Summary

For erythropoiesis it is of utmost importance that cellular processes like proliferation, differentiation and apoptosis are balanced in order to generate sufficient erythrocytes, but also to prevent an excessive production of erythrocytes. Especially the presence or absence of cytokines like erythropoietin (Epo) and stem cell factor (SCF) determines the faith of erythroid progenitor cells. These growth factors activate intracellular signal transduction cascades that ultimately result in the expression or silencing of the appropriate genes and turn the balance between proliferation, differentiation and apoptosis in the favorable direction.

In **chapter two**, we examined the role of the SH2-containing inositol phosphatase (SHIP) on proliferation and apoptosis in the Epo-dependent cell line AS-E2. Stable overexpression of wild-type SHIP did not affect Epo-induced proliferation, but apoptosis was strongly enhanced in the absence of Epo. Epo-deprived cells showed an increase in caspase-3 and -9 activity, without a distinct effect on caspase-8 activity or mitochondrial depolarization. The increase in apoptosis was not due to the inositol phosphatase activity of SHIP, since AS-E2 cells stably expressing catalytically inactive SHIP showed a similar increase in apoptosis. However, unlike overexpression of wild-type SHIP, overexpression of catalytically inactive SHIP did reduce proliferation, indicating that the 5'-phosphatase activity of SHIP is linked to erythroid proliferation. The decline in proliferation was associated with increased activation of protein kinase B (PKB) and the extracellular regulated kinase (ERK), suggesting a link between phosphatidyl inositol 5'-phosphatase activity of SHIP and Epo-mediated signal transduction pathways. Furthermore, these results were in line with data obtained from SHIP-deficient mice, which displayed reduced numbers of CFU-Es in *in vitro* colony assays (1).

One of the proteins that is highly activated in erythroid cells and has been linked to erythroid development and cell survival is the signal transducer and activator of transcription 5 (STAT5). In **chapter three**, we show that both Epo and prostaglandin-E_2 (PGE_2) contribute to STAT5-signaling, but with different mechanisms. While Epo itself strongly increases STAT5 transactivation, PGE_2 only contributes to increased STAT5-signaling when administered in combination with Epo. This PGE_2-costimulation did not affect the initial steps in the STAT5-signaling cascade, including tyrosine phosphorylation, nuclear translocation and DNA-binding. Instead, we identified a novel regulatory mechanism that modulates STAT5-signaling at the level of gene transactivation. This mechanism requires protein kinase A (PKA)-mediated phosphorylation of the cAMP-response element-binding protein (CREB) and is sensitive to E1A-mediated inhibition of the CREB-binding proteins CBP and p300. These findings suggest that phosphorylated CREB enhances the recruitment of CBP/p300 to the transactivation complex, which results in enhanced transcriptional activity of STAT5. In addition, we observed Epo-depe 726/731, but serine phosphorylatio

In **chapter four** we show t also reflected in a synergistic effe erythroid STAT5 activation, S administered in addition with Epo. mediated STAT5 transactivation wa synthetic PKA inhibitor H89 a Furthermore, the synergistic SCF e CREB, demonstrating that PKA-me between Epo and SCF.

Since there are many simi thrombopoietin (TPO)-signaling in STAT5 transactivation in **chapter fi** resulted in strong STAT5 transactiva with SCF. The underlying mechanis cells. In megakaryocytic MO7e cells mediated tyrosine phosphorylation o did not affect the synergistic STAT5 in megakaryocytic STAT5-signaling.

In **chapter six** an overview is cAMP and PKA in the regulation interaction between cAMP/PKA-sign addition, the cAMP-mediated regulatio

addition, we observed Epo-dependent phosphorylation of STAT5A/B serine residues 726/731, but serine phosphorylation did not increase the transactivational potential of STAT5.

In **chapter four** we show that the synergy between SCF and Epo on erythropoiesis is also reflected in a synergistic effect on STAT5-signaling. While SCF itself cannot induce erythroid STAT5 activation, SCF strongly enhances STAT5 transactivation when administered in addition with Epo. We observed that the synergistic effect of SCF on Epo-mediated STAT5 transactivation was dependent on PKA activation as it was inhibited by the synthetic PKA inhibitor H89 and by overexpression of PKA-inhibitor constructs. Furthermore, the synergistic SCF effect was inhibited by overexpression of serine-mutated CREB, demonstrating that PKA-mediated CREB phosphorylation is involved in the synergy between Epo and SCF.

Since there are many similarities between Epo-signaling in erythroid cells and thrombopoietin (TPO)-signaling in megakaryocytic cells, we examined the TPO-mediated STAT5 transactivation in **chapter five**. Stimulation of megakaryocytic MO7e cells with TPO resulted in strong STAT5 transactivation, which could be further enhanced by costimulation with SCF. The underlying mechanism of this synergy, however, is different from erythroid cells. In megakaryocytic MO7e cells SCF enhances STAT5-signaling by upregulating TPO-mediated tyrosine phosphorylation of STAT5 and STAT5 DNA-binding. Inhibition of PKA did not affect the synergistic STAT5 transactivation, demonstrating that PKA is not involved in megakaryocytic STAT5-signaling.

In **chapter six** an overview is given of the current knowledge regarding the role of cAMP and PKA in the regulation of erythropoiesis. Special attention is given to the interaction between cAMP/PKA-signaling and additional signal transduction pathways. In addition, the cAMP-mediated regulation of erythroid gene expression is discussed.