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Review

Molecular mechanisms of axon guidance

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Abstract

In order to form a functional nervous system, neurones extend axons, often over long distances, to reach their targets. This process is controlled by extracellular receptors and their ligands, several families of which have been identified. These proteins may act to either repel or attract growth cones and a given receptor may transduce either type of signal, depending on the cellular context. In addition to these archetypal axon guidance molecules, it is becoming apparent that molecules previously known for their role in patterning can also direct axonal outgrowth. The growth cone receptors do not act in isolation and combine with members of the same or other families to produce a graded response or even a complete reversal in its polarity. These signals can be further combined and/or modulated by processing of the molecule both directly at the cell surface and by the network of intracellular signalling pathways which are activated. The result is a sophisticated and dynamic set of cues that enable a growth cone to successfully navigate to its destination, modulating its response to changing environmental cues along its pathway.

Keywords: Axon guidance; Growth cone; Signalling; Ephrin; Semaphorin; Slit; Netrin; Morphogen; Second messenger

Introduction

A little over a hundred years ago, the pioneering neuroanatomist Ramón y Cajal, looking at a histochemical section, observed club-shaped structures at the end of processes emanating from nerve cells. He named them 'growth cones' and made the remarkably prescient observation that these might somehow burrow through the embryo, enabling nerves to connect with distant targets. The motility of growth cones was demonstrated a couple of decades later by Harrison, who grew frog neurones in lymph clots. For a detailed account of the early history of growth cone study, see Gordon-Weeks (2000). Despite advances in culturing neurones, the question remained as to how growth cones could be guided in vivo. In 1963, Sperry proposed a chemoaffinity hypothesis, which has become the basis for many subsequent models of axon guidance. He suggested that growth cones carried molecular tags to direct them to their destinations by responding to gradients of guidance cues, growing up an attractive one or down a repulsive one (Sperry, 1963). The advent of precise methods to label neuronal pathways using vital dyes allowed the mapping of neuronal circuits, and the trajectories taken by individual axons could be traced. Axonal processes were thus revealed to make abrupt changes in direction and to possess remarkable capacities for error correction (Guthrie and Lumsden, 1992; Harris, 1986; Lance-Jones and Landmesser, 1981). Many of the early candidates for axon guidance molecules, such as integrins, fasciclin and neural cell adhesion molecules (NCAMs), generally act in a permissive manner by providing a substrate that promotes outgrowth rather than by actively inducing growth cone turning (Lilienbaum et al., 1995). However, in the late 1980s and early 1990s, a series of genetic and biochemical screens identified proteins acting in an instructive manner which can actively attract or repel axons, and it is on these that this review will principally focus. Outgrowth is controlled by the concerted action upon the growth cone of attractive and repulsive cues working in a contact-dependent fashion or at a distance via secreted factors (Fig. 1). Recent data have revealed that, in certain contexts, molecules regarded as archetypal chemorepellents act attractively and vice versa. In addition to receiving inward signals, the growth cone can also initiate them itself and convey these outwards: it is not simply a passive receptor of instructions. These features, combined with alternative mRNA splicing and post-translational modifications of receptors and their ligands, result in a myriad of subtly

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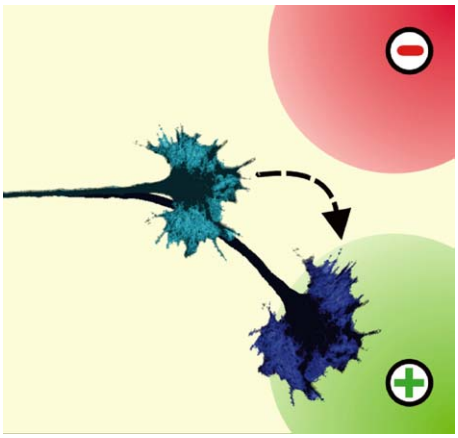

Fig. 1. When a growth cone (light blue) encounters guidance molecules, it extends away from chemorepellents (red) and towards chemoattractants (green). The net effect is to cause a turning of the growth cone (dark blue).

different signals that can be employed to ensure the precise wiring of the nervous system. The resultant molecular cues do not act in isolation but influence each other through interactions at the cell membrane and complicated networks of intracellular signalling cascades.

Fishing for guidance molecules

The following description of four well-characterised families of axon guidance molecule will, in addition to outlining their modes of action, demonstrate the variety of techniques that have been used to identify and characterise them.

