7. Summary

“Development“ represents an abstract term describing a fascinating natural process, which at the end creates a complex organism derived from a single zygote.

Focusing on the nervous system a multitude of challenging events, such as proliferation, cell lineage commitment, differentiation followed by migration and integration into the appropriate tissue, and, finally synaptogenesis have to be executed in a stereotyped manner. The precise regulation of individual steps orchestrates the continuous reciprocal cellular interactions on the one hand and the cooperation with the instructive surrounding cellular environment on the other hand. The resulting flow of information is processed and coordinated with intrinsic preconditions, which eventually results in coordinated cellular behaviors.

The present study investigated the cellular expression and biological relevance of the extracellular matrix molecules Phosphacan/RPTPβ/ζ and Tenascin C (Tnc) in the embryonic retina stem cell niche and further addressed the question, whether the transcription factor Pax6 is involved in the expression of these molecules and thereby indirectly mediates their functions during retinogenesis.

The examination of the cellular source of the chondroitin sulfate proteoglycans Phosphacan and RPTPβ/ζ-long was performed by using the monoclonal antibody (mab) 473HD in combination with other cell type-specific markers. The antibody recognizes a specifically sulfated carbohydrate epitope (DSD-1-epitope) of chondroitin sulfate GAG chains. Between embryonic day (E) 13.5 to E18.5 immunoreactivity of the 473HD-epitope was detected on cycling progenitors, partly associated with the cytoskeleton in close vicinity of separating chromosomes in the anaphase and early telophase. Dependent on the developmental stage chondroitin sulfates were additionally produced by early post-mitotic neurons. To address the functional relevance, two different approaches were carried out. After successfully establishing an appropriate model system for studying the influence on stem/progenitor cells in vitro, the CS chains were removed by exposure to the enzyme ChondroitinaseABC (ChABC). The degradation stimulated the differentiation to βIII-Tubulin-positive neurons, which indicates an implication of chondroitin sul-
fates in the regulation of accurate progenitor proliferation, in concert with inhibiting the induction of differentiation.

Microinjection of siRNA for all RPTPβ/ζ isoforms into the subretinal space of E14 embryos resulted in a reversed situation, reflected by an increase of cycling cells and radial glia at the expense of neurogenesis ex vivo. Obviously, this phenotype could be explained by the down-regulation of the core proteins. Nonetheless, it emphasized the function of RPTPβ/ζ with regard to keeping proliferation and differentiation in the appropriate balance. The knockdown by the siRNA further affected the Tnc expression, which was up-regulated under these conditions.

The involvement of Tnc in developmental processes was investigated in a Tnc knockout mouse model. It could be shown that Tnc-deficiency causes an increase of G2-phase cells, as well as increasing the amount of post-mitotic neurons in the embryonic retina. Further investigations provided evidence that Tnc regulates these processes via the Wnt-signaling cascade. Moreover, it was interesting to note a significant increase in the production of the 473HD-epitope in the Tnc-deficient ECM, because Tnc and RPTPβ/ζ are known to interact with each other. Remarkably, the alteration of Tnc or RPTPβ/ζ reciprocally modified the expression/production of these molecules.

Recently, it has been described that in the embryonic forebrain the expression of alternatively spliced variants of Tnc is regulated by the transcription factor Pax6. A Pax6 knockout mouse model (Sey) therefore should provide more information concerning the influence on the expression of Tnc and, concomitantly, the chondroitin sulfates exposed by RPTPβ/ζ in the embryonic retina. Indeed, both molecules are down-regulated in heterozygote Sey-mutants.

In summary, the studies presented in this thesis uncovered cellular sources expressing chondroitin sulfate chains of Phosphacan/RPTPβ/ζ, the functional implications of this molecule and of Tnc in distinct functions, and the mutual influences on their expression and functions. Intriguingly, the results demonstrated clearly that Tnc, chondroitin sulfate chains of Phosphacan and Pax6 are involved in the regulation of progenitor cell proliferation and the transition to a differentiation state. Regarding the generation of neurons, indications were obtained that these molecules cooperate to some extent, because the elimination or limited interactions of
Tnc and RPTPβ/ζ resulted in all approaches in an enhanced neurogenesis. These findings support the conclusion that the molecules inhibit the premature onset of differentiation in a subpopulation of the retinal progenitor cell pool.