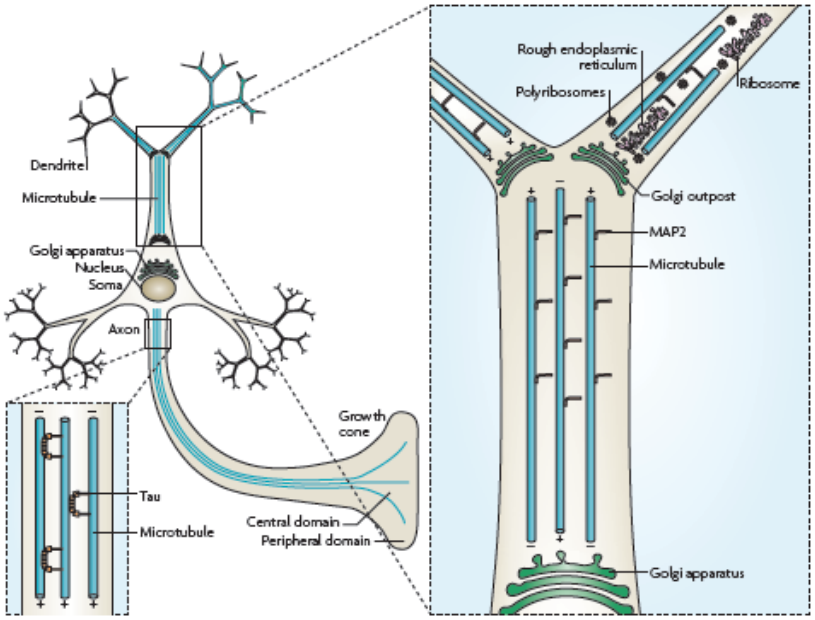
Cold Spring Harb Perspect Biol 2010;

Figure 2. Regulation of NgCAM transcytotic routing by spatial regulation of phosphorylation of the adaptor binding site (based on Anderson et al. 2005). NgCAM is sorted to the basolateral domain based on a tyrosine-based motif (YRSLE) in its cytoplasmic tail. This signal is recognized by the basolateral sorting adaptor AP1B. The site of action of AP1B-based sorting is either the TGN or the recycling endosome. NgCAM is then exocytosed on the basolateral plasma membrane. The clathrin adaptor AP2 binds to the YRSLE motif and mediates endocytosis into endosomes. At some point after endocytosis, the YRSLE is phosphorylated by a src family kinase. This phosphorylation event prevents binding of AP1B and thereby redirects the endocytosed NgCAM away from basolateral recycling into an apical-directed transcytotic route. In the presence of the kinase inhibitor herbimycin A, NgCAM accumulates on the basolateral, instead of the apical, domain. An analogous mechanism likely operates for NgCAM in neurons.

Neuronal cytoskeleton

- microtubules (MTs)
  - >10% of total brain protein; 25 nm diameter, > 100 mm length
  - a, b tubulin monomers, „dynamic stability"
  - intracellular transport, inner stabilisator of neurites: dynamic / stabile MTs
    - nucleation: g-tubulin (centrosome); cleavage (katanin, spastin)
    - +TIPs (*+end tracking proteins*): APC, DCX - axon
  - posttranlational modifications (Tyr, Ac, P_i)
  - bundled structure (mainly in axons)
    - axon, dist. dendrite: + end distally
    - prox. dendrite: + / - ends mixed
  - diverse, specific MAPs

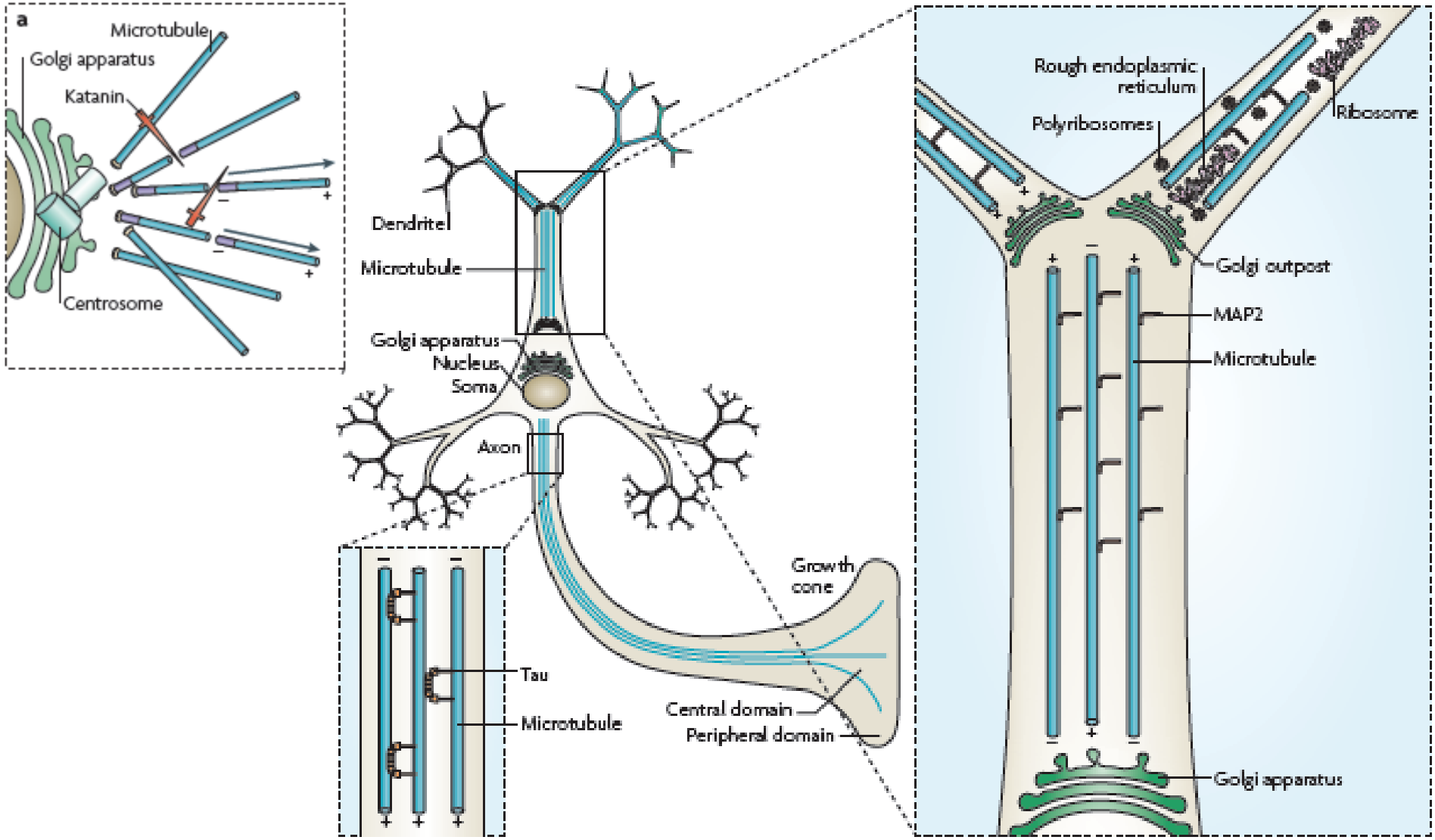




Microtubule assembly, organization and dynamics in axons and dendrites

Cecilia Conde and Alfredo Cáceres

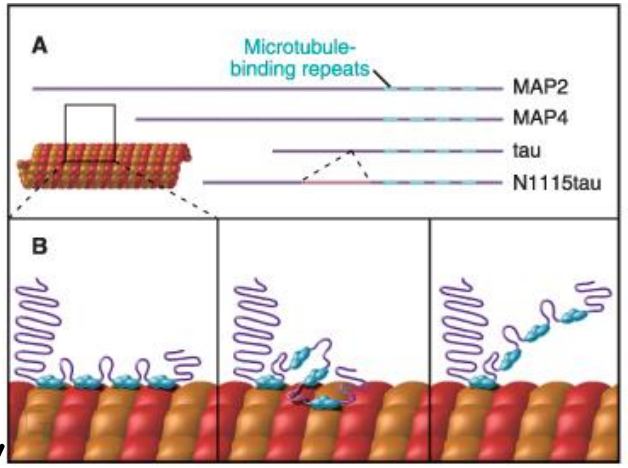
Neuronal MTs



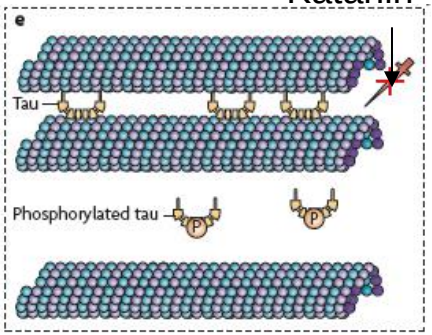
Microtubule organization and organelle distribution in axons and dendrites. Axons have tau-bound microtubules of uniform orientation, whereas dendrites have microtubule-associated protein 2 (MAP2)-bound microtubules of mixed orientation. Dendrites also contain organelles that are not found in axons, such as rough endoplasmic reticulum, polyribosomes and Golgi outposts.

