

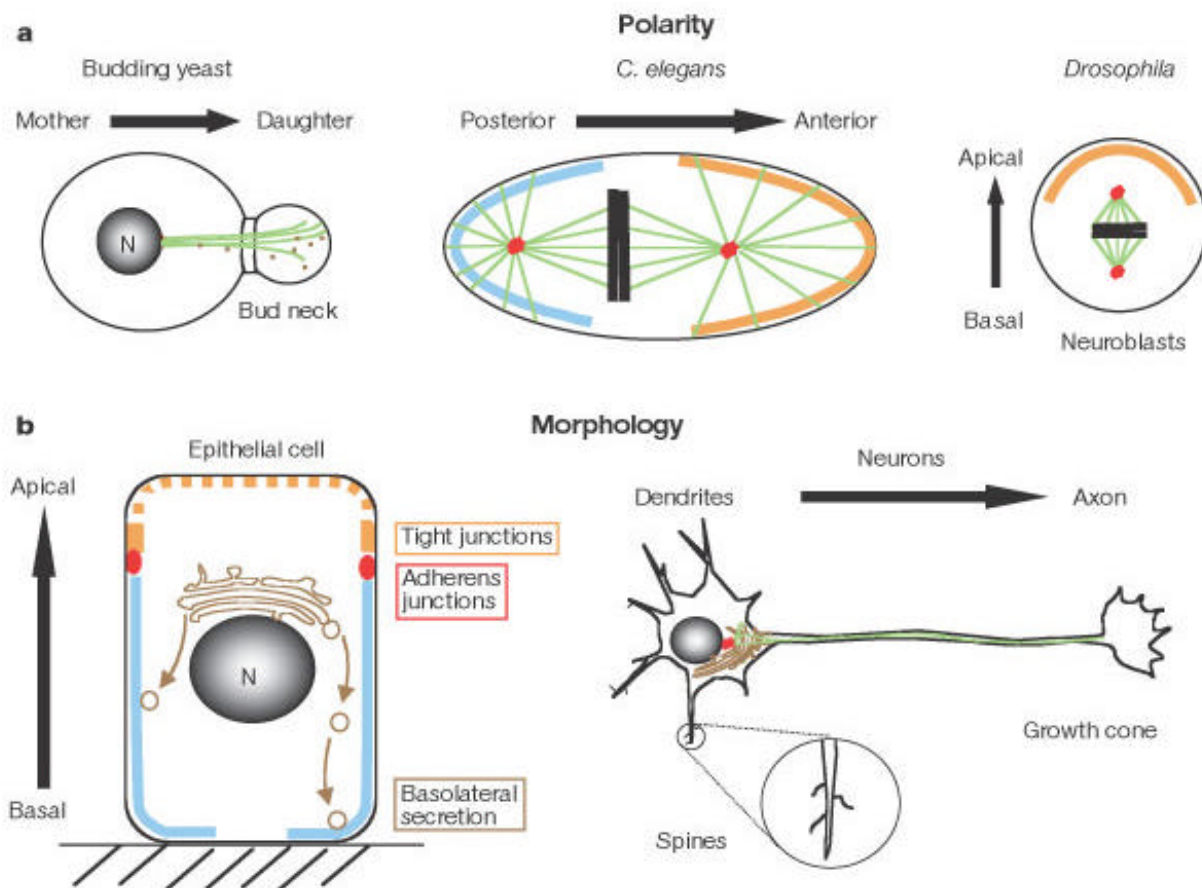
Neuronal cell biology, polarity, subcellular specialization

Neuronal polarity

Virtually all cells show some polarity and subcellular specialization *[any exceptions?]*

All receptors, ion channels (in fact, probably all membrane proteins) are distributed in a specific (often polarized) pattern on cell surface

Neuronal polarity can be considered an extreme example of polarized cell (cf epithelial cells).
[Are neurons epithelial cells?]

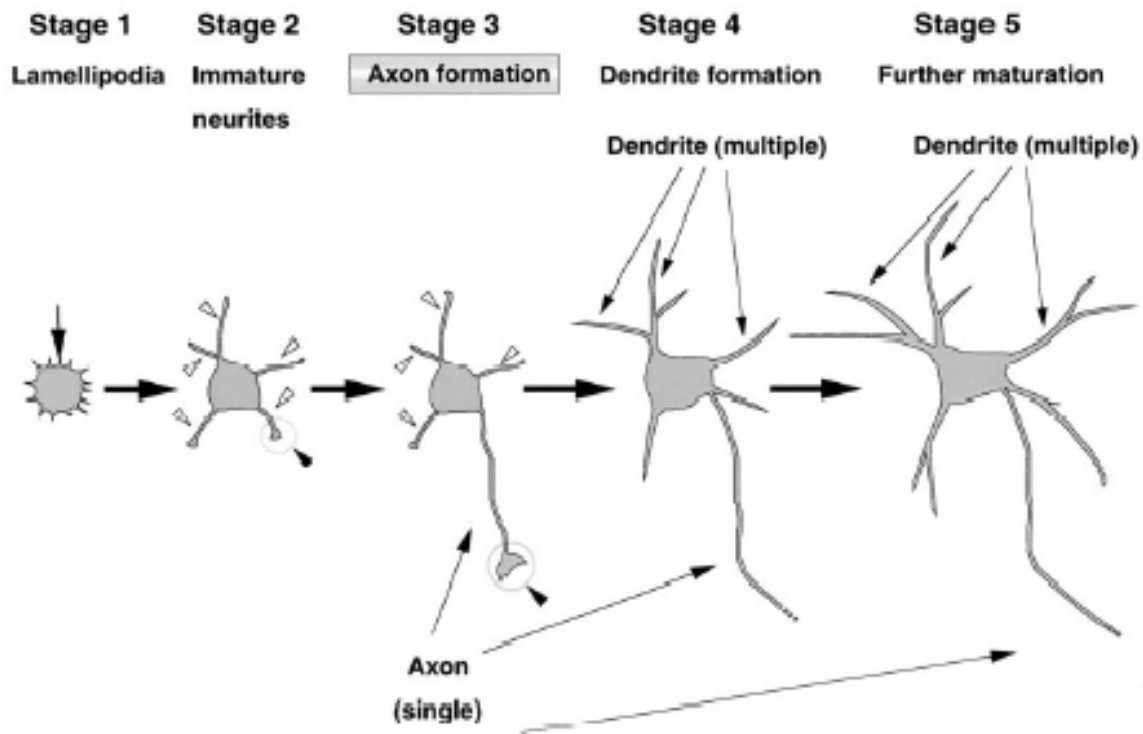


Cell Polarity. All eukaryotic cells are able to polarize in response to cues at the plasma membrane. a, Rho family GTPase Cdc42 is required for bud site assembly in yeast and, if deleted, cells expand isotropically. The polarized distribution of proteins in the *C. elegans* zygote or in *Drosophila* neuroblasts leads to asymmetrical cell division, a prerequisite for cell fate determination. In *C. elegans*, polarity establishment is controlled by Cdc42, the products of six par genes (PAR-1–6), and an atypical protein kinase C (PKC-3). PAR-6–PAR-3–PKC-3 localize at the anterior pole (orange line); PAR-1 and PAR-2 localize at the posterior pole (blue line). The microtubule organizing centre (MTOC) is shown in red and microtubules are in green. b,

Morphology. Polarity is also a major factor in determining cell morphology. In an epithelial cell, Cdc42, Par6, Par3 and atypical PKC regulate tight junction assembly to determine apical basolateral polarity. Cdc42, Rac and Rho are also involved in the assembly of adherens junctions and, along with basolaterally directed vesicle transport, this leads to the typical columnar shape. Junctions are further stabilized by associated actin filaments and, here too, Rho GTPases have an involvement. Rho GTPases are involved in determining the morphology of neurons; they affect axon, dendrite and spine growth, as well as axon guidance. It has not yet been shown whether Cdc42 has a role in establishing the polarity of the neuron.

