Funktionelle Analyse der *C. elegans* Gene *vang-1*, *atad-3* und *mics-1* in Bezug auf Alterung und Stressresistenz

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Sebastian Jakob Honnen

aus Geldern

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aus dem Institut für Toxikologie
der Heinrich-Heine-Universität Düsseldorf
und
dem Institut für Molekulare und Zelluläre Anatomie
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Abkürzungsverzeichnis

ADP	Adenosindiphosphat
AS	Aminosäure
ATP	Adenosintriphosphat
Bp	Basenpaar
C. elegans	*Caenorhabditis elegans*
C-Terminal	Carboxy-Terminal
ETC	*electron transport chain*
IIS	Insulin/IGF-1 Signalweg
MAPK	Mitogen aktivierte Protein Kinase
N-Terminal	Amino-Terminal
OXPHOS	oxidative Phosphorylierung
PI3K	Phosphatidylinositol 3-Kinase
PIP_2	Phosphatidylinositol 4,5-bisphosphat
PIP_3	Phosphatidylinositol 3,4,5-trisphosphat
RNAi	Ribonukleinsäure-Interferenz
ROS	„*reactive oxygene species*“ (reaktive Sauerstoffspezies)
S. cerevisieae	*Saccharomyces cerevisiae*

Zusammenfassung

Alterung stellt den größten Risikofaktor für Krankheiten wie z.B. Diabetes Typ-2 und Alzheimer dar. Seit Entdeckung der Möglichkeit diesen Prozess genetisch zu modulieren, hat sich der Nematode *Caenorhabditis elegans* als Modellorganismus bestens etabliert. So wurde der bis zum Menschen konservierte Insulin/IGF-1 Signalweg als zentraler Regulator der Lebensspanne in *C. elegans* entdeckt. Langlebige *C. elegans* Mutanten dienen auch als Modell, um mit Mitochondrien assoziierten Pathologien des Menschen zu untersuchen.

Ausgangspunkt der vorliegenden Dissertation bildete zunächst die Identifizierung zweier Interaktionspartner des Gerüstproteins DLG-1/Disc large, welches die epitheliale Kontaktstruktur in *C. elegans* organisiert. Bei VANG-1 handelt es sich um das Strabismus/Van Gogh Homolog, eine Schlüsselkomponente des planaren Zellpolaritäts-Signalwegs in anderen Systemen. ATAD-3 ist das Homolog des humanen mitochondrialen ATAD3 Proteins und besitzt eine AAA Domäne (***A**TPases **a**ssociated with a variety of cellular **a**ctivities*). Mit MICS-1/OMP25 (***o**uter mitochondrial **m**embrane **p**rotein **25***) konnte dann in dieser Arbeit ein neuer ATAD-3 Interaktionspartner isoliert werden. Ziel war es, die Funktion dieser drei Proteine in *C. elegans* in Bezug auf Lebensspanne und Stressresistenz zu untersuchen und in diesbezüglich bereits bekannte Signalwege einzuordnen.

Es wird gezeigt, dass VANG-1 über den Insulin/IGF-1 Signalweg, sehr wahrscheinlich in der Keimbahn, temperaturunabhängig die Lebensspanne von *C. elegans* reguliert. *Vang-1* Mutanten leben bis zu 40% länger als der Wildtyp und sind resistent gegen reaktive Sauerstoffspezies und Hitze. Sie zeigen weiterhin partielle Sterilität, verlangsamtes Wachstum und eine verzögerte Produktion des Alterspigments Lipofuszin. Aufgrund dieser Ergebnisse kann *vang-1* als Mitglied der Gerontogen-Familie eingeordnet werden. Dies ist die erste Beschreibung eines planaren Zellpolaritätsgens in Bezug auf die Lebensspanne in *C. elegans*.

Der Aufall des mitochondrialen AAA-Proteins ATAD-3 und seines Bindungspartners MICS-1/OMP25 führen sowohl einzeln als auch zusammen zu einem Anstieg der mittleren Lebensspanne um bis zu 84%. Dieser erfolgt hauptsächlich unabhängig von DAF-16/FoxO. In Bezug auf den intrazellulären ROS (***r**eactive **o**xygen **s**pecies*) Level verhalten sich die Interaktionspartner gegensätzlich. In der *mics-1* Mutante ist DAF-16/FoxO unter Hitzestress wahrscheinlich aktiver, was zu einem geringeren Anstieg des ROS Levels führt. Bei *atad-3(RNAi)* hingegen ist keine erhöhte DAF-16 Aktivität zu beobachten und der ROS Level steigt auf ~200%.

Summary

Aging is the major risk factor for pathologies like type 2 diabetes, Alzheimer´s disease among others. The nematode *Caenorhabditis elegans* has proved to be an invaluable modelsystem in the basic research on the genetic modulation of life span. In particular due to the finding that the insulin/IGF-1 signaling (IIS) pathway is central regulator of life span. Also long-lived *C. elegans* mutants are used as a model for human mitochondrial-associated diseases. Over the last decade, several hundred genes have been found to modulate the aging process.

In this thesis, two interacting partners of the scaffold protein DLG-1/Discs large VANG-1 and ATAD-3 have been investigated with regard to life span in *C. elegans*. DLG-1 is a key component of the apical junction and ensures the correct formation of junctional belts around the apex of epithelial cells in *C. elegans*. Here, we describe a novel role of VANG-1, the only *C. elegans* ortholog of the conserved planar cell polarity pathway component Strabismus/Van Gogh. We show that two alleles of *vang-1* and depletion of the protein by RNAi cause an increase of mean life span up to 40%. Mutations in *vang-1* also cause enhanced resistance to thermal- and oxidative stress. In addition, *vang-1* animals show defects like reduced brood size, decreased ovulation rate and prolonged reproductive span, which are typical to gerontogenes. Lifespan extension in *vang-1* mutants depends on DAF-2/Insulin receptor and DAF-16/FoxO transcription factor, which mainly seem to act in the germline but not the intestine or neurons.

Investigating the mitochondrial AAA-protein ATAD-3, the ortholog of human ATAD3, we identified the novel interacting partner MICS-1/OMP25. We demonstrate that *mics-1(RNAi)* animals or *mics-1* mutants display enhanced longevity with an increased mean lifespan of up to 54% compared to control animals. Of note, also *atad-3(RNAi)* promoted longevity, although to a lesser extend (29% compared to controls). Further studies revealed that MICS-1 and ATAD-3 associated longevity was predominantly independent of DAF-16/FoxO. This observation offers new options to investigate modulators of life span apart from the well-established key component DAF-16/FoxO.

1. Einleitung

1.1 *Alterung ist der größte Risikofaktor für zahlreiche Erkrankungen des Menschen und unterliegt klassischen, modulierbaren Signalwegen*

In Anbetracht des demografischen Trends westlicher Gesellschaften und den damit verbundenen Kosten für das Gesundheitssystem ist die Grundlagenforschung im Bereich Alterung zunehmend in den Fokus der wissenschaftlichen Welt geraten. Die Anzahl Publikationen unter dem Stichwort „Alterung“ in der „pubmed“ hat sich von 7550 für das Jahr 2000 zu 15139 für das Jahr 2010 verdoppelt. Das Gesamtvolumen der Finanzierung in diesem Sektor wächst stetig. Das biologische Alter ist der größte Risikofaktor vieler Krankheiten, wie beispielsweise Diabetes Typ-2, Alzheimer, Parkinson, Sarkopenie, Arteriosklerose und Bluthochdruck (Martin, 2010).

Wissenschaftlich betrachtet ist Alterung gekennzeichnet durch progressive, degenerative Veränderungen in der Gewebeorganisation und Funktion, welche die Wahrscheinlichkeit des Todes erhöhen (Collins et al., 2008). Ein Hinweis auf die genetische Modulierbarkeit der Alterung kann aus der Tatsache geschlossen werden, dass sehr nahe verwandte, genetisch ähnliche Arten teils dramatisch unterschiedliche Ausprägungen der Lebenserwartung aufweisen. Eines der berühmtesten Beispiele ist der Vergleich zwischen Mäusen, welche bis zu vier Jahre leben, und Nacktmullen mit einer Lebenserwartung von 28 Jahren (Buffenstein, 2008). Diese nahe verwandten Arten bewohnen vereinfacht dargestellt ähnliche Habitate und weisen eine vergleichbare Physiologie, Größe etc. auf. Trotzdem ist die Eigenschaft „Lebenserwartung“ offensichtlich sehr unterschiedlich selektiert worden.

Nach der ersten Beschreibung von langlebigen *C. elegans* Mutanten durch Klaas im Jahr 1983, entstand ein kontinuierlich wachsendes Bild der Genetik des Alterns (Klaas, 1983). Hunderte Proteine konnten mit der Modulation der Lebensspanne in Zusammenhang gebracht werden, welche häufig bereits als Bestandteil evolutionär hoch konservierter Signalwege bekannt waren (Kenyon, 2005). Eines der am besten untersuchten Spezies übergreifenden Beispiele ist die erhöhte Lebenserwartung durch kalorische Restriktion. Dem ersten Bericht von McCay et al. (1935), der die lebensverlängernde Wirkung auf Ratten zeigte, folgten weitere Studien und positive Ergebnisse mit Hefe, Fliegen, Würmern, Fischen, Mäusen und wiederum Ratten (Holloszy and Fontana, 2007; McCay, 1935).

Faszinierend sind zwei Studien an Rhesus Affen. Eine davon arbeitet mit *Macaca mulatta* Primaten, welche 93% Sequenzidentität mit dem menschlichen Genom haben und auf eine sehr ähnliche Art altern. Sie zeigten, dass durch kalorische Restriktion Entzündungsfaktoren

reduziert, Sarkopenie verzögert und Altersdiabetes verhindert werden (Colman and Anderson, 2011).

Diese Beobachtungen sind aufgrund der nahen Verwandtschaft zum Menschen von Interesse. Nähere Informationen bezüglich kalorischer Restriktion im Speziellen und auch über den Mechanismus der Alterung im Allgemeinen werden aus Studien am Nematoden *Caenorhabditis elegans* erlangt. Deshalb wurde auch in der hier vorliegenden Arbeit *C. elegans* als Modellorganismus für die Aufklärung von Fragestellungen aus dem Gebiet der Alterungsforschung verwendet. Dieser bietet die Möglichkeit guter experimenteller Erforschung und weist zugleich eine große Ähnlichkeit in Signalwegen, welche die Alterung modulieren, zu höheren Organismen auf (siehe Abb. 1).

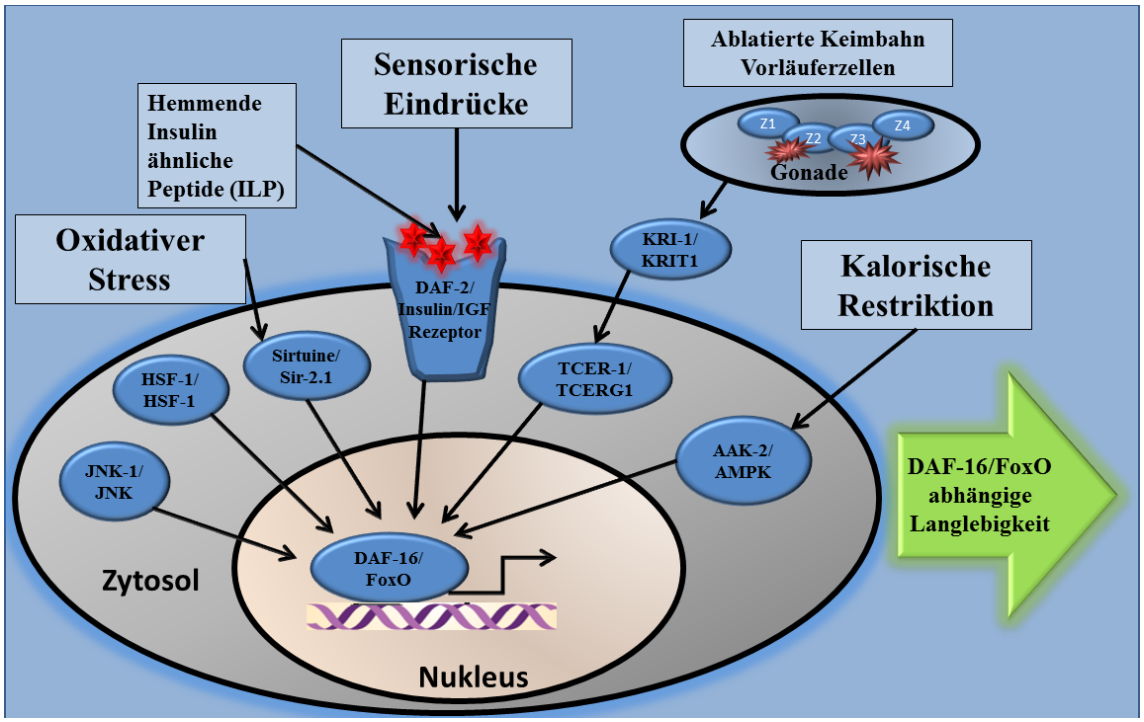

Abb. 1: Der Transkriptionsfaktor DAF-16/FoxO fördert die Lebensspanne in *C. elegans* abhängig von verschiedenen Signalen
Eine schematische Ansicht verschiedener Effektoren der Lebensspannemodulation, welche einen aktivierenden Einfluss auf DAF-16/FoxO haben. Abgesehen von DAF-2, welches dazu reprimiert wird, gilt es jeweils für die aktive Form der entsprechenden Proteine (modifiziert nach Kenyon, 2010).