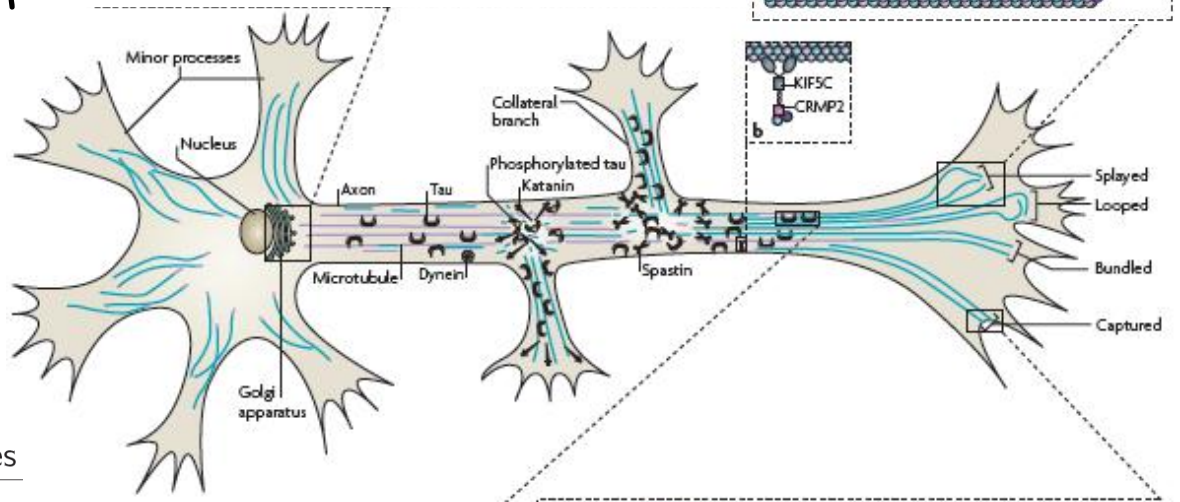
Neuronal cytoskeleton

- microtubulus associated proteins (MAPs)
  - HMW MAPs (>1300 KDa)
    - MAP1: axon, dendrite; MAP2: dendrite
    - sidebranches attached to microfilaments
    - morphology – regulates neuronal plasticity
  - tau
    - [neurofibrillary tangles]
    - MT stabilisation – P_i inactivates
    - dephosphorylated in the distal axon (spec. marker)



Katanin





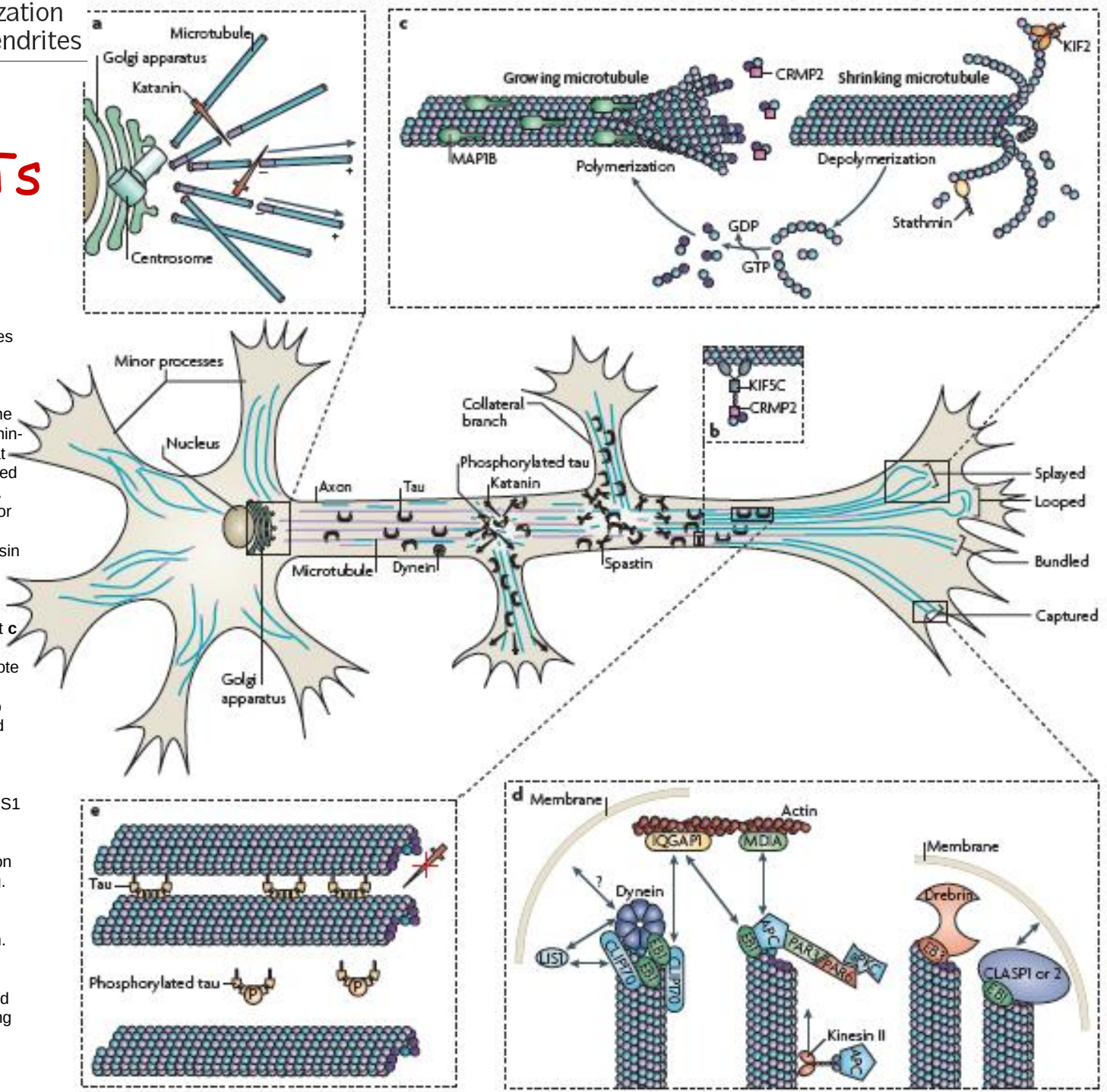
Microtubule assembly, organization and dynamics in axons and dendrites

Cecilia Conde and Alfredo Cáceres

Microtubule assembly, organization and dynamics in axons and dendrites

Cecilia Conde and Alfredo Cáceres

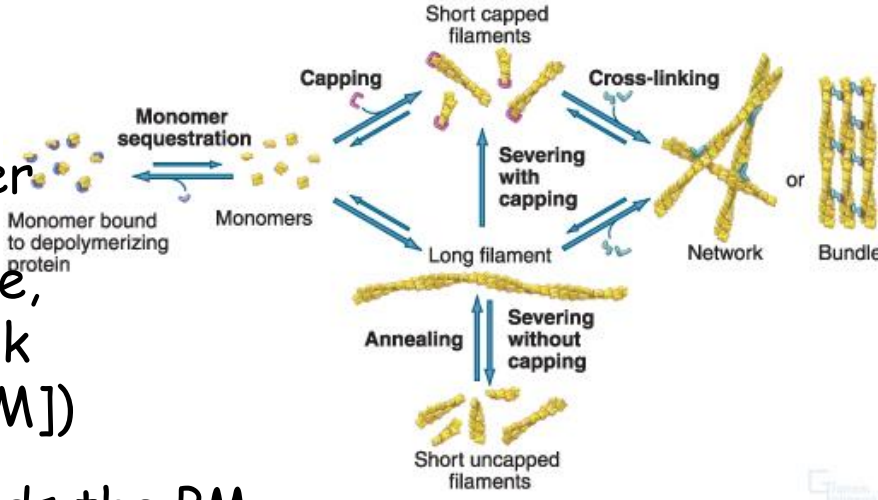
Neuronal MTs



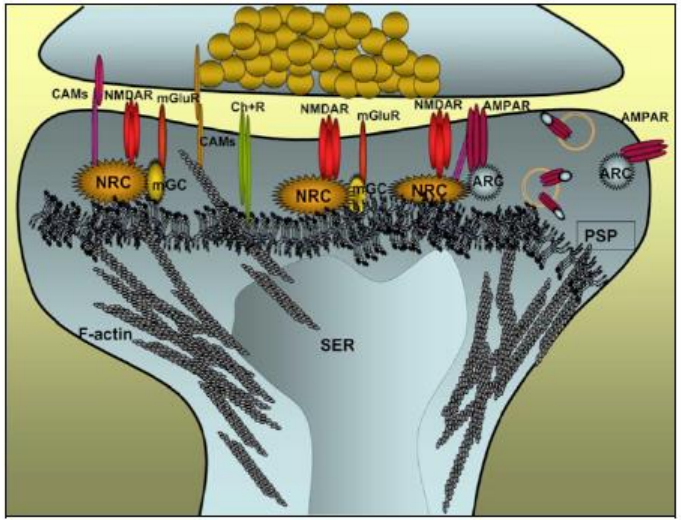
Microtubule organization in developing axons. The organization and regulation of microtubules in a stage 3 hippocampal pyramidal neuron. Dynamic (blue) microtubules predominate in minor processes (short, unbranched neurites) and at the distal end of the axon and collateral branches, whereas stable (purple) microtubules are enriched in the proximal part of the axon. Inset **a** shows katanin-mediated release of microtubules nucleated at the centrosome. Short polymers are transported along microtubules by motors such as dynein. Inset **b** shows the transport of tubulin dimers or oligomers to the growth cone by a complex of kinesin family member 5C (KIF5C) and collpasin response mediator protein 2 (CRMP2; also known as DPYSL2). On entering the axonal growth cone microtubules splay, bend, loop, bundle or get captured at the cell cortex. Inset **c** illustrates the dynamic behaviour of splayed microtubules. Proteins such as CRMP2 promote microtubule assembly, whereas microtubule-associated protein 1B (MAP1B) contributes to the maintenance of microtubule dynamics and KIF2 and stathmin induce microtubule depolymerization. Inset **d** shows the protein machinery that is involved in microtubule capture (see also Supplementary information S1 (table)). Inset **e** shows how tau protects microtubules from katanin-induced severing, thereby contributing to microtubule stabilization and preventing excessive collateral branching. In the axon shaft, the formation of collateral branches is regulated by the action of microtubule severing proteins such as spastin. APC, adenomatous polyposis coli; aPKC, atypical protein kinase C (also known as PRKCI); CLASP, cytoplasmic linker associated protein; CLIP170, CAP-GLY domain containing linker protein 170 (also known as CLIP1); EB, end-binding protein; IQGAP1, IQ motif containing GTPase activating protein 1.