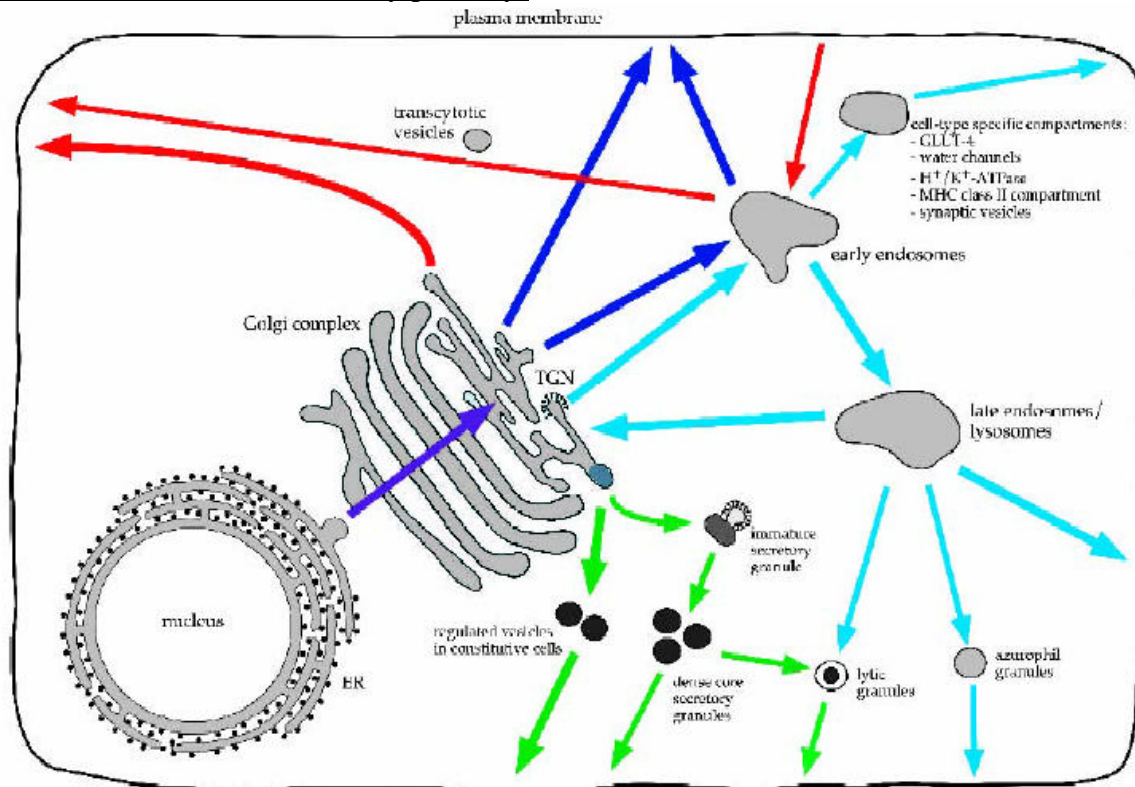
From Review by Etienne-Manneville S, Hall A. Rho GTPases in Cell Biology. Nature 2002; 420:629-35.

Establishment of neuronal polarity in cultured hippocampal neurons



Embryonic hippocampal neurons in culture acquire their characteristic polarized morphology in a sequence of well-defined stages. Stage 1, immediately after plating, lamellipodia (indicated by an arrow) surround the cell. Stage 2, within half a day, several short immature neurites (around 20 mm) emerge that are not distinguished either as the axon or as dendrites. Stage 3, within another 12_24 h, polarity first becomes evident, as one of the immature neurites shows enhanced elongation and acquires axonal characteristics. Stage 4, after 2_3 days in culture, the remaining neurites acquire dendritic morphology. Stage 5, the continued maturation of both axonal and dendritic arbors. Open arrowheads indicate the immature neurites. Black arrowheads indicate growth cones. Diagram from Dotti, C.G., Sullivan, C.A., Banker, G.A. 1988. The establishment of polarity by hippocampal neurons in culture. J. Neurosci. 8, 1454-1468.

A general schematic of the secretory pathway.



Hypothetical cell depicting post-Golgi biosynthetic pathways.

While pathways common to all cells are shown by thick arrows, pathways present in only a subset of cells are represented by thin arrows. The main sorting station of the biosynthetic pathway is the *trans*-Golgi network (TGN), from where direct routes to the apical surface (red), the basolateral surface (dark blue), and the endosomal system (light blue) emerge. In addition, the TGN gives also rise to 'classical' secretory granules, as well as to a *regulated secretory pathway* in specialized cells (green). The second sorting station in the biosynthetic pathway are early endosomes. They sort apical proteins in cells that use a transcytotic pathway for apical delivery (red) and might in addition be involved in the sorting of some basolateral proteins (dark blue). Early endosomes give also rise to a number of cell-type specific compartments, containing GLUT-4, MHC class II molecules, water channels, $H^+/K^+-ATPase$, or synaptic vesicle proteins.

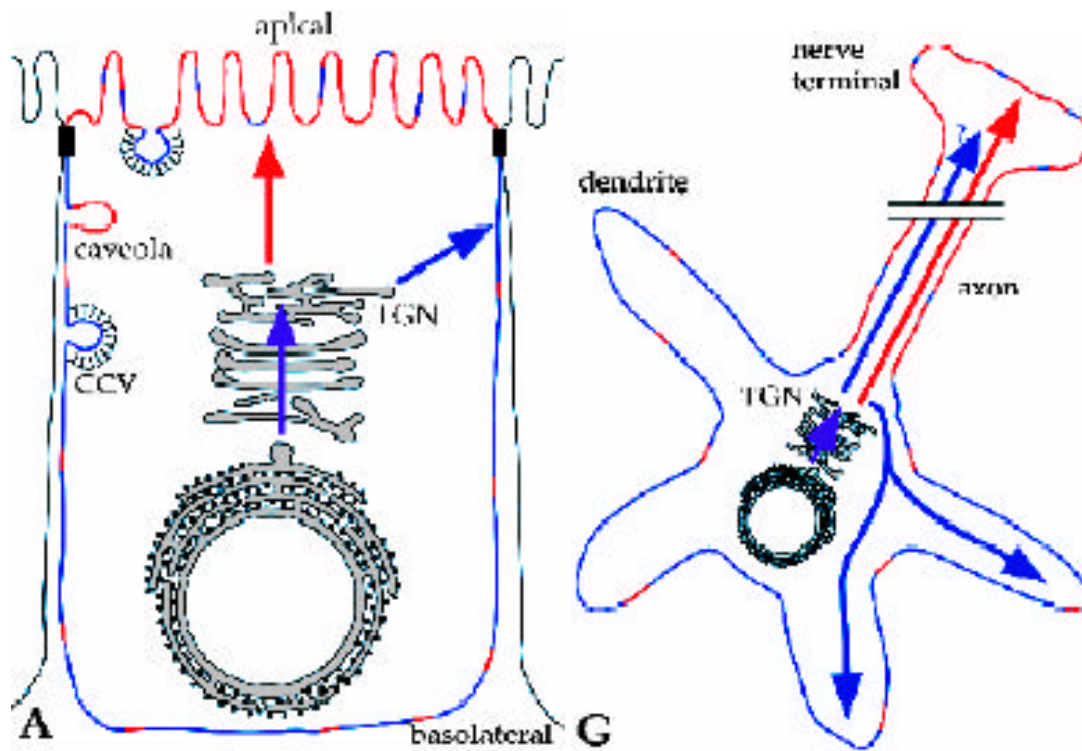


Fig. 2. Apical and basolateral pathways in a number of polarized and non-polarized cells. Membranes equivalent to the apical and basolateral surfaces of MDCK cells (A) are depicted in red and dark blue, respectively. In hippocampal neurons (G) the axon and somatodendritic surfaces have been proposed to be equivalent to the apical and basolateral domains of MDCK cells, respectively.

How are different membrane components sorted to distinct plasma membrane domains?

More extensively studied in polarized epithelial cells (e.g. MDCK cells) than in neurons.

Basolateral sorting in MDCK cells depends on sequence determinants (typically short motifs) in the cytoplasmic tails of the proteins. There is a wide variety of such “determinants”.

