

● PERSPECTIVE

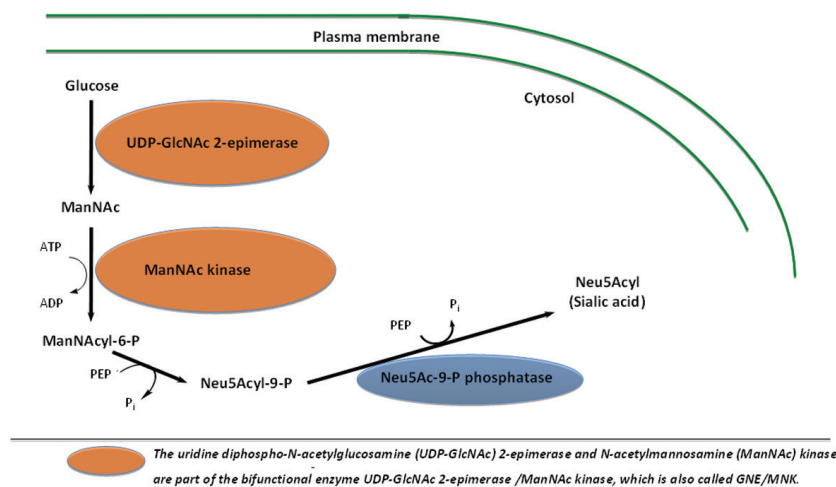
## N-Propionylmannosamine: using biochemical glycoengineering to promote peripheral nerve regeneration

The peripheral nervous system, in contrast to the central nervous system, is capable of spontaneous regeneration. However, nerve reconstruction in the peripheral nervous system remains a major challenge, as the functional outcomes following nerve repair are variable. Quantitative parameters such as the number of regenerating axons and degree of myelination are crucial, but correct axon target organ allocation, time to regeneration and target organ quality are also equally important.

After nerve transection, the distal part of the nerve undergoes Wallerian degeneration, while the Schwann cells change from a myelinating to a regenerating phenotype and form bands of Büngner. In doing so, they determine the conditions for the elongating axons to reach a target organ. On the other side, the injured nerve also goes into regeneration mode by activating regeneration-associated genes and elongating the transected axon until it reaches a target organ. This is a complex, time-consuming process that presents the regenerating axon with several obstacles. Crossing the coaptation site is one

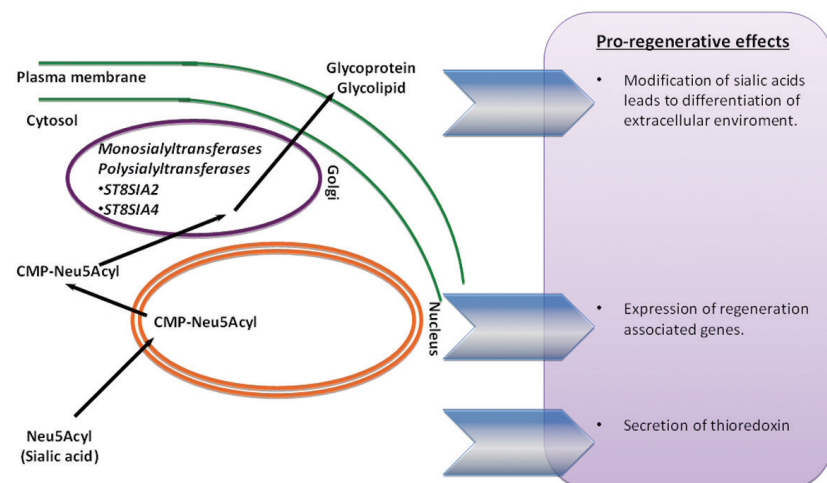
of the first. Once it has done this, the regenerating axon has to decide which Schwann cell tube it will enter to reach a target organ. Several different behavioral patterns have been observed here. Some axons penetrate the distal nerve segment as a single axon, mainly *via* a lateral movement. Others arborize into several branches that enter separate Schwann cell tubes and elongate towards the target organ. In our laboratory, we found that single axons crossing the site can reach as many as 142 Schwann cell tubes after 7 days, whereas arborizing axons can reach up to 68 Schwann cell tubes in the same period (Witzel et al., 2005). It is of note that axon branches are internal competitors for the supply of energy and structural materials, and external competitors for growth factors and Schwann cell tubes. According to our unpublished data, the mean number of branches per arborizing axon is limited to an average of one to two. This internal and external competition might be a regulation process designed to single out the stable branches (Smalheiser and Crain, 1984). Thus, misdirected branches are eventually pruned back so as to achieve specificity of regeneration (Brushart et al., 1998).

Sialic acid of glycoproteins and gangliosides plays an integral role in the development and regeneration of the nervous system, as well as in neural plasticity. Polysialylation of the neural cell adhesion molecule (NCAM) is an important posttranslational modification that is crucial to the development of the nervous system. Polysialylation of NCAM decreases during adulthood, but increases again after nerve injury and thus contributes to



**Figure 1 Biosynthesis of N-acetylneuraminic acid.**

The natural precursor of sialic acid, also known as N-acetylneuraminic acid (Neu5Ac), is N-acetylmannosamine (ManNAc). It is metabolized in the cytosol in several stages by the uridinediphospho-N-acetylglucosamine (UDP-GlcNAc) 2-epimerase and ManNAc kinase to Neu5Ac. The UDP-GlcNAc-2-epimerase and ManNAc kinase form the bifunctional enzyme UDP-GlcNAc-2-epimerase / ManNAc kinase (GNE/MNK). The result of this multi-stage procedure is Neu5Ac.



