7. Summary

Studies on kidney development provide insights into general processes of embryogenesis like inductive interactions, mesenchymal condensation, mesenchymal-epithelial interactions, cell fate determination as well as differentiation and thereby into the basis of congenital malformations. Once induced by the ureteric bud, the metanephrogenic mesenchyme gives rise to the functional units of the kidney - the nephrons - and the renal stroma. These morphogenetic processes rely on complex regulatory changes in gene expression, which to date are not understood in detail.

The present thesis aimed to identify known and primarily novel genes regulated within the metanephrogenic mesenchyme upon induction. Gene expression of induced versus uninduced mesenchyme from murine kidney anlagen was compared using ddPCR together with transfilter organ culture. Several candidates were assayed for differential expression by northern blot hybridization and selected for further characterization.
As one of the known genes, sFRP2 was verified to be induced within the metanephrogenic mesenchyme. *In situ* hybridization of whole-mount mouse embryos and paraffin sections revealed specific and dynamic expression patterns for sFRP2 as well as for the related genes sFRP1 and sFRP4 in the developing kidney and other tissues. The detailed sFRP gene expression analysis was performed to guide functional studies for this recently identified novel gene family.
Preliminary investigations of the ddPCR products C0-5, J6-3 and M2-4 revealed that they are all derived from novel genes with distinct expression patterns during kidney development. While C0-5 expression dynamically switches from the ureteric bud to the nephron precursors and the collecting system, J6-3 specifies the stromal cell lineage and M2-4 is already detectable in the condensing mesenchyme with subsequent expression in epithelial derivatives. Isolation and analysis of the C0-5 cDNA resulted in the identification of a collagen-like protein in mice and humans that is located upstream of the EWS gene of the human chromosome 22q12.
Additionally, a novel gene family related to hairy and the E(spl)-complex genes has been identified. Because of this relationship and a characteristic YRPW tetrapeptide they were designated as Hey genes for “hairy and E(spl) related with YRPW motif“. Compared to hairy/E(spl) or the mammalian Hes proteins they show novel features of DNA-binding and protein interaction. Moreover, their expression patterns frequently correlate with those of members of the Delta-Notch signaling pathway suggesting that Hey genes may participate in this pathway in cell fate decisions and boundary formation. Analysis of Dll1 knockout mice partly confirmed this assumption for Hey1 and Hey2 during somitogenesis.

This screen has shown that transfilter organ culture in combination with ddPCR is a powerful tool to identify genes regulated within the metanephrogenic mesenchyme upon induction. Expression and sequence analysis of the novel genes implies a function during development of the kidney and other tissues that can now be studied in further detail. The collection of more than 50 additional candidates for novel genes regulated during nephrogenesis provides a rich resource for future analysis of the networks governing kidney development.