6 Summary

Opitz BBB/G syndrome (OS) is a congenital disorder characterised by malformations of the ventral midline, with hypertelorism and hypospadias being the two cardinal phenotypic manifestations. OS is genetically heterogeneous, with an autosomal and an X-linked locus. While the gene at the autosomal locus remains to be identified, the X-chromosomal form has been shown to be caused by mutations in the *MID1* gene, which harbours mutations in approximately 68% of patients with X-linked OS. Most of the mutations identified in OS patients are located at the 3′ end of the MID1 open reading frame, thus affecting the C-terminus of the MID1 protein.

The MID1 protein belongs to the RFP subfamily of the RBCC family of proteins. At its N-terminal end it contains a RING finger, two Bboxes (Bbox1, Bbox2), and a coiled-coil domain (RBCC domain), which are followed by a FNIII domain and a B30.2 domain (RFP domain) at its C-terminal end. MID1 has been shown to form macromolecular cellular complexes, the components of which were, up to now, mainly unknown. Similar to other RBCC proteins, MID1 contains several putative protein-protein interaction domains. Recently, we have shown that the C-terminally microtubule-associated MID1 protein binds α4, a regulatory subunit of phosphatase 2A (PP2A), through the Bbox1 domain, thereby targeting the catalytic subunit of microtubule-associated PP2A (PP2Ac) towards ubiquitin-specific modification and degradation. MID1 mutations in the C-terminal end of the protein lead to disruption of microtubule association of MID1 and subsequent formation of clumps in the cytosol. Despite preserving its association with α4, C-terminally mutated MID1 can not approach the vicinity of microtubule-associated PP2Ac and, therefore, the ubiquitination and degradation of microtubule-associated PP2Ac becomes disrupted, leading to hypophosporylation of its downstream targets.

During this thesis, basic functions of Bbox1 and Bbox2, with respect to MID1-α4 and MID1-microtubule interactions, were studied in detail by immunofluorescence, immunoprecipitation and yeast two-hybrid experiments. In this way, a novel pathomechanism for OS could be identified based on mutations in Bbox1 or Bbox2 domains of MID1 rather than C-terminal mutations. While the Bbox1 was shown to be responsible for the interaction of MID1 with α4, the 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.

As the main focus of this thesis, the MID1 multiprotein complex was elucidated via affinity chromatography and mass spectrometry. Besides tubulin association, which has previously been reported, MID1 was shown to associate with several proteins of the small ribosomal subunit (S3, S8, p40) and other multifunctional proteins such as NPM, RACK1 and ANXA2,

which also associate with ribosomes and RNA. In addition, heat shock proteins, such as Hsp60, Hsc70, and the multifunctional chaperones Hsp90 and p32, were identified in the complex.

Through further characterisation of the MID1 protein complex, it could be demonstrated during this thesis that the MID1 protein, together with the mTOR target α4, forms a microtubule-associated mRNP that contains active polyribosomes and RNA, and thus links the translation regulatory mTOR pathway with a microtubule-associated translation unit. This complex 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 neural crest cells to migrate and polarised cells to step into epithelial-mesenchymal transition, both essential processes during ventral midline development. Therefore, the results of this thesis suggest a molecular basis for both the development of the ventral midline and the pathogenesis of OS.

Moreover, it could be shown that the MID1/α4 complex integrates mRNAs of ephrinB molecules (ligands and receptors) through G-quartet structures located in their 3´UTRs. Ephrins and Eph receptors participate in the regulation of essential processes during the development of the ventral midline, such as cell attachment, cell migration and embryonic patterning. Therefore, this thesis suggests a central role for the MID1/α4 protein complex in the microtubule-associated compartmentalised translation of EphB receptors and ephrins-B. Interaction of the MID1 protein complex with the mRNA of ephrin-B1 (*EFNB1*) is of particular interest since mutated EFNB1 leads to the development of craniofrontonasal dysplasia, a monogenic disorder with manifestations that are highly reminiscent of the OS phenotype. Consequently, the model proposed here also provides an attractive explanation for the conspicuous phenotypic overlap of the two disorders.