Neuronal cytoskeleton

- microfilaments (actin)

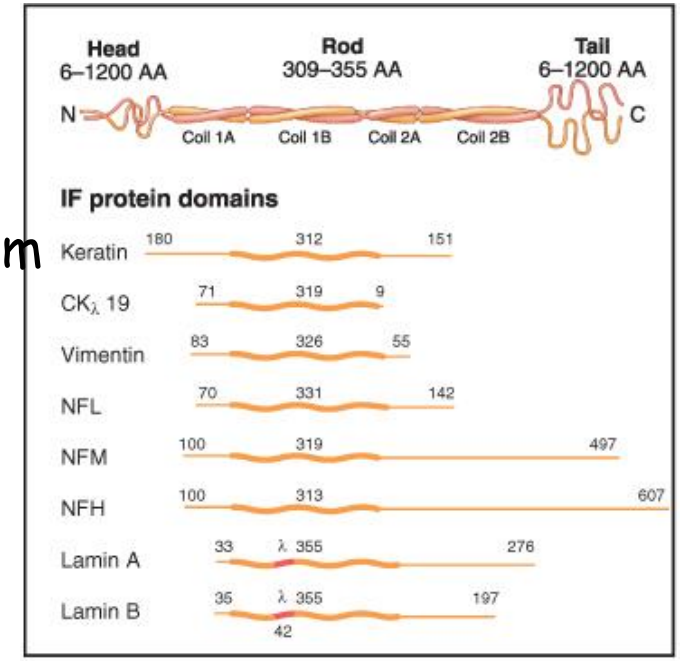


  - 400-800 nm length, 4-6 nm diameter
  - presynaptic terminal, dendritic spine, growth cone, cortical actin network (beneath the plasma membrane [PM])
  - pre/postsynapse: barbed end towards the PM
  - barbed-end capping (ezrin, radexin, moezin): Ranvier node
    - localisation; also to ECM components
  - indirect binding (spektrin, dystrophyin, a-actinin)
    - cortical actin network, PSD, receptors, ion channels
  - intensive, activity-dependent remodelling
  - no major differences between axonal / dendritic functions or structure



Neuronal cytoskeleton

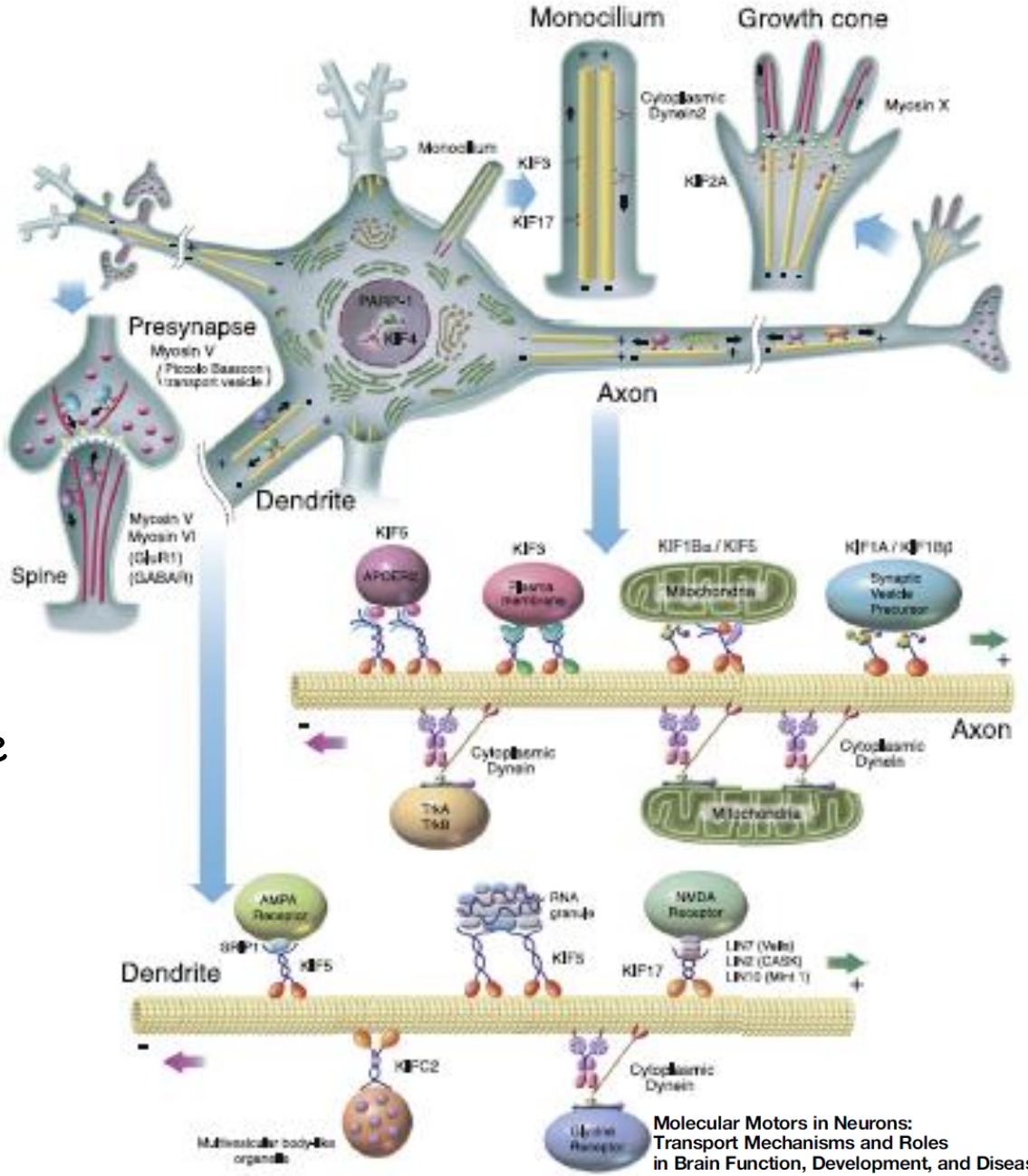
- intermedier filaments
  - few nm length, 8-12 nm diameter
  - lamin (nuclear membrane; type V) + cytoplasm
    - Type III: vimentin, GFAP
    - Type IV: neurofilament (NF), nestin
      - NFH ~180-200 KDa
      - NFM ~130-170 KDa
      - NFL ~60-70 KDa



  - stabile structure: providing cellular morphology
    - NF: side branches, highly phosphorylated (NFH, NFM mainly in axons)
    - spatially extended -> regulates axon diameter
  - mutations, injury: neuropathological changes

Neuronal motor proteins

- transport (cargo):
  - vesicule
  - multiprotein complex
  - organelles
  - RNA granules
  - cytoskeletal elements
- anterograde or retrograde
- along MTs or actin
- kinesin / dynein / myosin

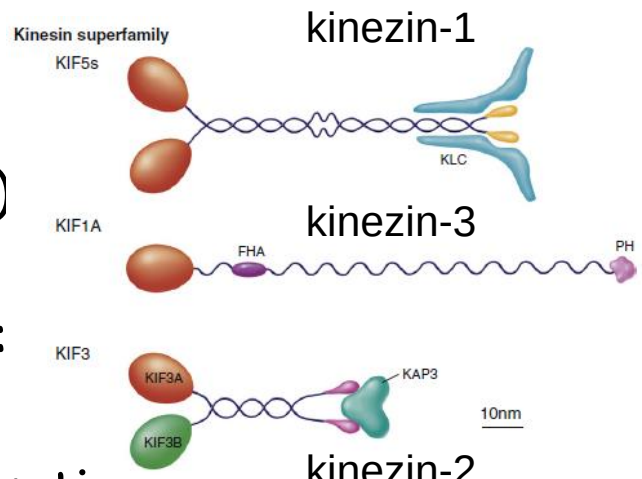


Molecular Motors in Neurons: Transport Mechanisms and Roles in Brain Function, Development, and Disease

Nobutaka Hirokawa,[1,*] Shinsuke Niwa,[1] and Yosuke Tanaka[1]

Neuronal motor proteins

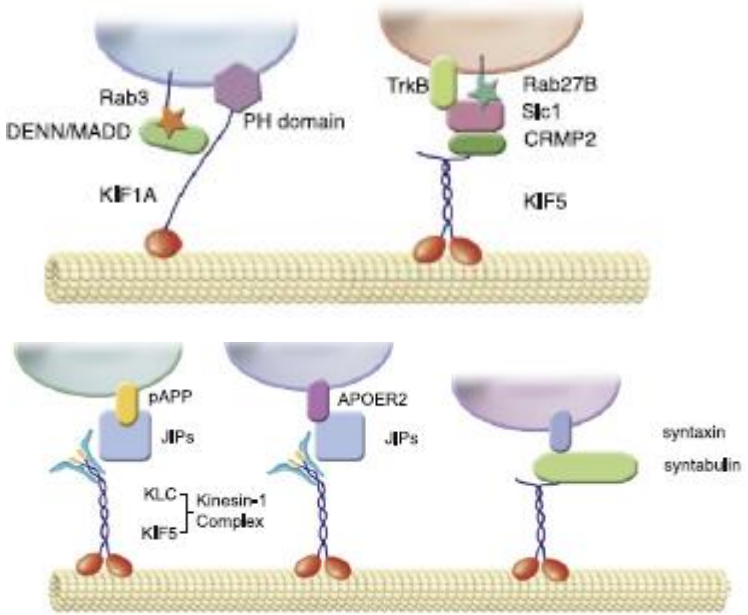
- kinesin superfamily (KIFs)
  - > 45 genes, 14 classes
    - N-KIFs (*N-term. motor domain*) mainly towards MT+ end
    - C-KIFs (*C-term. motor domain*): mainly towards MT- end
    - KIF2A, 2C: MT depolymerisation



