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Review

The specific targeting of guidance receptors within neurons: Who directs the directors?

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ABSTRACT

Guidance molecules present in both axonal and dendritic growth cones mediate neuronal responses to extracellular cues thereby ensuring correct neurite pathfinding and development of the nervous system. Little is known though about the mechanisms employed by neurons to deliver these receptors, specifically and efficiently, to the extending growth cone. A deeper understanding of this process is crucial if guidance receptors are to be manipulated to promote nervous system repair. Studies in other polarised cells, notably epithelial, have elucidated fundamental routes to the intracellular segregation of molecules mediated by endosomal pathways. Due to their extreme complexity and specialisation, neurons appear to have built upon these generic systems to evolve sophisticated trafficking networks. A striking feature is the axon initial segment which acts like a valve to tightly regulate the flux of molecules both entering and leaving the axon. Once in the growth cone, further controls operate to enhance the retention or rejection, as appropriate, of membrane receptors. We discuss the current state of knowledge regarding the intracellular trafficking of axon guidance receptors and how this relates to their developmental roles. We highlight the various facets still to be properly elucidated and by building on existing data regarding neuronal polarity and intracellular sorting mechanisms suggest ways to fill these gaps.

Introduction

Success is frequently fortuitous, a case of being in the right place at the right time. Indeed, cellular processes may even have evolved to exploit the random noise inherent in biochemical systems; yet not everything can be left to the vagaries of chance. For a cell to function properly it is imperative that, even within just the plasma membrane, certain proteins are restricted to specific compartments. Many of the body's cells are polarised and much of our knowledge of how this arises derives from studies of the apical and basolateral division of epithelial cells extrapolated to other cell types (Folsch, 2008). In nerve cells this is taken to an extreme. Electrical signals are received and integrated by the branched dendritic processes, relayed to the cell body and an appropriate output is then conveyed down the axon. Such specialisation manifests itself physically in the sinuous, ramified forms of axons and dendrites and reflects a high degree of molecular compartmentalisation. The correct ion channels and signalling proteins must be localised to the corresponding part of the neuron to translate form into function (Horton and Ehlers, 2003) but segregation of receptors is not solely an issue for mature neurons. In order to form the correct circuits, embryonic neurons must carefully regulate the enrichment of guidance receptors in their nascent axons. How axons and dendrites grow out in the right direction to find the appropriate synaptic target among a myriad of others has been a topic of intense empirical and theoretical investigation (Chilton, 2006; Kim and Chiba, 2004; Mortimer et al., 2008). Irrespective of the exact nature by which navigational cues are interpreted, it is clear that for correct axon pathfinding to occur, expression of guidance molecules must be under tight spatiotemporal control to ensure they are expressed on the right cell at the right time and place. Although thoroughly investigated at a population level, e.g. an entire nerve fascicle, this is often overlooked in the context of individual cells. Somewhere between examining initial axon specification and later the synaptic targeting of proteins in the mature neuron, the question of how axon guidance molecules reach (and are retained) in the growth cone is often neglected such that the underlying mechanisms remain essentially unknown.

For some of the major classes of axon guidance receptor these trafficking systems are beginning to be uncovered. However the available data are fragmentary with different parts of the process and different mechanisms being revealed for different proteins. Indeed, this may be of fundamental importance, a facet of how the growth cone produces a fine-tuned response rather than a generic collapse or outgrowth. For instance, a receptor that is rapidly removed from the growth cone after ligand binding may produce a transient stalling whereas one that is tightly embedded in the membrane could act for longer and induce a significant retraction. Nevertheless, there is no satisfactory explanation of how a given

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guidance receptor is both targeted to the growth cone and retained once there. We will begin by describing what is already known about the trafficking of axon guidance receptors to their site of action, using the examples of different proteins to highlight various parts of the process. We then speculate upon how these gaps in our knowledge may be filled by building upon what is already known about the development of neuronal polarity and its links to protein sorting within the neuron.

Identifying the molecules responsible for guiding growing axons to their target is only the first step; it is crucial to know how they are localised within the neuron itself. There is little point in loading neurons with guidance receptors if they are unable to leave the cell body. This is especially true of injured or diseased axons in which axonal transport is compromised or is even the root cause of the degeneration (Duncan and Goldstein, 2006).

Robo receptor targeting — switching responses

The best demonstration of both the importance of receptor trafficking for mediating axon guidance and the complexities yet to be unravelled is the Robo receptor. This system is a striking example of the need for the growth cone to change its response as it grows toward, through and beyond a guidance cue and the fundamental role played by the surface expression of receptors and their associated signalling components. In both vertebrates and invertebrates, surface expression of the Robo receptor on axons of longitudinally projecting neurons and on pre-crossed commissural ones prevents them approaching the embryonic midline. Robo is the receptor for the chemorepellent protein Slit which emanates from the midline (Brose et al., 1999; Kidd et al., 1999). Downregulation of Robo on commissural axons during midline crossing abrogates the repulsive effect of Slit so that a contralateral projection can be formed. Following crossing, Robo is then restored to the axonal growth cone; this is thought – though not proven – to prevent re-crossing (Dickson and Gilestro, 2006; Tamada et al., 2008). The specific mode of action of Robo at each stage is still to be clarified however mechanisms clearly exist to regulate its surface expression with high spatiotemporal specificity in flies, mice and humans (Jen et al., 2004; Kidd et al., 1998; Sabatier et al., 2004).

