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**FINAL SUMMARY REPORT FOR PIEF-GA-2011-300945 HEALTHYMYELIN**

**Summary of the Project Objectives**

Myelin sheath thickness and compact myelin depositions are affected in numerous peripheral neuropathies indicating that controlled growth of the myelin sheath is crucial during myelination and myelin maintenance. The diameter, length and organisation of the myelin sheath are important determinants of nerve conduction velocity and myelin maintenance, but the basic molecular mechanisms that contribute to longitudinal extension of the myelin sheath have not been defined.

The polarity protein Crumbs3 (Crb3) is expressed exclusively in the SC microvilli that surround the node and as such is ideally placed to play a role in the regulation of myelin extension. So the objective of this project was to examine the role of Crb3 in myelin formation, extension and node of Ranvier formation using targeted silencing or overexpression of Crb3, and its potentially interacting proteins, in mouse Schwann cells. **Objective 1- Crb3 in Schwann cell myelination, extension and in the formation of the node of Ranvier: 1-** Characterize the role of Crb3 in mSC elongation.  **2-** Characterize the molecular complex associated with Crb3 in microvilli and describe the molecular mechanisms involved. **3-** Investigate the role of Crb3 and its complex in the formation of the node of Ranvier. Work conducted in the first year of this grant funding period uncovered a previously unknown link between a tumour suppressor pathway, Hippo, and myelination. Therefore the second objective was modified (as outlined in the mid-term report) to **Objective 2- Crb3/HPO/YAP in myelination: 1-** Generation of viral constructs to target HPO pathway components and YAP. **2-**Visualise the consequence of manipulating HPO/YAP on myelination and **3-** Investigate the impact of HPO/YAP pathway in a model of peripheral neuropathy using a mouse model of human muscular dystrophy.

 **Summary of Results**

**Objective 1:** By targeting Crb3 we have uncovered the existence of a pathway that negatively regulates myelin sheath elongation, an entirely new concept in the field of myelin research. Furthermore, by identifying the pathway involved in the negative regulation of myelin elongation, the Hippo/YAP pathway, we have identified a new pathway regulating myelination. We used varied techniques to examine, on a cell by cell basis *in vivo*, the consequences of manipulating elements of this pathway, finding that modulation of YAP, via Crb3, provides a unique tool to control the quantity and location of myelin deposition in peripheral nerves.

**Objective 2:** By examining the role of the Crb3/Hippo/YAP pathway in myelinating Schwann cells we have greatly extended our understanding of how the mSC and axon are intimately linked. We demonstrate, for the first time, the link between axonal stretch (as takes place during postnatal development) and the activation of YAP in SCs. This work enabled us to propose a model describing how the myelin segments on an axon form harmoniously in concert with the growing nerve (Figure 1). Furthermore we demonstrated that a defect in the Hippo/YAP pathway underlies the phenotype described in a mouse model of human muscular dystrophy. We successfully applied our proposed model to the problem of shortened internode myelin length in these dystrophic mice and were able to significantly correct the observed deficit to recover myelin elongation, via Crb3.

**Figure 1. Schematic illustrating the model of myelin elongation in mSC.** Top: A myelinating Schwann cell is exposed to input (green arrow) from the stretching axon in the elongating nerve which stimulates the translocation of YAP to the nucleus (green dotted line). YAP nuclear activity promotes myelin gene expression (blue arrow), which increases myelination and myelin elongation (blue dotted arrows). YAP nuclear translocation is negatively regulated by Crb3/Hippo pathway signaling originating from the cells extremities (long red arrow) which phosphorylates YAP, sequestering it in the cytoplasm which consequently limits myelin elongation (short red line). Bottom: the combination of signals promoting YAP activity (green arrows) and inhibiting it (red lines) allows the harmonious growth (blue dotted lines) of all myelin segments along the axon.

**Final Results and Impact of Completed Project**

The final results of this project is the generation of a model proposing the mechanisms and pathways underlying myelin sheath elongation along the nerve, in concert with limb elongation and it’s neighbouring Schwann cells (see Figure 1). The impact of this work is three fold. Firstly the understanding of myelin elongation in itself is an entirely new concept in the field of myelin research and this has implications for the understanding of how myelin is formed during postnatal development. Secondly, the description of the activity of the HPO/YAP pathway is an entirely novel finding and whose impact is unlikely to be limited to just myelin elongation. Finally we demonstrate that the Crb3/HPO/YAP pathway can be engaged to reverse the peripheral neuropathy phenotype of short internodes in a mouse model of human muscular dystrophy. This raises the possibility that this pathway can be engaged for therapeutic ends in other peripheral neuropathies.

The manuscript outlining these results, titled ‘Yap transcription cofactor controls myelin sheath geometry in healthy and diseased peripheral nerves’ has received favourable reviewer comments from *Nature Neuroscience*. These comments are currently being addressed and an the manuscript will be resubmitted to *Nature Neuroscience* in the first half of 2015. This work has also spawned new projects and avenues of research within the host research group including the testing of drugs to target Crb3/Hippo/YAP pathway and the potential role of YAP in myelination in the central nervous system (CNS).

**Socio-economic impact**

Peripheral neuropathies are a heterogeneous collection of hereditary and acquired disorders that are characterized by peripheral nerve abnormalities. They are common, with a population prevalence of around 2400 per 100 000 (2.4%) up to 8% in the elderly population. Due to the relative simplicity of the PNS composition and its easy access makes this system particularly advantageous to test and develop new biotherapies. In addition PNS diseases are relatively well defined. Together this suggests that new cell and gene therapies are likely to emerge in this field. But in order for this to happen it is imperative to characterise and define the basic cellular mechanisms involved. The work conducted during this project identifies a completely new pathway controlling myelination. In doing so, we have uncovered previously unknown targets and their function in the peripheral myelination, which can now be investigated for their therapeutic potential.