protein pair to interact, thereby allowing specific interactions and allosteric regulatory mechanisms to evolve relatively quickly. Scaffold proteins can therefore act as "catalysts" for the evolution of specific interactions between the proteins that are bound to them. They can also acquire the ability to directly control the activities of the docked proteins, as illustrated here by the action of the scaffold protein Ste5 on its clients Ste7 and Fus3. This incisive mechanistic analysis of MAPK signaling by Good and coworkers may well change our view of scaffold proteins as the boring partners of catalytically active kinases. These results also show us that unexpected relationships can develop when evolution tinkers with molecules that are tethered together.

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A Gate Keeper for Axonal Transport

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DOI 10.1016/j.cell.2009.03.003

The axon and dendritic arbor of neurons require different sets of membrane proteins to carry out their functions. In this issue, Song et al. (2009) describe how a cytoplasmic diffusion barrier in the axon initial segment of rat hippocampal neurons ensures that only axonal (and not dendritic) membrane proteins enter the axon.

Neurons are polarized cells harboring two distinct subcellular domains: the axon and the somatodendritic region including the dendritic arbor. The dendritic arbor receives signals from neighboring neurons, whereas the axon sends signals to neighboring neurons, providing the basic building blocks of the neuronal circuitry. Consistent with their specialized functions, the axon and dendrites have different protein and lipid compositions, including distinct sets of membrane proteins. The localization of membrane proteins to their sites of action in the specialized subdomains of the neuron is crucial for the maintenance of proper neuronal polarity and function. In this issue of *Cell*, Song et al. (2009) report the discovery of a cytoplasmic barrier in the axon initial segment of the neuron that prevents the free diffusion of macromolecules between the dendritic arbor and axon subdomains. This cytoplasmic selectivity filter allows entry into the axon of only axonal proteins (Figure 1).

Mechanisms for specific targeting of membrane proteins in neurons include selective sorting and transport along the secretory and endosomal pathways, selective retention of proteins at the membrane, and barriers in the membrane that prevent unwanted mixing of proteins (Horton and Ehlers, 2003) (Figure 1). Vesicular transport of membrane proteins to axons and dendrites is generally carried out by molecular motors of the kinesin and dynein superfamilies, which move along microtubules to transport vesicles toward the plus and minus ends of microtubules, respectively (Hirokawa and Takemura, 2005). Different molecular motors of the kinesin superfamily (KIFs) recognize specific transmembrane cargo proteins and direct them from the cell body to the axon or dendrites. Interestingly, the motor protein KIF5 is responsible for both the dendritic targeting of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors and the axonal transport of certain membrane proteins including amyloid precursor protein and VAMP2, a synaptic vesicle protein. This raises the question of how a motor protein determines whether its cargo is destined for the axon or for dendrites.

Maintaining the asymmetric distribution of membrane proteins in the different neuronal subdomains is crucial for proper neuronal function. A membrane diffusion barrier that restricts the lateral mobility of membrane proteins and lipids has been identified in the axon initial segment of neurons (Nakada et al., 2003; Winckler et al., 1999). The axon initial segment harbors a high density of ankyrin G, adaptor proteins that connect the spectrin-actin cytoskeleton with integral membrane proteins. In their new study, Song et al. (2009) now find that in