The rapid change in responsiveness to Robo is best understood in *Drosophila* and is achieved by the action of the *commissureless* (*Comm*) gene product (Tear et al., 1996). In the presence of Comm, Robo is prevented from travelling down the axon due to being conveyed directly from the Golgi apparatus to endosomes and then lysosomes (Keleman et al., 2002, 2005). Using Comm as a filter for the Robo protein is a rapid mechanism to temporarily block the ingress of Robo to the axon. Furthermore, by dragging Robo to its destruction and following it into the lysosomes, the Comm itself is also degraded thereby ensuring that it does not accumulate in the cell and potentially impair the subsequent upregulation of Robo. A striking feature of Robo localisation that profoundly reinforces the need for accurate subcellular targeting of guidance receptors during development is that it is locally removed from the portion of the axon lying across the midline (Kidd et al., 1998). Therefore it is not sufficient for Comm to simply block all access of Robo to the entire axon. As will be described in later sections, evidence emerging from other axon guidance receptors, such as Neuropilins and Ephrins, suggests that they may be transported directly to the growth cone for insertion there and that the limits of their diffusion are then tightly regulated by a combination of endocytic turnover, cytoskeletal tethers and phospholipid anchors. One or more of these mechanisms, acting in response to the Slit signal itself, could then induce or inhibit the enrichment of Robo at discrete domains along the axon. Currently the majority of work examining Comm function in the soma has been carried out either *in vitro* or *in vivo* in peripheral nervous system neurons which do not normally express Comm and may thus be lacking in some of the cellular machinery necessary for its function. High resolution studies using commissural axons will lead to a better understanding of the role of Comm in Robo trafficking.

All searches for vertebrate orthologues of Comm have proved fruitless (Dickson and Gilestro, 2006). Instead it seems that a similar net effect is achieved by the vertebrate Robo3 receptor which prevents Robo1 and 2 from responding to Slit (Sabatier et al., 2004). Mutations in Robo3 cause the human syndrome Horizontal Gaze Palsy with Progressive Scoliosis, in which specific neuronal tracts fail to decussate correctly (Jen et al., 2004), a rare example of a human disorder directly attributable to mutations in an axon guidance receptor. Rather than affecting the intracellular trafficking or surface expression of Robo1 and Robo2, Robo3 appears to interfere directly at the membrane, possibly by forming heteromeric complexes which are unable to transduce the Slit signal (Sabatier et al., 2004). This does not explain how vertebrate Robo1 and Robo2 are absent from the midline portion of commissural axons whereas Robo3 is enriched there. The parallels and divergences between *Drosophila* and vertebrate regulation of Robo localisation are intriguing. On the one hand they may be an example of convergent evolution resulting in homologous wiring of the nervous system using differing tools. On the other, they may reflect opposing ends of the spectrum of mechanisms employed to regulate receptor targeting with such exquisite precision. There may be a vertebrate Comm, albeit with no sequence similarity, which performs an analogous endosomal sorting function. Likewise, other transmembrane receptors could also interact with *Drosophila* Robo receptors to form regulatory heteromeric complexes. Between these processes in the cell body and at the axonal membrane, there probably exist a host of shared mechanisms, such as those described below for Neuropilins and Ephrins, which further refine the subaxonal localisation. This is given further credence by the expanding body of evidence that close association and crosstalk between axon guidance receptors is required to regulate developmental decisions such as midline crossing (Stein and Tessier-Lavigne, 2001; Zou et al., 2000).

Motoring into the axon — transport of transmembrane receptors

Following axonogenesis, it has been proposed that the proximal section of the axon forms a diffusion impermeable barrier termed the axon initial segment (AIS; Winckler et al., 1999). The AIS acts as a barrier through which the mobility of membrane proteins is greatly reduced. It is not a complete block on all membrane diffusion because lipophilic dyes can cross; although single molecule imaging has revealed that the rate of diffusion, even of phospholipid, is reduced up to 800-fold. This barrier increases as the axon develops and is thought to be composed of a fence of transmembrane proteins anchored via proteins such as ankyrin to the actin cytoskeleton (Hedstrom et al., 2008; Nakada et al., 2003; Winckler et al., 1999). Thus the AIS acts like a valve to control the flux of proteins in and out of the axon. They can get in if they are transported up the inside of the axon by vesicles but once inserted into the membrane cannot diffuse back into the soma. These vesicular packages are labelled, during transit through the Golgi apparatus, with tags recognised by a system of molecular motors which pick up their load and travel along microtubules to deliver it to the extremities of the neuron (Fig. 1).

Microtubules have an intrinsic polarity with a minus-end and a plus-end, the latter being where polymerisation of tubulin occurs. In axons, microtubules are lined up with their plus-ends pointing distally whereas in dendrites they display mixed orientation (Baas et al., 1988). This is the starting point of a model of neuronal polarity based upon selective delivery to axons or dendrites by motor proteins (Black and Baas, 1989). The motor proteins move in specific directions with dynein travelling towards the minus-end and kinesin family members (KIFs) usually moving towards the plus-end, although there are some exceptions. As more becomes known about the many different types of KIF motor protein, the more it seems likely that they have a major role in actually organising the underlying axonal cytoskeleton in