- mainly indirect cargo binding: polarised transport
  - Rab GTPases

    synaptic vesicule precursor

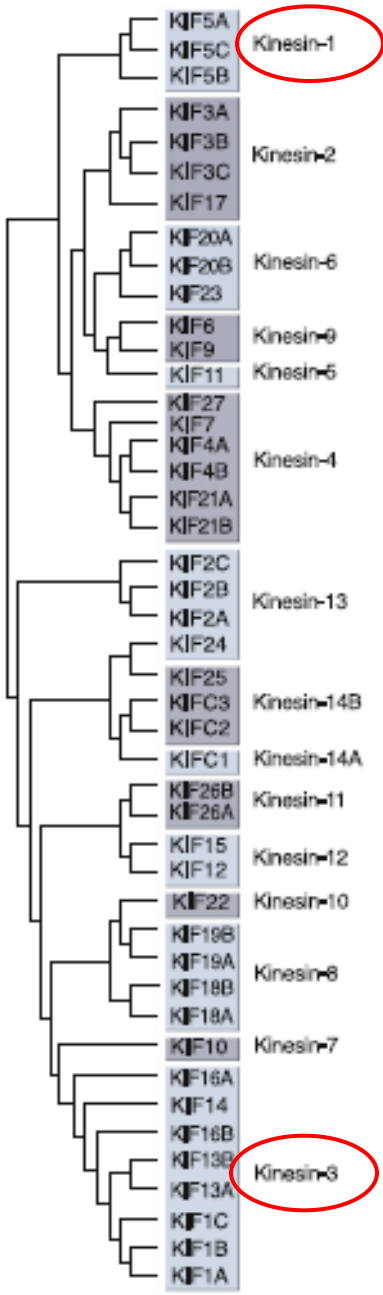
  - adaptor proteins



axon

Molecular Motors in Neurons: Transport Mechanisms and Roles in Brain Function, Development, and Disease

Nobutaka

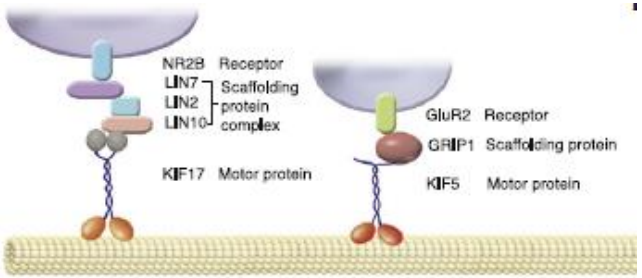


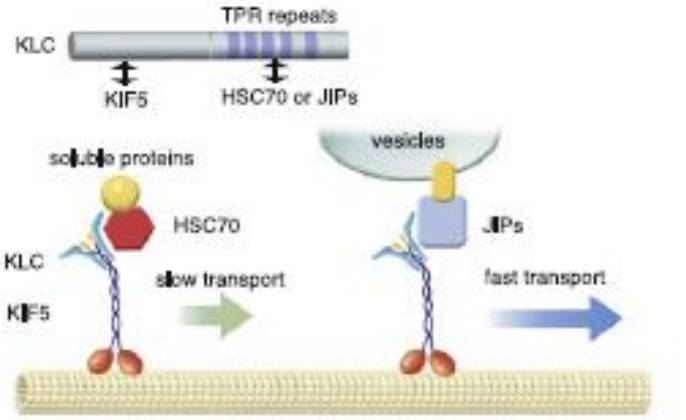
Neuronal motor proteins

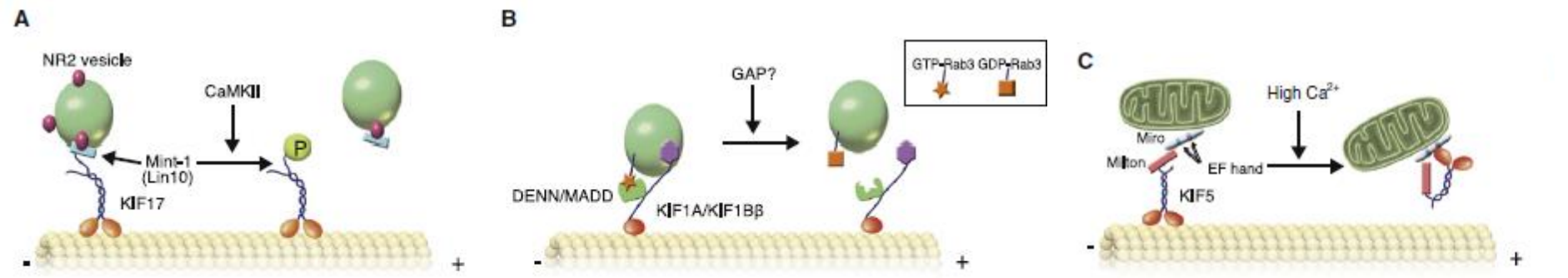
Molecular Motors in Neurons:
Transport Mechanisms and Roles
in Brain Function, Development, and Disease

Nobutaka Hirokawa,[1,*] Shinsuke Niwa,[1] and Yosuke Tanaka[1]

- kinesin superfamily (KIFs)
  - mainly indirect cargo binding: polarised transport (cont.)
    - scaffold proteins
  - interaction regulates the transport itself, as well
  - regulation of cargo binding (e.g.)
    - phosphorylation
    - Rab GTPases (GEFs, GAPs)
    - Ca^{2+} level.....







Kinesin superfamily

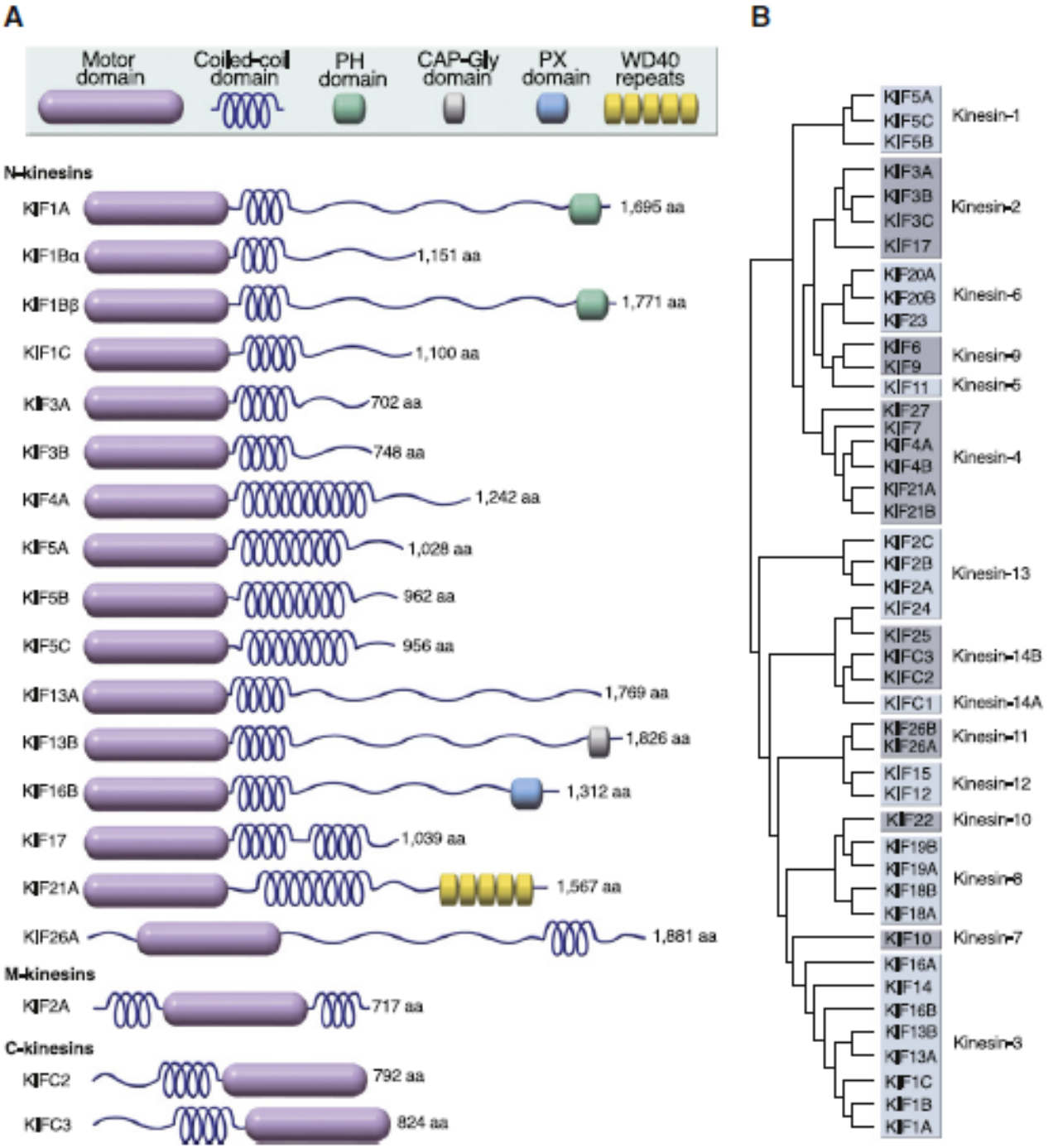


Figure 4. Diversity among KIFs
(A) Structure of KIFs.
(B) Phylogenetic tree of KIFs.