1.2 *C. elegans ist als Modellorganismus der Alterungsforschung etabliert*

C. elegans weist einen sehr kurzen, wohldefinierten Lebenszyklus auf. Er ist nur ca. 1 mm lang und einfach in nahezu isogenen Populationen auf kostengünstigen Agarplatten mit hoher Individuen- und Nachkommenzahl zu kultivieren. Bei *C. elegans* wurden mittlerweile hunderte Gene identifiziert, deren Funktion mit einer veränderten Rate der Sterblichkeit im Zusammenhang steht. Damit wurde *C. elegans* zum führenden Modellorganismus der

Biogerontologie (Antebi, 2007; Johnson, 2008; Tissenbaum, 2012). Weitere Vorteile des Modells sind seine Transparenz und der invariable Zellstammbaum, wodurch altersbedingte Veränderungen *in vivo* dokumentiert werden können (Collins et al., 2008; Sulston et al., 1983). Hervorzuheben ist zudem die Möglichkeit nahezu jedes Genprodukt zum gewünschten Entwicklungszeitpunkt über RNA vermittelte Interferenz sogar gewebespezifisch zu drücken (Calixto et al., 2010; Fire et al., 1998; Minois et al., 2010; Pilipiuk et al., 2009; Sijen et al., 2001).

Bei *C. elegans* wurde eine Klasse von Mutanten beschrieben, welche ein spezieller Katalog von Phänotypen wie verzögertes Wachstum, mindestens 20% verlängerte mittlere Lebensspanne und die Resistenz gegen verschiedene Stressfaktoren wie Hitze oder reaktive Sauerstoffspezies (***r**eactive **o**xygen **s**pecies* [ROS]) vereint. Die entsprechenden Gene dieser „Alterungs-Mutanten" werden Gerontogene genannt (Johnson et al., 2002). Häufig wird auch eine reduzierte Akkumulation von Lipofuszin beobachtet. Dies sind oxidierte und kreuzvernetzte Moleküle, welche sich im Zuge der Alterung bei *C. elegans* hauptsächlich als Granula im Darm ablagern. Bei vielen Organismen sind sie ein Maß für das biologische Alter (Garigan et al., 2002; Yin, 1996).

Im Fokus der vorliegenden Arbeit stehen insbesondere der Insulin/IGF-1 Signalweg sowie Effekte der Mitochondrien und assoziierter Proteine auf die Alterung. Im Folgenden wird zuerst näher auf den sehr gut untersuchten Insulin/IGF-1 Signalweg in *C. elegans* eingegangen, der auf den Transkriptionsfaktor DAF-16/FoxO in der Modulation der Lebensspanne zurückgreift (siehe Abb. 1).

1.2.1 *Der Insulin/IGF-1 Signalweg in der Modulation der Lebensspanne*

Die Funktion des Insulin/IGF-1 Signalweg (IIS) in der Modulation der Lebensspanne wurde zuerst in *C. elegans* entdeckt. Er wird diesbezüglich heute auch in *Drosophila melanogaster*, *Mus musculus* und dem Menschen intensiv erforscht (Holzenberger et al, 2003; Kenyon, 2005; Kenyon, 2011; Kenyon, 2010; Tissenbaum, 2012).

Die erste in *C. elegans* entdeckte Lebensspanne modulierende Komponente war *age-1*, welche eine **P**hosphatidyl**i**nositol **3**-Kinase (PI3K) kodiert (Friedman and Johnson, 1988; Morris et al., 1996). In der Folge wurde das Insulinrezeptor Homolog DAF-2 in der Modulation der Lebensspanne identifiziert (Kenyon et al., 1993; Kimura et al., 1997). In der Larvalentwicklung beeinflusst DAF-2 den Übergang zum alternativen L3 Stadium, der Dauerlarve. Bei schlechten Wachstumsbedingungen (Mangel an Nahrung, kritische Populationsdichte, Hitzestress) beschreitet *C. elegans* nach dem L2 Stadium nicht den reproduktiven

Entwicklungsweg, sondern bildet nicht-reproduktive, extrem langlebige Dauerlarven (Riddle et al., 1981). Bei Funktionsverlust von *daf-2* gehen die Individuen auch bei guten Umweltbedingungen konstitutiv in dieses Dauerstadium. *Daf-16* Mutanten hingegen sind nicht mehr in der Lage ein solches auszubilden, selbst im *daf-2* negativen Hintergrund (Gottlieb and Ruvkun, 1994). Das Labor von Cynthia Kenyon brachte diese Ergebnisse mit der Modulation der Lebensspanne zusammen. Sie zeigten, dass der Transkriptionsfaktor DAF-16, das einzige **Fo**rkhead Bo**x O** (FoxO) Homolog in *C. elegans,* essentiell für die Langlebigkeit von *daf-2* und *age-1* Mutanten ist. Damit wurde erstmals eine direkte Regulation des Alterungsprozesses demonstriert (Dorman et al., 1995; Kenyon et al., 1993).

Mittlerweile wurden weitere Faktoren dieses Signalwegs beschrieben, welche zum Großteil bis zum Menschen konserviert sind (Christensen K., 2006). FoxO DNA Varianten konnten in verschiedenen Studien mit hoher Lebenserwartung beim Menschen in Zusammenhang gebracht werden (Flachsbart et al., 2009; Kenyon, 2011; Willcox et al., 2008).

Es gibt in *C. elegans* 40 aktivierend oder repremierend wirkende Insulin-ähnliche Peptide (***i****nsulin-****l****ike* ***p****eptides* [ILP]), welche an DAF-2 binden können. Die aktivierte Rezeptor Tyrosin-Kinase DAF-2 induziert eine Kinase Kaskade über die PI3K AGE-1 und die Serin/Threonin Kinase PDK-1 hin zu den AKT-Kinasen (AKT-1/AKT-2/SGK-1). Diese phosphorylieren DAF-16/FoxO, so dass der Transkriptionsfaktor im Zytoplasma verbleibt. Dort wird er durch die E3 Ubiquitin Ligase RLE-1 polyubiquitiniert und im Proteasom degradiert (Li et al., 2007b). Eine DAF-2 Hemmung oder direkte Aktivierung von DAF-16 führen zur Translokation in den Zellkern (Fielenbach and Antebi, 2008).

Im Zellkern aktiviert DAF-16 verschiedene Sets nachgeschalteter Gene wie beispielsweise antioxidativer Proteine (z.B. **S**uper**o**xid **D**ismutase/*sod-3*), Chaperone (z.B. **H**itze**s**chock **P**rotein/*hsp-16.2*) und antimikrobielle Proteine (Murphy et al., 2003). Die konzertierte Aktivität dieser Proteine, von denen jedes für sich nur einen geringen Effekt hat, trägt zur Verdopplung der Lebensspanne von *C. elegans daf-2* Mutanten bei.

Neben DAF-16 ist auch der **H**itze**s**chock **F**aktor HSF-1 essentiell für die Verdopplung der Lebensspanne durch *daf-2* (Hsu et al., 2003).

Die Translokation von FoxO in Säugern ist in der Regel durch eine Interaktion mit Proteinen der 14-3-3-Familie reguliert. Hier führt die Phosphorylierung durch Akt-Kinasen zu einer erhöhten Affinität zu 14-3-3 Proteinen, wodurch FoxO von der DNA gelöst und ins Zytosol relokalisiert (Brunet et al., 1999; Cahill et al., 2001). Dort blockieren die 14-3-3 Proteine die für die Kernlokalisation wichtigen Domänen und verhindern so eine erneute Translokation (Brunet et al., 2002; Burgering and Kops, 2002; Daitoku et al., 2004). Kürzlich konnte in

C. elegans ein ähnlicher Zusammenhang zwischen DAF-16/FoxO und den 14-3-3 Proteinen PAR-5 und FTT-2 gezeigt werden (Berdichevsky et al., 2006; Li et al., 2007a; Wang et al., 2006).

Parallel zu DAF-16 reprimiert der IIS auch SKN-1, welches in einem positiven feedback loop unter anderem die Expression von DAF-2 reprimierenden ILPs (*ins-39*; *daf-28*) fördert (Okuyama et al., 2010). Das Nrf Homolog SKN-1 aktiviert die Phase-II Detoxifizierung in *C. elegans* zum Schutz gegen oxidativen Stress (Tullet et al., 2008). Es wird im Darm durch die MAPK PMK-1 (*human*: p38) phosphoryliert, wodurch es im Zellkern akkumuliert und die Expression nachgeschalteter Gene wie verschiedener Glutathion S-Transferasen reguliert (An and Blackwell, 2003).

Neben der Regulation der Langlebigkeit fördert der IIS unter optimalen Ernährungsbedingungen die Proliferation der Keimbahn (Michaelson et al., 2010). Signale dieses Gewebes nehmen selbst auch Einfluss auf die Lebensspanne.

1.2.2 *Signale der Keimbahn nehmen über zwei parallele Wege Einfluss auf die Translokation von DAF-16/FoxO in die Zellkerne des Darms*

Das Labor von Cynthia Kenyon zeigte 1999, dass bei Ablation der Keimbahn-Vorläuferzellen Z2 und Z3 die Lebensspanne um 60% gegenüber unablatierten Tieren steigt (siehe Abb. 1; Hsin and Kenyon, 1999). Ein endokrines Signal der mitotischen Keimbahnstammzellen aktiviert das FERM-Domänen und „*Ankyrin-repeats*“ enthaltende Protein KRI-1 im Darm. Dieses wirkt aktivierend auf die Expression des Transkriptionselongationsfaktor TCER-1 und wird, zusammen mit der microRNA *mir-71,* für die DAF-16 Kernlokalisation benötigt (siehe Abb. 1; Berman and Kenyon, 2006; Boulias and Horvitz, 2012). Gemeinsam mit TCER-1 aktiviert DAF-16 die Expression eines speziellen Sets von Genen (beispielsweise: *gpd-2*/GAPDH; *nnt-1*/Nikotinamid Nukleotid Transhydrogenase), so dass ein positiver Einfluss auf die Lebensspanne ausgeübt wird (Arantes-Oliveira et al., 2002; Ghazi et al., 2009).

Ein paralleler Signalweg, welcher den Lebensspanne-Effekt der Keimbahnstammzellen vermittelt, läuft über den nukleären Hormonrezeptor DAF-12. Durch Ablation von Z2/Z3 werden in Abhängigkeit von DAF-9/Zytochrome P450 sogenannte „***d**afachronic **a**cids*“ (DAs) gebildet. Die DAs wirken als Liganden für das Vitamin D Rezeptor Homolog DAF-12 und stimulieren eine DAF-16/FoxO Translokation in die Zellkerne des Darms (Berman and Kenyon, 2006; Motola et al., 2006, Wollam et al., 2012). Der Darm gilt als allgemein sehr wichtig für die Koordination der Stressantwort und Lebensspannemodulation (Libina et al., 2003).

1.3 *Der Darm ist das zentrale Gewebe der Reaktion auf Stress in C. elegans*

Bedingt durch das Habitat von *C. elegans* ist von den Geweben des Nematoden an erster Stelle der Darm für die Reaktion auf Toxine und chemischen Stress verantwortlich (McGhee, 2007). Außerdem wurde beispielsweise anhand des Insulin/IGF-1 Signalwegs, des Keimbahnsignalwegs und auch SKN-1/Nrf gezeigt, dass diese Signalwege der Stressresistenz und Lebensspannemodulation letztlich hauptsächlich im Darm wirken (An and Blackwell, 2003; Lin et al., 2001).

Der *C. elegans* Darm besteht aus 20 Zellen, welche in neun Einheiten, den „ints" (intestinal rings), organisiert sind (Sulston et al., 1983; Leung et al., 1999). Der erste „int" besteht aus vier Zellen, die nach posterior folgenden je aus zwei gegenüberliegenden. An den Kontaktpunkten befindet sich die „*C. elegans* apical junction (*Ce*AJ)" (McMahon et al., 2001). Auch Vertebraten und *Drosophila* besitzen solche interzellulären Kontaktstrukturen. Dort verteilen sich die Eigenschaften und Proteinkomposition zwar auf mehrere Komplexe, aber gemeinsam ist ihnen unter anderen ein Gerüstprotein der *Discs large (dlg)* Proteinfamilie (Knust and Bossinger, 2002). Es gilt als Schlüsselkomponente und sein Ausfall führt bei *C. elegans* Embryonen zu strukturellen Defekten und Lethalität (McMahon et al., 2001).

Diese Proteinfamilie wurde zuerst für ihre Funktion in den Imaginalscheiben der *Drosophila melanogaster* Larve beschrieben. In rezessiven *dlg*-Allelen kommt es durch krebsartige Neubildung von Gewebe innerhalb der Imaginalscheiben zu larvaler Lethalität (Woods and Bryant, 1991). *Dlg* wurde als Tumorsuppressor-Gen eingestuft und hat weitere Funktionen für beispielsweise Zellpolarität, Adhäsion und Lokalisation von Signalproteinen (Yamanaka and Ohno, 2008).

Die *dlg*-Proteine gehören zur Familie der „**M**embrane **A**ssociated **GU**anylate **K**inase"-Proteine (MAGUK), welche speziesübergreifend als kritische Komponenten von Signalzentren betrachtet werden (Thomas et al., 2010).

C. elegans DLG-1 besitzt mehrere Protein-Protein Interaktions-Domänen: drei PDZ-Domänen (**P**SD95, **D**iscs Large, **Z**O-1), eine SH3-Domäne (**S**rc **H**omolog **3**) und die GUK-Domäne (**GU**anylate **K**inase Homolog; Bossinger et al., 2001).

Innerhalb einer Hefe 2-Hybrid Analyse zeigten 19 Proteine eine Interaktion mit den PDZ Domänen 1-3 von DLG-1 (Hoffmann et al., 2010). Zwei davon wurden im Rahmen dieser Arbeit in Bezug auf Lebensspanne und Stressresistenz untersucht. Zum einen das einzige Homolog der Kernkomponente planarer Zellpolarität Strabismus/Van Gogh in *C. elegans*, VANG-1. Zum anderen das Homolog des mitochondrialen AAA-Proteins ATAD3, ATAD-3.