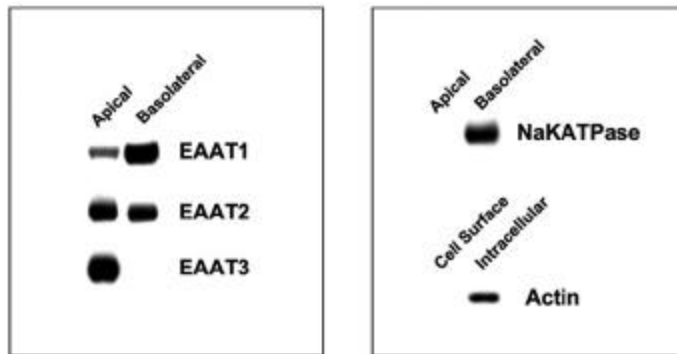
Apical sorting is less well understood. Some proposed apical sorting mechanisms include specific sequence motifs, glycosylphosphatidylinositol (GPI) linkage, association with cholesterol-rich lipid rafts, and glycosylation (Winckler and Mellman, 1999+).

Early studies showed that apically and basally targeted proteins in epithelial cells also localized to axons and somatodendritic compartments in neurons, respectively, but this is not universally true.

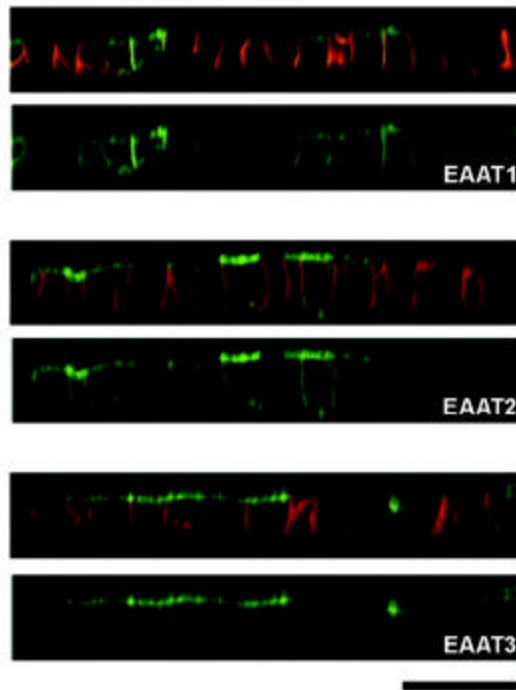
How do you identify a targeting signal / determinant in polarized cell?

Example: Differential localization of EAAT1-3 in MDCK cells.

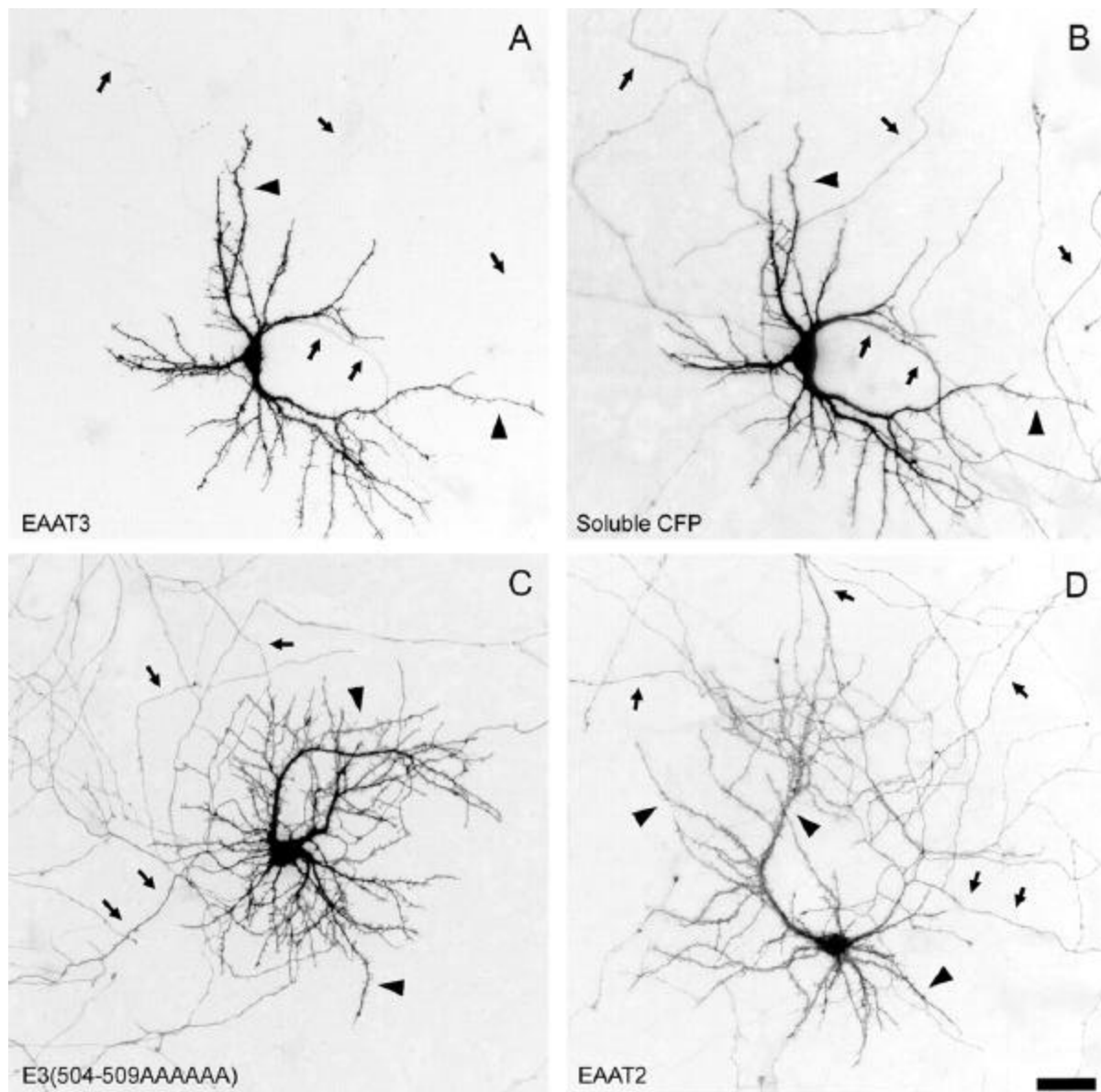
A



B



MDCK cells were stably transfected with EAAT1-3 tagged with GFP at the N terminus and grown on transwell filters. *A*, Representative Western blots from a cell surface biotinylation assay. This assay assessed the stable expression level of each EAAT at each cell surface by the application of membrane-impermeant biotinylating agent at the apical or basolateral surfaces. The *control blots* show that Na⁺/K⁺-ATPase is detected at the basolateral but not the apical cell surface fraction, and actin is only detected in the intracellular fraction. *B*, Representative confocal images of EAAT1-3 in a vertical section (*z*-series) illustrating the localization of GFP-EAAT (*green*) and a basolateral marker, E-cadherin (*red*). The *top panel* of each construct is a composite image illustrating both GFP and basolateral marker signals, and the *bottom panel* contains only the GFP signal. In the vertical section, a fluorescence signal at the apical surface appears as a *horizontal line* at the *top* of the cell, whereas a signal at the basolateral surface appears as *vertical lines* at the *sides* of the cell. All EAATs are expressed at both surfaces, except EAAT3, which is restricted to the apical surface. Scale bar, 20 μ m.



Distribution of wild-type and mutant EAATs in polarized hippocampal neurons. Hippocampal cultures were transiently transfected with GFP- or YFP-tagged EAATs, and expression of the constructs was assessed in 11-d-old hippocampal neurons. The EAAT3-YFP signal (*A*) is completely restricted to dendrites, whereas cotransfected soluble cyan fluorescent protein (*CFP*; *B*) fills the entire cell, including its axon. Mutant *E3(504-509AAAAAA)* with a six-alanine replacement in the apical sorting domain is present in both axons and dendrites (*C*), as is *EAAT2* (*D*). Arrows indicate axons; arrowheads indicate dendrites. Scale bar, 100 μ m.