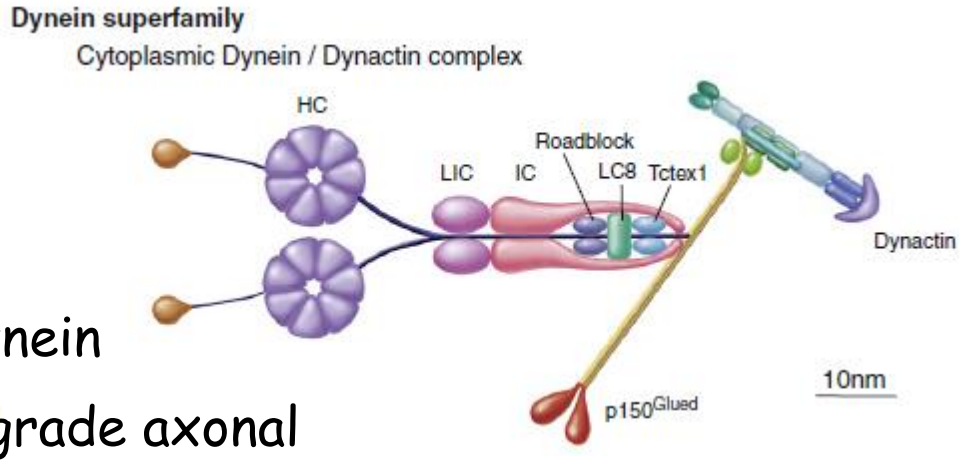
Molecular Motors in Neurons: Transport Mechanisms and Roles in Brain Function, Development, and Disease

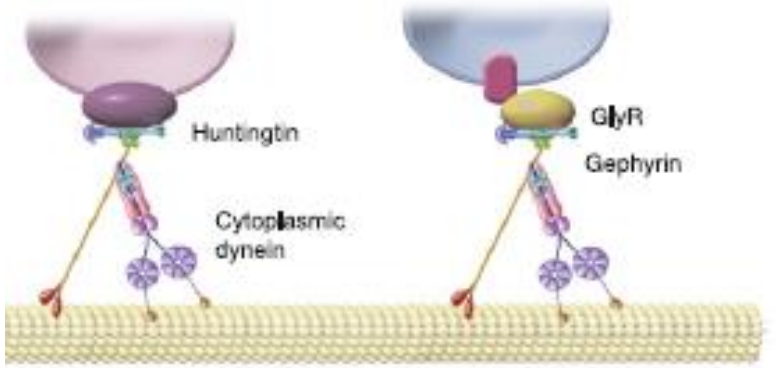
Nobutaka Hirokawa,[1,*] Shinsuke Niwa,[1] and Yosuke Tanaka[1]

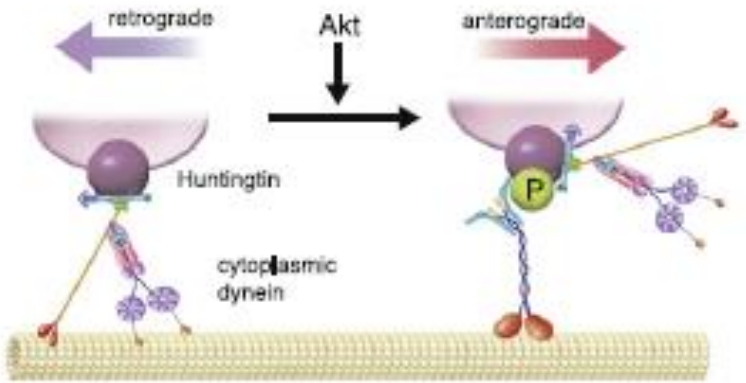
Neuron 68, November 18, 2010 ©2010

Neuronal motor proteins

- dynein superfamily
  - citoplasmic dynein
    - > 1,5 MDa protein complex
  - axonemal (ciliary / flagellar) dynein
  - mainly towards MT- end; retrograde axonal transport
  - cargo-binding via associated proteins (dynactin)
  - competition for cargo binding: eg, BDNF vesicle-transport depends on the phosphorylation of the adaptor

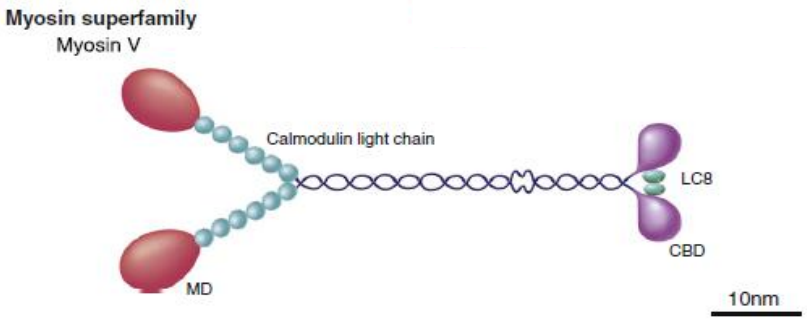




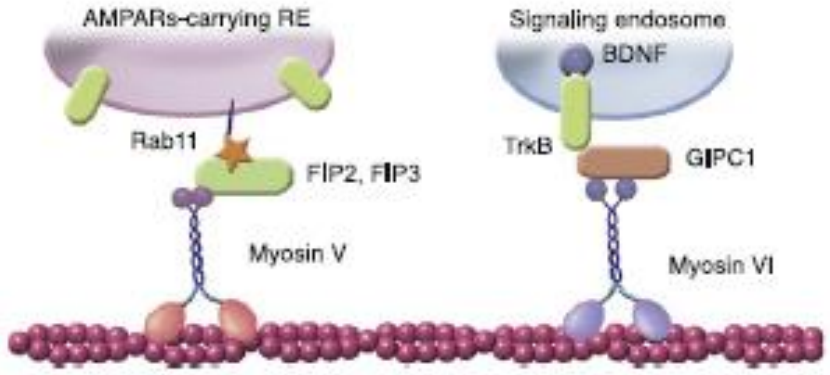


Neuronal motor proteins

- myosin superfamily
  - 18 classes; in neurons, myosin V (II, as well)
  - actin-dependent movement

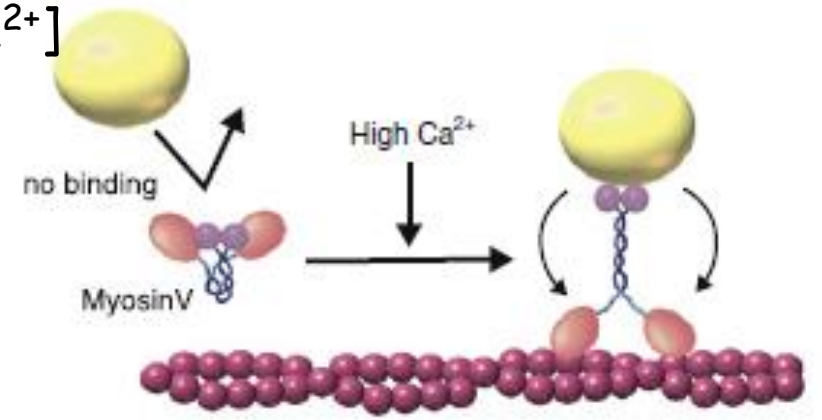


  - cargo-binding via associated proteins adaptors

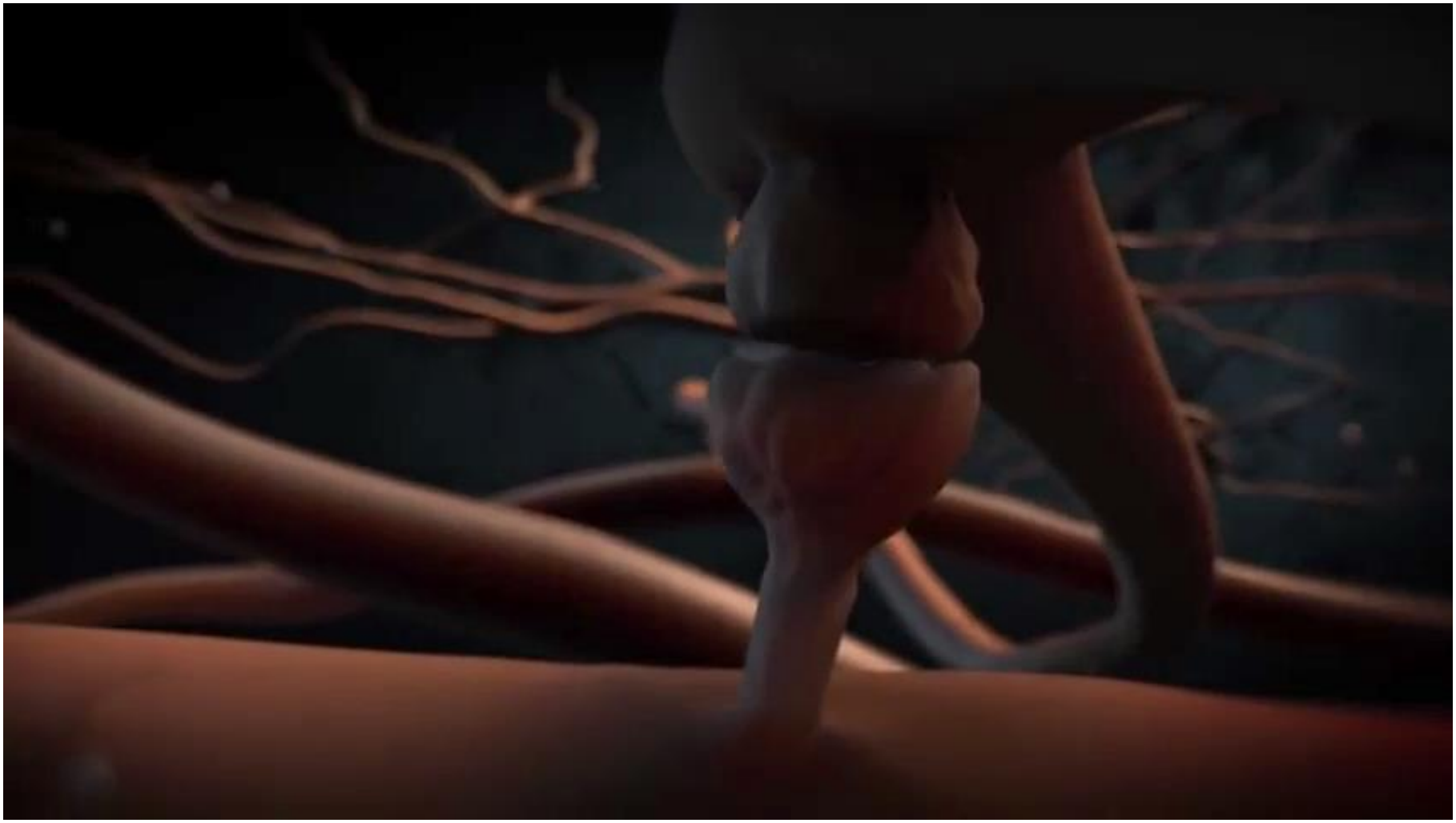


dendritic spine

  - cargo-binding normally depends on [Ca^{2+}]