1.3.1 *vang-1 ist das einzige Homolog der Strabismus/Van Gogh Familie in C. elegans*

Die erste Beschreibung dieser Genfamilie erfolgte aufgrund eines Funktionsverlustphänotyps im Auge (Strabismus) bzw. im Flügel (Van Gogh) von *Drosophila*. Strabismus/Van Gogh wirkt im planaren Zellpolaritätssignalweg (*planar cell polarity* [PCP]) zur zellübergreifenden Etablierung einer Gewebepolarität und sein Ausfall führt zu proximalen Defekten (Taylor et al., 1998; Wolff and Rubin, 1998).

Der PCP ist einer von drei Wnt Signalwegen, neben Wnt/β-Catenin und Wnt/Calcium (Nelson and Nusse, 2004). Neben Strabismus/Van Gogh wurden fünf weitere Proteine des PCP in eine Gruppe von Kernkomponenten eingestuft (siehe Abb. 2; Singh and Mlodzik, 2012). Diese Proteine vermitteln im Gewebe durch gegenüberliegende Verteilung am Zellkortex Positionsinformationen zur Polarisierung in anterior-posterior oder proximo-distale Achsen. Das Ergebnis ist eine differentielle Polarisierung von nachgeschalteten Effektoren (zum Beispiel des Aktin Zytoskeletts) und somit eine Organisation innerhalb der 2-dimensionalen Oberfläche der Epithelien (Goodrich and Strutt, 2011; Jenny, 2010).

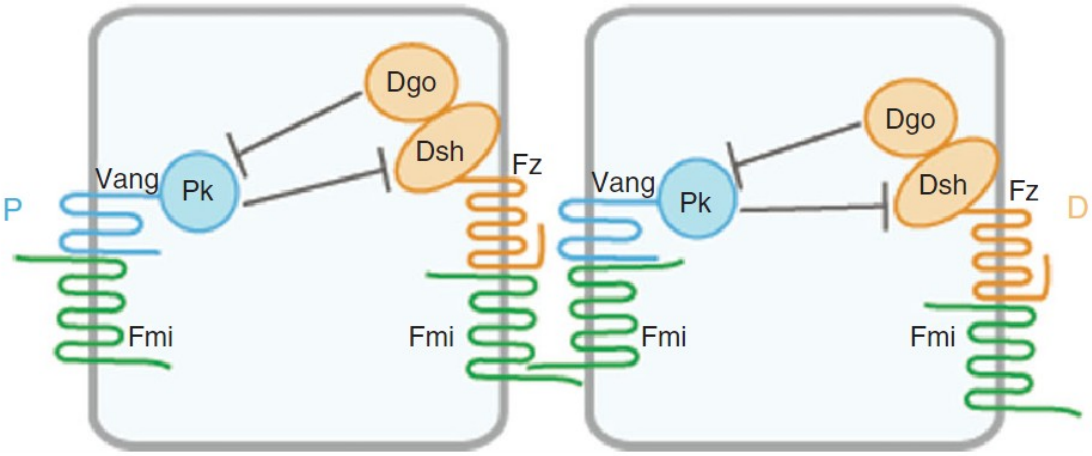

Abb. 2: Schema der asymmetrischen Verteilung von PCP Kernkomponenten und ihrer molekularen Wechselwirkungen am Beispiel von *Drosophila* Flügelepithelzellen

Frizzled (Fz)-Dishevelled (Dsh)-Diego (Dgo)-Flamingo (Fmi) sind distal (D) als Komplex angereichert, während der Vang-Prickle (Pk)-Flamingo (Fmi) Komplex proximal (P) konzentriert ist. Fz (orange) bindet hauptsächlich über die Cystein-reiche Domäne an Vang (blau). Diese Interaktion wird vom atypischen Cadherin Fmi (grün) stabilisiert. Fz bildet einen intrazellulären Komplex mit Dsh (orange) und Dgo (orange), während Vang intrazellulär mit Pk (blau) interagiert. Dsh und Dgo interagieren physisch miteinander um das Signal von Fz-Dsh zu fördern. Sie können als Gegenspieler zu Pk wirken. Dgo kompetetiert mit Pk um die Bindung an Dsh und wirkt somit gegen den inhibierenden Effekt von Pk auf Dsh (Singh und Mlodzik, 2012).

In den letzten Jahren wurden durch neue PCP Komponenten zunehmend Verbindungen zu konservierten Signalwegen gefunden, welche die Entwicklung von Invertebraten und Vertebraten beeinflussen (Green et al., 2008; Hoffmann et al., 2010; Kim et al., 2012; Mirkovic et al., 2011).

Strabismus/Van Gogh-Proteine sind membranständige Signalproteine mit vier N-terminalen Transmembrandomänen. Am C-Terminus befindet sich ein PDZ-Bindemotiv aus den Aminosäuren Ser/Thr-X-Val (Hoffmann et al., 2010). C- und N-Terminus befinden sich intrazellulär und besitzen neben mehrerer putativer Phosphorylierungsstellen (serinreiche Regionen) die zwei typischen Strabismus-Homologie Domänen (Axelrod, 2002; Katoh, 2002).

In *C. elegans* wirkt VANG-1 im Aufbau des Darms über eine Verbindung zum Fibroblasten Wachstumsfaktor Signalweg (***f**ibroblast **g**rowth **f**actor* [FGF]) mit dem Rezeptor *egl-15* (Säuger: FGFR 1-4; Hoffmann et al., 2010). In der Orientierung der Vulva Vorläuferzellen (***v**ulva **p**recursor **c**ell* [VPC]) wurde das erste Mal dokumentiert, dass eine Verbindung von VANG-1 zu einem β-Catenin (SYS-1) besteht. Hier wirken mehrere Wnt-Signalwege zusammen um die Polarität einzelner Zellen während des Aufbaus komplexer Strukturen zu etablieren (Green et al., 2008). Kürzlich wurde ein konservierter PCP-ähnlicher Signalweg bestehend aus VANG-1, PRKL-1/Prickle und DSH-1/Dishevelled in *C. elegans* beschrieben. Er ist verantwortlich für die Aufrechterhaltung der polarisierten Morphologie einer Klasse von Neuronen (Sanchez-Alvarez et al., 2011).

Neben VANG-1 wurde mit ATAD-3, dem Homolog des mitochondrialen AAA-Proteins ATAD3 der Vertebraten, ein zweiter DLG-1 Interaktionspartner untersucht.

1.3.2 *Das mitochondriale AAA-Protein ATAD3 ist in C. elegans konserviert und ebenfalls ein Bindungspartner des CeAJ Gerüstproteins DLG-1*

ATAD3 (**AT**Pase mit **A**AA-**D**omäne) ist ein AAA-Protein der inneren Mitochondrienmembran, welches zuerst im Rahmen eines Screens zur Proteinzusammensetzung von Mitochondrien aus murinen Leberzellen gefunden wurde (Mootha et al., 2003). Bei Funktionsverlust kommt es zu einer veränderten Morphologie der Mitochondrien und der Organisation der mtDNA-Protein Komplexe (Nukleoide; Da Cruz et al., 2003; Hubstenberger et al., 2010; Rebelo et al., 2009).

Die AAA (**A**TPase **a**ssoziiert mit verschiedenen zellulären **A**ktivitäten) Domäne, welche diese Proteinfamilie auszeichnet, ist in *C. elegans* hochkonserviert (Maurizi and Li, 2001).

Mit dem mitochondrialen ATAD-3 konnte ein Homolog zu ATAD3 aus Vertebraten in *C. elegans* beschrieben werden (Hoffmann et al., 2009). Sein Funktionsverlust führt zu

embryonaler Letalität, larvalem Arrest, Fehlfunktion der Gonade, verringertem Fettspeicher und zu einer gestörten Morphologie der Mitochondrien (Hoffmann et al. 2009). ATAD-3 ist ebenfalls ein Bindungspartner der Schlüsselkomponente der *Ce*AJ DLG-1 (Hoffmann et al., 2010).

Im Rahmen der zweiten vorliegenden Publikation wird gezeigt, dass das mitochondriale AAA-Protein ATAD-3 die Lebensspanne von *C. elegans* moduliert und ein neuer Interaktionspartner identifiziert. Bei diesem handelt es sich um MICS-1, das *C. elegans* Homolog zum Säuger Protein OMP25 (*mitochondrial **o**uter **m**embrane **p**rotein **25***).

OMP25 hat eine einzelne PDZ-Domäne, welche hohe Ähnlichkeit zu den DLG-1 PDZ-Domänen 1/2 zeigt. Seine mitochondriale Lokalisation wird über einen hydrophoben Bereich am C-Terminus vermittelt. Bei Säugern scheint OMP25 eine Rolle in der korrekten Verteilung der Mitochondrien zu haben (Nemoto and De Camilli, 1999).

Es wurde gezeigt, dass OMP25 zusammen mit der MAPK SAPK3 (**s**tress **a**ctivated **p**rotein **k**inase **3**) in Mitochondrien lokalisiert und diese hemmt (Court et al., 2005).

Mitochondrien haben allgemein einen Einfluss auf Stressresistenz und die Lebensspanne in verschiedenen Spezies. Denn wie der *C. elegans* Darm Stress durch die Außenwelt abwehren muss, bestehen durch die Atmungskettenaktivität innerhalb der Mitochondrien ständig Stressbedingungen. Die Menge an produzierten- und abgebauten reaktiven Sauerstoffspezies muss stets im Gleichgewicht sein, da sonst vermehrt Zellschäden auftreten können.

1.4 *Die Störung mitochondrialer Komponenten und assoziierter Proteine beeinflusst die Lebensspanne verschiedener Spezies*

1.4.1 *Die reaktiven Sauerstoffspezies der Mitochondrien gelten als Motor der Alterung und stehen im Zusammenhang mit Erkrankungen des Menschen*

Mitochondrien sind als Bestandteil beinahe aller Eukaryontenzellen als sogenannte „Zellkraftwerke“ zu finden. Über den Zitronensäurezyklus produzieren sie neben CO_2 die Elektronendonoren $FADH_2$ und NADH, welche ihre reduzierenden Äquivalente an die Elektronentransportkette (***e**lectron **t**ransport **c**hain* [ETC]) innerhalb der Mitochondrienmembran abgeben. Dies bietet Energie für die oxidative Phosphorylierung (OXPHOS), bei welcher die Elektronen letztlich auf O_2 als Elektronenakzeptor übertragen werden. Der hierbei erzeugte Protonengradient treibt die ATP-Synthase an, welche Adenosindiphosphat (ADP) mit anorganischem Phosphat (P_i) zu Adenosintriphosphat (ATP) synthetisiert, der allgemeinen „Energiewährung“ der Zellen (Jastroch et al., 2010).

Als Nebenprodukt der OXPHOS entstehen auch immer reaktive Sauerstoffspezies (ROS). Dies sind zum einen freie Radikale (z.B. Superoxid-Anion), zum anderen stabilere Oxidantien wie Wasserstoffperoxid. ROS sind aufgrund ihrer hohen Reaktivität in der Lage Makromoleküle und Organellen zu beschädigen. Normalerweise halten sich die Schäden mit der Detoxifizierung und Reparatur bis zu einem gewissen Grad die Waage (Rossignol et al., 2003; Yang and Hekimi, 2010a). ROS gelten laut der „*mitochondrial free radical theory of aging*“ nach Denham Harman durch die über die Zeit akkumulierten Makromolekül- und Organellschäden als Motor der Alterung (Harman, 1956).

Erkrankungen des Menschen durch Veränderung der Mitochondrienkonstitution (Mitochondriopathien) stellen ein Spektrum teils im frühen Kindesalter letaler Erbkrankheiten dar. Viele Syndrome zeichnen sich durch schwere Schädigung stark energieverbrauchender Gewebe aus (Muskeln, Nervensystems). Das Leigh Syndrom (sub-akute nekrotisierende Enzephalopathie) wurde bereits 1951 sehr genau dokumentiert (Leigh, 1951). Meist findet sich dort eine Komplex-I Defizienz. Diese führt zu einer Dysbalance zwischen ROS-Produktion und –Abbau, wodurch die oxidative Schädigung stark ansteigt (DiMauro and De Vivo, 1996). Ähnliches gilt für die neurodegenerative Krankheit „Friedreich´s Ataxia“, bei welcher die Expression des mitochondrialen Frataxins vermindert ist. Die Mutante des *C. elegans* Homologs von Frataxin, *frh-1*, wird als Modell zur Untersuchung dieser Krankheit genutzt (Ventura et al., 2006a; Zarse et al., 2007).

1.4.2 *C. elegans ist als Modellsystem zur Untersuchung mitochondrialer Komponenten und assoziierter Proteine etabliert*

In *C. elegans* wurde eine Klasse von Mutanten auf genetischer Ebene oder innerhalb von RNA Interferenz Screenings entdeckt, welche trotz Störungen in Komponenten der ETC langlebig sind (Dillin et al., 2002; Lee et al., 2003; Rea et al., 2007). Sie werden als **mit**ochondriale (Mit-) Mutanten zusammengefasst (Rea, 2005; Rea et al., 2007; Ventura and Rea, 2007; Ventura et al., 2006b).

Die zuerst beschriebene dieser Art ist *clk-1* (Lakowski and Hekimi, 1996). CLK-1 ist eine Demethoxyubiquinon Monoxygenase. Bei einem Funktionsverlust von CLK-1 sind die Tiere nicht in der Lage DMQ_9 (5-Demethoxyubiquinon-9) weiter zu synthetisieren und es kommt über die Anhäufung der Ubiquinon-Vorstufe zur Komplex-I Hemmung (Yang et al., 2011). *Clk-1* zählt zu den langlebigen Mit-Mutanten, ebenso wie *isp-1*, *tpk-1*, *irs-2* und *cco-1* (Feng et al., 2001; Rea et al., 2010). In diesen Mutanten ist die ETC inhibiert, was generell zu einer Erhöhung des ROS-Levels führt. (Ventura et al., 2006b). Mit-Mutanten nutzen Wege zur ATP

Produktion, welche nicht Sauerstoff als finalen Elektronenakzeptor benutzen (Butler et al., 2010). Sie sind generell resistenter gegenüber sauerstoffarmen (hypoxischen) Bedingungen, weshalb die Hypothese besteht, dass HIF-1 (***h**ypoxia-**i**nducible **f**actor-1*) in diesen Tieren aktiver ist.