[Better experiment would be to double label for specific markers of axons (tau) and dendrites (e.g. MAP2)]

Table 1. Examples of known transport signals

Type	Location	Representative examples
Basolateral sorting signals		
YXXØ	Cytoplasmic	Influenza virus hemagglutinin (Tyr mutant) Vesicular stomatitis virus glycoprotein
LL/IL	Cytoplasmic	FcRII-B2 receptor
Others	Cytoplasmic	Transferrin receptor LDL receptor distal domain Polymeric immunoglobulin receptor
Apical sorting signals		
GPI-anchor	Membrane, luminal leaflet	Placental alkaline phosphatase Thy-1
N-glycosylation	Luminal	gp-80 Erythropoietin
Transmembrane domain	Membrane	Influenza virus neuraminidase Influenza virus hemagglutinin
Late endosomes/lysosomes		
Mannose-6-phosphate	Luminal	Cathepsin B Cathepsin D
GYXXØ	Cytoplasmic	Lamp-1, Lamp-2 CD63

Neuronal cytoskeleton and motor-based transport

The polarized morphology of neurons depends on underlying cytoskeleton

Specific motors utilize microtubules (kinesins, dynein) or F-actin (myosins) as tracks for hauling cargos in ATP-dependent manner

Amazingly dynamic movements of organelles, proteins and subcellular structures

Other than motors, how else could you power vectorial movement within cells?

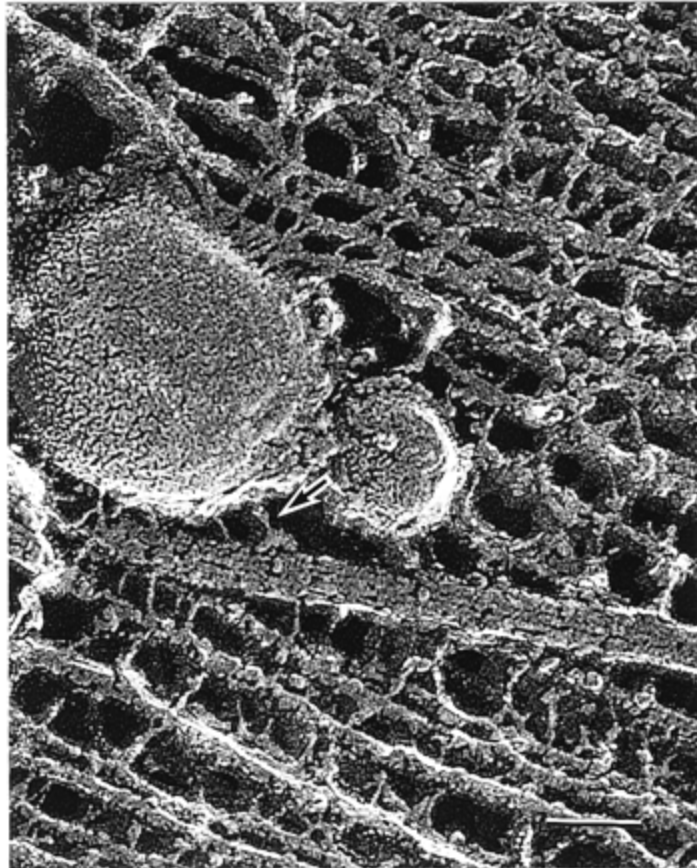


Figure. Quick freeze-deep etch electron micrograph of mouse axon. A membranous organelle conveyed by fast transport is linked with a microtubule by a short cross-bridge (arrow), which could be a motor molecule. Scale bar, 50 nm.

From Hirokawa N, Science 1998; 279:519-26.

Microtubules can be considered long-distance transport tracks

MTs usually concentrated in central core of dendrite shaft

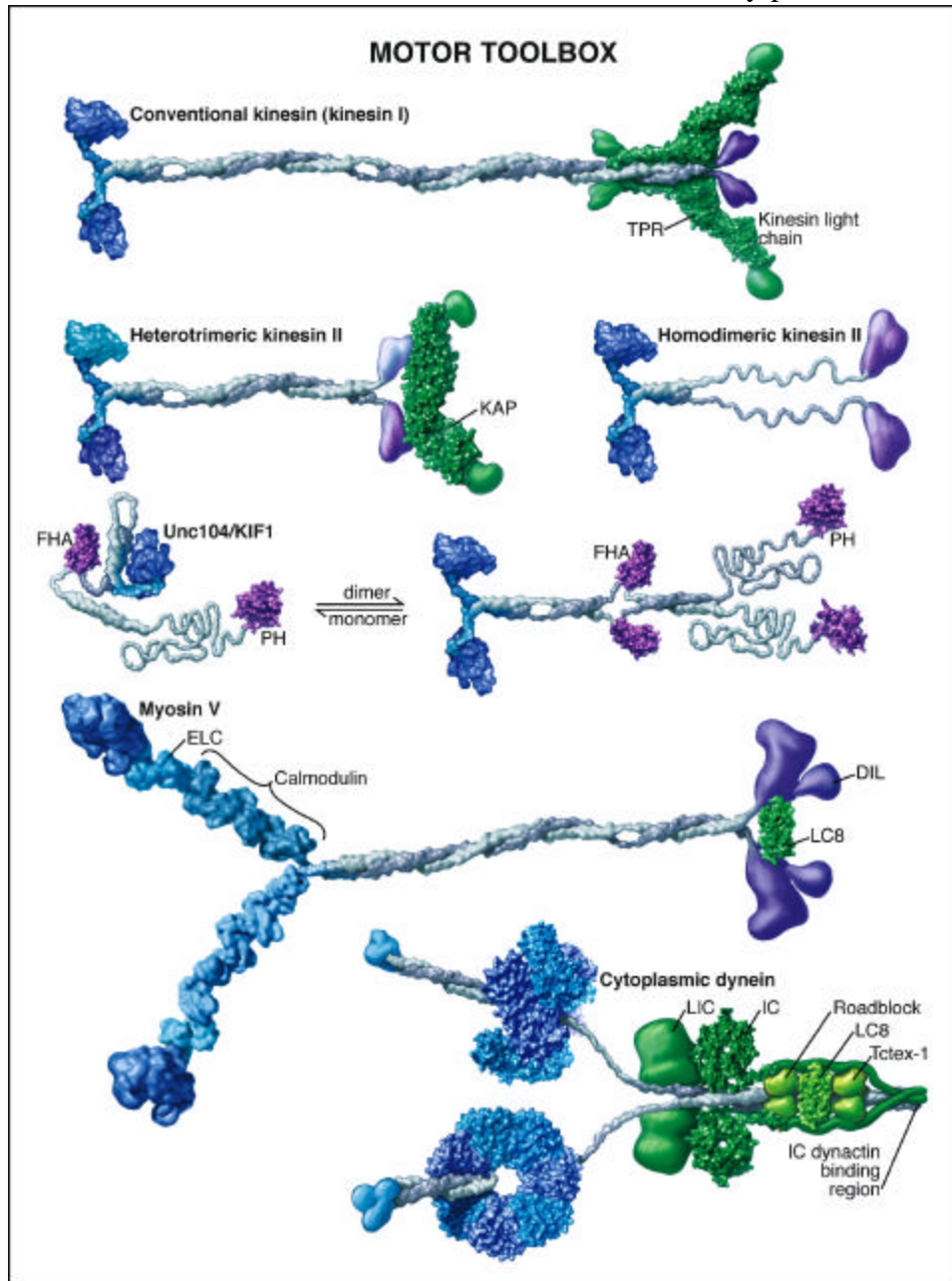
F-actin concentrated at the cell cortex and at cell-cell or cell-matrix contacts

can be considered short-distance transport track, especially for trafficking at or to the cell surface

(e.g. dendritic spines lack microtubules but are enriched with F-actin).

[analogy with central hub sorting?]

Motor proteins generally consist of ATPase domain that binds to filament, and tail domain that mediates multimerization and/or interaction with accessory proteins and cargos.

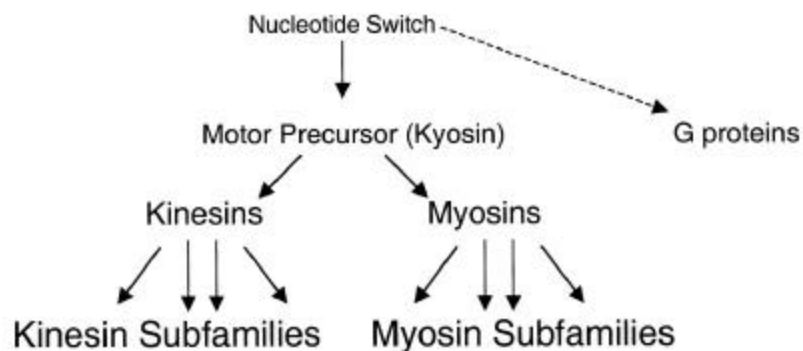


Examples of Cargo-Transporting Motor Proteins

Surface features are rendered based upon atomic resolution structures when available and appear as smooth images for domains of unknown structure. The motor catalytic domains are displayed in blue, mechanical amplifiers in light blue, and tail domains implicated in cargo attachment are shown in purple. Dynein is shown in mixed purple, blue shading to illustrate the distinct domains that comprise the motor head, four of which are likely to be functional ATP binding AAA^+ domains. The stalks extending from the ring bind to microtubules at their globular tips.

