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Analysis of the Role of MicroRNAs in the Control of *C. elegans* Aging. **Konstantinos Boulias**, Ezequiel A. Alvarez-Saavedra, Ala Berdichevsky, Bob Horvitz. HHMI, Dept. Biology, MIT, Cambridge, MA 02139 USA.

MicroRNAs constitute a recently discovered class of small (20-24 nt) non-coding RNAs found in *C. elegans*, *Drosophila*, plants, mammals and other organisms. Studies over the past few years indicate that microRNAs are critical regulators of gene expression in diverse biological processes, including developmental timing, cell-fate specification, cell proliferation and differentiation. The first microRNAs discovered were *lin-4* and *let-7*, which control the timing of developmental processes in *C. elegans*. Since aging can be regarded as a temporally-regulated developmental process, it is plausible that microRNAs also control aging. The genetic basis of *C. elegans* aging has been studied extensively and genes that define conserved regulatory pathways that affect lifespan have been characterized.

To identify microRNAs that might function in the regulation of the aging process, we are using microarrays that contain most known *C. elegans* microRNAs to determine microRNA expression patterns during aging. In parallel, we are analyzing deletion alleles of 91 microRNA genes to identify microRNA mutants with an abnormal aging phenotype. To this end, we are assessing the lifespan, response to stress and accumulation of a lipofuscin-like intestinal pigment, a well-characterized marker of aging, for each strain.

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Functional Characterization of *pnc-1* in C. elegans. **Juan J. Carmona**[1,2,3], Anne C. Hart[1,2], David A. Sinclair[1,3]. 1) Department of Pathology, Harvard Medical School, Boston, MA, 02115; 2) Massachusetts General Hospital Cancer Center, Charlestown, MA, 02129; 3) Paul F. Glenn Laboratories for the Biological Mechanisms of Aging, Harvard Medical School, Boston, MA, 02115.

In yeast, Pnc1 (nicotinamidase), an enzyme in the NAD^+ salvage pathway that converts nicotinamide to nicotinic acid to assist in regenerating NAD^+, has been shown to be necessary and sufficient for lifespan extension under caloric restriction and mild stresses. Furthermore, a Pnc1-GFP fusion reporter has shown that Pnc1 is nuclear, cytoplasmic, and peroxisomal in yeast and that caloric restriction and mild stress, like heat, causes a significant increase in fluorescence intensity. Given these data, therefore, attention has now turned to Pnc1 homologues in multi-cellular organisms, such as PNC-1 in *C. elegans*, specifically with the desire to study functional conservation of this enzyme with respect to longevity and stress pathways. Recently, it has been reported that RNAi-mediated knockdown of the *C. elegans* homologue *pnc-1* significantly decreases adult lifespan and that increased gene dosage enhances survival during oxidative stress treatment. However, the tissue-specific expression pattern of PNC-1 remains unknown and a protective role for PNC-1 during heat shock, a common stress paradigm in *C. elegans*, has not been considered. To this end, we have generated GFP fusion constructs to pinpoint PNC-1 expression. Upon first examination, GFP is expressed in pharyngeal muscle and ASK neurons, with the latter playing a role in insulin signaling and longevity. Furthermore, we have generated polyclonal antibodies against PNC-1 and have determined by Western blot that stresses, in particular heat, increase PNC-1 protein levels. Finally, we have produced PNC-1 over-expressing lines which we are actively characterizing for a longevity phenotype. Our preliminary data suggest that these over-expressing lines are markedly thermotolerant; other forms of stress are under analysis. Collectively, it is anticipated that all of these studies will help to elucidate a functional role for *pnc-1* in animals, to further expand our understanding of conserved longevity and stress pathways across organisms.

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Uncoupling *daf-2* lifespan extension: pathway specificity, demographic, and movement rate analysis of gene inactivations identified in a functional genomic screen for accelerated aging. Andrew Samuelson[1,2], **Christopher Carr**[1,3], Gary Ruvkun[1,2]. 1) Department of Molecular Biolog, Massachusetts General Hospital, Boston, MA; 2) Department of Genetics, Harvard Medical School, Boston, MA; 3) Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, MA.

The *daf-2* insulin-like signaling pathway is the most potent pathway for lifespan extension in *C. elegans*, converging on the DAF-16 transcription factor to regulate a large number of genes. We sought to identify the full range of genes which are necessary for the long lifespan induction in a *daf-2* insulin signaling deficient mutant. To this end, we have identified 143 genes whose inactivation shortened the lifespan of long-lived *daf-2* mutant animals when inactivated through a systematic genome-wide RNAi screen. We classified gene inactivations which only shortened the lifespan of a *daf-2* mutant, and those which shortened the lifespan of wild type as well as a *daf-2* mutant. Detailed measurements of lifespan allowed the calculation of the relative mortality rate doubling time (MRDT, proportional to the reciprocal of the rate of aging) and initial mortality rate (IMR) for each gene inactivation. Additionally, analysis for each gene inactivation includes longitudinal scoring for changes in movement rate ("activespan"), one measure to distinguish genes that accelerate the aging process versus those that show signs of poor health.

Analysis of these phenotypes has allowed us to segregate the gene inactivations into refined classes based on whether there is accelerated aging or poor health. For instance, some of the gene inactivations that shorten lifespan in both control and *daf-2* mutant animals, such as the previously described heat shock transcriptional regulator *hsf-1*, accelerated MRDT with only a modest increase in IMR. Results from this detailed analysis will be presented.