**Figure 2 Neuraminic acid and nerve regeneration.**

After biosynthesis of Neu5Ac, as described in Figure 1, Neu5Ac is activated in the nucleus by the cytidine monophosphate-N-acetylneuraminic acid hydroxylase to cyclic monophosphate N-acetylneuraminic acid (CMP-Neu5Ac). Hereafter, the activated CMP-Neu5Ac is used at the Golgi apparatus for posttranslational modification in the form of polysialylation of proteins to glycoproteins, and of lipids to gangliosides.

regeneration success.

Several therapeutic strategies show promise for promoting the quantity, quality, and duration of peripheral nerve regeneration. The newly described metabolic glycoengineering (MGE) seems capable of stimulating nerve regeneration. MGE involves the metabolic modification of the N-acetyl side chain of N-acetylneuraminic acid (= sialic acid, Sia). The modification of Sia is achieved *via* a simple biochemical procedure using new unphysiological precursors of Sia. Whereas the N-acetyl group of N-acetylneuraminic acid originates from the physiological precursor N-acetylmannosamine, slight modifications of N-acetylneuraminic acid are obtained *via* the use of analogs of N-acetylmannosamine with elongated N-acyl side chains. In the simplest manner, it is elongated by one or more methylene groups, leading to N-propionyl derivatives, N-butanoyl derivatives or an azido group leading to N-azido Sia. The promiscuity of the enzymes that catalyze the biosynthesis of sialic acid is essential to the success of this new biochemical method; most modifications of the N-acyl side chain are tolerated (Keppler et al., 2001) (Figure 1). The biosynthetic pathway of polysialic acid and posttranscriptional modification of proteins and lipids to glycoproteins and glycolipids continues from the cytosol to the nucleus and then to the Golgi apparatus, as shown in Figure 2. The enzymes that perform polysialylation of specific glycoproteins are the two polysialyltransferases ST8SIA2 and ST8SIA4.

The enzymes' ability to incorporate unnatural precursors during Sia biosynthesis creates new scope for engineering N-acetylneuraminic acid to N-acylneuraminic acid *in vitro* and *in vivo* (Buttner et al., 2002). This biochemical glycoengineering has revealed unexpected biological characteristics of Sia. N-Propionylmannosamine (ManNProp), one unnatural precursor, is metabolized to the new N-propionylneuraminic acid (Neu5Prop) and replaces partially physiological N-acetylneuraminic acid (Keppler et al., 2001). Recent *in vitro* results showed that feeding PC12 cells with ManNProp resulted in increased neurite outgrowth, and that feeding organotypical *in vitro* co-cultures with ManNProp accelerated the reestablishment of functional synapses (Buttner et al., 2002). We recently found that a systemic application of ManNProp increased the distance of axonal elongation and the number of arborizing axons in a murine injury model (Witzel et al., 2015 in press). We also showed that the polysialyltransferase ST8SiaII is substantially involved in stimulating axonal elongation and arborization. Using nerve grafts from ST8SiaII<sup>-/-</sup> knockout mice in a sciatic nerve transection and transplantation mouse model, we showed that the absence of the polysialyltransferase ST8SiaII significantly reduced the regeneration distance and number of arborizing axons during early peripheral nerve regeneration (Koulaxouzidis et al., 2015). The presence of ST8SiaII in the distal nerve graft appears to be a pivotal factor in axonal extension and arborization induced by ManNProp.

Others have shown that a deficiency of ST8SiaII reduced the number and size of regenerating fibers without impairing remyelination. PolySia was up-regulated by the polysialyltransferases ST8SiaII and ST8SiaIV. The cellular localization of polySia seems to be crucial for peripheral nerve regeneration (Jungnickel et al., 2009).

However, several mechanisms appear to be involved in the beneficial effect that ManNProp has on nerve regeneration. Firstly, the incorporation of N-propionylneuraminic acid (Neu5Prop) and partial replacement of N-acetylneuraminic acid modulates the glycan structure of glycoproteins (such as NCAM) and gangliosides (Buttner et al., 2002; Franz et al., 2005). This is followed by differentiation of the extracellular

environment and might be a further interface between regenerating axons and their environment (Brushart et al., 1998). Secondly, the resulting unnatural sialic acids lead to expression genes involved in cell differentiation, such as the transcription factors c-Jun and TOAD-64/Ulip/CRMP. These stimulate the phosphorylation of erk1/2 within the nucleus, a process that enhances the activation of regeneration-associated genes (Kontou et al., 2009; Horstkorte et al., 2010). Thirdly, ManNProp stimulates the secretion of thioredoxin, a small protein that promotes neurite outgrowth and has neuroprotective and neurotrophic effects (Horstkorte et al., 2010) (Figure 2).

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