Chapter 6

Summary
Aging is a complex phenomenon, resulting from damage accumulation, the increased deregulation of biological pathways, and a loss of cellular homeostasis, all of which lead to a functional decline in the organism over time. Interestingly, aging is not a static process but it is highly regulated by an interconnected signaling network that predominantly regulates the activity of aging-preventive stress response and longevity promoting pathways. In this thesis, I provided new mechanistic insights into the transcriptional regulation of genes that promote stress resistance and longevity. For this work, I used the model organism *C. elegans* and focused on maybe the most central aging-regulatory signaling pathway known to date, namely insulin/IGF-like signaling (IIS) with its downstream transcription factor DAF-16/FOXO. By showing and evaluating genetic and biochemical interactions between DAF-16 and some of its essential cofactors and regulators, we gained important new insights into how DAF-16 is regulated and targets its downstream genes. Hopefully, this information provides new mechanistic avenues towards interventions against the aging process and resultant age-related diseases.

In the first part of the thesis in Chapter 2, we gave an overview of the relationship between the epigenome, aging, and aging-regulatory signaling pathways, with a particular focus on how the epigenome may influence aging-regulatory transcription factors and vice versa. We emphasized the importance of aging-regulatory target gene accessibility, which is strongly influenced by the epigenome and the resulting chromatin state at these loci. Moreover, we pointed out how aging-regulatory signaling pathways can also actively shape the epigenome and thus use it to confer phenotypes at the transcriptional level.

In Chapter 3, we characterized the combinatorial roles of two transcription factors, DAF-16 and the helix-loop-helix transcription factor HLH-30/TFEB, which is a well-known master regulator of autophagy and lysosome biogenesis. We newly observed that HLH-30 actually acts as a broad regulator of aging and stress resistance, in very close interplay with DAF-16/FOXO. We could show that under harmful conditions, DAF-16 and HLH-30 both translocate into the nucleus, form a complex, and co-occupy many target promoters, often co-regulating many downstream target genes. Interestingly, the genetic interaction between these transcription factors depends on the upstream stimulus and together they orchestrate the physiological outcomes for the animal. For instance, they function in the same pathway and there depend on each other to promote longevity under low IIS or in germline-deficient animals or to induce resistance to oxidative stress, but they provide heat stress responses independently, and they even oppose each other during dauer formation. In the end, we showed that their cooperation and cross-talk ensures customized transcriptional responses to diverse stimuli, leading to stress resistance, certain decisions during development, and the promotion of longevity.

In Chapter 4, we focused on an essential positive regulator of DAF-16/FOXO, called SMK-1/SMEK, and elucidated the mechanism by which it influences the expression of many DAF-16 target genes. To find this mechanism, we first determined binding partners of SMK-1 by large scale IP followed by mass spectrometry-based identification of co-purifying proteins. We could show that SMK-1 is part of a specific Protein Phosphatase 4 (PP4) complex, and that it fulfills its aging-regulatory role as part of this complex. Loss of PP4^SMK-1^ under low IIS led to mildly delayed nuclear entry of DAF-16 and mildly reduced binding of DAF-16 to its target promoters. However, these defects did not appear sufficient to explain the important role of PP4^SMK-1^ in aging regulation, i.e. for the expression of DAF-16 target genes. To get a closer look, we investigated the behavior of RNA polymerase II (Pol II) by ChIP-Seq under
low IIS and found a PP4<sup>SMK-1</sup>-dependent defect in Pol II recruitment and transcriptional initiation at DAF-16 activated genes, i.e. those co-activated by DAF-16 and PP4<sup>SMK-1</sup>. In search of the relevant substrate of PP4<sup>SMK-1</sup> which leads to this transcriptional initiation defect, we first tested DAF-16 itself, but we found neither a physical interaction between PP4<sup>SMK-1</sup> and DAF-16, nor could we observe any SMK-1-dependent change in the phosphorylation status of DAF-16 under low IIS. Thus, we used mass spectrometry to identify the full SMK-1-dependent phospho-proteome, followed up the emerging substrate candidates by genetic screening and settled on a top candidate, the transcription initiation/elongation factor SPT-5. Based on already published knowledge on SPT-5 and further own analyses we eventually arrived at the following model: SPT-5 is essential for transcription, being required in different phosphorylation states during Pol II recruitment (dephosphorylated), transcriptional initiation (dephosphorylated), and transcriptional elongation (phosphorylated). At the end of transcription, SPT-5 needs to be dephosphorylated again, so it can be recycled back to a promoter and catalyze another round of transcription. PP4<sup>SMK-1</sup> is the phosphatase conferring this dephosphorylation; and in its absence, there is a lack of dephosphorylated SPT-5, impairing Pol II recruitment and transcriptional initiation. We believe that this preferentially affects DAF-16 target genes under low IIS, because their expression is particularly dependent on transcriptional initiation as a rate-limiting step.

In summary, we filled a crucial gap in the mechanistic picture of how DAF-16 regulates its target genes to promote stress resistance and longevity.