SUMMARY

The lack of germline-competent ES cells makes gene inactivation through homologous recombination impossible in the rat. The main goal of this thesis was to solve this problem by establishing a new gene down regulation technology using shRNA, a natural phenomenon and a powerful tool for gene knockdown in mammalian cells and mice. Three DNA constructs harbouring hairpin cassettes against the EGFP gene and controlled by the U6 promoter were generated. After standard pronuclear microinjection into zygotes of EGFP transgenic rats more than three hundred rats were born. Surprisingly, only one animal was found to carry the construct integrated into the genome. No GFP silencing was observed in this transgenic founder nor was any shRNA expression detectable. Even the use of BAC constructs based on the mouse *Rosa26* locus to achieve single transgene integration did not result in successful generation of shRNA transgenic rats.

Suspecting embryo-toxicity by abundant production of shRNA molecules an *in vitro* study was carried out. By assaying embryonic development and survival rate of rat and mouse embryos after introduction of a hairpin construct it was shown that shRNA expression does not disturb embryonic development at early preimplantation stages. Consequently, the problem may appear later during pregnancy.

Since pronuclear microinjection of ubiquitously and permanently active shRNA constructs does not work in the rat as shown in this thesis, a novel strategy to achieve conditional gene knockdown was tested. For this purpose, a tetracycline activatable system was used to control the expression of shRNA against the insulin receptor (InsR), resulting in an inducible model of insulin resistance. Consequently, an inducible and chronic model of type II diabetes including several hallmarks of this disease was established.

In addition, a novel inducible expression system for shRNA was developed using a ligand-dependent chimeric transcription factor. As demonstrated in cell culture, a mutated TATA-binding protein (mTBP) activates a U6 promoter with a corresponding TATA box mutation. The fusion of a mutated ligand binding domain of an estrogen receptor to the mTBP leads to a chimeric protein. The translocation of the fusion protein into the nucleus should depend on the presence of tamoxifen. However, this ligand-inducibility of the mTBP could not be achieved in this thesis.

In conclusion, with the use of the doxycycline inducible shRNA expression system, time and/or dose dependent gene inhibition in rats becomes possible. This flexible gene manipulation strategy in the preferred animal model for physiopathological studies may provide a powerful tool for the understanding of gene function.