Heterotrimeric kinesin II contains two distinct motor subunits, which is reflected in the two different color shadings. Tightly associated motor subunits (light chains) are shown in green.

Evolution of Motor Proteins



Examples of Kinesin Subfamilies

Conventional	(Dimer)	Direction	Plus End	Processivity	Yes	Biological Activities	Membrane Transport
Ncd	(Dimer)		Minus End		No		Meiotic/Mitotic Spindle Function

Examples of Myosin Subfamilies

Myosin I	(Monomer)	Direction	Barbed End	Processivity	No	Biological Activities	Cell Motility/Membrane Functions
Myosin II	(Dimer)		Barbed End		No		Muscle Contraction/Cytokinesis
Myosin V	(Dimer)		Barbed End		Yes		Membrane/mRNA Transport
Myosin VI	(Dimer)		Pointed End		Unknown		Membrane Transport

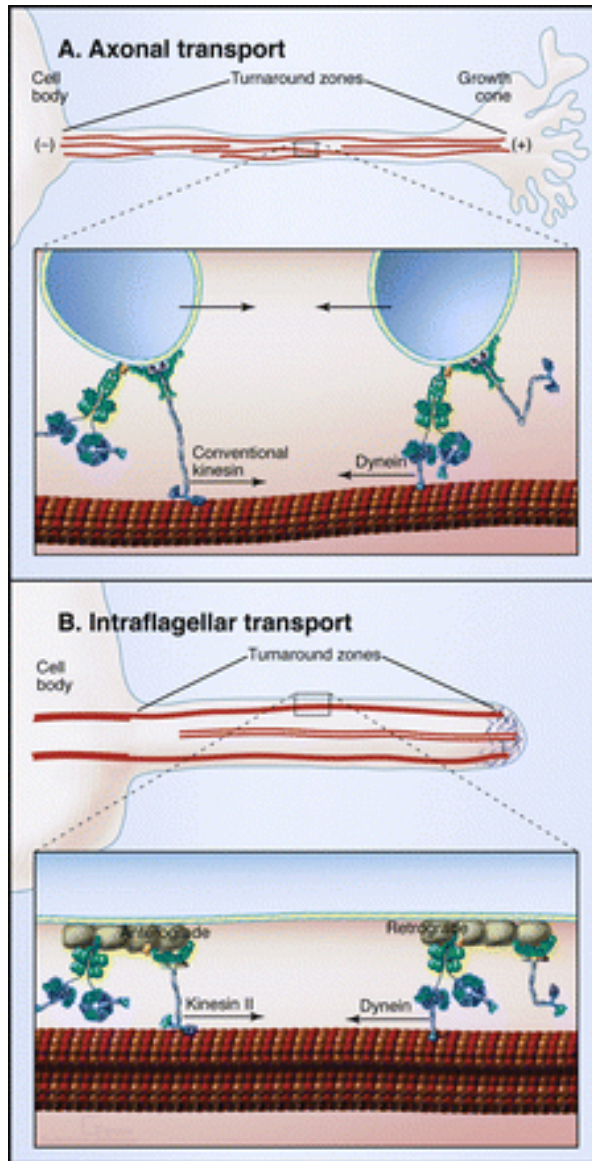
Dynein

MT-based multisubunit motor of completely different structure – also minus end directed motor.

How many motors are there?

Human:	kinesins 45	dyneins 14-15	myosins 40
Drosophila	25	13	13
C. elegans	20	2	17
S. cerevisiae	6	1	5

The problem of changing direction of transport



Coordination of Opposite Polarity Motors in Axonal and Intraflagellar Transport

In both transport systems, kinesin motors carry cargo (membrane organelles in the axon and submembranous protein particles in the flagellar axoneme) along a unipolar array of microtubule toward the plus ends. Dynein is carried along with this anterograde cargo in a repressed form, and reversals in the direction of movement are infrequent. At a "turnaround" zone at the tip of these structures, dynein is activated and kinesin is repressed, and the processed cargo then can be transported back toward the cell body. The opposite activation/inactivation of the motors is believed to occur at the base near the cells body. Molecules that mediate the coordination of these opposite polarities motors (here depicted as hypothetical yellow proteins located near the motors at the site of cargo attachment) as well as the switching mechanisms at the "turnaround" zones remain to be elucidated.

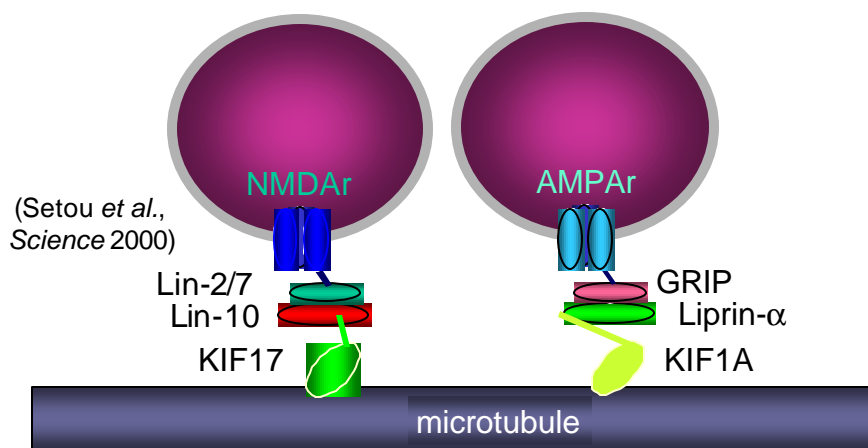
Motor-cargo interactions

Different cargoes are carried by different motors via specific protein interactions between motor (typically tail domain) and cargo receptor.

There may be overlap of cargos and hand-off from one to another, e.g. from kinesin to myosin.

[How do you identify the specific cargos of a particular motor?]

Transport of glutamate receptors by kinesin superfamily motors



NMDA and AMPA receptors are carried by distinct kinesin motors

NMDA receptors by KIF17 and AMPA receptors by KIF1A and kinesin [not shown here], in different vesicles.