HIF-1 ist ein konservierter Transkriptionsfaktor, welcher durch die Wahrnehmung einer hypoxischen Umgebung aktiviert wird (Powell-Coffman, 2010). Bei Säugern wurden die Gene mit einem HIF-responsivem Element, welche kurz- und langfristig die Reaktion auf hypoxische Bedingungen bestimmen, auch für Angiogenese, Vaskularisierung, Steuerung des Axonwachstums und Alterung für wichtig befunden (Kaelin and Ratcliffe, 2008; Leiser and Kaeberlein, 2010; Pocock and Hobert, 2008; Semenza, 2003).

Die Theorie einer Akkumulation oxidativer Schädigung von Makromolekülen und Organellen durch ROS als Grundlage der Alterung kann in Anbetracht neuerer Evidenzen nicht mehr generell herangezogen werden. Vielmehr konnte gezeigt werden, dass gerade langlebige Mutanten wie *nuo-6* und *isp-1* einen erhöhten ROS Level haben und dieser essentiell für ihre Langlebigkeit ist (Lee et al., 2010; Yang and Hekimi, 2010b). ROS sind funktionell in der Aktivierung von sekundären Botenstoffen (z.B. 8-nitro-cGMP), JNK und HIF-1 (Ahmed et al., 2012; Lee et al., 2010; Shen and Liu, 2006). Hypoxie führt kurzfristig zu einem intrazellulären Anstieg des ROS-Levels (Chandel et al., 1998; Vanden Hoek et al., 1998). Dieser Anstieg könnte HIF-1 unter sauerstoffarmen Bedingungen auch in *C. elegans* aktivieren (Lee et al., 2010; Yang and Hekimi, 2010a). Dies passt zur Beobachtung, dass eine moderate Erhöhung des ROS-Levels positive Effekte hat, aber eine zu hohe adverse zeigt. Demonstriert wurde dieser Zusammenhang anhand von Substanzen, welche als „*redox cycler*“ wirken und so intrazellulär ROS erzeugen (z.B. Juglone oder Paraquat). Vorinkubation mit einer subletalen Dosis führten bei *C. elegans* zu einer Erhöhung der Stressresistenz gegen ansonsten letale Konzentrationen des „*redox cyclers*“ sowie zu Langlebigkeit (Przybysz et al., 2009). Für dieses Phänomen der positiven Effekte einer nicht tödlichen Stressmenge wurde der Begriff „*Hormesis*“ geprägt (Cypser and Johnson, 2002).

Über *C. elegans* hinaus konnte eine beeinträchtigte mitochondriale Atmung auch in *Saccharomyces cerevisiae*, *Drosophila melanogaster* und *Mus musculus* mit einer ausgedehnten Lebensspanne verknüpft werden (Copeland et al., 2009; Dell'agnello et al., 2007; Kirchman et al., 1999).

2. Thesen der Publikationen

2.1 *Thesen der Publikation (Honnen et al., 2012. PLoS One)*

These 1: VANG-1 moduliert die Lebensspanne von *C. elegans* temperatur-unabhängig

Die *vang-1(tm1422)* Mutante lebt im Mittel um bis zu 40% länger als der Wildtyp (WT) unter gleichen Bedingungen. Die mittlere Lebensspanne von *tm1422* Tieren ist sowohl bei 18 °C, 20 °C als auch bei 25 °C signifikant gegenüber dem WT unter gleichen Bedingungen erhöht. Für ein weiteres Allel, *ok1142*, und RNAi gegen VANG-1 gilt ähnliches. Demnach handelt es sich bei der beobachteten Langlebigkeit nicht um einen Sekundäreffekt erhöhter thermaler Resistenz, sondern um eine Verlangsamung des Alterungsprozesses.

These 2: Eine Mutation im *vang-1* Lokus bedingt Resistenz gegen Hitze- und oxidativen Stress

Im Vergleich zum WT zeigen *tm1422* Tiere eine erhöhte Resistenz gegenüber Hitzestress und reaktiven Sauerstoffspezies (ROS). Der unter Hitzestress-induzierte ROS-Level ist auf dem Niveau der langlebigen Insulin/IGF-1 Rezeptor Mutante *daf-2(e1370)*. Weitere Defekte von *tm1422* Tieren, wie verlangsamtes Wachstum (Larve→Adult), verringerte Nachkommenschaft und weniger Akkumulation von Lipofuszin (Alterspigment) komplementieren, neben der Erhöhung der mittleren Lebensspanne (s. These 1), das Bild von *vang-1* als ein Gerontogen (Altersgen).

These 3: Die Modulation der Lebensspanne in *vang-1* Mutanten verläuft keimbahnspezifisch über den Insulin/IGF-1 Signalweg (IIS)

Die erhöhte mittlere Lebensspanne von *tm1422* Tieren wird maßgeblich über den DAF-16/ FoxO Transkriptionsfaktor vermittelt, eine Schlüsselkomponente des IIS. Dieser transloziert nach *vang-1* Funktionsverlust vermehrt in den Zellkern. Weiterhin entwickeln sich *tm1422* Tiere, ähnlich wie *daf*-2 Insulinrezeptor Mutanten, konstitutiv zu Dauerlarven, wozu ebenfalls die erhöhte Aktivität von DAF-16 im Zellkern Voraussetzung ist (Fielenbach and Antebi, 2008). Der Einfluss von SKN-1/Nrf, ein Transkriptionsfaktor für Phase-II-Detoxifikationsenzyme, auf die Langlebigkeit von *tm1422* Tieren kann durch eine positive Rückkopplung auf den IIS erklärt werden. Gewebespezifische RNAi und *in-situ* Hybridisierungsdaten lassen bezüglich der Lebensspanne auf eine gemeinsame Funktion von VANG-1, DAF-2 und DAF-16 in der Keimbahn von *C. elegans* schließen.

2.2 *Thesen der 2. Publikation (Hoffmann, Honnen et al., 2012. Exp Gerontol)*

These 1: ATAD-3 interagiert mit MICS-1/OMP25 in der Modulation der Lebensspanne von *C. elegans*

Die *in silico* vorhergesagte Interaktion zwischen der PDZ-Domäne von MICS-1 und dem PDZ-Bindemotiv von ATAD-3 kann experimentell *in vitro* mittels Hefe 2-Hybrid Analyse und Co-Immunopräzipitation bestätigt werden. Der einzelne Funktionsverlust beider Proteine erhöht die mittlere Lebensspanne von *mics-1(ok1451)* Tieren und nach *atad-3* RNAi um bis zu 54% beziehungsweise 29% im Vergleich zum WT. Der gleichzeitige Verlust wirkt sich additiv auf die Erhöhung der Lebensspanne aus.

These 2: ATAD-3 und MICS-1 haben gegensätzliche Effekte auf den Hitzestress-induzierten intrazellulären ROS Level

RNAi gegen ATAD-3 führt zu einer Verdopplung des ROS Levels im wildtypischen Hintergrund und zu einer 31%igen Steigerung im *daf-2* mutanten Hintergrund. Der Funktionsverlust von MICS-1 führt hingegen zu einem niedrigeren ROS Level gegenüber dem WT. ATAD-3 verhält sich wie andere mitochondriale Modulatoren der Lebensspanne, z.B. *nuo*-6 oder *isp-1*, deren Funktionsverlust trotz eines erhöhten intrazellulären ROS-Level zu Langlebigkeit führt (Lee et al., 2010; Yang and Hekimi, 2010b), wohingegen MICS-1 sich entsprechend der klassischen Annahme der „*mitochondrial free radical theory of ageing*“ (Harman, 1956) verhält.

These 3: Nach Funktionsverlust von ATAD-3 und MICS-1/OMP25 unterliegt die Modulation der Stressresistenz, aber nicht der Lebensspanne, DAF 16/FoxO

Unter Hitzestress und RNAi gegen MICS-1 lokalisiert DAF-16::GFP vermehrt in den Zellkernen. Weiterhin zeigen *mics-1*(*ok1451)* Tiere einen konstitutiven Dauerlarven-Phänotyp, was ebenfalls mit erhöhter DAF-16 Aktivität vereinbar ist. Dahingegen wird die erhöhte mittlere Lebensspanne zum größten Teil DAF-16 unabhängig vermittelt. In *atad-3(RNAi)* Populationen gibt es unter Hitzestress und bezüglich der ROS Produktion keinen Hinweis auf eine erhöhte DAF-16 Aktivität. Die Langlebigkeit von *atad-3(RNAi)* Tieren ist ebenfalls nur partiell von DAF-16 abhängig.

3. Publikationen

Übersicht über die Publikation: ***Honnen et al., 2012: „C. elegans VANG-1 modulates life span via Insulin/IGF-1-like signaling".*** *PLoS One* **7,** e32183**:**

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C. elegans VANG-1 modulates life span via insulin/IGF-1-like signaling

Running Head:
„VANG-1 modulates life span"

by
Sebastian J. Honnen [1,2]
Christian Büchter [2]
Verena Schröder [2]
Michael Hoffmann [3]
Yuji Kohara [4]
Andreas Kampkötter [2,5]
and
Olaf Bossinger [1,§]

[1]Institute of Molecular and Cellular Anatomy, Medical School, RWTH Aachen University, D-52074 Aachen, Germany

[2] Institute of Toxicology, Heinrich-Heine-University Düsseldorf, D-40225 Düsseldorf, Germany

[3] Department of General Pediatrics, University Children's Hospital, Heinrich-Heine-University Düsseldorf, D-40225 Düsseldorf, Germany

[4] Genome Biology Laboratory, National Institute of Genetics, 1111 Yata, Mishima, 411-8540, Japan

[5] Bayer Animal Health GmbH, Research and Development D-40789 Leverkusen, Germany

[§]Correspondence should be addressed to:

Olaf Bossinger
Institute of Molecular and Cellular Anatomy
Medical School, RWTH Aachen University
D-52074 Aachen
GERMANY
Fone: +49-241-808-8928
Fax: +49-241-808-2508
E-Mail: olaf.bossinger@rwth-aachen.de

ABSTRACT

The planar cell polarity (PCP) pathway is highly conserved from *Drosophila* to humans and a PCP-like pathway has recently been described in the nematode *Caenorhabditis elegans* (Green et al., 2008; Hoffmann et al., 2010; Wu and Herman, 2006; Wu and Herman, 2007). The developmental function of this pathway is to coordinate the orientation of cells or structures within the plane of an epithelium or to organize cell-cell intercalation required for correct morphogenesis (Wang and Nathans, 2007; Wu and Mlodzik, 2009). Here, we describe a novel role of VANG-1, the only *C. elegans* ortholog of the conserved PCP component Strabismus/Van Gogh. We show that two alleles of *vang-1* and depletion of the protein by RNAi cause an increase of mean life span up to 40%. Consistent with the longevity phenotype *vang-1* animals also show enhanced resistance to thermal- and oxidative stress and decreased lipofuscin accumulation. In addition, *vang-1* mutants show defects like reduced brood size, decreased ovulation rate and prolonged reproductive span, which are also related to longevity genes. The germline, but not the intestine or neurons, seems to be the primary site of *vang-1* function. Life span extension in *vang-1* mutants depends on the insulin/IGF-1-like receptor DAF-2 and DAF-16/Foxo transcription factor. RNAi against the phase 2 detoxification transcription factor SKN-1 also reduced *vang-1* life span that might be explained by gradual inhibition of insulin/IGF-1-like signaling in *vang-1*. This is the first time that a key player of the PCP pathway is shown to be involved in the insulin/IGF-1-like signaling dependent modulation of life span in *C. elegans*.

HIGHLIGHTS

- *vang-1* modulates life span and stress resistance in *C. elegans*
- *daf-16* is fully epistatic to *vang-1*
- *vang-1* seems to act coordinately with *daf-2*
- *vang-1* populations are dauer constitutive

KEYWORDS

- *C. elegans*
- insulin
- longevity
- PCP
- vang

INTRODUCTION

Wnt/planar cell polarity (PCP) is one of three identified Wnt signaling pathways, along with Wnt/β-Catenin and Wnt/Calcium (Nelson and Nusse, 2004). These signaling pathways are abundant in various developmental processes across the animal kingdom (Axelrod, 2009; Cadigan and Nusse, 1997; Wang and Nathans, 2007; Wodarz and Nusse, 1998; Wu and Mlodzik, 2009). PCP is extensively studied in the *Drosophila* wing, or in the organization of ommatidia in the fly eye or hair follicles in mammalian skin. Six proteins were placed in the core PCP pathway, Frizzled (Fz), Dishevelled (Dsh), Diego (Dgo), Strabismus/van Gogh (Stbm/Vang), Prickle (Pk) and Flamingo (Fmi). The signaling mediated by PCP core proteins during development contributes to the polarization alongside the epithelial anterior-posterior or proximo-distal axis and requires contrary clustering of PCP components at the respective cell cortex. As a consequence of PCP signaling, downstream effectors e.g., the actin cytoskeleton are polarized within individual cells that finally lead to well organized structures within the two-dimensional epithelial surface (Goodrich and Strutt, 2011; Jenny, 2010). PCP processes also shape three-dimensional tissues that do not exhibit obvious signs of planar polarity. Here, individual cells have to move in a specific direction or divide with a specific orientation, hence showing transient planar polarization e.g. during mediolateral cell intercalation required for morphogenesis of the neural tube in vertebrates (Keller, 2002; Keller and Shook, 2008). Novel components of PCP signaling have been identified in the recent years, and the number of crosslinks to other conserved pathways required for development is rising (Green et al., 2008; Hoffmann et al., 2010; Mirkovic et al., 2011).

