Metabolic control of aging in C. elegans

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CHAPTER 7

General Discussion and Future Perspectives
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The objective of the studies presented in this thesis was to investigate the metabolic control of aging in the worm *C. elegans* from different aspects and elucidate how interactions between genes and environments influence the aging process.

In **Chapter 3**, we optimized and validated a targeted metabolomics platform that is specifically suited for identification of major metabolite classes in worms. We applied this platform to study physiological relevance of metabolite alterations related to the aging process and dietary interventions. In the metabolite profiles of worms collected at different developmental stages and adulthood from day 1 to day 10, we observed age-related changes in both fatty acid and amino acid profiles. The majority of fatty acids showed an age-dependent increase in the wild-type N2 worms, while this lipid accumulation was blunted in *aak-2* mutants, suggesting an involvement of AAK-2 in the lipid metabolism in aged worms. Interestingly, a recent study on lifespan extension mediated by caloric restriction and AMPK activity highlights an important role for mitochondrial dynamics and peroxisome remodeling in longevity (Weir et al., 2017). In addition, they also found an energy source shifting to fatty acid oxidation in aged worms that is regulated by AAK-2. The lifespan extension observed in worms expressing a constitutively active form of AAK-2 was attenuated when mitochondrial fatty acid oxidation was blocked (Weir et al., 2017). In contrast to the lipid profile, the abundance of most amino acids was high in young worms and decreased with age. Strikingly, we found two amino acids, glycine and aspartic acid, that showed a marked accumulation in aged worms. To elucidate the underlying mechanisms behind this observation, future work will study the causal link between these two amino acids and the aging process. For instance, one way to identify the role of these two amino acids is by supplementation of either glycine or aspartic acid to worm culture medium and determine the effect on worm lifespan. A recent study determined the effect of 20 amino acids on worm lifespan and reported such lifespan increase or decrease was in a dose-dependent manner (Edwards et al., 2015). Although supplementation of different amino acids could influence worm lifespan, the underlying mechanisms remain to be elucidated. Together with our findings, future work will continue on this aspect with the aim to identify the mechanisms of how glycine and aspartic acid influence worm lifespan.

In **Chapter 4**, we used a cross-omics strategy to determine key pathways and metabolite signatures of longevity. We collected both transcriptomics and metabolomics data of two well-known long-lived mutant strains, including the *daf-2* mutant (impaired IGF/insulin-like pathway) and the caloric restriction model *eat-2* mutant strains. Although both mutants have been studied independently at their transcriptome, proteome, and metabolome level, few studies focused on the shared regulatory pathways and metabolite changes that define these
regulators in long-lived worms. From the transcriptomics analysis, we detected shared pathways that were upregulated in both long-lived mutants, including stress defence response and lipid storage, while synthesis of macromolecules and developmental processes were downregulated. At the metabolite level, a number of shared metabolite signatures was found between the two long-lived worm strains, such as glycerol-3P, adenine, xanthine and AMP. Additionally, both long-lived mutants had lower levels of the fatty acids C18:0 and C17:1, as well as an overall lower level of amino acids. Using a cross-omics approach, our data suggests that downregulation of pyrimidine metabolism and upregulation of purine metabolism are common longevity mechanisms. A previous study has reported a causal involvement of the pyrimidine metabolic gene K02D7.1 as a longevity regulator in the daf-2 mutants (Martell et al., 2016). In fact, we also detected an upregulation of this gene in both daf-2 and eat-2 mutants based on our cross-omics analysis. We screened and found a number of pyrimidine metabolic genes that were upregulated in both long-lived worms and we will continue to investigate whether or not these genes also function as mediators for longevity in both daf-2 and eat-2 mutants.