Transport within the neurons

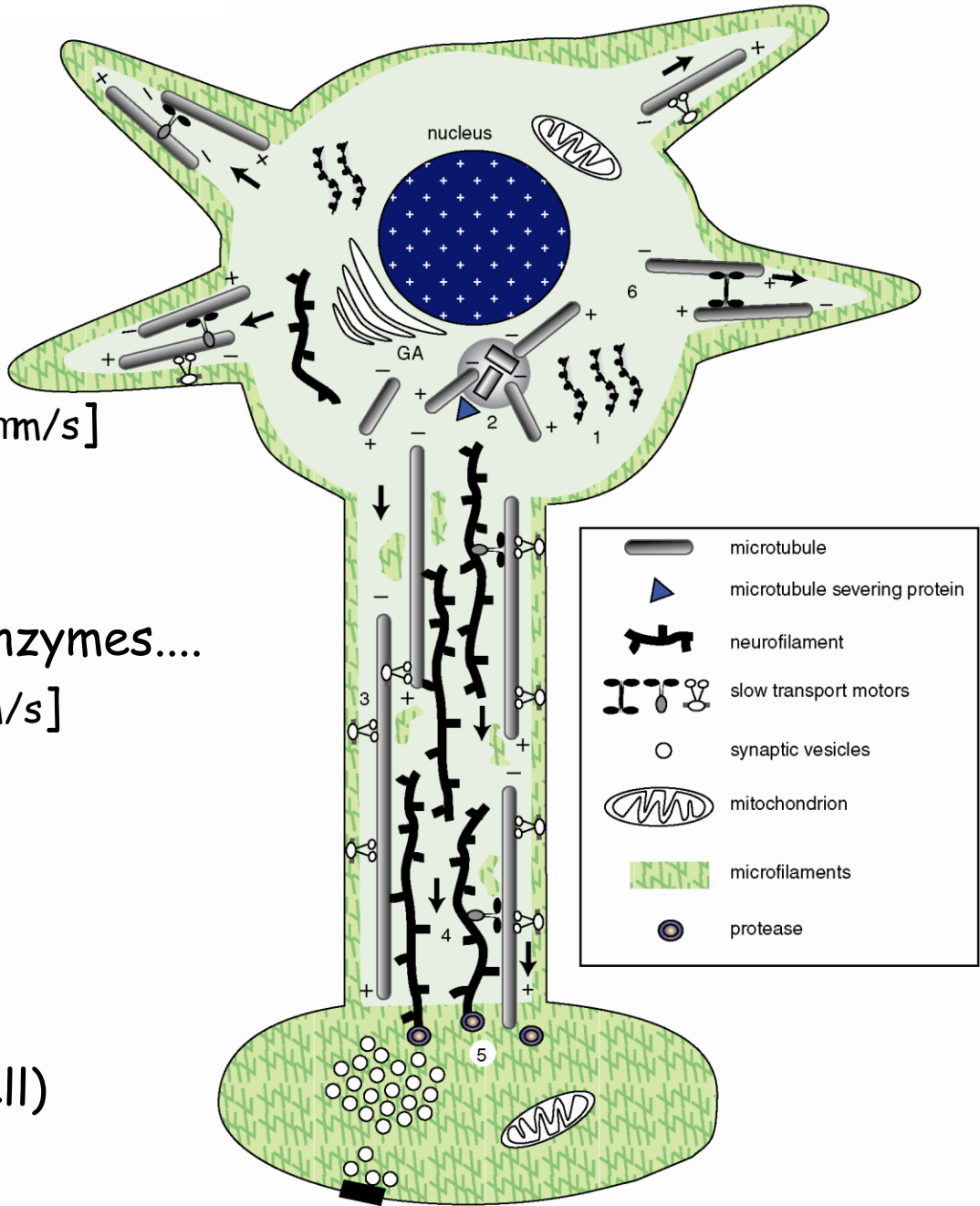


Transport inside the brain: The basic mechanisms of neuronal trafficking. Hoogenraad Lab

https://www.youtube.com/watch?v=RRfH4ixgJwg

Slow axonal transport

- directs axonal elongation, regeneration
- SCa (slow component a): cytoskeletal structures
  - NF, MT
  - 0.1 – 1 mm/day [0,001 – 0,01 mm/s]
- SCb (slow component b): cytoplasmic proteins
  - actin, tubulin monomers, enzymes....
  - 2 – 4 mm/day [0,02 – 0,04 mm/s]
- normally stop-and-go movement
- uni-directed, anterograde
- KIF5: Hsc-70 adaptor
  myosin Va: NF-L
  dynein: MT (can bind NFs, as well)

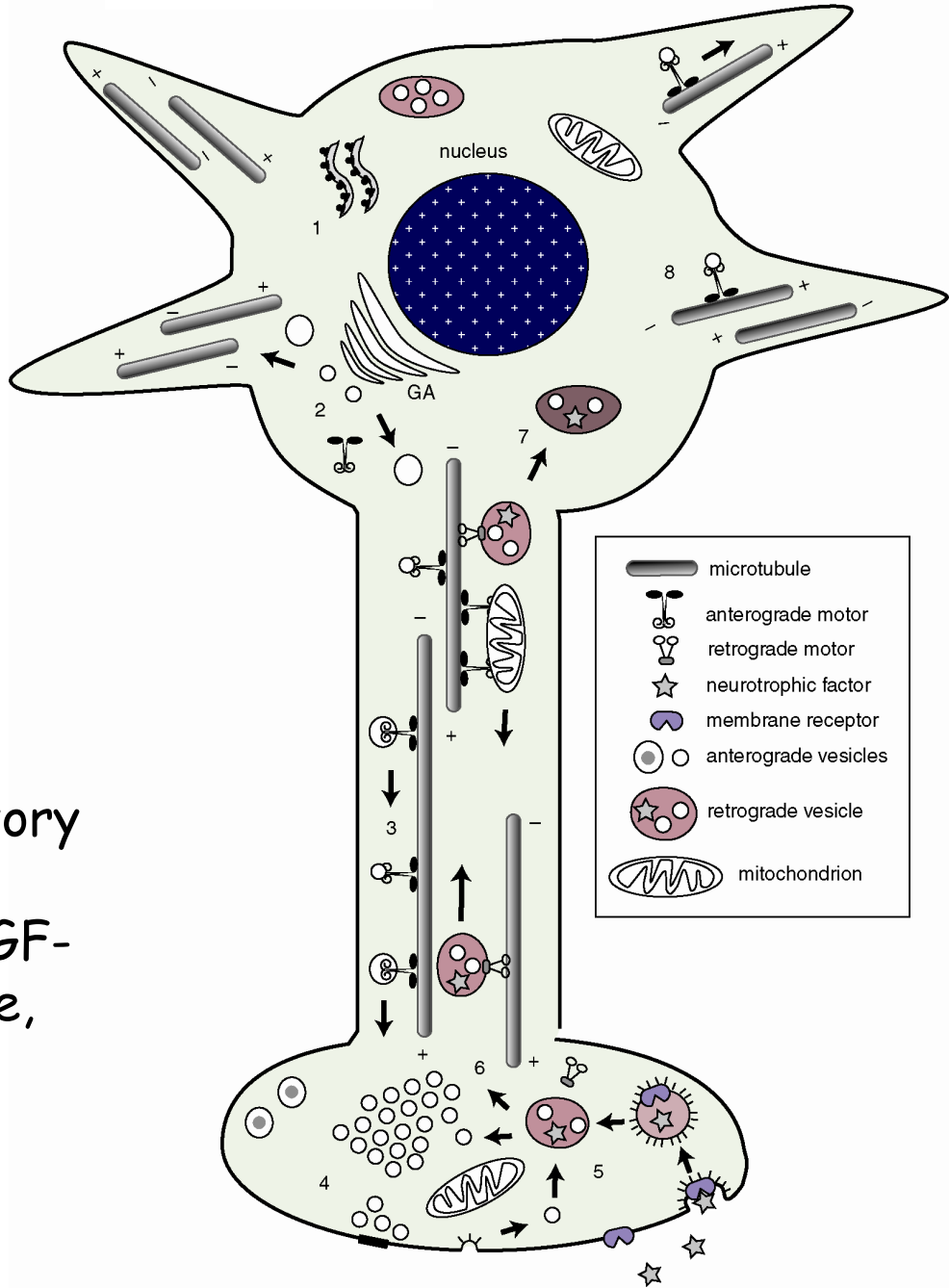


Slow axonal transport

Figure 2.8 Slow axonal transport represents the delivery of cytoskeletal and cytoplasmic constituents to the periphery.
Cytoplasmic proteins are synthesized on free polysomes and organized for transport as cytoskeletal elements or macromolecular complexes (1). Microtubules are formed by nucleation at the microtubule-organizing center near the centriolar complex (2) and then released for migration into axons or dendrites. Slow transport appears to be unidirectional with no net retrograde component. Studies suggest that cytoplasmic dynein may move microtubules with their plus ends leading (3). Neurofilaments may move on their own or may hitchhike on microtubules (4). Once cytoplasmic structures reach their destinations, they are degraded by local proteases (5) at a rate that allows either growth (in the case of growth cones) or maintenance of steady-state levels. The different composition and organization of cytoplasmic elements in dendrites suggest that different pathways may be involved in delivery of cytoskeletal and cytoplasmic materials to dendrites (6). In addition, some mRNAs are transported into dendrites, but not into axons.