The *C. elegans* genome (http://www.wormbase.org) encodes a sole four-pass transmembrane protein, VANG-1 showing sequence similarities and conservation of overall domain architecture compared to the Strabismus/Van Gogh/Ltap proteins identified in *Drosophila*, *Xenopus* and mammals. Like in *Drosophila* and mammals, VANG-1 contains four hydrophobic transmembrane domains at its N-terminus and a consensus PDZ binding motif at its C-terminus (Hoffmann et al., 2010). VANG-1 was implicated in playing a minor role in B cell polarity in the *C. elegans* male tail (Wu and Herman, 2006). However, it plays a major role in organ formation either by mediating correct intercalation of intestinal primordial cells during embryogenesis (Hoffmann et al., 2010; Leung et al., 1999) or by establishing ground polarity in vulval development (Green et al., 2008). Whereas PCP signaling required for morphogenesis is generally well understood, a more physiological role of this pathway with effects on metabolism has not been described so far.

C. elegans is a well-established model to study genes that contribute to the process of ageing. The corresponding genes of "Age" mutants are referred to as gerontogenes (Johnson et al., 2002). These mutants share a specific catalogue of defects, e.g. a minimum of 20% life span increase and resistance against certain stress factors like reactive oxygene species or heat. The *C. elegans* homolog of insulin receptor in mammals, *daf-2*, is one of the best described gerontogenes, and the signaling mediated by DAF-2 is well understood (Antebi, 2007; Kenyon, 2010; Wolff and Dillin, 2006). DAF-2 is capable to phosphorylate target substrates e.g. AGE-1/AAP-1, a PI3 kinase that generates PI(3,4,5)P3 (Friedman and Johnson, 1988; Morris et al., 1996; Wolkow et al., 2002). Via a phosphorylation cascade, downstream kinases PDK-1, AKT-1, AKT-2 and SGK-1 (Hertweck et al., 2004; Paradis et al., 1999; Paradis and Ruvkun, 1998) are activated and in turn negatively regulate the forkhead transcription factor (FOXO), DAF-16 (Lin et al., 1997; Ogg et al., 1997). Inhibition of DAF-2 signaling, e.g. by *daf-2* mutations or active insulin peptide signaling, leads to dephosphorylation, activation and accumulation of DAF-16 to the nucleus (Huang and Tindall, 2007). Consequently, transcription of DAF-16 targets that include genes involved in defence against stresses, DNA repair and metabolism lead to a higher resistance against stresses and significantly extension of lifespan (Kenyon et al., 1993). Besides DAF-16, inhibiting insulin/IGF-1-like signaling also activates heat-shock transcription factor HSF-1 and phase 2 detoxification transcription factor SKN-1, a Nrf1/2/3 protein ortholog (An and Blackwell, 2003; Hsu et al., 2003).

In the present study, we identify VANG-1, the only *C. elegans* ortholog of the conserved PCP protein Strabismus/Van Gogh, as a gerontogene with a typical phenotype, including extended life- and reproductive span, multiple stress resistances, slow growth, reduced brood size and reduced lipofuscin accumulation. The *vang-1*–dependent life span extension and stress defences seem to be coordinated in the germline and mostly require *daf-16* and *skn-1* gene functions.

RESULTS AND DISCUSSION

vang-1 increases life span, stress resistance and reproductive span in C. elegans

The *C. elegans* genome (http://www.wormbase.org) contains a sole four-pass transmembrane protein with homology to the Strabismus/Van Gogh/Ltap proteins identified in *Drosophila*, *Xenopus* and mammals (Darken et al., 2002; Goto and Keller, 2002; Kibar et al., 2001). During analysis of *vang-1(tm1422)*, in which 188 amino acids of the N terminus are missing (including three of the four transmembrane domains; see supplementary data; (Hoffmann et al., 2010)), we noticed several defects (Figs.1-2) with regard to the postembryonic phenotype, e.g. slow growth (data not shown) and reduced fecundity (Fig.2A) that are also associated with loss of function phenotypes of certain ageing genes in *C. elegans* (Luscombe et al., 2002). Life span assays in *vang-1(tm1422)* at 25 °C (Fig.1A), 20 °C and 18 °C (Table 1) detected a significant increase of mean life span of up to 40% compared to wild type (WT) animals. Furthermore, we tested life span of another *vang-1* deletion mutation, *ok1142*, lacking 162 amino acids of the C terminus (including a predicted phosphorylation site; see supplementary data; (Hoffmann et al., 2010)) and of animals depleted of VANG-1 by RNAi (Fig.1A). Again, we noticed a significant extension of *C. elegans* mean life span up to 27% and 20% in comparison to WT controls either kept on standard OP50 or RNAi HT115 bacteria with the empty "feeding"-vector. In addition, the *tm1422* phenotype was not enhanced by RNAi against *vang-1* C terminus (Table 1). With regard to longevity we assume *tm1422* to be a null mutation whereas *ok1142* seems to be a hypomorphic allele and RNAi does not generate the complete loss-of-function phenotype, as reported for other genes (Guo and Kemphues, 1996). Hence, we used *tm1422* in all further experiments.

Next, we tested increased resistance of *tm1422* against various stressors, a typical feature of longevity genes. First, we measured thermoresistance in semi-automated and manual assays using SYTOX® Green nucleic acid stain under temperature lethal conditions of 37 °C. Both assays revealed increased thermoresistance of *tm1422* of about 40% (Fig.1B and not shown), which is in the similar range as extension of mean life span. Second, we tested the resistance of *tm1422* against reactive oxygen species (ROS) and determined intracellular ROS accumulation in living worms. In a stress assay with juglone (from *Juglans niger*) as a redox cycler, we found the fraction of *tm1422* animals that survived the induced ROS stress conditions was about four fold higher than WT (Fig.1C). Furthermore, a 60% decrease in ROS accumulation, which is in the similar range of *daf-2* mutant population (Fig.1D), was

found in *tm1422* worms in comparison to WT using a fluorescence well-plate reader to measure DCF fluorescence (see "experimental procedures" for details). According to the "free radical theory of ageing" (Harman, 1956), ROS are a crucial factor for ageing, and the intracellular amount of ROS can be correlated to stress. Organisms developed inducible detoxification systems like catalases, peroxidases and superoxide dismutases to reduce ROS levels (Braeckman et al., 2002). The competence to keep intracellular ROS levels low is considered to be one possibility for the extension of life span (Finkel and Holbrook, 2000; Van Raamsdonk and Hekimi, 2010). Thus, the diminished amounts of ROS in *tm1422* may explain the increased survival rate at lethal thermal stress conditions. Recent findings suggest that the relationship between ROS and the aging process is more complex than what was originally thought. The generation of ROS cannot be longer seen as the initial trigger of the aging process (Hekimi et al., 2011). Nevertheless, in case of *tm1422* population reduced ROS generation indicates a lower stress level that finally may account for the extension of life span.

In order to estimate the biological age of *vang-1* mutants, we measured the amount of lipofuscin, a product of oxidative damage and autophagy. In *C. elegans*, lipofuscin is detectable as autofluorescent granules in the intestine and its accumulation is a well-established marker to judge the biological age of *C. elegans* (Garigan et al., 2002; Gerstbrein et al., 2005). In comparison to WT, *ok1142* and *tm1422* animals showed a significantly decreased accumulation of lipofuscin after five (Fig.2B) and even after ten days (Fig.2C).

In addition to the longevity phenotype, we observed a significantly reduced brood size in *tm1422* animals (Fig.2A), a decreased ovulation rate (Fig.2D) and a dramatically prolonged reproductive span (Fig.2E). Normally, the reproductive system of *C. elegans* ages significantly during the first week of adulthood (Hughes et al., 2007), reflected by germ line degeneration and a decline in oocyte quality (Andux and Ellis, 2008; Garigan et al., 2002). Individual *tm1422* mothers continue to produce viable progeny as they age (Fig.2D). This phenotype also points to *vang-1* being a typical longevity gene. Some of the known mutations (e.g. *daf-4* or *daf-7*) that extend *C. elegans'* reproductive period also regulate longevity, suggesting that there is a link between reproductive span and life span (Luo et al., 2009).

Taken together these results suggest that loss of the planar cell polarity ortholog VANG-1 causes robust temperature independent extension of life span, increases stress resistance and extends reproductive period in *C. elegans*.

Life span modulation by VANG-1 depends on the insulin/IGF-1-like signaling pathway

The main regulator of longevity and stress resistance in *C. elegans* is insulin/IGF-1-like signaling with its effector DAF-16. This FOXO transcription factor is translocated into the nucleus where it activates gene expression for distinct processes e.g. resistance against different stressors and longevity when insulin/IGF-1-like signaling is inhibited (Kenyon, 2005; Lin et al., 2001). To gain further insight into the pathway operating in *tm1422*, we disrupted FOXO/DAF-16 transcription factor by RNAi in *tm1422* and WT worms and compared the mean life span (Fig.3A and Table 1). As expected (Samuelson et al., 2007), mean life span in WT animals depleted of DAF-16 slightly decreased in comparison to the control. Surprisingly, *daf-16* RNAi in *tm1422* eliminated *vang-1* induced life span extension at 20 °C and 25 °C (Table 1), suggesting that *daf-16* is epistatic to *vang-1*.

The activation of the DAF-16 transcription factor can be easily observed by a functional DAF-16::GFP fusion (Henderson and Johnson, 2001). After *vang-1* RNAi at room temperature and 27 °C we observed 16% and 57% DAF-16 translocation into the nucleus, respectively (Fig.S1), suggesting that complete nuclear localization of DAF-16 is not a prerequisite for increased life span and stress resistance. This phenomenon has also been observed in case of *age-1* at 20 °C, which is well known for modulating lifespan in a DAF-16 dependent manner (Henderson and Johnson, 2001; Lin et al., 2001).

To further validate our *daf-16* RNAi life span result, we investigated other parameters of high DAF-16 activity, e.g. developmental arrest. In *C. elegans*, the activity of DAF-16 is sufficient and necessary for L1 diapause and dauer formation (Baugh and Sternberg, 2006; Paradis and Ruvkun, 1998). Hatching L1 larvae stay in diapause, a developmental arrested state with reduced metabolism, until they start feeding. Dauer formation is an alternative third larval stage (beside the normal L3 larval stage) that is introduced under harsh environmental conditions, high temperature, low food or overcrowding (Gerisch et al., 2001).

We performed our dauer assay in comparison to WT, *daf-2(e1370)* and *daf-16(mu86)* at 27 °C (Hu et al., 2006). Consistent with the literature, we found that *daf-2(e1370)*, encoding the sole insulin receptor homologue in *C. elegans* (Friedman and Johnson, 1988), is dauer constitutive (~99% arrest), while *daf-16(mu86)* is dauer defective (< 10% arrest; Fig.3B; Baugh and Sternberg, 2006; Gottlieb and Ruvkun, 1994). *tm1422* animals showed four times more developmental arrest compared to WT (Fig.3B), which is inhibited by RNAi against *daf-16* (*tm1422*: 7.8% dauer, 92.2% “other”, n=64; WT: 1.2% dauer, 98.8% “other”, n=160;). While 21% of WT animals developed into dauers, 58% and 18% of *tm1422* animals arrested as

dauers and in L1 diapause, respectively (Fig.3B). A noteworthy difference concerning the dauer constitutive phenotypes of *daf-2* and *tm1422* is the percentage of L1 diapause arrests, which is also induced by DAF-16 (Baugh and Sternberg, 2006) and suggests higher activity of DAF-16 in *tm1422* during early development.

We further investigated the role of the receptor tyrosine kinase DAF-2 (Friedman and Johnson, 1988), which acts upstream of FOXO/DAF-16 transcription factor to modulate life span and stress resistance in the conserved insulin/IGF-1-like signaling pathway (Landis and Murphy, 2010). Inhibition or loss of DAF-2 function leads to induction of alternate dauer formation (see above) early in life and life span extension of up to 100% late in life both depending on DAF-16 function (Kenyon et al., 1993). RNAi against *daf-2* in WT and *tm1422* worms resulted in nearly identical survival curves with no significant difference in mean life span (Fig.3A and Table 1), indicating that *vang-1* may function in the insulin/IGF-1-like signaling pathway, rather than in parallel pathways, e.g. through regulation of DAF-16 by *kri-1* and lipophilic-hormone signaling (Berman and Kenyon, 2006; Hsin and Kenyon, 1999).

We also tested the longevity promoting factor SKN-1, which orchestrates the phase II detoxification response including defense against oxidative stress (Tullet et al., 2008). RNAi against *skn-1* did reduce *tm1422* life span significantly about 17% (Table 1). Inhibition of insulin/IGF-1-like signaling in *tm1422* may explain this result. Like DAF-16, SKN-1 is also repressed by DAF-2 downstream kinases, AKT-1/2 and SGK-1 and possibly acts as a key player in a positive feedback loop to extend life span (Okuyama et al., 2010; Tullet et al., 2008).

To further specify how *vang-1* functions in the extension of life span, we performed specific knock downs of *vang-1* first in the intestine (McGhee et al., 2009; Pilipiuk et al., 2009), second in the germline (Sijen et al., 2001) and third, because of its expression in ventral cord neurons (Green et al., 2008; Sanchez-Alvarez et al., 2011), in strains showing enhanced neuronal RNAi (Calixto et al., 2010).

The intestine is highly exposed to environmental toxins and pathogens and it has been speculated to be the major site of stress response (Libina et al., 2003). To further support this hypothesis, we depleted DAF-2 (as a control) by RNAi only in the intestine and found a 60% extension of life span (Table 1). In contrast, *vang-1* RNAi in the intestine did not result in a significant extension of mean life span (Fig.4A; Table 1), suggesting that the intestine is not where VANG-1 is acting to modulate life span.

