SUMMARY

In the last decade it has become clear that a number of the molecular mechanisms that are required for proper navigation of axons in complex nervous systems are also employed to guide muscles to their appropriate attachment sites. Examples of gene families that mediate these diverse processes are the highly conserved Slit and the Roundabout gene families, and the Ryk family, the latter being the topic of this thesis. The fruit fly (*Drosophila melanogaster)* has three RYK members: DRL, DRL-2 and DNT. The aim of our research is to advance the understanding of the mechanisms by which the *Drosophila* RYKs operate in the nervous system and the musculature using biochemical and genetic approaches.

In **Chapter 2,** we demonstrate that DRL can form homodimers and heterodimers with its orthologues DRL-2 and DNT in a *Drosophila* cell line, suggesting that the partly overlapping temporal and spatial expression patterns of the different Ryk family members *in vivo* have important consequences for their function. RYKs act as Wnt receptors in mammals and in invertebrates. We find that the *Drosophila* WNT5 protein is able to significantly increase homodimerization of DRL causing a subsequent increase in the recruitment of SRC64B, a non-receptor tyrosine kinase, previously identified as a downstream effector of DRL. We further show that ligand independent homodimerization of DRL is mediated by a TLIGV motif in its transmembrane domain. The formation of the DRL/SRC64B complex requires the DRL PDZ-binding motif and the SH2 domain of SRC64B. In addition, several mutations in the putative inactive DRL kinase domain prevent formation of the DRL/SRC64B complex indicating that WNT5 induced conformational changes are likely essential for their interaction. Using an *in vivo* axon guidance assay we find that that the DRL Wnt-binding domain, a juxtamembrane extracellular tetrabasic cleavage (TBC) site and the PDZ-binding domain are needed for axon repulsion by DRL. These results suggest that activation of DRL as an axon guidance receptor involves a number of molecular events including dimerization and proteolytic processing.

In **Chapter 3** we study the function of *Drosophila* RYKs in the mushroom body (MB): a brain structure implicated in learning and memory acquisition. The α- and β-axons of MBs connect different regions of the brain required for these processes. We show that DRL is needed during *Drosophila* brain development to guide the MB α-branch in order to connect to its target neurons. However, DRL is not expressed by MB neurons themselves but instead can be detected in a neuronal cell lineage that adjoins the outgrowing MB α-branch. We find that the MB α-branch expresses DRL-2, another *Drosophila* RYK orthologue, and that correct MB α-branch guidance is dependent on its presence. The DRL-2 cytoplasmic domain is required for this function. This result indicates that DRL-2 can act as an axon-intrinsic guidance receptor

in this cellular context. Interestingly, the DRL cytoplasmic intracellular domain is not needed in the adjacent cells for a-branch guidance. DRL receptor function necessitates its cleavage at the TBC site for appropriate a-branch guidance, resulting in the extracellular shedding of DRL's Wnt binding domain. DRL, therefore, acts extrinsically to capture and present WNT5 to MB axons that express DRL-2 on their surface and transduce a subsequent intracellular signal. This model is consistent with our biochemical data supporting the existence of a ternary complex consisting of the shed DRL ectodomain, WNT5, and transmembrane DRL-2. Therefore, we conclude that MB-extrinsic and –intrinsic RYKs guide MB-α axons via their common ligand WNT5.

Drosophila RYKs do not only act as guidance receptors for axons; they are also involved in the guidance of the lateral transverse muscles (LTMs) to their final muscle attachment site (MAS) at the body wall in the *Drosophila* embryo. In **Chapter 4,** we show that in an embryo lacking *wnt5*, as has been shown for its receptor *drl,* these muscles often overshoot their target and attach more ventrally at an ectopic site in the body wall. Rescue experiments demonstrate that WNT5 expressed in either the muscle or the tendon cell can restore muscle attachment but that DRL has to be present at the approaching muscle fiber. Strikingly, *dnt*, but not *drl-2*, mutants also exhibit the muscle bypass phenotype. Moreover, in absence of both *drl* and *dnt* the number of overshooting fibers dramatically increases, indicating that *drl* and *dnt* act together to guide the LTMs to its appropriate MAS. We also show that *wnt5*, *drl* and *dnt* are likely in the same pathway since removing a copy of *wnt5* enhances the *drl/dnt* phenotype significantly. The approaching muscle fiber and the prospective tendon cell exchange a number of molecular signals enabling the formation of a stable extracellular matrix-mediated myotendinous junction. Surprisingly, the majority (65%) of the novel ectopic MASs do not express Stripe, a nuclear protein postulated to be both necessary and sufficient for tendon cell differentiation. Furthermore, we provide evidence that the overshooting fibers interact extensively with their prospective Stripe-positive tendon cell yet proceed to attach more ventrally. We conclude that an interplay of DRL, DNT and WNT5 is responsible for generating a stop signal to allow the muscle attachment to be established. Why and how attachment does occur at an ectopic ventrally located epidermal cell is not clear at this moment and future studies will be needed to identify the actual stop signal.

The studies described in this thesis enhance our understanding of the basic biological roles and operating mechanisms of Ryks in such diverse processes as axon guidance and muscle attachment site selection. We hope that this work will also contribute to future therapies for diseases in which these proteins are likely implicated such as cancer and poor regeneration after nervous system trauma.