Summary

The mammalian pancreas is comprised of several cell populations, the exocrine cells, which are organized into acini, the endocrine cells, which form the islets of Langerhans, as well as the ductal cells, endothelial cells, and neurons. A key function of the endocrine pancreas is the control of blood glucose homeostasis. Loss or defects of the insulin-producing ß-cells in the pancreas lead to the pathological condition *diabetes mellitus*. One possible therapy for *diabetes mellitus* is the development of a culture system to generate replacement ß-cells *in vitro*. However, to develop such replacement therapy, we first need to identify the factors, which control ß-cell differentiation. It has been shown that several classes of tissue restricted transcription factors have crucial functions in the pancreatic endocrine cell differentiation. HMG box proteins are a class of transcription factors, whose function has not yet been explored in the pancreas. The overall goal of this research project was to analyze the expression of HMG-domain transcription factors in the developing mouse pancreas, and to study their function in pancreas development.

The HMG box class contains two transcription factor gene families, the *Sox* and *Tcf/Lef* transcription factors. In mammals, the *Sox* family of HMG box transcription factors is comprised of twenty members, which are classified into nine groups on the basis of sequence similarity and genomic organization. Sox transcription factors have been shown to control the development of numerous tissues and cell types during embryogenesis. However, little is known about their expression and function in the pancreas. One goal of this research project was to characterize the expression and function of Sox genes in the mouse pancreas. Expression of thirteen different Sox genes, which belong to groups C, D, E, F, G and H, was found in the developing pancreas or in adult endocrine islets. Subsequently, the expression patterns of seven Sox genes (*Sox4, Sox11, Sox5, Sox13, Sox8, Sox9, Sox10*) were analyzed in detail by *in situ* hybridization at several stages of pancreas development. Sox transcription factors were detected in pancreas from as early as E9.5 to adulthood. In the pancreatic epithelium, different Sox genes were often expressed in overlapping domains, suggesting that there may be functional redundancy. To study the function of Sox genes in pancreas development, two *Sox* mutant mouse strains, *Sox8* and *Sox10* mutant mice, were analyzed for defects in overall pancreatic morphology and in the expression of different cell lineage markers. Neither homozygous *Sox8* nor *Sox10* mutant mice displayed any defects in

pancreatic endocrine or exocrine differentiation, suggesting that both *Sox8* and *Sox10* are dispensable for endocrine and exocrine pancreas development. However, in *Sox10*-/- mice, Schwann cells, which are the islet-sheathing glial cells in the pancreas, were completely absent from the neonatal pancreas. Since endocrine or exocrine development was not affected in *Sox10*-/- mice, this finding suggests that pancreatic Schwann cells are not required for endocrine and exocrine differentiation. In summary, this novel information on the expression of Sox transcription factors in the embryonic and adult mouse pancreas will be the necessary basis for studying *Sox* gene functions in the pancreas.

In the second part of this research project, the role of TCF/LEF transcription factors in murine pancreas development was explored. TCF/LEF proteins are downstream effectors of the canonical Wnt signaling pathway. The canonical Wnt signaling pathway controls cell differentiation in numerous tissues during embryogenesis and has also been implicated in the control of stem cell maintenance in regenerating tissues, such as the hematopoietic cell lineage, skin and intestine. Stimulation of the Wnt signaling pathway results in the nuclear translocation of ß-catenin, which forms a complex with TCF/LEF proteins to activate Wnt target genes in the nucleus. To date, it is still unclear whether the pancreas contains true stem cells and if so, whether Wnt signals control their maintenance. As a first step to identify a possible role of Wnt signaling in the pancreas, the expression of *Tcf/Lef* genes as well as of other components of the canonical Wnt signaling pathway was studied during pancreatic development. It was found that all four *Tcf/Lef* genes, as well as genes coding for the Wnt ligands, the Frizzled receptors and other key factors of the canonical Wnt pathway were expressed in the developing pancreas from the earliest stages through adulthood.

Next, to study if Wnt signaling is active in the pancreas, pancreata of two independent Wnt reporter mouse lines, in which formation of an active TCF/LEF/ß-catenin complex leads to the expression of ß-galactosidase, were analyzed by enzymatic ß-galactosidase staining. The analysis showed that the canonical Wnt pathway is active from early formation of the pancreatic anlage until birth. However, no activity was detected in the adult pancreas. To address if the canonical Wnt signaling controls pancreas development, transgenic mice were generated, in which the canonical Wnt cascade was either blocked, or ectopically activated in early pancreatic progenitors. To block Wnt signaling, a dominant negative form of TCF4 (dnTCF4) was expressed under control of an early pancreas specific promoter, while a constitutively active form of TCF4 (caTCF4) was used to stimulate Wnt signaling. Late stage

embryos of neither one of the two transgenic strains displayed detectable pancreatic defects. Since the promoter which was used to drive the transgene, only targets a small population of cells at later developmental stages, the lack of transgene expression in appropriate cell populations could account for the absence of a phenotype. To overcome this problem, a bigenic *Cre-loxP* based system was employed, which through matings with different *Cre*-recombinase expressing mouse lines, allows for expression of a dnTCF4 or caTCF4 in various cell populations of the pancreas. In the mice expression of dnTCF4 or caTCF4 is controlled by the ubiquitous *ROSA26* locus, but expression is prevented by a STOP cassette. Only removal of this STOP cassette by *Cre* recombinase leads to heritable and stable expression of the TCF4 transgene. Thus far, double transgenic $Rosa26^{dnTcf4}$*:ins-*$cre^{+/tg}$ mice, in which canonical Wnt signaling is inhibited in mature ß-cells, have been generated.

The results of this study show that HMG box transcription factors of both the Sox and Tcf/Lef families are expressed during murine pancreas development. Moreover, detection of TCF/LEF/ß-catenin-mediated transcription in Wnt reporter mouse lines demonstrates that pancreatic progenitor cells receive canonical Wnt signals. The generation of a flexible, bigenic *Cre-loxP* based transgenic system will allow us to now study the role of Wnt signaling in pancreatic development, and adult ß-cell function.