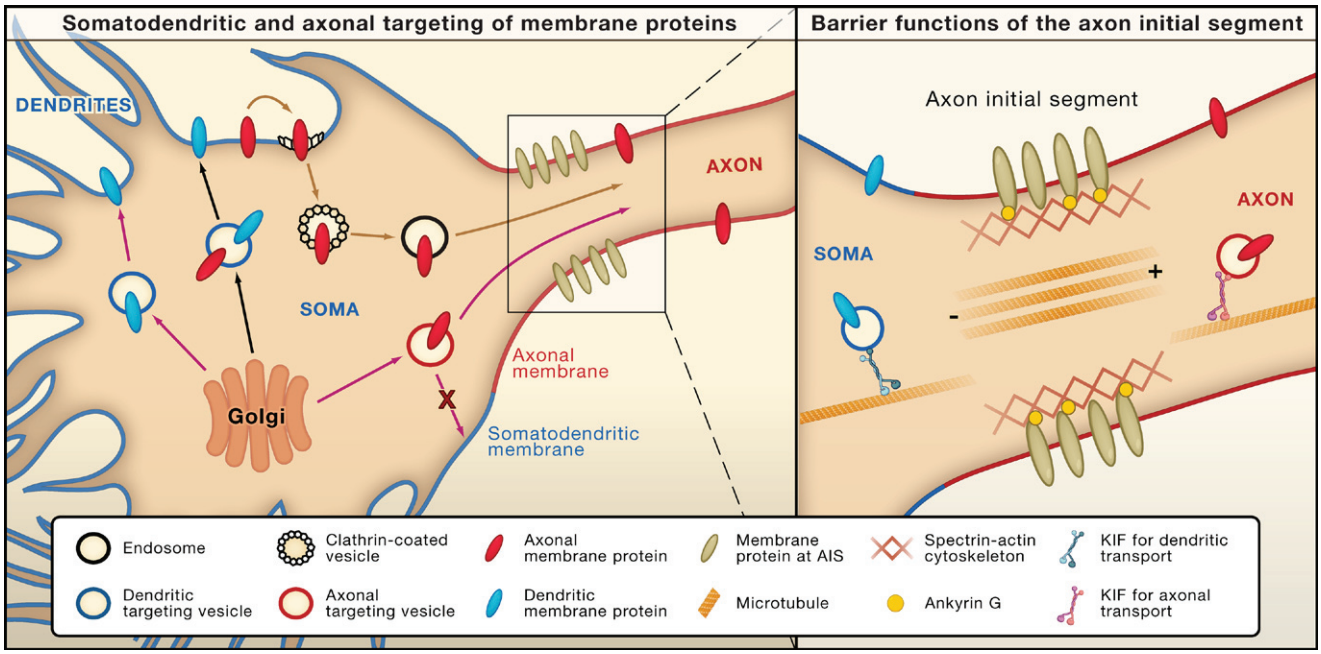

Figure 1. Domain-Specific Targeting of Protein Cargo in Neurons

(Left) Somatodendritic and axonal membrane proteins can be differentially localized in several ways. They can be sorted into distinct vesicles in the trans-Golgi network for selective delivery to dendrites or the axon (purple arrows). This requires mechanisms that prevent fusion of vesicles with the incorrect plasma membrane region (blocked purple arrow). Alternatively, dendritic and axonal proteins may be nonselectively packaged into vesicles and inserted into the plasma membrane (black arrows). At the plasma membrane, the correctly targeted proteins are selectively retained, whereas the mislocalized proteins are endocytosed into clathrin-coated vesicles and transported by transcytosis to the correct location (brown arrows). Segregation of dendritic and axonal membrane proteins is further maintained by a physical diffusion barrier in the plasma membrane of the axon initial segment (AIS).

(Right) Song et al. (2009) now show that in addition to the membrane diffusion barrier for protein segregation, a cytoplasmic filter also exists in the axon initial segment, which prevents the kinesin motor (KIF)-driven transport of vesicles containing somatodendritic proteins into the axon. The axon initial segment is enriched in voltage-gated sodium channels, ankyrin G, spectrins, actin, and cell adhesion molecules. Spectrins and actin assemble into the subplasmalemmal skeleton supporting the plasma membrane. Ankyrin G further links the membrane skeleton with several transmembrane proteins. A bundle of microtubules differing from those found in the cell body and dendrites is also present in the axon initial segment (Nakata and Hirokawa, 2003).

addition to this membrane diffusion barrier, there is also a cytoplasmic barrier in the axon initial segment of hippocampal neurons isolated from rat embryos and cultured for 3 to 5 days. By monitoring the distribution in cultured rat hippocampal neurons of fluorescent proteins and fluorescently labeled dextrans of various molecular weights, Song et al. observe that large molecules freely diffuse into the axon after 3 days in culture but are hampered in their movements by 5 days. Macromolecules could not move along the axon more than 60 μm from the cell body, which corresponds to the location of the axon initial segment. The authors find that, like the membrane diffusion barrier, the cytoplasmic barrier that blocks diffusion of large dextrans is dependent on both intact F-actin and ankyrin G. Both disruption of F-actin by latrunculin A and downregulation of ankyrin G expression by small-interfering RNAs result in axonal distribution of large dextrans. Moreover, the accumulation of F-actin and ankyrin G in the axon initial segment occurs between 3 and 5 days of culture, the time period during which the free diffusion of large molecules into the axon is known to be blocked.