Fast axonal transport

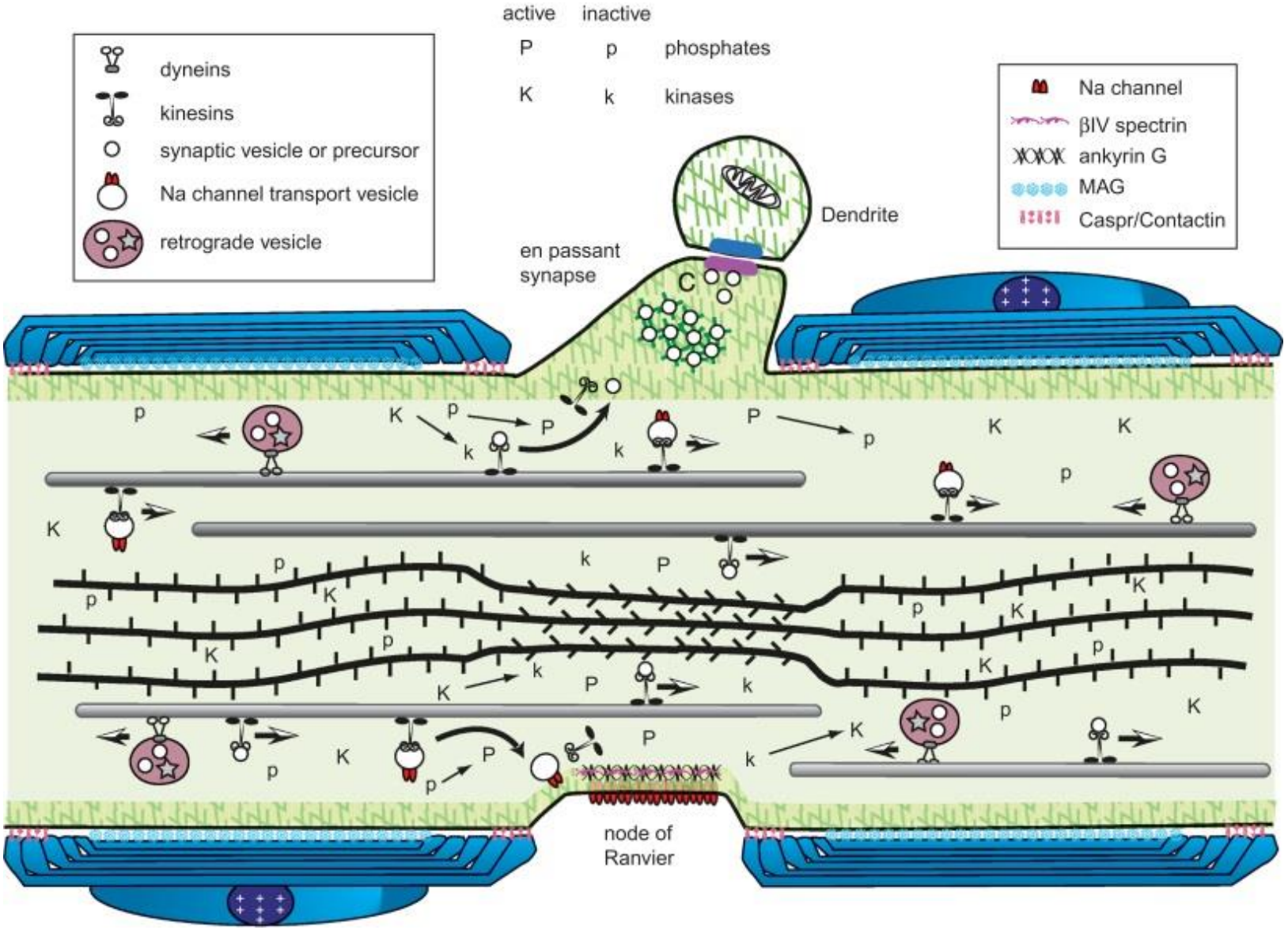
- vesicular trafficking:
  mitochondrium, transmembrane receptors, synaptic vesicles, neuropeptides, neurotrophines, viruses...
- anterograde and retrograde
- 100 – 400 mm/day [1 – 4 mm/s] (retrograde transport is normally slower)
- KIF1, KIF5: mitochondrium
  myosin Va: synaptic vesicles, secretory granules, mRNA granules
  dynein: signalling endosomes (eg. NGF-TrkA); BDNF; Rab5 endosome, myosin V (retrograde)



Fast axonal transport

Figure 2.9 Fast axonal transport represents transport of membrane-associated materials, having both anterograde and retrograde components.
For anterograde transport, most polypeptides are synthesized on membrane-bound polysomes, also known as rough endoplasmic reticulum (1), and then transferred to the Golgi for processing and packaging into specific classes of membrane-bound organelles (2). Proteins following this pathway include both integral membrane proteins and secretory polypeptides in the vesicle lumen. Cytoplasmic peripheral membrane proteins such as kinesins are synthesized on free polysomes. Once vesicles are assembled and appropriate motors associate with them, they move down the axon at a rate of 100–400 mm per day (3). Different membrane structures are delivered to different compartments and may be regulated independently. For example, dense core vesicles and synaptic vesicles are both targeted for presynaptic terminals (4), but release of vesicle contents involves distinct pathways. After vesicles merge with the plasma membrane, their protein constituents are taken up in coated vesicles via the receptor-mediated endocytic pathway and delivered to a sorting compartment (5). After proper sorting into appropriate compartments, membrane proteins are either committed to retrograde axonal transport or recycled (6). Retrograde moving organelles are morphologically and biochemically distinct from anterograde vesicles. These larger vesicles have an average velocity about half that of anterograde transport. The retrograde pathway is an important mechanism for delivery of neurotrophic factors to the cell body. Material delivered by retrograde transport typically fuses with cell body compartments to form mature lysosomes (7), where constituents are recycled or degraded. However, neurotrophic factors and neurotrophic viruses act at the level of the cell body and escape this pathway. Vesicle transport also occurs into dendrites (8), but less is known about this process.