In *C. elegans* and mice, VANG-1 and Vangl2^{Lp} have been connected with correct uterine epithelium development in the reproductive tract (Green et al., 2008; vandenBerg and

Sassoon, 2009), but its function in meiotic maturation and ovulation is still ellusive. Both processes are regulated by intense signaling between the germline and the proximal gonadal sheath cells, specialized myo-epithelial cells that surround and form gap junctions with oocytes (Greenstein et al., 1994; McCarter et al., 1997; Miller et al., 2003; Rose et al., 1997). During ovulation, sheath cells contract rapidly, the distal constriction of the spermatheca dilates, and sheath cells pull the distal spermatheca over the mature oocyte (McCarter et al., 1999). The decreased fertility/brood size, ovulation rate, and the increased reproduction span of *tm1422* animals (Fig.2 A,D-E) suggests VANG-1 being involved in the communication between germline and somatic gonad. To test if *vang-1* also acts in the germline to control life span by insulin/IGF-1-like signaling, we performed germline-specific RNAi (Sijen et al., 2001). *vang-1(RNAi)* in *rrf-1* led to a significant increase in lifespan (13.5%, Fig.4B; Table 1), which is about two third of whole life span extension observed in *vang-1* RNAi animals (Fig.1A; Table 1). In contrast, depletion of VANG-1 in the enhanced-neuronal RNAi strains TU3311 and TU3401 (Calixto et al., 2010), has no effect on *C. elegans* life span extension (Fig.4C; Table 1). As suggested by Calixto et al. (Calixto et al., 2010) the neuronal expression of *sid-1* in TU3311 might serve as a sink for double-stranded RNA used by non-neuronal RNAi and thus could explain why *vang-1(RNAi)* in TU3311 leads not to the same life span extension as in WT. Additionally, *vang-1(tm1422)* individuals have an intact chemosensory apparatus and are "open" to the environment (personal comunication with N.J. Storm - it has been testet two times with up to 30 individuals per experiment for uptake of DiI [Mukhopadhyay et al., 2005]). Dye-fill defective (dyf-phenotype) mutants have previous been found long-lived (Apfeld and Kenyon, 1999). Taken together, our findings of tissue-specific RNAi against VANG-1 in combination with in-situ hybridization data of *vang-1*, *daf-2* and *daf-16* (Fig.S2) implicate the germline to be the primary site of *vang-1* action concerning longevity in *C. elegans*. A previous study has already been implicated components of the insulin/IGF-1-like signaling pathway to act in the germline. Michaelson et al. found that the effect of reducing *daf-2* signaling on larval germline proliferation is dependent on *daf- 16* (Michaelson et al., 2010).

In summary, we have identified a link between the *C. elegans* planar cell polarity key player *vang-1* and insulin/IGF-1-dependent extension of lifespan. Mutations in *vang-1* show the typical phenotype of age-mutants, including longevity, slow growth, multiple stress resistances, reduced lipofuscin accumulation, and reduced brood size. The germline, but not the intestine or neurons seems to be the primary site of *vang-1* function, which may operate in the same pathway as *daf-2* and *daf-16* to extend life span of about 40% in *C. elegans*.

METHODS

C. elegans strains and alleles

Maintenance and handling of *C. elegans* were carried out as described previously (Brenner, 1974). Bristol N2 was used as the WT strain. WT or mutant worms were synchronized as described previously (Lewis and Fleming, 1995).

Single mutants were as follows:

TM1422: *vang-1(tm1422)X* (outcrossed x3); RB1125: *vang-1(ok1142) X*; CB1370: *daf-2(e1370) III*; CF1038: *daf-16(mu86) I*; NL2098: *rrf-1(pk1417) I*;

Transgenic strains were as follows:

OLB11: *rde-1(ne219);[pOLB11(elt-2p::rde-1) + pRF4(rol-6(su1006)]*; TU3311: *[unc-119p::YFP + unc-119p::sid-1]*; TU3401: *sid-1(pk3321) V; [pCFJ90(myo-2p::mCherry) + unc-119p::sid-1]*; TJ356: integrated DAF-16::GFP roller strain (Henderson and Johnson, 2001) (for further details see: https://cgcdb.msi.umn.edu/strain.php?id=13306)

RNA-mediated interference (RNAi)

RNAi by "feeding" was performed essentially as described by others (Kamath et al., 2001). In brief, after amplification of a single colony overnight (37 °C, $LB_{amp\ tet}$ medium), HT115(DE3) bacteria (RNase III-deficient *E. coli* strain, carrying IPTG-inducible T7-polymerase) (Kamath et al., 2001; Timmons et al., 2001) were diluted to an OD_{600} of 0.9, and after addition of IPTG (1 mM) seeded on $NGM_{amp\ tet}$ plates, that contained IPTG (1 mM), too. Bacteria were further incubated overnight at room temperature (~22 °C) to allow the expression of double-stranded RNA. HT115(DE3) bacteria harboring the empty KS+ based vector L4440 (containing two T7 promoters flanking a polylinker) were used as a control for RNAi "feeding" experiments. RNAi clones against *vang-1* and *daf-16* were obtained from the Ahringer RNAi "feeding" library (Geneservice Limited, Cambridge, UK) while *daf-2* "feeding" clone was kindly provided by Dr. Andrew Dillin (Cohen et al., 2006) (see supplementary data for sequencing results of RNAi "feeding" clones).

Life span assay

Life span was determined at 25 °C, if not stated otherwise. Because *vang-1(tm1422)* shows a delayed egg laying phenotype, synchronization was performed as follows: embryos were randomly collected from cut-off worms, transferred and grown on plates (three plates per trial) either seeded with OP50 (Brenner, 1974) or HT115(DE3) (Kamath et al., 2001;

Timmons et al., 2001) bacteria harboring the empty L4440 “feeding”-vector or L4440 with a fragment of the gene of interest (Kamath et al., 2001). Worms were transferred to fresh plates every day during time of reproduction but at least every third day. Individuals were considered as dead when stopped moving and not responded to gentle touches. When dying upon “rupture” / “bag of worms” phenotypes or disappearance occurred, the animal was censored on that day. The resulting data sets were analyzed using Kaplan-Meier survival test or weighted log-rank tests (Woolson and Clarke, 2002).

Determination of progeny

C. elegans populations were synchronized and hatched on NGM Agar plates at 25 °C. On day three, single worms were transferred as L4 larvae to 35-mm NGM-plates with NGM agar. Adult worms were transferred to fresh plates and then their progenies were counted each day. The experiment was stopped when production of progeny ceased.

Dauer assay

The assay was performed as described elsewhere (Hu et al., 2006). In brief, some gravid adults were put on individual tagged 60-mm NGM-plates where they laid eggs for 4-6 h at 20 °C before they were removed again. Plates were shifted to the assay temperature of 27 °C. After 60 h the stages were scored for L1 diapause and dauers. Farther grown worms (individuals larger than L2 larvae but not predauer/dauer stages) were pooled as “other”.

Reproductive span of self-fertile animals (modified after Luo et al., 2009)

Ten hermaphrodites per trial were individually transferred to fresh 35 mm NGM-plates seeded with OP50 daily. No production of progeny for 48 h marked reproductive cessation. Individuals were censored if they died or matricide occurred. All trials were conducted at 20 °C with age synchronized populations. Unpaired t-test was used to test null hypothesis.

Determination of ovulation rate (modified after McCarter et al., 1999)

Documentation of ovulation rates were performed using a Zeiss Axioplan 2 microscope. Age-synchronized worms with more than six oocytes in-utero were transferred to small agarose pads on a microscope slide and coated with a cover slip. The number of ovulated oocytes per animal was counted for 3 h and slides were kept in a moisture chamber at room temperature.

DAF-16::GFP translocation

Synchronized populations of TJ356 (DAF-16::GFP) (Henderson and Johnson, 2001) worms were kept for 72 h at 25 °C on NGM plates seeded with RNAi HT115 bacteria either carrying the empty feeding vector or a fragment of *vang-1* cDNA. 15 Individuals per trial were transferred to small agarose pads (3%) on a microscope slide, anesthetized with levamisole (1%), coated with a cover slip, illuminated with UV light under an Axiolab fluorescence microscope (Zeiss, Göttingen, Germany) and dedicated to three categories concerning DAF-16::GFP translocation: "cytoplasmatic" (uniform distribution of DAF-16::GFP), "intermediate" (clearly distinguishable DAF-16::GFP in some nuclei), and "nuclear" (DAF-16::GFP in nearly all nuclei with low background fluorescence).

Lipofuscin accumulation

WT *C. elegans* were synchronized, hatched on NGM Agar plates at 20 °C and transferred to fresh plates every second day. At day five and day ten, individuals were placed on microscope slides, anaesthetized with 20 mM sodium azide in M9 buffer (Lewis and Fleming, 1995) and coated with a cover slip. Epifluorescence (excitation, 365 nm; emission, 420 nm) images were taken with Image ProPlus software (Version 4.5, MediaCybernetics, Silver Spring, MD, USA) using a CoolSnap CF Digital Monochrome Camera (Intas, Göttingen, Germany) mounted on an Axiolab fluorescence microscope (Zeiss, Göttingen, Germany) and using a 100x oil immersion objective. The fluorescence intensity of individual worms was determined densitometrically as relative fluorescence units (RFU: $OD_{individual} - OD_{background}/mm^2$).

Assessment of resistance to thermal/oxidative stress and determination of intracellular ROS accumulation in C. elegans

The resistance of WT and mutant animals to thermal stress was assessed by a semi-automated assay according to (Gill et al., 2003) with some modifications described in (Kampkötter et al., 2007b). After synchronization (Lewis and Fleming, 1995) both strains were cultured on NGM plates with OP50 bacteria (Brenner, 1974) for five days at 20 °C. Worms were then washed in PBST (PBS/0.1% Tween 20) and individually transferred with 1 µl PBST to the wells of a 384-well microtiter plate (Greiner Bio-One, Frickenhausen, Germany, #788096) containing 9 µl PBST with $1x10^7$ OP50 bacteria/ml (Kampkötter et al., 2007b). Immediately after transfer 10 µl of 2 µM SYTOX® Green nucleic acid stain (Molecular Probes Inc., Leiden, Netherlands) in PBS was added to the wells and the plate was sealed using BackSeal-96/384 Black (Perkin Elmer, Wellesley, USA, #6005189) to avoid evaporation. SYTOX® Green can

only enter cells with compromised plasma membranes and exerts a bright fluorescence in the DNA-bound state. Therefore, the fluorescence intensity is an indicator for cellular damage and hence for the viability of worms (Gill et al., 2003). For the application of thermal stress the fluorescence reader (Wallace Victor^2 1420 multilabel counter, Perkin Elmer, Wellesley, USA) was preheated to 37 °C. The measurement of each well through the transparent bottom of the microtiter plate (excitation, 485 nm; emission, 535 nm) was carried out for a minimum of 13 h with intervals of 15 min and a 0.2 s integration time. Fluorescence curves for every single well were obtained and individual cut off values were determined by multiplying the background fluorescence (average of the first four measurement readings) by a factor of three (Gill et al., 2003; Kampkötter et al., 2007b; Kampkötter et al., 2007c). The time point when fluorescence exceeded the cut off value was defined as the point of death of the corresponding worm and the survival curves as well as the mean life spans were assessed from these individual times of death.

To compare resistance to oxidative stress WT and mutant animals synchronized (Lewis and Fleming, 1995) and L4 larvae were incubated for approximate 5 h at 20 °C in liquid NGM containing 200 µM juglone, a redox cycler that generates intracellular oxidative stress (de Castro et al., 2004). Worms were then allowed to regenerate on NGM plates with OP50 bacteria (Brenner, 1974) for about 20 h at 20 °C before viability was determined by touch provoked movement (Lithgow et al., 1995).

For the determination of the intracellular amount of ROS synchronized WT and mutant larvae were cultured as described above and individually transferred with 1 µl PBST to the wells of a 384-well microtiter plate containing 7 µl PBS (Kampkötter et al., 2007a). After the complete transfer of the individual worms 2 µl 250 µM 2,7-dichlorodihydrofluorescein diacetate (H_2DCF-DA; Molecular Probes Inc., Leiden, Netherlands) in PBS (final concentration, 50 µM) was added to the wells and the plate was sealed (see above). After entering cells H_2DCF-DA is intracellular converted to membrane-impermeable, non-fluorescent H_2DCF, which then can be oxidized by ROS to yield fluorescent DCF and thus is a marker for the individual amount of intracellular ROS in a single worm (Kampkötter et al., 2007a; Kampkötter et al., 2007b; Kampkötter et al., 2007c). The fluorescence of each well is then measured through the transparent bottom in a fluorescence reader (see above) every 15 min for a minimum of 13 h at 37 °C (1.0 s integration time; excitation, 485 nm; emission, 535 nm).

