UNIVERSITY OF AMSTERDAM

Aging in Caenorhabditis elegans; the Role of Wnt Signalling

M. Lezzerini

Summary

Aging is a universal biological process characterized as the gradual decline of a multitude of physiological functions that leads to an increasing probability of death. To date we have a very poor understanding of what actually causes aging. A commonly held view is that aging is the result of damage accumulation over a lifetime, although recent results in worms and mice have led researchers to question the free radical theory as the primary or exclusive cause of aging. Another theory proposes that aging is driven by genetic programs that are useful for the organisms early in life, becoming detrimental for the survival al later stages (antagonistic pleiotropy theory of aging).

The scope of this thesis was to investigate whether one of the most important developmental pathways, the Wnt signalling, plays any functional role in *C. elegans* during natural aging. To do so, we tested whether the different components participating in the signalling cascade are still active past development and they affect the overall survival of the animals. We found that the Wnt pathway is actually involved in age-regulation in *C. elegans*, and although it does not affect the stress resistance of the animals, its functions cannot be entirely linked to the antagonistic pleiotropy view.

Chapter 1 introduces in the first part the concept of aging and presents the most recent findings in the aging research field together with the major theories to explain this process. The second part explains the architecture and functions of the Wnt signalling during development and aging, and summarizes the most recent discoveries connected to the aging process in *C. elegans* and others model organisms. The chapter concludes with an outline of the contents presented in this thesis.

Chapter 2 describes the analysis of the five Wnt ligands present in *C. elegans* and their effect on longevity. It presents data that demonstrate that all the Wnt ligands are expressed past developmental stages and keep on signalling during adulthood, keeping the Wnt activity during adulthood. These proteins regulate the previously known aging genetic circuit composed by the *elt-5*/*elt-3* transcription factors. The observed expression pattern suggests that the down-regulation of any of the 5 Wnt ligands would favor a prolonged life span. However, we found that only 2 Wnt ligands (*mom-2* and *cwn-2*) are detrimental for longevity, whereas other 2 (*lin-44* and *egl-20*) are beneficial. Interestingly, we provide evidence that these changes in life span are not linked to altered stress resistance in the animals.

Chapter 3 focuses on the non-canonical Wnt pathway and on its major components MOM-2 and WRM-1. We found that this pathway is overall harmful for longevity since down-regulating the gens involved prolong the life span of the animals. We performed RNA sequencing (RNA-seq) experiments discovering that the long-lived mutants are characterized by an altered fat metabolism and increased production of collagen. We also found that Wnt promotes aging starting from the beginning of adulthood and its detrimental effects cannot be reversed past day 5. Further analysis indicated that Wnt acts in the intestine to regulate the longevity and it is modulated by signals coming from the germline that possibly activate the signalling cascade.

The **chapter 4** characterizes the effects on gene expression mediated by the canonical Wnt pathway, by analyzing the transcriptome changes in a *bar-1*/β-catenin mutant. In this chapter, we also compared the gene expression changes of several Wnt mutants and discuss the biological processes most affected by these mutations in the context of aging.

Chapter 5 presents an alternative method to perform RNAi by feeding in *C. elegans*. We developed an RNAi system that uses *Bacillus subtilis* instead of *Escherichia coli* as bacterial host strain. As food source, *B. subtilis* represents a healthier option since the nematodes fed this kind of bacteria live on average 60% longer [130].

This work provides a new genetic tool to the *C. elegans* community and, in aging studies, offers the chance to address new research questions.

The **chapter 6** discusses the findings presented in this study. It summarizes the effects mediated by the different components participating in both, canonical and noncanonical Wnt signalling cascade, and highlights the double nature of Wnt pathway in aging. The chapter concludes by discussing the role of Wnt signalling in the context of the aging theories, in particular in relation to the antagonistic pleiotropy theory of aging.