Summary

Parkinson's disease (PD) is a progressive neurodegenerative movement disorder. Neuropathological changes in sporadic PD involve multiple neuronal systems (Braak et al., 2003). Neurodegeneration of dopaminergic (DAergic) neurons in the substantia nigra (SN) is one of the most widely studied neuropathological hallmarks of the disease, as it leads to the development of the clinical motor symptoms. Only 5% of the PD cases are familial, while the rest of the patients suffer from a sporadic form of the disease for which the causes are largely unknown (Dauer and Przedborski, 2003). The current treatments, both pharmacological and surgical, diminish the symptoms of both familial and sporadic forms of the disease, but merely temporarily improve the quality of life of the patients (reviewed in Olanow et al., 2009). As of yet, no genuine treatments ceasing the progression or curing the disease are available, although currently cell replacement and gene therapy approaches are being investigated. These approaches aim to either replace the damaged cells or prevent neuronal degeneration by stimulating regenerative and neuro-protective mechanisms in the affected tissue (reviewed in Chapter 1).

Mitochondrial activity, protein aggregation and the oxidative stress response have been linked to the development of PD based on mutations found in familial forms of the disease (reviewed in Hardy et al., 2006; Bonifati, 2007; Maguire-Zeiss et al., 2008; Hardy, 2010). However, for sporadic PD the specific molecular alterations leading to the typical neuropathological changes in the SN still need to be elucidated. The etiology of the disease appears to be multifactorial, involving both biological and environmental components (Dauer and Przedborski, 2003; Olanow et al., 2009). More research is required into the basic cellular and molecular changes that occur in the brains of sporadic PD patients.

The work presented in this thesis is based upon a gene expression study identifying 287 genes differentially expressed in the SN of PD patients compared to matched controls (Bossers et al., 2009). Since the tissue used for this study was from end stage PD patients these gene expression changes may be either causal to the development and/or progression of sporadic PD, or may be the consequence of the disease process. The main challenge of the research described in this present thesis was therefore to translate the alternations in gene expression into concrete biological mechanisms that may underlie the degeneration of DAergic neurons in the SN of PD patients, with a potential for development of novel therapeutic targets.

The first step to identify the key players in the neurodegenerative process of PD was the selection of target genes (from the total set of 287 genes) that are potentially involved in this process based on Ingenuity pathway analysis, gene ontology analysis, and literature search (Figure 1, Chapter 2). We based our gene selection on the potential roles that these genes play in cell death, axon guidance, neurotrophic support, synaptic transmission, mitochondrial function...
and cellular metabolism. This approach resulted in the identification of 79 genes that are linked to one or more of these processes and may therefore be specifically involved in the development of PD neuropathology.

The second stage was to 1) identify the cell type in which these dysregulated genes are expressed in the human brain, and determine whether their cellular localization changes during the disease process (Chapter 2), and 2) establish a suitable cell model to study target gene function (Chapter 3). Cellular localization studies of 34 target genes, using in situ hybridization and immunohistochemistry, revealed that these genes are almost exclusively expressed in neurons in the SN, and not in reactive astrocytes or activated microglia (Figure 1, Chapter 2). The neuronal localization of the majority of the target genes motivated us to use retinoic acid (RA) differentiated SH-SY5Y neuroblastoma cells as a cellular platform for functional high content screens (HCS). Genome-wide transcriptional profiling combined with gene ontology and pathway analysis demonstrated that these cells 1) contain the main cellular and molecular properties that are characteristic of DAergic cells, 2) are sensitive to environmental factors that are known to contribute to PD, and 3) are readily available and can be easily expanded in culture (Chapter 3).

The SH-SY5Y cellular model was used to functionally validate the role of 62 target genes in cell viability, neurite outgrowth and mitochondrial activity in HCS (Figure 1, Chapter 4). This integrative approach, using siRNA mediated knockdown and LV-mediated overexpression, identified 12 genes significantly affecting one or more of these parameters. The most dramatic effects on both cellular viability and neurite outgrowth were observed after overexpression of 4 genes that are also highly upregulated in PD SN neurons: CTDSP1, RGMA, PTMA and WWC1.

As a first step to study the role of one of these target genes in vivo, we overexpressed RGMA in the mouse SN (Figure 1, Chapter 7). In order to do this, we first performed a comparative study of four adeno-associated viral (AAV) vector serotypes and found that AAV7 is the best tool to use for targeted gene delivery to the mouse SN (Chapter 6). Overexpression of RGMA in the SN of mice appeared to induce degeneration of DAergic neurons, behavioral abnormalities that are typical for the loss of striatal DA input, and microglia and astroglial activation in the SN. Below we will discuss how this result may enhance our understanding of development of PD pathology.

In the future, we would also like to examine the other three candidate gene targets (CTDSP1, PTMA and WWC1) in vivo and additionally test their function in a mouse model that mimics the early stages of PD based on a chronic low dose MPTP treatment (Chapter 5).
Figure 1. The experimental approach: PD genes were selected that may play a key role in the neurodegenerative process of PD. The subsequent steps that were undertaken to select the most promising target genes to be tested in vivo are indicated.