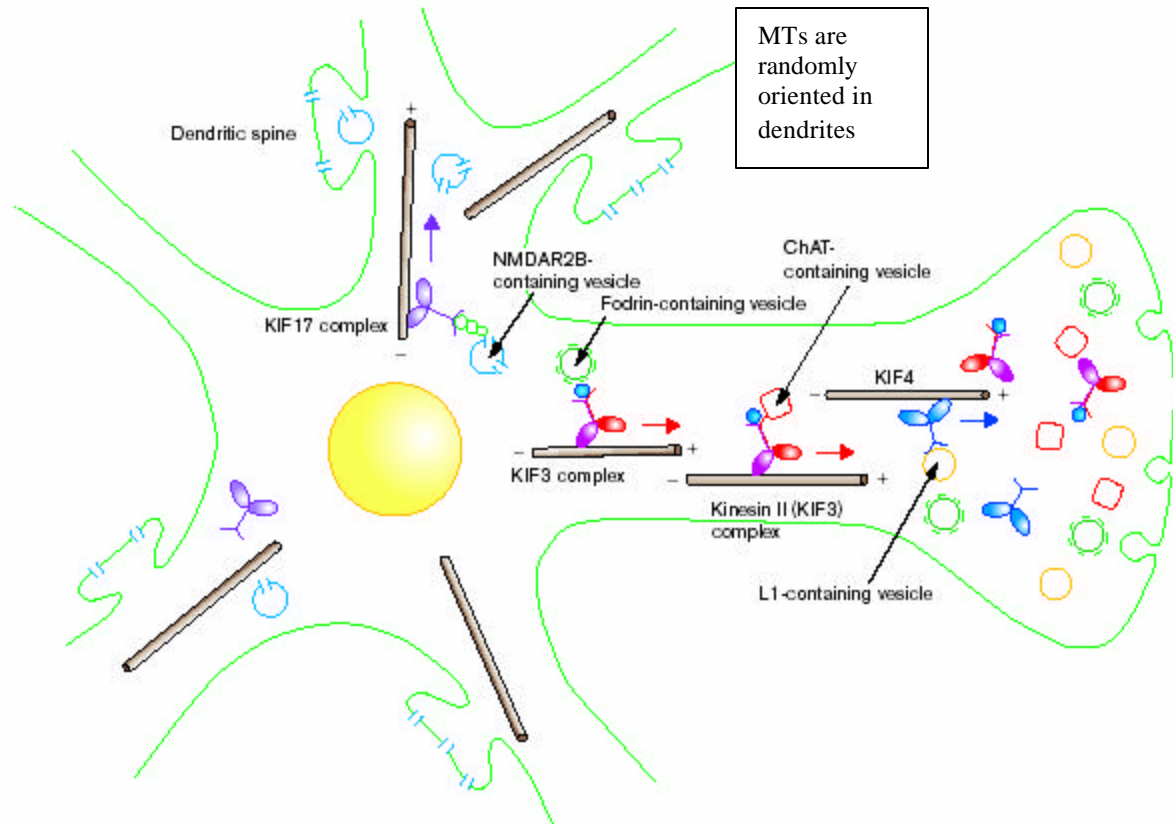
Distinct transport mechanism enables differential trafficking and regulation of AMPA versus NMDA receptor trafficking.

Note that motor proteins do not usually interact directly with receptor cargo, but rather with an adaptor protein (often a PDZ protein scaffold) that links the motor to a large protein complex. Other components of the vesicle can still be carried as cargo even if not interacting (directly or indirectly) with the motor protein.

Setou et al Glutamate-receptor-interacting protein GRIP1 directly steers kinesin to dendrites. Nature. 2002 May 2;417(6884):83-7.

Setou et al Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. Science. 2000 Jun 9;288(5472):1796-802.

Shin et al Association of the kinesin motor KIF1A with the multimodular protein liprin-alpha. J Biol Chem. 2003 Jan 8 [epub ahead of print]



In

axons, microtubules are oriented + end distal.
In dendrites, microtubule orientation is mixed.

Binding partners and cargos of kinesin superfamily proteins are now being elucidated.

Biochemical observations suggest that the cargoes of the motor proteins seem to be in the form of vesicles that possess specific functional marker molecules. The KIF3 complex conveys fodrin-containing vesicles through the direct interaction of KAP3 with fodrin and plays a role in neurite elongation [20·], whereas the kinesin II (KIF3) complex associates with other vesicles containing ChAT [21·], although the factors linking the vesicle and the motor are unknown. KIF4 transports L1-containing vesicles and assists in axonal elongation [24·]; in this case, also, the exact players between L1 and KIF4 remain obscure. KIF17 conveys NMDAR2B-containing vesicles via a complex of mLin-10, mLin-2 and mLin-7, and may modulate the efficacy of synaptic transmission [22·].

From Terada and Hirokawa. Moving on to the cargo problem of microtubule-dependent motors in neurons. *Curr Opin Neurobiol.* 2000 Oct;10(5):566-73.

Subcellular domains and microdomains of neurons

Further subdivision of major compartments into microdomains: e.g.

Axon: Usually one axon

initial segment / trunk / branches / nodes of Ranvier / terminal / active zone / peri-active zone
(although typically thin, axons are long and branching and usually account for the majority of the volume of the neuron)

Dendrite: Usually multiple dendrites (basal versus apical)

proximal / distal / shaft / dendritic spine / inhibitory synapse / excitatory synapse / PSD / peri-PSD

Proximal dendrite (thickest) probably similar to cell body in composition and function

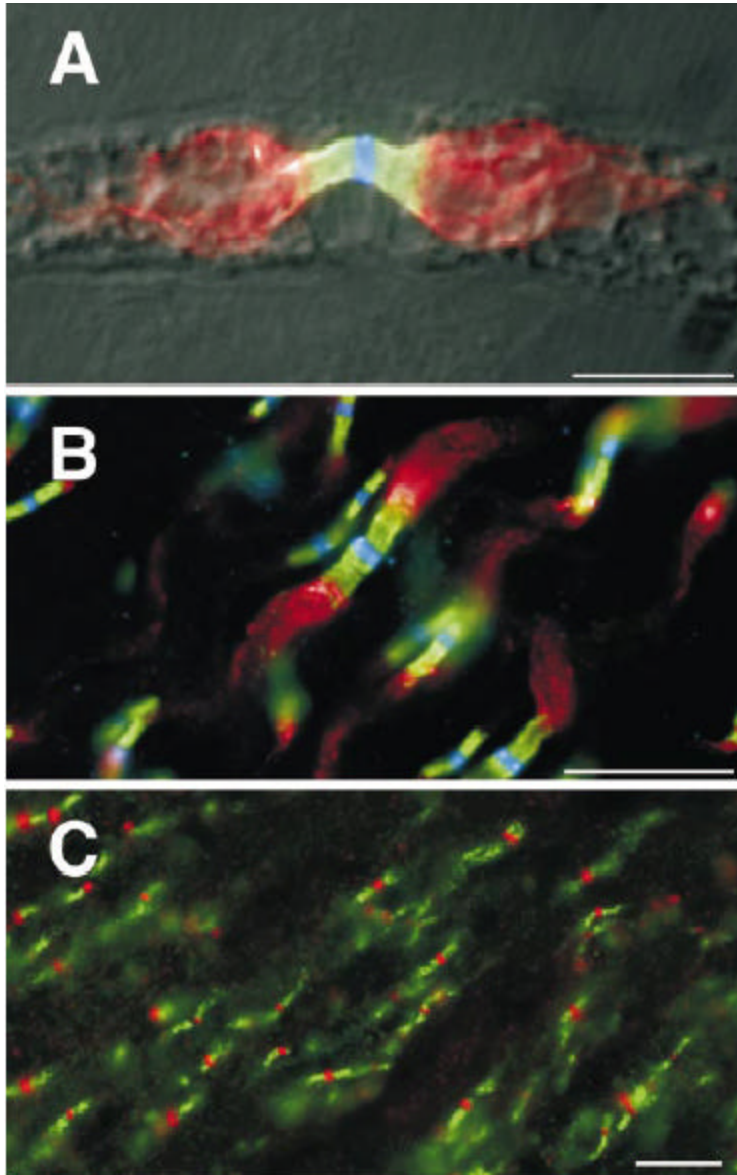
Cell body:

