

Lincoln's Inn Fields

Molecular Neuropathobiology

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The morphology and signalling capacity of a neuron is based on the strict polarisation into axonal and dendritic compartments, and is crucial for its function and survival. This unique architecture is ensured by a network of transport pathways, which allow efficient communication over long distances, and regulate the spatio-temporal pattern of receptor stimulation by trophic and morphogenetic factors. Despite its importance for the development, differentiation and maintenance of the nervous system, characterisation of the mechanisms controlling the targeting of specific ligands to axonal transport routes remains in its infancy.

The goal of our laboratory is to build up a functional map of the organelles responsible for the endocytic sorting and axonal transport of neurotrophin receptors and selected virulence factors in neurons. By studying the basic mechanisms controlling neuronal membrane dynamics, we aim to provide new insights into how neurons control the uptake and sorting of ligands in health and disease.

An RNAi screen using neuronally-differentiated embryonic stem cells

The crucial role of axonal transport in cell homeostasis is underscored by the finding that mutations in genes encoding components of this machinery, such as motor complexes, cause neuronal death. Work from our laboratory has established that an atoxic fragment of tetanus toxin (H_C), which is transported in axonal carriers together with neurotrophins and their receptors, is a useful probe to monitor the dynamics of these complexes in neurons. The uptake of H_C occurs via a specialised clathrin-dependent pathway involving a sorting step in which lipid microdomain-associated markers, such as polysialogangliosides, are retained on the plasma membrane. This route is thus a concerted microdomain- and clathrin-dependent endocytosis, two pathways usually viewed as mutually exclusive. Although our studies shed light on the role of the GTPase dynamin and clathrin adaptors in this process, our strategy has been largely based on low-throughput assays. A major obstacle in the implementation of unbiased strategies for gene discovery, such as high-throughput siRNA screenings, to the dissection of this process, is the paucity of cell lines that can be used as valid alternatives to primary neurons. Major advances in the field of embryonic stem (ES) biology have recently lifted this shortcoming by providing protocols for the differentiation of ES cells into specific neuronal subpopulations. We have optimised methods for the differentiation of an ES cell line expressing GFP under the control of a motor neuron (MN) promoter and the isolation of large amounts of ES-derived MNs. These cells are indistinguishable from primary MNs in terms of the expression of specific markers (Figure 1), ligand-specific endocytosis or axonal transport. These features make them a valid alternative to primary neurons and a unique resource for high-throughput screens using siRNA-based approaches. Using a library of siRNA pools targeting genes involved in endocytosis and membrane traffic, and a high-

throughput transfection method, we have performed a screen based on the endocytosis of a fluorescently-labelled H_C and an antibody directed against the extracellular domain of the neurotrophin receptor p75^{NTR}. High content image analysis has been optimised to detect the uptake of the ligands and to identify primary hits that have been subsequently re-screened using independent siRNA pools. Validated genes have been selected for further analyses to investigate the phenotype observed by RNAi. These results will greatly contribute towards building a road map of neurotrophin receptor endocytosis in primary neurons, providing at the same time insights into how a single receptor is able to mediate different signals leading to distinct physiological outputs.

Distinct axonal transport pathways target pathogens and vectors for gene therapy to the central nervous system

Pathogens exploit anterograde and retrograde transport pathways to enter and exit the central nervous system. In addition to the aforementioned studies using the H_C fragment of tetanus toxin, we have recently characterised the internalisation and axonal transport of canine adenovirus serotype 2 (CAV2) and poliovirus, both of which preferentially infects neurons *in vitro* and *in vivo*.

Adenoviruses are widespread human pathogens and have been associated with brain tumours and severe CNS infections. Their relevance in human health and their potential use as vectors for gene therapy makes the understanding of the neuronal trafficking of these viruses very important. Major advantages of CAV2 vectors are their selective MN targeting when injected intramuscularly, and their long-lasting transgene expression *in vivo*. In addition to their potential for addressing fundamental questions in neurobiology, these features make CAV2-based vectors an ideal choice to treat CNS pathologies. In spite of their neurotropism, the trafficking of CAV2 has only been studied in epithelial cells or fibroblasts. We have recently filled this gap, exploring the determinants for CAV2 sorting and transport in motor and sensory neurons. CAV2 binding to axons was strictly dependent on the presence of its receptor CAR. Using fluorescently-labelled CAV2, we found that the virus moves bidirectionally in axons, with a bias for the retrograde direction. In contrast to the targeting observed in fibroblasts, the majority of CAV2 transport occurs in Rab7-positive compartments that display a neutral pH. CAR was found associated with these axonal organelles, which also contain other cargoes as diverse as H_C and neurotrophin receptors. These results suggest that a single axonal carrier is capable of transporting cargoes targeting different membrane compartments in the soma. Moreover, our data suggest that CAR is a component of axonal carriers and that it promotes the entry of CAV2 into this compartment.

In an independent investigation, we also examined the

trafficking of human poliovirus (PV) in neurons expressing its human receptor hPVR. Retrograde axonal transport is required for PV dissemination through the sciatic nerve of mice expressing hPVR, and for the appearance of a paralytic syndrome recapitulating the landmarks of the human poliomyelitis. Surprisingly, the axonal transport of PV was not completely abolished in the absence of hPVR, indicating that several different pathways for PV endocytosis axonal transport exist *in vivo* and *in vitro*. Altogether, our data demonstrates that distinct receptor-mediated endocytic events determine the sorting of diverse cargoes to non-degradative organelles, which are then recruited to long-range retrograde transport routes, in a process that allows endogenous ligands, pathogens and virulence factors to reach the CNS.

Publications listed on page 126

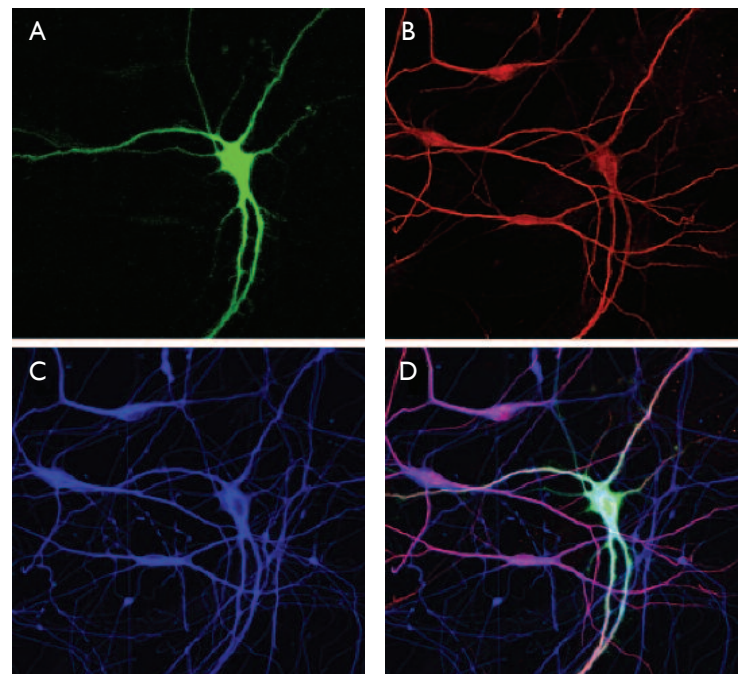


Figure 1. Differentiation of mouse ES cells into MNs. An optimised protocol allows the differentiation of primary MNs from a mouse ES cell line. MN specification is determined by the addition of retinoic acid and sonic hedgehog, whilst MN maturation is achieved in the presence of neurotrophins. ES cell-derived MNs are characteris