In the adult mammalian spinal cord, nerve tracts do not regenerate following injury. The inability to re-establish functional connections is caused by both the lack of a sufficient growth response in central nervous system (CNS) neurons and by the formation of a neural scar at the lesion site that forms a major obstacle for regrowing axons. In contrast to the regenerative failure of the spinal cord, the primary olfactory nervous system is able to recover from injury. Following a lesion to the neuroepithelium, primary olfactory neurons project new axons towards the olfactory bulb to reconnect with CNS neurons in the glomeruli. The regenerative potential of the olfactory system is based on two unique characteristics. First, primary olfactory neurons are continuously replaced by new neurons that originate from stem cells located at the basal part of the epithelium. Neurogenesis is enhanced after the massive loss of olfactory neurons as a result of injury. Second, the olfactory axons are enfolded by olfactory ensheathing glia (OEG), which play an important role in providing a growth-supportive environment for the axons. OEG in the olfactory nerve layer (ONL) facilitate the entrance of axons into the CNS and contribute to axonal outgrowth and targeting. Since OEG are believed to play a significant role in the successful regeneration of the olfactory system, several studies focused on the potential of OEG to promote regeneration in the injured spinal cord as well. OEG transplantation studies in various experimental spinal cord injury models indeed resulted in increased axonal sparing and sprouting and an improved functional outcome. Furthermore, OEG reduced the formation of cavities and they intermingled with astrocytes (ACs) at the lesion site. This is a pronounced difference with Schwann cells (SCs), which induce increased hypertrophy of ACs. In Chapter 1, the function of OEG in the regeneration of the olfactory system as well as the effects of OEG after transplantation in the spinal cord are reviewed extensively.

Several growth-promoting molecules and extracellular matrix molecules have been identified that are probably involved in the neuroregeneration-promoting properties of OEG. To identify novel molecular mechanisms that are involved in the neuroregeneration-supporting effects of OEG, we have first studied the transcriptional changes in OEG following an injury to
the olfactory system as well as transcriptional differences between cultured OEG, cultured SCs and native OEG (directly isolated from the ONL). Second, we have undertaken a medium-throughput screening approach to functionally validate identified target molecules. The different steps in the target discovery and validation process are schematically summarized in the upper part of Fig. 1.

In the ONL, we analyzed gene expression profiles following a lesion of the neuroepithelium (Chapter 2). Functional data mining revealed that the main biological processes, overrepresented within the differentially expressed genes, were immune response and cholesterol biosynthesis. A pathway analysis of the genes revealed the coordinated expression of genes involved in complement system activation and phagocytosis of cellular debris. This suggested that OEG in the ONL contribute to the regeneration process by removing axon debris and thereby clearing the pathway for new axons. The downregulation of many cholesterol biosynthetic enzymes and concomitant upregulation of genes involved in cholesterol efflux indicated local cholesterol recycling as part of the phagocytosis process. Cholesterol may be transported out of the cell where it is incorporated in lipoproteins and taken up by nearby axons, which are not able to synthesize cholesterol themselves. In addition, several extracellular matrix (ECM) and cell adhesion molecules showed a characteristic profile of downregulation followed by upregulation in time, which may represent the initial loss of axonal contact and the subsequent synthesis of new ECM to support the growth of new axons. Some ECM-associated genes are associated with the invasive potential of tumors, which suggested that the creation of a permissive environment for axonal outgrowth may be similar to the creation of a permissive environment for tumor invasion. Most of the differentially expressed ECM and cell adhesion molecules have not been reported before in relation to regeneration of the olfactory system and these proteins may represent novel molecules involved in the axonal outgrowth-supporting properties of OEG.

In addition to OEG in their natural environment, we studied the gene expression profile of cultured OEG just before they would be transplanted in the spinal cord. The transcriptome of
cultured OEG was compared to the transcriptome of cultured SCs and to native OEG, obtained from the intact ONL (Chapter 3). GO overrepresentation analysis showed that GO-classes related to tissue repair, such as ‘response to wounding’, ‘blood vessel development’, ‘cell adhesion’ and GO-classes associated with the ECM were overrepresented within the differentially expressed genes of both comparisons. A comprehensive literature study of the genes belonging to these GO-classes indicated that cultured OEG have tissue repair properties that are distinct from SCs and from native OEG. With respect to the effects of OEG and SCs after transplantation in the spinal cord, it is important to know the interaction behavior of both cell types with cells from the neural scar. The neural scar consists mainly of ACs but, when the injury causes a rupture of the meninges, proliferating meningeal cells invade the lesion site and form an important component of the neural scar as well. As mentioned before, whereas OEG and ACs are able to intermingle, SCs and ACs form separate territories upon cellular contact and ACs become highly hypertrophic when in contact with SCs. Little is known about the interaction effects of OEG and SCs with MCs and we therefore studied the interaction behavior of OEG and SCs with MCs (Chapter 4). OEG intermingled with MCs in cocultures, comparable to the intermingling behavior of OEG with ACs. However, in cocultures of SCs and MCs, SCs aggregated and eventually formed dense clusters. Our data suggested that both secreted factor(s) as well as cellular contact play a role in MC-induced cluster formation of SCs.

The ultimate aim of this thesis was to discover novel molecules that play a role in the neuroregeneration-promoting properties of OEG (Chapter 5). We focused particularly on two specific properties of OEG, namely their neurite outgrowth-promoting properties and their intermingling properties with MCs as compared to the cluster formation of SCs. We selected 178 target genes from the two microarray experiments on OEG in the ONL following a lesion and on cultured OEG that potentially played a role in stimulating neurite outgrowth or in the different interaction behavior of OEG and SCs with MCs. The cocultures, as described in chapter 4, were scaled down and used as a bioassay to measure cluster formation. In addition, an outgrowth
bioassay was established consisting of dissociated DRG neurons plated on OEG. The expression of an initial set of 60 genes was silenced by siRNA-mediated gene knockdown in one or both bioassays to study the role of each individual gene in the outgrowth-promoting properties of OEG or in the interaction behavior of either OEG or SCs with MCs. We identified 7 genes that showed an effect in the outgrowth assay and 2 genes that showed an effect in the SC-cluster assay. These genes are potential novel molecules involved in the molecular mechanisms underlying neurite outgrowth stimulation by OEG and the different interaction behavior of OEG and SCs with MCs.