Transport within the axons

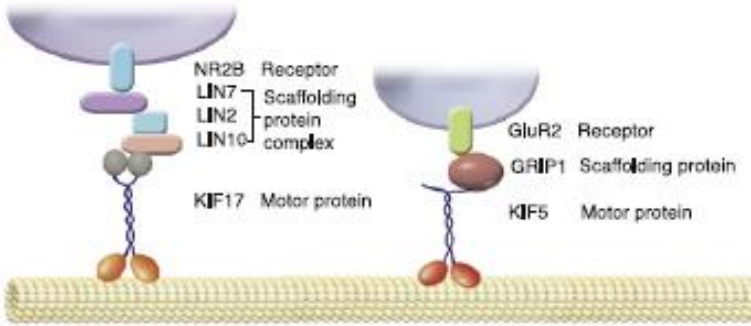


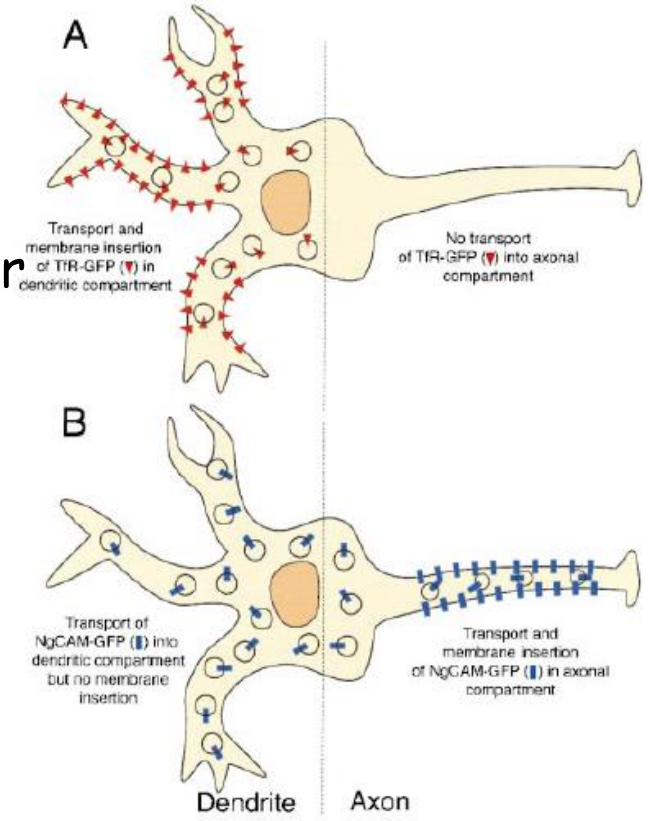
Transport within the axons

Figure 2.10 Axonal dynamics in a myelinated axon from the peripheral nervous system (PNS).
Axons are in a constant flux with many concurrent dynamic processes. This diagram illustrates a few of the many dynamic events occurring at a node of Ranvier in a myelinated axon from the PNS. Axonal transport moves cytoskeletal structures, cytoplasmic proteins, and membrane-bound organelles from the cell body toward the periphery (from right to left). At the same time, other vesicles return to the cell body by retrograde transport (retrograde vesicle). Membrane-bound organelles are moved along microtubules by motor proteins such as the kinesins and cytoplasmic dyneins. Each class of organelles must be directed to the correct functional domain of the neuron. Synaptic vesicles must be delivered to a presynaptic terminal to maintain synaptic transmission. In contrast, organelles containing sodium channels must be targeted specifically to nodes of Ranvier for saltatory conduction to occur. Cytoskeletal transport is illustrated by microtubules (rods in the upper half of the axon) and neurofilaments (bundle of rope-like rods in the lower half of the axon) representing the cytoskeleton. They move in the anterograde direction as discrete elements and are degraded in the distal regions. Microtubules and neurofilaments interact with each other transiently during transport, but their distribution in axonal cross sections suggests that they are not stably cross-linked. In axonal segments without compact myelin, such as the node of Ranvier or following focal demyelination, a net dephosphorylation of neurofilament side arms allows the neurofilaments to pack more densely. Myelination is thought to alter the balance between kinase (K indicates an active kinase; k is an inactive kinase) and phosphatase (P indicates an active phosphatase; p is an inactive phosphatase) activity in the axon. Most kinases and phosphatases have multiple substrates, suggesting a mechanism for targeting vesicle proteins to specific axonal domains. Local changes in the phosphoryation of axonal proteins may alter the binding properties of proteins. The action of synapsin I in squid axoplasm suggests that dephosphorylated synapsin cross-links synaptic vesicles to microfilaments. When a synaptic vesicle encounters the dephosphorylated synapsin and actin-rich matrix of a presynaptic terminal, the vesicle is trapped at the terminal by inhibition of further axonal transport, effectively targeting the synaptic vesicle to a presynaptic terminal. Similarly, a sodium channel-binding protein may be present at nodes of Ranvier in a high-affinity state (i.e., dephosphorylated). Transport vesicles for nodal sodium channels (Na channel vesicle) would be captured upon encountering this domain, effectively targeting sodium channels to the nodal membrane. Interactions between cells could in this manner establish the functional architecture of the neuron.

Transport within the dendrites

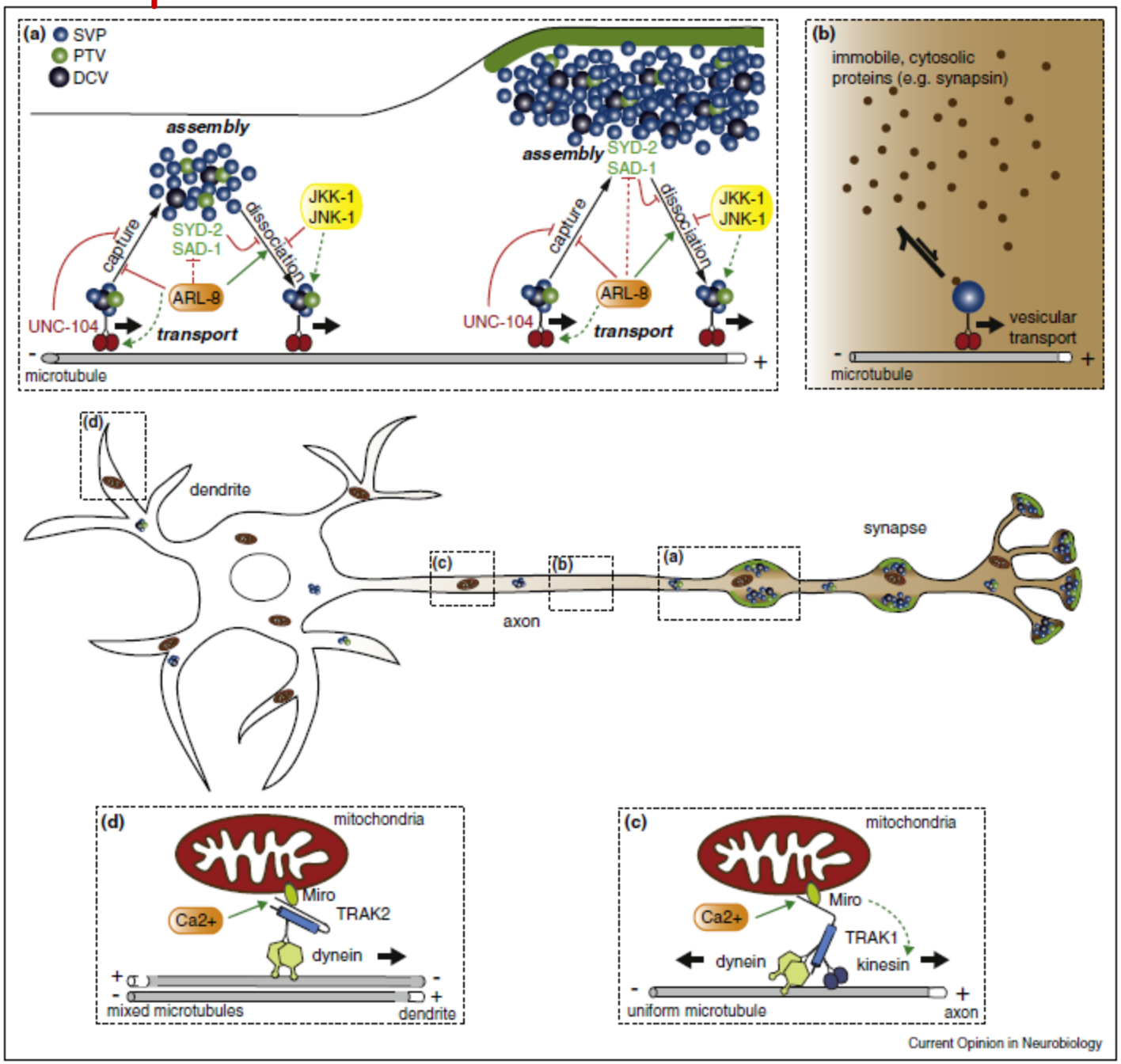
- local protein synthesis + bidirectional transport for shorter distances
- dendrite-specific motor:
  - KIF17: NMDA receptors
  - KIFC2 (C-KIF); MT- end: multivesicular bodies
- motors within the axons:
  - KIF5: AMPA, GABA receptors; mRNA
  - dynein: Rab5, 7 endosome; glycine receptor
  - myosin Va, Vb: AMPA receptor
- how can the axonal / dendritic membrane receptors be sorted specifically?
  - „smart" and „dumb" motor proteins
  - „dumb" motors are helped by selective endocytosis, too....





"Smart" versus "Dumb" Motor Transport

Transport within the axons and dendrites



Transport within the axons and dendrites

Transport and regulation of neuronal cargoes to axons and dendrites. **(a)** The balance between transport and assembly is regulated by a molecular network consisting of the small G-protein ARL-8, the active zone molecules the kinesin motor UNC-104 and the JNK MAP kinase pathway. **(b)** Slow axonal transport of cytosolic proteins is facilitated by their stochastic and transient association with fast moving vesicles. **(c and d)** Mitochondria employ different transport machinery for their delivery either to the axon or the dendrite. **(c)** TRAK-1 steers mitochondria into axons through its ability to bind to both kinesins and dyneins. **(d)** Adaptor protein TRAK2 binds preferentially to dynein and mediates dendritic targeting of mitochondria.

Axon and dendritic trafficking

Celine I Maeder[1], Kang Shen[1] and Casper C Hoogenraad[2]

Current Opinion in Neurobiology 2014, **27**:165–170

Essay questions (choose one)

Describe the major steps and factors regulating early neuronal polarisation, thus, the initial axon/dendrite specification! Ismertesse azokat a meghatározó szabályozási lépéseket, amik a neuronális polarizációt, azaz a korai axon / dendrit elkülönülést irányítják!

Compare the major axonal and dendritic features, highlighting similarities as well as specific differences! / Hasonlítsa össze az axon, illetve a dendritek főbb jellemzőit! Milyen tulajdonságaikban hasonlítanak vagy különböznek?

Explain the major axon-specific transport pathways! How do membrane or cytoplasmic proteins get transported into the axons? / Milyen axon-specifikus transzportútvonalakat ismer? Hogyan jutnak el az axonba a membrán- és a citplazmás fehérjék?

Characterise the major types of neuronal cytoskeletal proteins! Explain which features are characteristic to the axons or to the dendrites! / Sorolja fel a neuronális vázfehérjék fő típusait! Röviden jellemezze, hogy az axonokban és a dendritekben mely komponensek jellemzőek és ezeknek milyen jellegzetességei figyelhetőek meg!

Characterise the major types of neuronal motor proteins! Explain which features are characteristic to the axons or to the dendrites! / Sorolja fel a neuronális motorfehérjék fő típusait! Röviden jellemezze, hogy az axonokban és a dendritekben melyek jellemzőek és ezek milyen jellegzetességekkel bírnak!

Describe and compare the characteristics of fast and the slow axonal transport! / Ismertesse és hasonlítsa össze a lassú és a gyors axonális transzport jellegzetességeit!

Recommended literature

The cellular mechanisms that maintain neuronal polarity

Marvin Bentley and Gary Banker

NATURE REVIEWS | NEUROSCIENCE

Trafficking Guidance Receptors

Bettina Winckler[1] and Ira Mellman[2]

Cold Spring Harb Perspect Biol 2010;

Microtubule assembly, organization and dynamics in axons and dendrites

Cecilia Conde and Alfredo Cáceres

NATURE REVIEWS | NEUROSCIENCE VOLUME 10 | MAY 2009 |

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Neuron 68, November 18, 2010 ©2010

Axon and dendritic trafficking

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Current Opinion in Neurobiology 2014, **27**:165–170