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FIGURES AND FIGURE LEGENDS

Figure 1

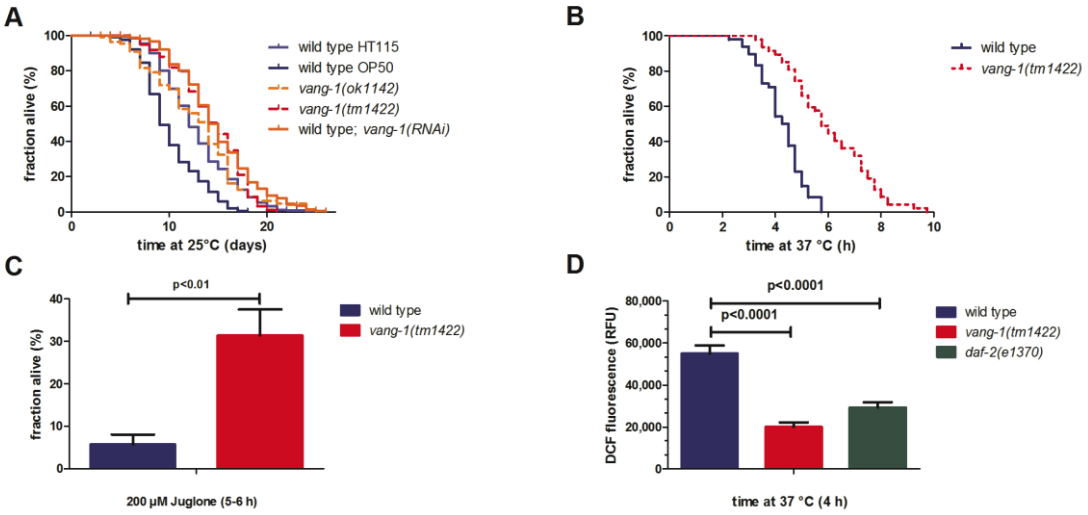

Fig. 1: *vang-1* function interferes with life span extension and resistance against high temperature and reactive oxygen species in *C. elegans*

(A) *vang-1* function interferes with life span extension in *C. elegans*

tm1422 (red), *ok1142* (green) and *vang-1(RNAi)* (orange) animals showed a significantly extended mean life span (**14.3±0.4 d**, n=174*; **12.9±0.5 d**, n=114*; and **14.9±0.2 d**, n=576*, respectively) in comparison to controls: WT animals either grown on OP50 bacteria (light blue; **10.2±0.2 d**, n=214*, p<0.0001**) or RNAi HT115 bacteria (blue; **12.8±0.1 d**, n=936*, *p*<0.001**).

(B-D) *vang-1(tm1422)* increases resistance to thermal/oxidative stress in *C. elegans*

(B) At 37 °C, the mean survival time of *tm1422* (red, **6.2±0.3 h**, n=48*) was significantly increased (*p*<0.01**) in comparison to WT (blue, **4.3±0.1 h**, n=48*).

(C) After 5-6 h under oxidative stress (induced by 200 µM juglone), a significantly larger fraction of *tm1422* animals survived (*p*<0,05***) (red, **34.46%**, n>100*) in comparison to WT (blue, **5.7±2%**, n>100*).

(D) After 4 h at 37 °C, *tm1422* animals (red, **20,020±2,148**, n=48*) and *e1370* animals (green, **29,243±2,528**, n=52*) showed a significantly lower DCF (2,7-dichlorodihydrofluorescein) fluorescence (p<0.001***) in comparison to WT (blue, **54,911±3,940**, n=48*).

(*three or more independent trials, **Mantel-Cox log rank test, ***unpaired t-test; animals grown on OP50 bacteria, if not stated otherwise; results are shown as mean±SEM)

Figure 2

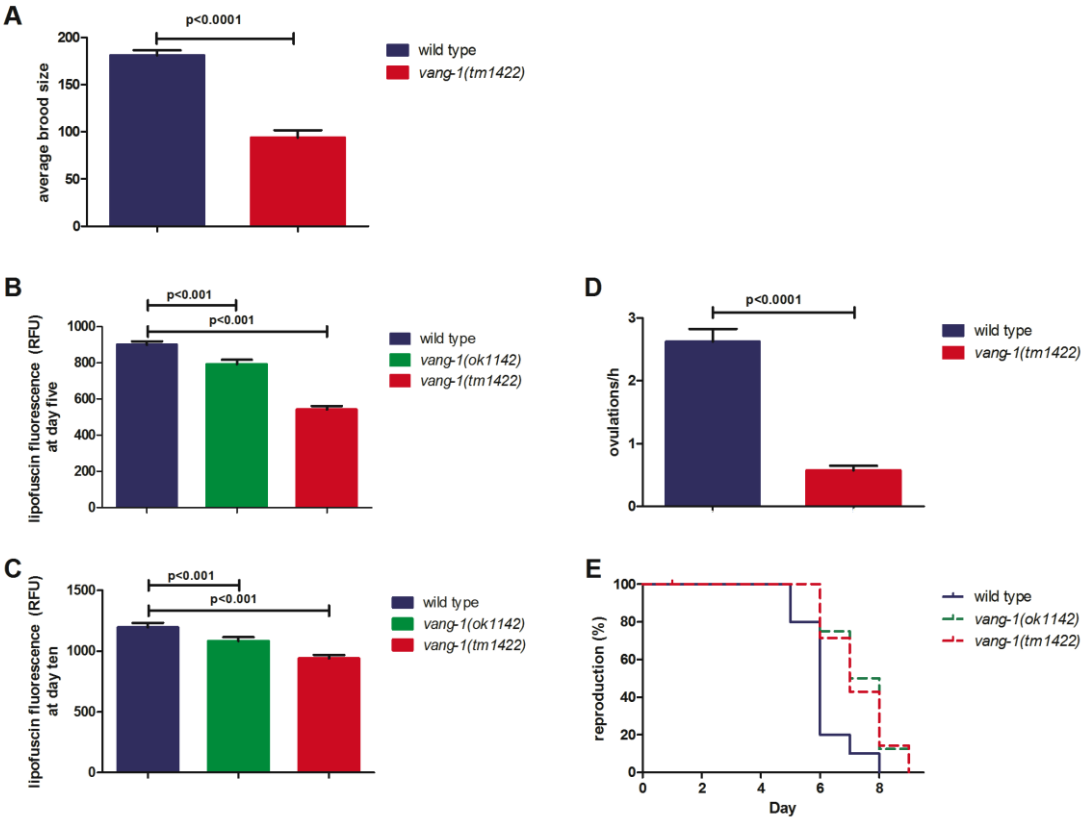
A
p<0.0001
average brood size
200
150
100
50
0
wild type
vang-1(tm1422)
B
p<0.001
p<0.001
lipofuscin fluorescence (RFU) at day five
1000
800
600
400
200
0
wild type
vang-1(ok1142)
vang-1(tm1422)
C
p<0.001
p<0.001
lipofuscin fluorescence (RFU) at day ten
1500
1000
500
0
wild type
vang-1(ok1142)
vang-1(tm1422)
D
p<0.0001
ovulations/h
3
2
1
0
wild type
vang-1(tm1422)
E
reproduction (%)
100
80
60
40
20
0
0
2
4
6
8
Day
wild type
vang-1(ok1142)
vang-1(tm1422)

Fig. 2: *vang-1* shows reproduction- and ageing-related defects

(A) *vang-1(tm1422)* populations have a reduced brood size

The average brood size at 25 °C in *vang-1(tm1422)* (red, **111±41** progeny; n=28*) is significantly reduced (p<0.0001**) in comparison to WT (blue, **194±50** progeny; n=56*). Results are shown as mean±standard deviation.

(B-C) *ok1142* and *tm1422* show decreased lipofuscin accumulation five and ten days after hatching

(B) Five days after hatching, *ok1142* (green, **RFU=792.35±25**, n=31, p<0.001**) and *tm1422* (red, **RFU=543.1±18**, n=37, p<0.001**) accumulate significantly less lipofuscin in comparison to WT (blue, **RFU=900.4±17.27**, n=45).

(C) Ten days after hatching, *ok1142* (green, **RFU=1083±32**, n=33, p<0.05**) and *tm1422* (red, **RFU=940.9±27**, n=29, p<0.01**) still accumulate significantly less lipofuscin in comparison to WT (blue, **RFU=1196±37**, n=27). Results are shown as mean±SEM of relative fluorescence units (RFU: $OD_{individual} - OD_{background} / mm^2$).

(D) In *tm1422* the ovulation rate is reduced in comparison to WT

tm1422 has a ovulationrate of **0.7±0.1** (n=25***) and the WT shows **2.3±0.7** (n=15***) what is significantly more (p<0.05**). Ovulations were counted per gonad arm per hour at 20 °C for synchronous WT and mutant populations.

(E) *vang-1* populations have a prolonged reproductive span

The reproductive span in *ok1142* (green, **6.6** d; n=20***) and *tm1422* (red, **6.9** d; n=20***) is significantly prolonged (p<0.05$^{\#\#}$) in comparison to WT (blue, **5.7** d; n=20***).

(*three independent trials, **unpaired t-test, ***two independent trials; animals grown on OP50 bacteria, $^{\#\#}$Mantel-Cox log rank test)

Figure 3

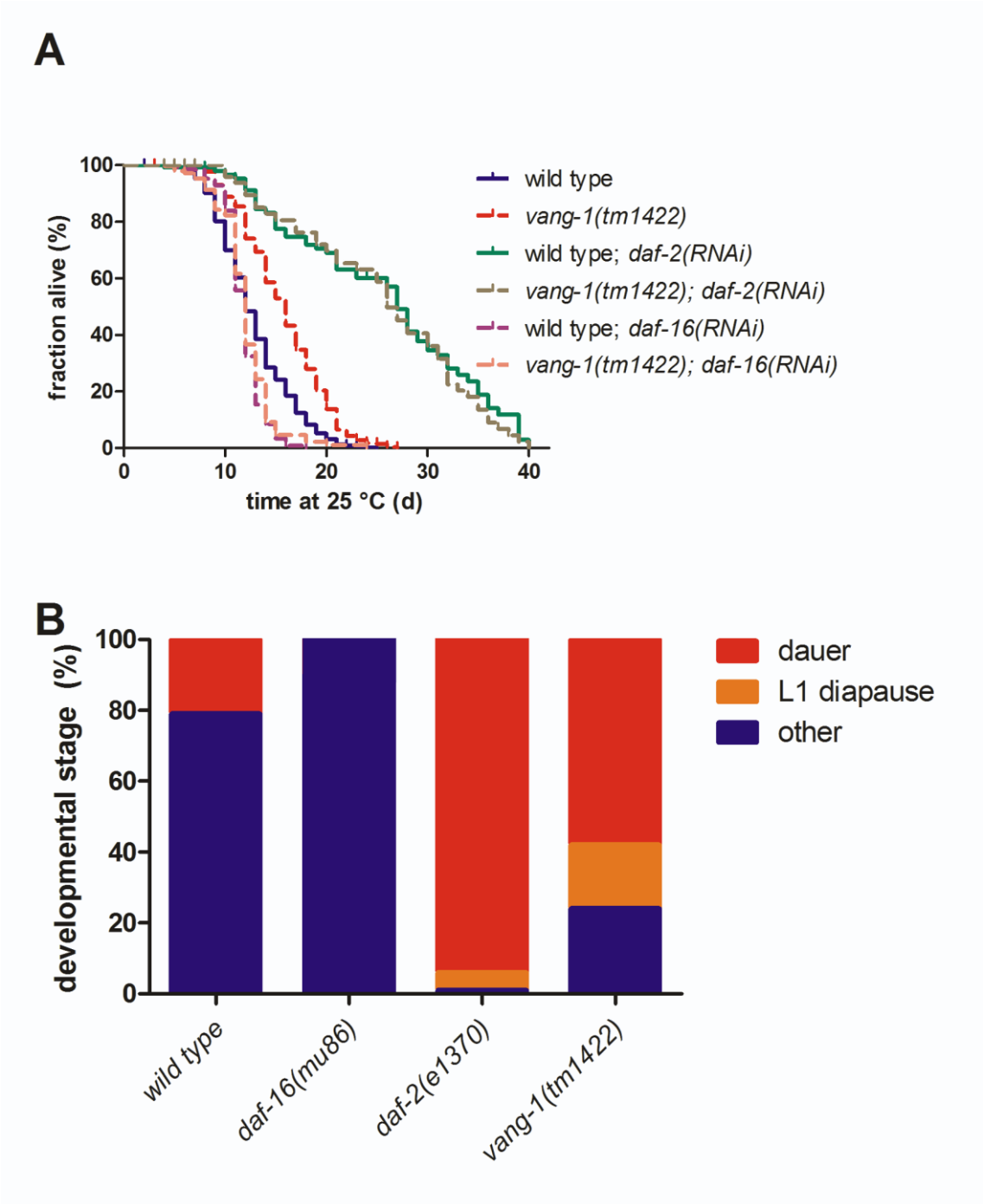
A
fraction alive (%)
100
80
60
40
20
0
0
10
20
30
40
time at 25 °C (d)
wild type
vang-1(tm1422)
wild type; daf-2(RNAi)
vang-1(tm1422); daf-2(RNAi)
wild type; daf-16(RNAi)
vang-1(tm1422); daf-16(RNAi)
B
developmental stage (%)
100
80
60
40
20
0
wild type
daf-16(mu86)
daf-2(e1370)
vang-1(tm1422)
dauer
L1 diapause
other

Fig. 3: *vang-1(tm1422)* life span modulation depends on Insulin/IGF-1 like signaling and leads to higher DAF-16 activity

(A) *vang-1(tm1422)* induced life span extension interferes with RNAi against *daf-2* and *daf-16*

Depletion of DAF-2 by RNAi in *tm1422* (brown spotted line) and WT (green solid line) causes an increase of mean life span to **25.6±1.3 d** (n=135*) and **25.8±1.1 d** (n=126*), respectively (p<0.62**), which is in agreement with published results for *daf-2* mutants (Hertweck et al., 2004). In contrast, depletion of DAF-16 by RNAi in *tm1422* (rose spotted line) and WT (purple solid line) causes a decrease of mean life span to **12±0.3 d** (n=247*) and **11.9±0.2 d** (n=142*), respectively (p<0.96**). Life spans of WT (blue solid line) and *tm1422* (red spotted line) fed with RNAi HT115 bacteria carrying the empty "feeding"-vector are **12.8±0.1 d** (n=936*) and **15.8±0.2 d** (n=480*), respectively (p<0.001**).

