SUMMARY

Canonical Wnt signalling regulates muscle stem cell/myoblast differentiation, but there have been conflicting reports about the requirement for β-catenin in adult regenerative myogenesis. Wnt is also known to be a key player in fibrosis in many tissues. That Wnt may be a double edged sword in muscle repair, promoting myogenesis but also contributing to pathogenic fibrosis, suggests a pressing need to better understand the molecular processes of Wnt signalling in these contexts.

To better understand the role of β-catenin in myogenesis we used CRISPR to generate β-catenin null primary adult mouse myoblasts *in vitro*. β-catenin null myoblasts showed greatly impaired spontaneous and Wnt3a-induced differentiation. RNA-seq analysis showed a strong delay in activation of the global myogenic differentiation program after Wnt treatment, thus confirming the requirement for β-catenin in myogenesis. β-catenin interacts with TCF/LEF factors but also with the muscle regulatory factor MyoD, and it was unclear which regulatory complex may be involved in myogenesis. Using ChIP-seq analysis, we showed that Wnt induced activating histone modifications at genomic regions that contain MyoD (E-box) binding elements, but not TCF/LEF elements. We also found that Wnt increased binding of MyoD to E-box elements in wild-type myoblasts but not β-catenin null cells. Among the gene targets that were found to be controlled by MyoD and β-catenin is the membrane fusion protein Myomaker, which we propose as a novel effector of Wnt signalling in myoblasts. To explicitly test whether TCF/LEF is required for myogenic differentiation, we used a variant of β-catenin that cannot interact with TCF/LEF in rescue studies in β-catenin null myoblasts. The mutant β-catenin variant rescued the differentiation capacity of null myoblasts as effectively as wild-type β-catenin. Together these data indicate that Wnt promotes adult

myogenesis in a β-catenin-dependent and likely MyoD-dependent, but TCF/LEF-independent, manner.

β-catenin-TCF/LEF-CBP complexes are known to be involved in fibrosis in many contexts. Because we found no requirement for β-catenin-TCF/LEF complexes in myogenesis, we postulated that inhibition of this complex would not impair myogenesis, but might reduce fibrosis mediated by muscle fibroblasts/FAPs. In support of this, preliminary evidence suggests that a small molecule inhibitor of β-catenin-TCF/LEF-CBP complexes inhibits activation of fibroblasts *in vitro* and *in vivo* without inhibiting myogenic differentiation. In contrast, similar chemical inhibitor studies suggest that β-catenin-MRFs/MEFs-p300 complexes may be required for myogenic differentiation.

Wnt/β-catenin signalling was shown to inhibit Pax7 expression at the protein level in myoblasts. Wnt also induced expression of the myogenic miRNAs miR-133b and miR-206 in a β-catenin-dependent manner. miR-206 was previously reported to post-transcriptionally repress Pax7; our studies showed that miR-133b is likely to be a more potent inhibitor of Pax7 and confirmed that β-catenin is absolutely required to relieve Pax7-mediated inhibition of differentiation.

Overall, the findings reported in this thesis prompt the following conclusions about the role of Wnt/β-catenin signalling in myogenesis: 1. β-catenin regulates muscle stem cell differentiation by inducing miRNA-mediated Pax7 degradation to relieve Pax7's inhibitory effect on differentiation; 2. β-catenin works in concert with MyoD and p300 to positively regulate the differentiation-associated transcriptional network comprised of transcription factors, miRNAs, and mechanochemical effectors such as cytoskeletal remodelling and membrane fusion proteins. Moreover, given that the pro-differentiation and pro-fibrotic programs driven by Wnt/β-catenin are likely

mediated by different coactivators, these might be functionally separated as part of a therapeutic strategy for muscle fibrosis associated with muscle degenerative diseases such as DMD.