contains nucleus and transcription, translation machinery and other organelles

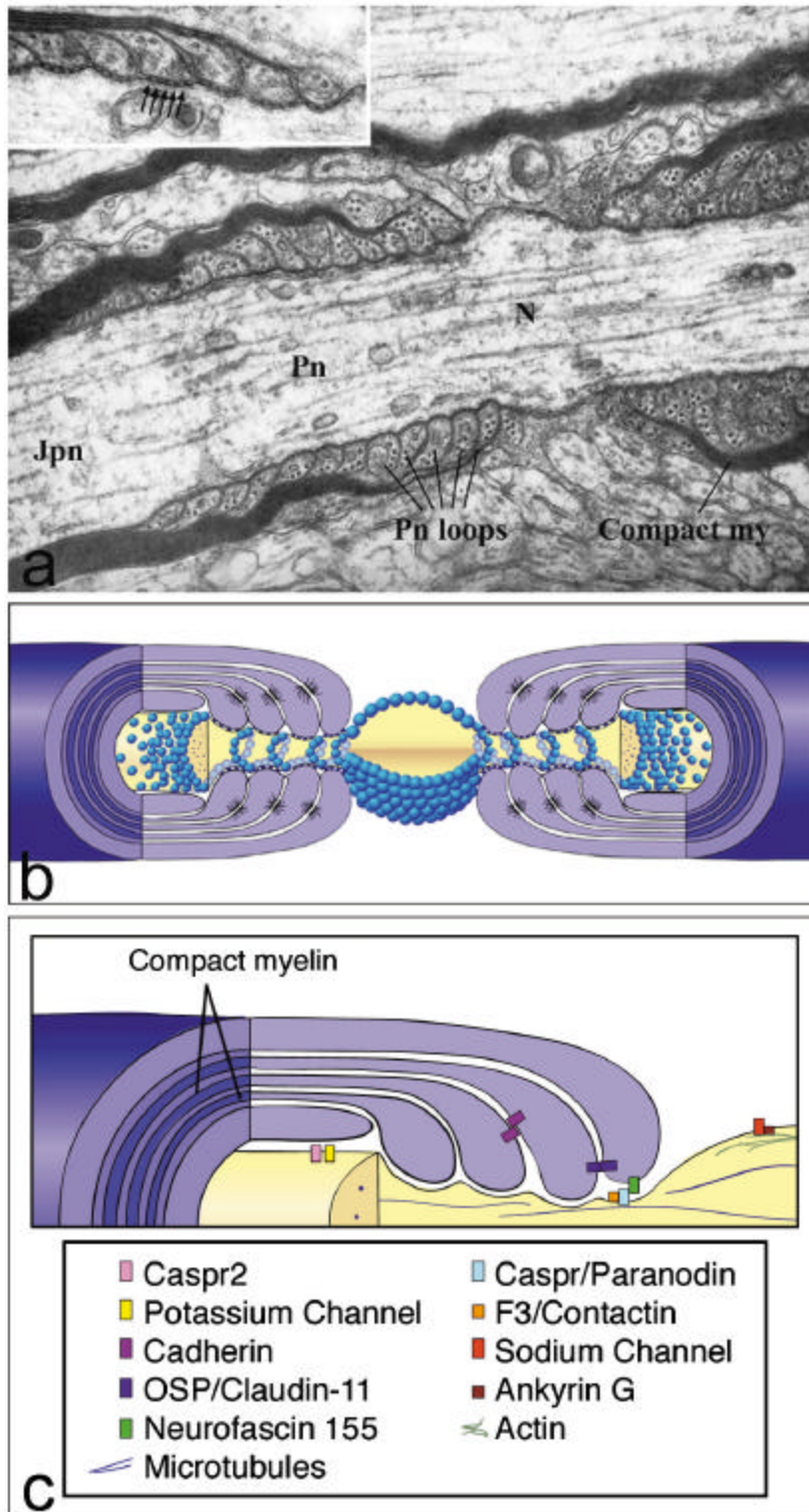
translational machinery extends into dendrites and local dendritic translation may occur for a subset of mRNAs that are specifically targeted to dendrites.

Node of Ranvier, a subcellular specialization of myelinated axons

[nodal / paranodal / juxtaparanodal]



Nav and Kv channels are clustered in distinct subcellular domains at the node of Ranvier. (A, B) Nodes of Ranvier from rat sciatic (A, PNS) and optic (B, CNS) nerves triple-labeled for Nav channels (blue), Caspr (green), and Kv1.2 subunit-containing Kv channels (red). The fluorescence image from the PNS node of Ranvier has been merged with a Hoffman-modulated contrast image to show the myelin sheath. (C) Rat optic nerve doublelabeled with antibodies against Nav1.6 (red) and Caspr (green). Scale bars, 10 micrometer. From Rasband and Trimmer. Developmental clustering of ion channels at and near the node of Ranvier. *Dev Biol.* 2001, 236: 5-16. Review.



From Pedrazza et al
Neuron, Vol 30, 335-344,
May 2001

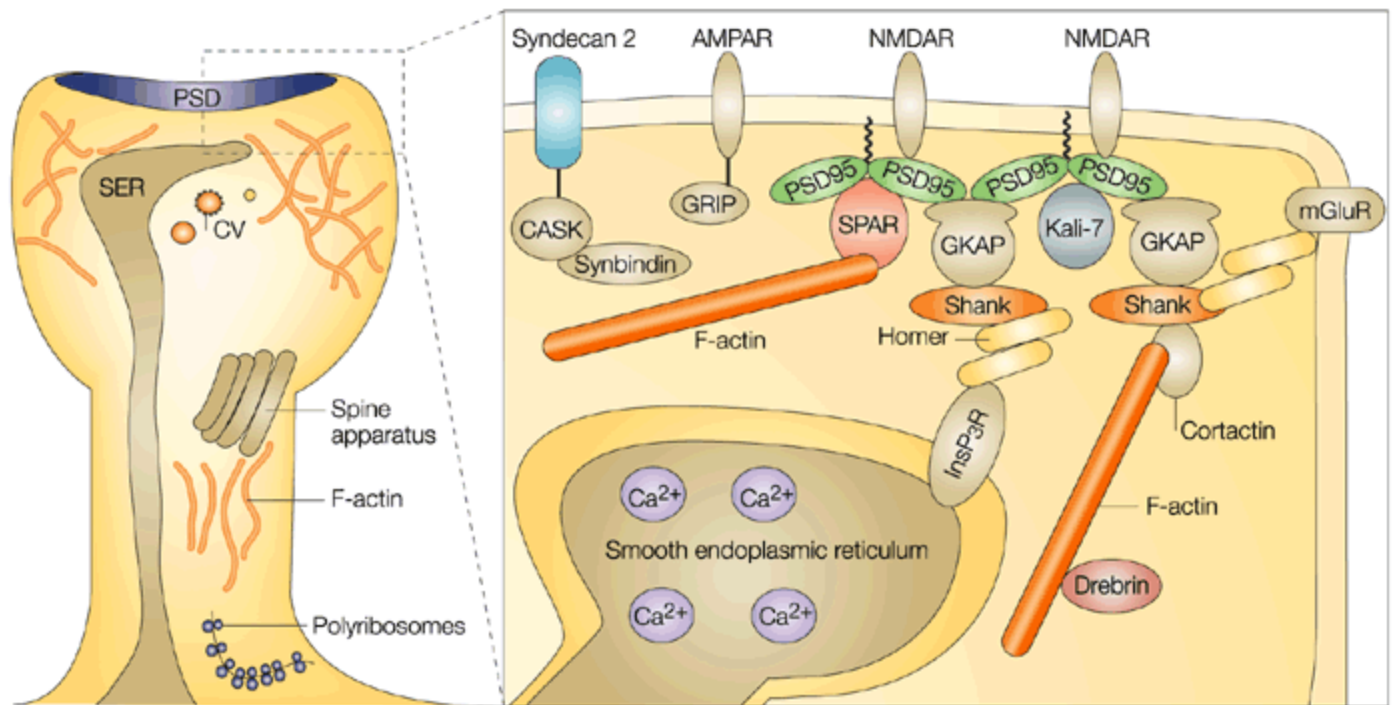
(a) The axoglial apparatus consists of the node of Ranvier (N), recognized as the bare axonal segment, flanked by paranodal (Pn) loops, formed by the terminal expansions of myelinating (My) cells, and the juxtaparanode (Jpn), which is located distal to the paranodal domain. The inset demonstrates the electron-dense "septa" (arrows) of the axoglial junction, whose functions may include intercellular adhesion, as well as molecular sieving. Adapted from Peters et al. (1991 +).

(b) Freeze-fracture electron microscopy reveals intramembranous particles, restricted to the nodal region, the narrow "interjunctional" region between the paranodal loops, and the juxtaparanodal region. The particles are largest in the nodal region, almost 20 nm in diameter and are likely to correspond to sodium channels and their interacting protein partners. Smaller particles in the paranodal and juxtaparanodal regions may participate in the adhesive linkage between the myelinating cell and the axon, and in barrier formation. Adapted from Rosenbluth (1976 ♣).

(c) The node comprises sodium channel subunits and their interacting partners, including neurofascin 186, NrCAM, and ankyrin G. The glial paranodal loops engage in two types of epithelial-like junctions: tight and adherens junctions. OSP/claudin-11 is a constituent of the tight junctions in the CNS, and E-cadherin mediates adhesion at the adherens junctions between the paranodal loops in the PNS. The proteins of the axoglial junction include caspr/paranodin, F3/contactin in the axon, and neurofascin 155 in myelinating glia. The juxtaparanode zone contains the potassium channel subunits Kv1.1, Kv1.2, and Kv2.1, and putative interacting proteins, such as caspr2 (Poliak et al., 1999 ♣).