Ephrin/Eph

Since the time of Sperry, it had been known that a topographic representation of the chick retina exists in the tectum, mapping the visual field onto a defined neural field. In terms of neuronal projections, this means that retinal ganglion cells (RGCs) from the nasal retina form synapses in the posterior tectum whereas temporal RGCs terminate in the anterior tectum (Fig. 2). Such a stereotyped linkage formed the basis for many theoretical and empirical investigations of axon guidance (Gierer, 1983; Sperry, 1963). In co-culture systems, retinal axons collapse in the presence of membranes derived from the inappropriate half of the tectum, a functional specificity strikingly demonstrated by the stripe assay (Walter et al., 1987). Retinal explants were placed across a series of parallel stripes of anterior and posterior tectal membranes, and emerging axons were thus confronted with the choice of which one to grow along. Temporal axons exhibited a definite preference to grow on the anterior membranes, their natural substrate, and this selectivity diminished as progressively more posterior tectal membranes were encountered. Furthermore, this effect is lost after treatment of the membranes with phosphatidylinositol-specific phospholipase C (PI-PLC), implying that the molecule responsible is linked to the membrane by a glycosylphosphatidylinositol (GPI) anchor. This protein was isolated by comparing the spots present on two-dimensional electrophoresis gels derived from specific regions of the tectum before and after PI-PLC treatment (Drescher et al., 1995). EphrinA5, as it is now known, is indeed expressed in an increasing anteroposterior gradient across the tectum, as is the related gene EphrinA2 (Monschau et al., 1997). Ectopic expression of EphrinA2 in the anterior tectum causes temporal axons to avoid this area (Nakamoto et al., 1996). Conversely, the removal of EphrinA5 in knockout mice leads to temporal axons overshooting into posterior regions (Frisen et al., 1998). EphrinAs are also required for patterning eye-specific projections to the appropriate layers of the lateral geniculate nucleus (LGN) in both mice and ferrets (Huberman et al., 2005; Pfeiffenberger et al., 2005). This is a striking demonstration of the same axon guidance molecules projecting RGC axons to a topographic map in one locality, the superior colliculus, and to discrete layers in another, the LGN. Both Ephrins and their partners, the Eph receptors, are divided into A and B families. EphrinAs have a GPI anchor, whereas EphrinBs are linked to the cell by a transmembrane domain. The EphA and EphB proteins are receptor tyrosine kinases, named based on their preferential binding to the EphrinA and EphrinB family respectively (Pasquale, 2005). Whereas gradients of EphA and EphrinA determine topographic mapping along the anteroposterior tectal axis, EphB and EphrinB gradients control the dorsoventral projection pattern, even acting as chemoattractants via their effect on topographic branching (Hindges et al., 2002; Mann et al., 2002).

The EphB2 null mouse has a diminished anterior commissure; however, this tract is normal in mice in which the EphB kinase domain has been replaced by β-galactosidase,

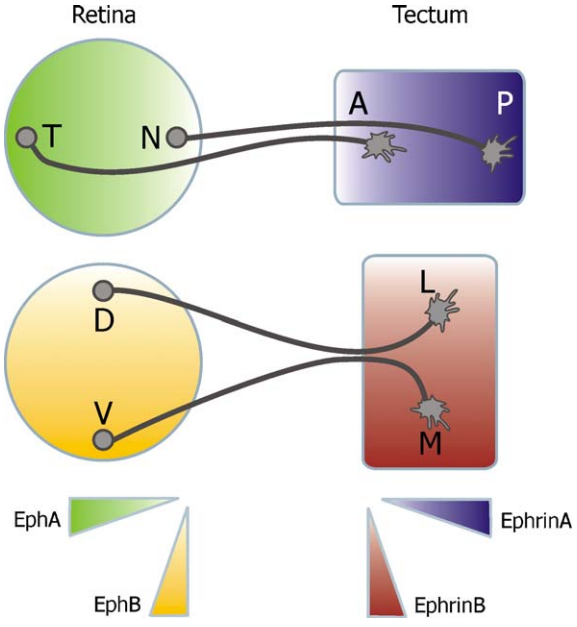

Fig. 2. Topographic maps are set up by opposing gradients of Eph receptors and their Ephrin ligands. Axons from the temporal (T) retina express high levels of EphA (green) and are repelled by the high levels of EphrinA (blue) in the posterior (P) tectum and terminate anteriorly (A). Nasal (N) retinal axons extend into the posterior tectum. However, ventral (V) axons expressing high levels of EphB (yellow) are attracted to the high EphrinB (red) levels in the medial tectum. Dorsal (D) axons terminate in the lateral (L) tectum.