As metabolic homeostasis is primarily subjected to genetic regulations, we applied a quantitative genetics approach to determine genomic regions that are important in the regulation of metabolism in worms. In Chapter 5, we applied a systems genetics approach to identify important genomic loci that regulate the level of metabolite in C. elegans. We analyzed amino acid and fatty acid data of 199 recombinant inbred lines (RILs) of C. elegans that were derived by crossing two wild-type strains, including N2 (Bristol) and CB4856 (Hawaii) (Li et al., 2006). Based on our analysis on metabolite-metabolite correlations, we detected co-regulated metabolites as well as the causal genomic loci that explains the variations we observed in the metabolite profiles of RILs. Using the targeted metabolomics platform introduced in Chapter 3, we were able to measure 20 amino acids and 44 fatty acids in the RILs and observed a large variation in the metabolite levels with 32-82% heritability. From the metabolite-metabolite correlation analysis, we observed strong co-correlated metabolite clusters in both fatty acids and amino acids. Some of the strongly correlated clusters have been reported in case studies of metabolic diseases in human. For instance, we observed a strong positive correlation between five amino acids, including aromatic amino acids tyrosine, phenylalanine, and three branched-chain amino acids valine, leucine and isoleucine. Elevated levels of these five amino acids were suggested as promising indicators to predict the development of type II diabetes in a Framingham Offspring cohort (Wang et al., 2011). Based on our results, the strong correlation between these amino acids indicated a shared mode of regulation. Next, we performed quantitative trait loci (QTL) mapping and detected 36 QTL that were at least in part responsible for the metabolite variations we observed in the RILs. We focused on the QTL that
showed high significance and heritability, i.e. a QTL for the fatty acid C14:1 on chromosome I and another QTL for the fatty acid C18:2 on chromosome IV. We then used introgression lines to narrow down the size of both QTL to a region of 1.4Mbp and 3.6 Mbp, respectively. This systems approach promises considerable advances to study the genetic basis of metabolism. In the future, we planned to use the RIL panel to study the interactions between environment and genetics and their influence related to the aging process and metabolism. For instance, we will expose RILs on different diets, such as a sugar diet or fat bacterial diet. Moreover, this RIL panel could also be used for screening potential compounds that are beneficial for health. We believe that this systems approach with QTL analysis will enable us to explore complex traits in relation to the genetic background.

The aging process is controlled by metabolic networks that are influenced by not only genetic or environmental factors, but is also regulated by gene and environment (GxE) interactions. In Chapter 6, we studied how ech-6 RNAi remits fat-induced accelerated aging in worms as an example of the influence of GxE interactions on metabolism and lifespan. The dietary fat we used in this study is polysorbate 80 (P-80), which is a soluble compound derived from a hydrophilic polyoxyethylene group and oleic acid. Worms fed on a P-80 diet have a decreased lifespan, whereas reduced expression of ech-6, a gene encoding enoyl-CoA hydratase, rescued the lifespan shortening from the P-80 diet. We applied several other soluble lipid derivatives as fat diets in worms, including span-80 (sorbitan hydrophilic group + oleic acid, S-80), and polysorbate 20 (polyoxyethylene + lauric acid, P-20) with different concentrations. Interestingly, ech-6 RNAi worms could rescue the shortened lifespan induced by P-80 and S-80, but not P20, suggesting an oleic acid effect only. To explore this further, we performed characterization of ech-6 RNAi worms and investigated the potential mechanisms that mediate the restored lifespan. Among these investigations, we noted an interesting upregulation in lipl-4, a gene encoding lysosomal lipase that has been showed previously to mediate longevity in germline-less long-live mutant worms (Lapierre et al., 2011). We then tested the role of lipl-4 on the lifespan extension in ech-6 RNAi worms fed a P-80 diet via two routes: 1) we analysed lifespan effect in lipl-4 overexpressing (lipl-4 OE) strains with a reduced expression of ech-6 on a P-80 diet; 2) as autophagy was previously shown to correlate with lipl-4 on longevity of germline-less worms, we also monitored the activation of autophagy in ech-6 RNAi worms fed on a P-80 diet using LGG-1:GFP strain. Interestingly, lipl-4 OE strain with ech-6 RNAI showed an even better lifespan extension on a regular diet, whereas these worms have a shorter lifespan when fed on a P-80 diet. This suggests that overexpression of lipl-4 extends the lifespan of ech-6 RNAi worms possibly by releasing more fatty acids in worms. In future studies, we will analyse the fatty acid profiles of lipl-4 OE worms under these conditions. However, we did not detect a strong autophagy in ech-6 RNAi worms fed on a P-80 diet, further experiments
are required to confirm this finding. Although we have shown an increased level of C18:1 and also proven that fatty acid oxidation is required for *ech-6* RNAi worms to cope better with the P-80 diet, it is important to determine where these dietary fats are stored in worms. To test this, we will also perform Oil Red O staining and lipidomics (developed in Chapter 3) in *ech-6* RNAi worms in both N2 and *lipl-4* OE strains that are fed on a P-80 diet.

Although metabolism has been known for decades to play a central role in the regulation of aging, the actual underlying mechanisms are still not fully understood. Metabolic networks consist of complex interactions between different signalling pathways, which requires regulation at different levels. With the advances of different “omics” approaches in the field, we now start to understand these different regulatory levels and put the pieces of biological information together to get a comprehensive picture of what is going on. In this thesis, we used different types of systems approaches to exploit the full spectrum of possible mechanisms relevant to the metabolic control of the aging process, and we believe that those valuable findings will help the field move forward and provide great ideas for future research.

Reference


