# Post-transcriptional control of C/EBPα and C/EBPβ proteins

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# CHAPTER I

# Aim and outlines of the thesis

## Aim

C/EBPα and C/EBPβ proteins are transcription factors playing important roles in the control of cellular proliferation, differentiation and metabolism of different tissues. Their functions are strictly regulated at the translational and post-translational levels. One objective of this thesis is to investigate whether C/EBPα is acetylated, which lysine acetyl-transferases and de-acetylases are involved in this process and how acetylation can regulate C/EBPα function in cell metabolism and energy homeostasis. A second objective is to convert the upstream open reading frame (uORF)-dependent translation control mechanism of C/EBPβ into a high-throughput screening system to look for compounds that decrease C/EBPβ-LIP levels and by that act as calorie restriction mimetics based on the calorie restriction like phenotype found in C/EBPβ$^{\Delta uORF}$ mice. As a third objective, we aimed to investigate whether C/EBPα and C/EBPβ translation is regulated by the SBDS ribosomal maturation protein and whether their translational deregulation is involved in the Shwachman Diamond Syndrome (SDS) which is caused by mutations in SBDS.

## Outlines of the thesis

**Chapter II** I start this thesis with a general introduction about C/EBPα and C/EBPβ transcription factors highlighting their expression, structure, dimerization and DNA binding ability. I explain how three protein isoforms are translated from their mRNA by a uORF dependent translation initiation/re-initiation mechanism and how this translation is regulated. I describe how the truncated isoforms (C/EBPα-p30 and C/EBPβ-LIP) negatively regulate the function of the full-length isoforms (C/EBPα-p42 and C/EBPβ-LAP) pointing to the importance of the isoform ratio in determining their biological functions. Then, I discuss how the function of C/EBPα and C/EBPβ is regulated by different post-translational modifications including methylation, phosphorylation, acetylation and sumoylation. Finally, I demonstrate how C/EBPα and C/EBPβ regulate the differentiation in adipocytes and hematopoietic cells and how their function and expression are modulated during cellular proliferation and in different types of cancers.

**Chapter III** We investigate if C/EBPα is acetylated and whether the acetylation of C/EBPα regulates its function in cell metabolism and energy homeostasis. We found that C/EBPα is acetylated at several lysine residues by the p300 lysine acetyl-transferase and deacetylated by the SIRT1 lysine deacetylase. We observed that acetylation has no effect on C/EBPα subcellular localization or DNA binding ability but modulates its transcriptional activity in a cell reporter assay. Transcriptome analysis performed with the Hepa1-6 mouse hepatoma cell line expressing C/EBPα mutants mimicking acetylation or deacetylation showed that 43% of the differentially expressed genes are genes involved in metabolic processes. Further analysis showed that Hepa1-6 cells expressing a C/EBPα deacetylation mimicking mutant have a higher mitochondrial mass and an increased oxygen consumption rate comparing to cells expressing the wt and acetylation mutant C/EBPα. This finding is in agreement with the results of the transcriptome analysis revealing mitochondrial genes to be significantly upregulated in cells expressing the mutant that mimics deacetylated C/EBPα. In this study, we show the importance of C/EBPα acetylation in the regulation of cell metabolism and uncover SIRT1 as critical modulator of the transcriptional function of C/EBPα.

**Chapter IV** We develop a reporter system that is applicable for a screening to find new compounds that downregulate the C/EBPβ-LIP isoform and thereby might function as anti-cancer and calorie restriction mimetic drugs. This project was based on the phenotype observed in the C/EBPβ$^{\Delta uORF}$ knockin mice that have constitutively low C/EBPβ-LIP levels due to a mutation in a cis-regulatory upstream open reading frame and show increased health- and lifespans combined with reduced tumor incidence upon aging. For this goal we converted the uORF dependent translation control mechanism of C/EBPβ into a dual luciferase-based reporter system to screen for compounds that translationally decrease the C/EBPβ-LIP/LAP ratio. The reporter system was validated following genetic and pharmacological approaches and then used to screen an FDA approved drugs library following a high throughput screening strategy. Drugs that decrease the translation reinitiation index as readout of the reporter were identified and selected ones were further validated in cell culture experiments for the effect on the C/EBPβ-LIP/LAP isoform ratio and downstream effects on cellular metabolism. In this study we produced a tool to screen for compounds that specifically affect the uORF dependent translation process and might have calorie restriction and anticancer effects.

**Chapter V** We investigate whether deregulated C/EBPα and C/EBPβ isoform expression might play a role in the Shwachman–Diamond syndrome (SDS) which is a disorder caused by mutations in the *SBDS* gene and which is characterized by bone marrow failure with neutropenia, exocrine pancreatic insufficiency and skeletal abnormalities. We ask the question if the failure of the ribosomal maturation protein SBDS is affecting the translation of C/EBPα and C/EBPβ mRNAs and to which extent this impaired translation is contributing to SDS phenotypes. We found that the SBDS protein plays an important role specifically in translation re-initiation and its loss of function by mutations or knockdown resulted in downregulation of the truncated isoforms, C/EBPα-p30 and C/EBPβ-LIP, while the translation of the full length isoforms, C/EBPα-p42 and C/EBPβ-LAP, was not affected. Furthermore, we found that deregulation of C/EBPα and C/EBPβ translation upon SBDS malfunction resulted in lower levels of MYC expression leading to decreased proliferation of progenitor cells which potentially contributes to the hematological phenotype of SDS.

Our results provide the first evidence that the phenotypes of SDS can be caused by deregulation of specific mRNA translation due to SBDS failure.

**Chapter VI** I discuss the work done in this thesis and critically analyze how successful I was in achieving the primary goals and how valuably I could contribute to scientific progress with this thesis. Furthermore, I discuss future plans and the follow up research that should be initiated or continued based on our results and also address many questions that are still open and need to be solved.