Dendritic spines, a specialization of dendrites

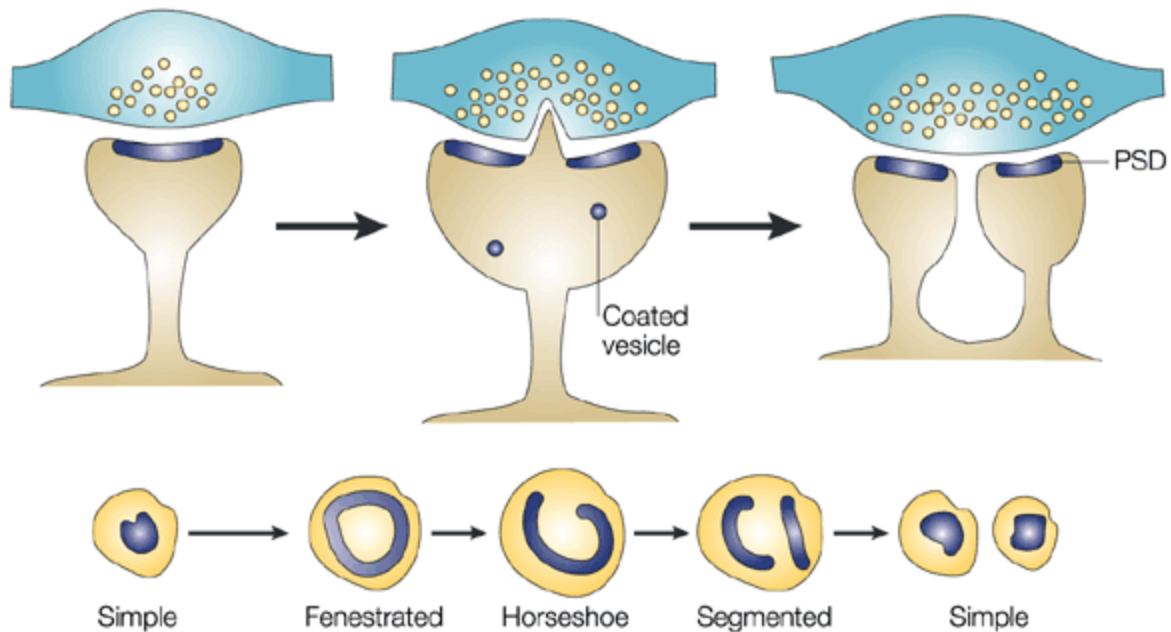
- Dendritic spines are morphological specializations that protrude from the main shaft of dendrites of principal neurons [examples of neurons that have and lack spines?].
- Most excitatory synapses in the mature mammalian brain occur on spines. Spines usually have a single excitatory synapse located on head. Thus, spines represent the main unitary postsynaptic compartment for excitatory input. [What is the function of spines?]



Nature Reviews | Neuroscience

From Hering and Sheng. Dendritic spines: structure, dynamics and regulation. *Nature Reviews Neuroscience* 2, 880-888 (2001)

- However, while most spines exhibit a single, continuous postsynaptic density (PSD), some PSDs are discontinuous or perforated (possibly in transition to multiple synapse spines). Thus spines may divide?

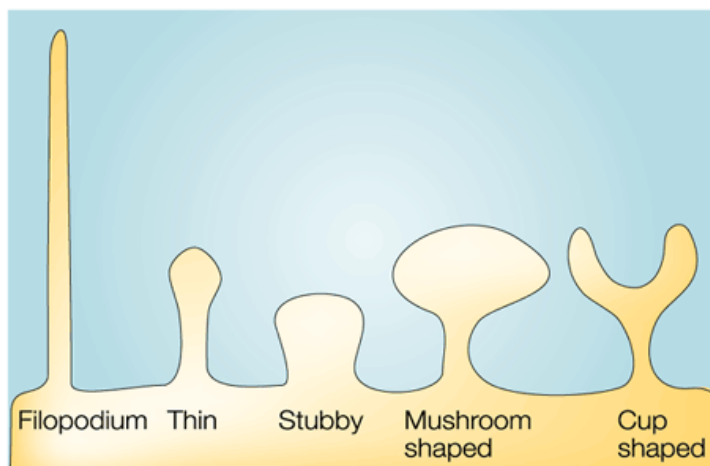


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Figure | **A model of changes in spine and PSD morphology after LTP.**

From Hering and Sheng Dendritic spines: structure, dynamics and regulation. *Nature Reviews Neuroscience* **2**, 880-888 (2001)

● Spines have been classified by shape as thin, stubby, mushroom- and cup-shaped. However, spine morphology is not static; spines change size and shape over variable timescales.



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● What is the significance of dendritic spines? There is no definitive answer to this question, but the prevailing view is that their primary function is to provide a microcompartment for segregating postsynaptic chemical responses, such as elevated calcium. Electrical resistance of the neck is generally considered too low to provide electrical compartmentalization. Because of

the tiny volume of spines, only small fluxes of ions through a few channels can drastically alter intracellular concentration within the spine.

- Dendritic filopodia are widely believed to be the precursors of dendritic spines—filopodia more abundant during early development, more motile, frequently not associated with synapses.. They may be considered an exploratory process of dendrites “searching” for presynaptic axon (?). However, a simple developmental relationship between filopodia and spines does not seem to exist. So, the filopodium–spine transition is unlikely to be a predestined process, but instead one that is reversible and regulated by factors such as synaptic activity. Spine-filopodia transitions probably occur in mature CNS as well as during development.

- Regulated changes in spine number might reflect mechanisms for converting transient changes in synaptic activity into long-lasting alterations. Indeed, changes in spine density have been observed in response to changes in the efficacy of neurotransmission. In general terms, spines seem to be maintained by an 'optimal' level of synaptic activity: spine density increases when there is insufficient activity, and decreases when stimulation is excessive. Moreover, spine morphology is markedly influenced by the activity of glutamate receptors.

Table 1 Environmental and disease factors that affect spine density		
Factor/stimulus	Associated change in spine density	References
Visual deprivation	Reduction in spine numbers and spines with abnormal morphology along apical shafts of pyramidal cells in the rabbit visual cortex	87
Visual stimulation	Increased spine density in the rat visual cortex	88
Rearing in complex/ 'enriched' environment	Increased spine density in the CA1 region of the hippocampus or dorsolateral striatum of the rat	52,89,90
Hibernation	40% loss of spines in the squirrel hippocampus during hibernation; spine numbers are recovered within hours of arousal	91
Sex steroid hormones	Female rats have more spines than males in a subset of hypothalamic nuclei and in the CA1 region of the hippocampus; spine density changes with the oestrus cycle in females	92–95
Stress	Spine density is enhanced in the hippocampus of male rats, but reduced in the female hippocampus in response to an acute stressful event	93
Fragile-X syndrome	Spines are abnormally long and thin, and of increased density in the cerebral cortex	96,97
Down's syndrome	Spine density is markedly decreased in the hippocampus and cortex	98,99
Epilepsy	Spine density is decreased on hippocampal and neocortical pyramidal cells	100

Table from Hering and Sheng Dendritic spines: structure, dynamics and regulation. *Nature Reviews Neuroscience* **2**, 880-888 (2001)

- Dendritic spines exhibit rapid motility. Most spines can change shape in seconds; appear or

disappear in minutes to hours; or be stable for weeks and longer. The shape change involves a remodelling of the actin cytoskeleton in the spine, and actin-based protrusive activity from the spine head. The underlying molecular mechanisms of this motile behaviour (except that it depends on F-actin), and its functional significance, are unknown.

● Considerable progress has been made in identifying the molecules that control spine growth and maturation. The cytoskeleton is crucial for their development and stability, and an expanding set of actin-binding and actin-regulatory molecules has also been implicated in these processes. They include GTPases of the Rho/Rac/Cdc42 family, the small GTPase Ras, and a series of receptors and scaffold proteins and actin-binding proteins.

● Several questions remain to be answered in this nascent field. For example, what is the actual function of spines in brain plasticity and behaviour? What are the intrinsic and extrinsic factors that determine the formation of spines? Why do some cells have spines and others not? [*how would you approach this?*] What is the relationship between the structural plasticity of spines, and the movements of molecules and membranes into and out of this postsynaptic compartment?