Song et al. further report that the cytoplasmic transport of membrane proteins, which is driven by microtubule-associated motor proteins, is selectively affected in the axon initial segment by 5 days in culture. Concurrent with the appearance of the cytoplasmic diffusion barrier in the axon initial segment, the surface and cytoplasmic distribution of NR2B—an NMDA (N-methyl-D-aspartate) receptor subunit targeted to dendrites—becomes restricted to the somatodendritic domain in an F-actin-dependent manner. Analysis by FRAP (fluorescence recovery after photobleaching) reveals that although all vesicle transport is slowed in the axon initial segment, the transport of vesicles carrying NR2B along the axon initial segment is much slower than for vesicles carrying the synaptic vesicle protein VAMP2. These observations suggest that the axon initial segment acts as a gate that prevents entry of vesicles destined for the dendrites, allowing through only those vesicles that are targeted to the axon.

To address how motor proteins and their associated vesicles are selectively inhibited or permitted to pass through the cytoplasmic filter in the axon initial segment, Song and colleagues measured the transport efficacy (transport rate) of motor-cargo complexes. As the dendritic transport of NR2B and the axonal targeting of VAMP2 are driven by the motor proteins KIF17 and KIF5B, respectively (Hirokawa and Takemura, 2005), they examined the transport rates of these molecular motors with and without cargo proteins. The authors find that the motor domain of KIF5B seems to move faster than that of KIF17 in the axon initial seg-

ment, consistent with the observation that unlike KIF17-NR2B, KIF5B-VAMP2 is able to pass through this region. However, as the truncated KIF17 and KIF5B proteins containing only the motor domains are distributed equally throughout the neuron, it seems that the motor domains alone do not possess a particular bias for the axon or dendrites. Furthermore, because KIF5 can drive both axonal and dendritic transport (Hirokawa and Takemura, 2005), it seems likely that some aspect of the cargo or of the motor-cargo complex dictates the location of cargo delivery.

To test whether cargo proteins are sufficient to determine vesicle targeting and selective entry to the axon, the authors swapped the tail domains between KIF17 and KIF5B to enable one motor protein to carry the other's cargo. The chimeric KIF17 protein harboring the tail domain of KIF5B is able to carry and deliver VAMP2 cargo, whereas the chimeric KIF5B protein harboring the KIF17 tail domain can carry and deliver NR2B cargo. Song et al. find that NR2B carried by the chimeric KIF5B motor shows a higher transport rate and is found distributed along the axon in addition to the somatodendritic region. VAMP2, when carried by the chimeric KIF17 protein, is largely absent from the axon where it normally accumulates. Thus, it seems unlikely that the cargo alone is sufficient to determine the selectivity of targeting for the axon or dendritic arbor (at least in the case of NR2B and VAMP2 cargo), suggesting that it is the motor-cargo complex itself that most likely dictates targeted transport.

The findings by Song et al. (2009) raise intriguing questions for future studies. What determines the transport rate for vesicles along the axon initial segment and what are the relative contributions of cargo and motors to the overall transport rate? The fact that KIF5 can drive both axonal and dendritic trafficking (Hirokawa and Takemura, 2005) suggests that cargo may play a role in determining the localization of motor-cargo complexes. However, Song et al. show that the dendritic protein NR2B was not excluded from the axon when transported by a different motor. Thus, it remains to be seen whether there is a hierarchy in the determinants that govern how cargo-motor complexes are targeted. It is also conceivable that components of the axon initial segment actively promote axonal entry of transport vesicles destined for the axon. Indeed, it has been reported that microtubules and their organization are different in the axon initial segment compared to the cell body and dendrites (Nakata and Hirokawa, 2003). Moreover, ankyrin G has been shown to facilitate the axonal targeting of the Kv3.1b potassium channel (Xu et al., 2007). Regardless of the precise mechanism of cargo-motor complex targeting, the study of Song and colleagues uncovering the formation of a cytoplasmic filter in the axon initial segment during axon development represents an exciting step toward achieving full understanding of how distinct subcellular domains in neurons are maintained.

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Extreme Genome Repair

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DOI 10.1016/j.cell.2009.03.008

Slade et al. (2009) describe in this issue how the genome of the bacterium *Deinococcus radiodurans* gets reassembled after being shattered by high-dose radiation. In contrast to the extreme nature of the damage, the steps of repair appear surprisingly ordinary. So, why can't all organisms carry out extreme genome repair?

If its naming had followed, rather than preceded, molecular analyses of its DNA, the extremophile bacterium *Deinococcus radiodurans* might have been called Lazarus. After shattering of its 3.2 Mb genome into 20–30 kb pieces by desiccation or a high dose of ionizing radiation, *D. radiodurans* miraculously reassembles its genome such that only 3 hr later fully reconstituted nonrearranged chromosomes are present, and the cells carry on, alive as normal. In its ability to repair