5 Conclusions and Outlook

During this thesis, a novel pathomechanism for OS was identified with a basis in mutations in Bbox1 or Bbox2 domains of MID1. Bbox1 was shown to be responsible for the interaction of MID1 with α4, a regulatory subunit of PP2A, and Bbox2 was demonstrated to act as a regulatory arm that couples the MID1 ubiquitin ligase function to the microtubules by regulating the association of MID1 with both α4 and microtubules. The inefficient MID1-α4 binding observed with mutations in any of the Bboxes causes accumulation of PP2Ac at the microtubules and hypophosphorylation of its downstream proteins, thus leading to the development of OS. A possible explanation for the striking dependence of Bbox1 function on Bbox2 could be a putative zinc atom shared by both domains. Consequently, zinc-binding stechiometry has to be analysed in next series of experiments.

As main focus of this thesis, MID1 was demonstrated to form part of an mRNP complex, which associates to microtubules and RNA, and is likely to participate in the transport of mRNAs to the poles of the cell, providing asymmetric mRNA localisation and protein production. Compartmentalised protein translation is an important prerequisite for NCC to migrate and polarised cells to step into EMT, both essential processes during ventral midline development. Therefore, the data obtained during this thesis suggest a molecular basis for both the development of the ventral midline and the pathogenesis of OS.

However, several lines of investigation remain open. The exact pathomechanism derived from MID1 mutations in other domains rather than the C-terminus or, now, the Bboxes remains unknown. Therefore, in further experiments, the effects of diverse MID1 forms carrying mutations in different domains on RNA or ribosome binding should be studied and hopefully, regions involved in this association will be narrowed down.

In addition, direct association of MID1 with RNA could not be proven during this work. Further experiments will also have to show which members, if not MID1 itself, are involved in the incorporation of RNA into the complex. As mentioned in the discussion, attractive candidates would be the RNA helicase identified by yeast two-hybrid experiments or any of the other RNA-binding proteins contained in the complex. The study of RNA-MID1 complex association should be also extended to the identification of further mRNAs that associated with the complex, via for instance protein-RNA experiments. In theory, gene products of the identified mRNAs could collaborate with ephrins during ventral midline development.

Despite having all the characteristics of a cargo transport, no motor protein that provides movement along the microtubules was found to associate to the complex. Further experiments, such as immunoprecipitations with the different complex members or candidate specific co-immunoprecipitations with motor proteins, should allow the identification of such a protein

integrated in the complex. Furthermore, immunofluorescence experiments in living cells should be done, thus aiming to visualize movement of the whole mRNP *in vivo*.

In addition, the role of the MID1 complex in cell migration would also open a new area of study, in which the involvement of the complex in focal adhesions and cytoskeleton remodelling should be studied. Moreover, polarisation studies, including activation of integrin receptors by different effectors or by activation of EFNB1, could shed light on the functions of the MID1 complex in cell polarisation and migration. These studies could be extended to OS patient cell lines, which might show defects in migration compared to control cell lines.

While underlying genetic defects in patients with autosomally inherited OS remain totally unknown, mutations in MID1 have only been found in 68% of cases with X-linked OS. Since during this thesis it was shown that ephrin mRNAs are integrated into the MID1 complex via G-quartet structures, these structures appear as good candidates to harbour the “missing” mutations. Therefore, mutation analysis of G-quartet structures in ephrin mRNAs in OS patients not holding mutations in MID1 should be done.

Last but not least, direct influence of the MID1 complex on EFNB1 mRNA translation should be studied, and it should be checked whether it is affected in OS patients.