(B) *vang-1(tm1422)* populations are dauer constitutive

Synchronous populations were scored after 60 h at 27 °C (OP50 bacteria) for dauers and L1 in diapause. All farther grown and adult animals were pooled as "other". WT animals developed **21%**, **0%** and **79%** dauers, L1 diapause and "other", respectively (n=390*). *daf-2(e1370)* animals showed **94.7%**, **4.6%** and **0.7%** dauers, L1 diapause and "other", respectively (n=281*). *daf-16(mu86)* animals developed **100%** "other" (n=111*). *tm1422* showed **57.6%**, **18%** and **24.4%** dauers, L1 diapause and "other", respectively (n=205*, p<0.05[§]).

(*three or more independent trials, **Mantel-Cox log rank test, animals grown on OP50 bacteria, if not stated otherwise, [§]Data analyzed by Chi-square test)

Figure 4

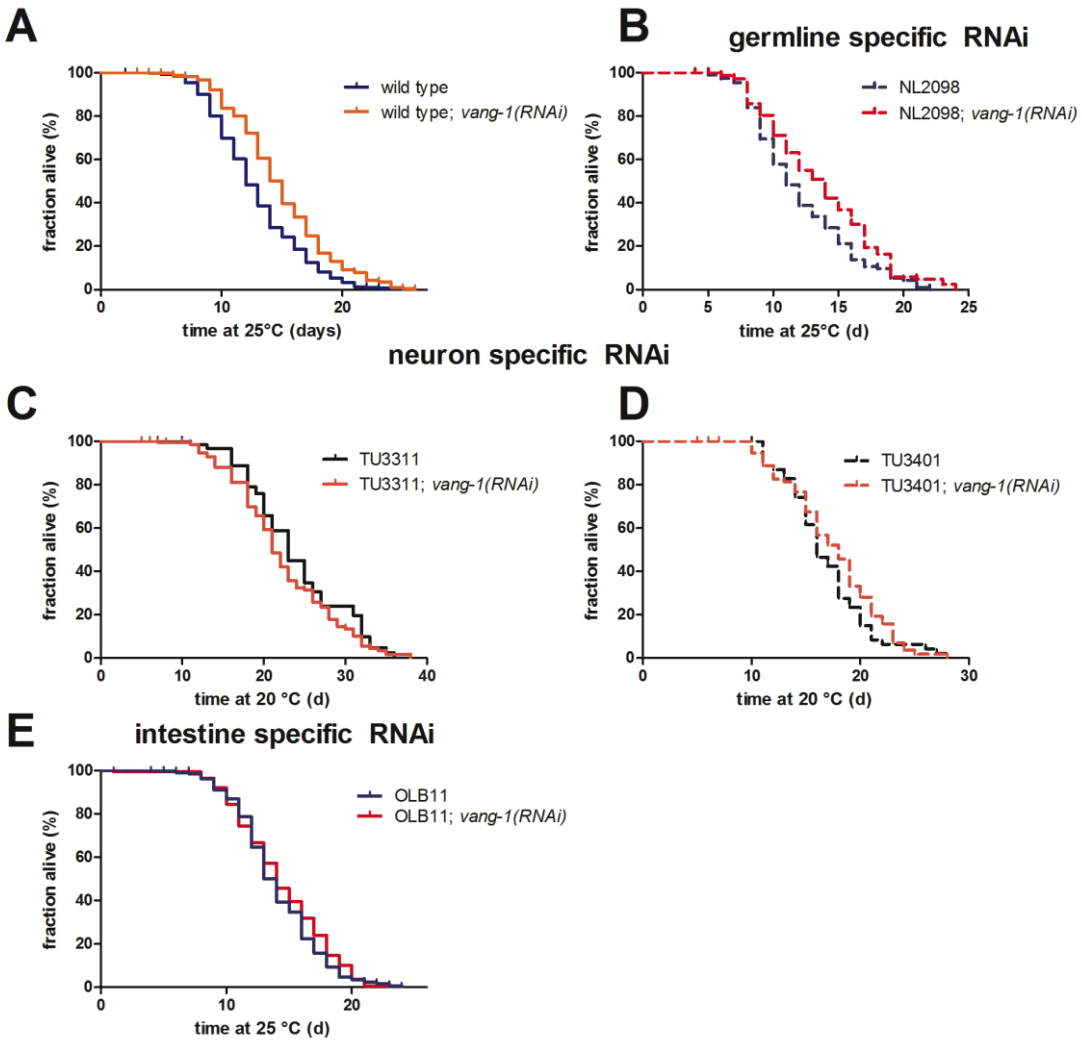
A
fraction alive (%)
time at 25°C (days)
wild type
wild type; vang-1(RNAi)
B
germline specific RNAi
fraction alive (%)
time at 25°C (d)
NL2098
NL2098; vang-1(RNAi)
neuron specific RNAi
C
fraction alive (%)
time at 20 °C (d)
TU3311
TU3311; vang-1(RNAi)
D
fraction alive (%)
time at 20 °C (d)
TU3401
TU3401; vang-1(RNAi)
E
intestine specific RNAi
fraction alive (%)
time at 25 °C (d)
OLB11
OLB11; vang-1(RNAi)

Fig. 4: Tissue specific RNAi against *vang-1*

(A) *vang-1* function interferes with life span extension in *C. elegans*
vang-1(RNAi) (orange) animals showed a significantly extended mean life span (**14.9±0.2 d**, n=576*) in comparison to control: RNAi HT115 bacteria (blue; **12.8±0.1 d**, n=936*, $p<0.001$**).

(B) Germline-specific RNAi against *vang-1* effects *C. elegans* life span
After *vang-1* RNAi in germline-specific RNAi strain NL2098 a significant increase (13%) of mean life span (**14.6±0.3 d**, red spotted line, n=274*) in comparison to the control (NL2098 kept on RNAi HT115 bacteria carrying the empty "feeding"-vector) can be observed (**12.9±0.3 d**, blue spotted line, n=309*, $p<0.01$**).

(C,D) Neuron-specific RNAi against *vang-1* does not effect *C. elegans* life span After depletion of VANG-1 in the enhanced-neuronal RNAi strain TU3311 *([unc-119p::YFP + unc-119p::sid-1])*, the mean life span is **21.1±0.4 d** (orange solid line, n=280*) compared to **19.5±0.5 d** (green solid line, n=92*, $p=0.02$**) in the control (TU3311 kept on RNAi HT115 bacteria carrying the empty "feeding"-vector). The same is true in the neuron-specific RNAi strain TU3401 *(sid-1(pk3321) V; [pCFJ90(myo-2p::mCherry) + unc-119p::sid-1])*, which only has SID-1 in neurons. Depletion of VANG-1 in this strain leads to a mean life span of **17.7±0.3 d** (red spotted line, n=284*) and **17±0.3 d** (blue spotted line, n=380*, no significant difference**) in the control (TU3401 kept on RNAi HT115 bacteria carrying the empty "feeding"-vector).

(E) Intestine-specific RNAi against *vang-1* does not effect *C. elegans* life span
After depletion of VANG-1 in the intestine-specific RNAi strain OLB11 {*(rde-1(ne219);[pOLB11(elt-2p::rde-1) + pRF4(rol-6(su1006)]*}, the mean life span is **14.4±0.3 d** (red solid line, n=195*) compared to **14.0±0.3 d** (blue solid line, n=250*, no significant difference**) in the control (OLB11 kept on RNAi HT115 bacteria carrying the empty "feeding"-vector).

(*three or more independent trials, **Mantel-Cox log rank test)

TABLES

Table 1

	Background	Conditions	LS +/- SEM	N	Significance
1	WT	OP50	10.2 +/- 0.2	214	
2	*tm1422*	OP50	14.3 +/- 0.4	174	*(1)
3	*ok1142*	OP50	12.9 +/- 0.5	114	*(1)
4	WT	18°C / OP50	19.6 +/- 0.8	61	
5	*tm1422*	18°C / OP50	27.1 +/- 0.9	43	*(4)
6	WT	20 °C / HT115	21.4 +/- 0.4	70	
7	*tm1422*	20 °C / HT115	25.6 +/- 0.4	71	*(25)
8	WT	20 °C / *daf-16(RNAi)*	19.5 +/- 0.5	75	*(25)
9	*tm1422*	20 °C / *daf-16(RNAi)*	22.5 +/- 0.5	71	*(26) 0.72(25)
10	WT	HT115	12.8 +/- 0.1	936	*(1)
11	*tm1422*	HT115	15.8 +/- 0.2	480	*(10)
12	WT	*vang-1(RNAi)*	14.9 +/- 0.2	576	*(10)
13	*tm1422*	*vang-1(RNAi)*	14.9 +/- 0.4	242	0.37(11)
14	WT	*skn-1(RNAi)*	13.0 +/- 0.2	133	0.76(10)
15	*tm1422*	*skn-1(RNAi)*	13.2 +/- 0.5	138	*(11)
16	WT	*daf-16(RNAi)*	11.9 +/- 0.2	142	*(10) / 0.27(17)
17	*tm1422*	*daf-16(RNAi)*	12 +/- 0.3	247	*(11)
18	WT	*daf-2(RNAi)*	25.8 +/- 1.1	126	*(10) / 0.62(19)
19	*tm1422*	*daf-2(RNAi)*	25.6 +/- 1.3	135	*(11)
20	OLB11	HT115	14 +/- 0.3	250	*(10)
21	OLB11	*daf-2(RNAi)*	22.6 +/- 0.7	85	*(20)
22	OLB11	*vang-1(RNAi)*	14.4 +/- 0.3	195	0.24(20)
23	NL2098*(rrf-1)*	HT115	12.9 +/- 0.3	309	
24	NL2098*(rrf-1)*	*vang-1(RNAi)*	14.6 +/- 0.3	274	*(23)
25	TU3401	20 °C / HT115	17.0 +/- 0.3	380	
26	TU3401	20 °C / *vang-1(RNAi)*	17.7 +/- 0.3	284	0.86(25)
27	TU3311	20 °C / HT115	19.5 +/- 0.5	212	
28	TU3311	20 °C / *vang-1(RNAi)*	21.1 +/- 0.4	280	*(27)

Table 1: Summary of **l**ife **s**pans (**LS**±SEM, standard error of the mean, at 25 °C, if not stated otherwise) under different experimental conditions in WT, two different alleles of *vang-1* (*tm1422* and *ok1142*), the intestine-specific RNAi strain OLB11 (McGhee et al., 2009; Pilipiuk et al., 2009), germline-specific RNAi strain NL2098 (Sijen et al., 2001) and the neuron-enhanced and neuron-specific strains TU3311 and TU3401 (Calixto et al., 2010). OP50 (Brenner, 1974) and RNAi HT115 (Kamath et al., 2001; Timmons et al., 2001) indicate standard and RNAi *E. coli* strains, respectively. Comparison of significant results are indicated by *($p<0.01$; Mantel-Cox log rank test) with corresponding experiments in parenthesis (the *p*-value is stated, if not significant). All the life span assays were repeated at least three times. Data shown is a sum of all experiments.

Supplementary Data

Figure S1:

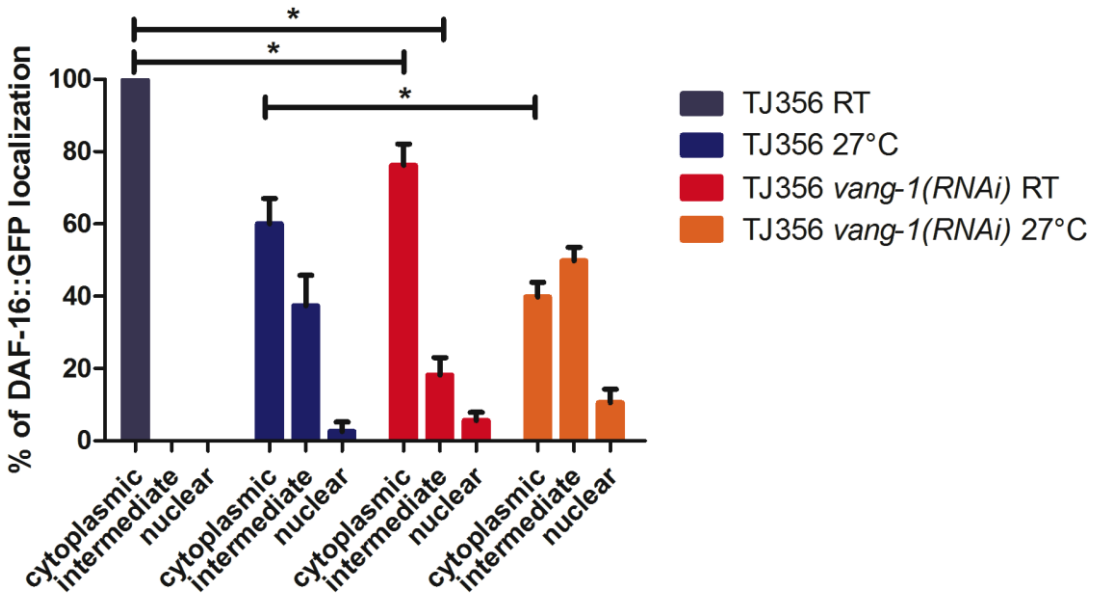

Fig. S1: DAF-16::GFP translocation into the nucleus

In TJ356 (DAF-16::GFP) worms (Henderson and Johnson, 2001), RNAi against VANG-1 at room temperature (RT) led to **12%** and **4%** intermediate and nuclear localization of DAF-16::GFP, respectively (n=49*). In contrast, TJ356 control animals fed with RNAi HT115 bacteria, carrying the empty "feeding" vector, showed **100%** cytoplasmic localization of DAF-16::GFP (n=70*). Under heat stress condition (27 °C), *vang-1(RNAi)* causes **45%** and **12%** intermediate and nuclear localization of DAF-16::GFP, respectively (n=42*). In comparison, TJ356 control animals showed **42%** intermediate- and **3%** nuclear localization of DAF-16::GFP (n=95*).

*(p<0.05 by two-way ANOVA with Bonferroni's post hoc test; three or more independent trials)

Figure S2:

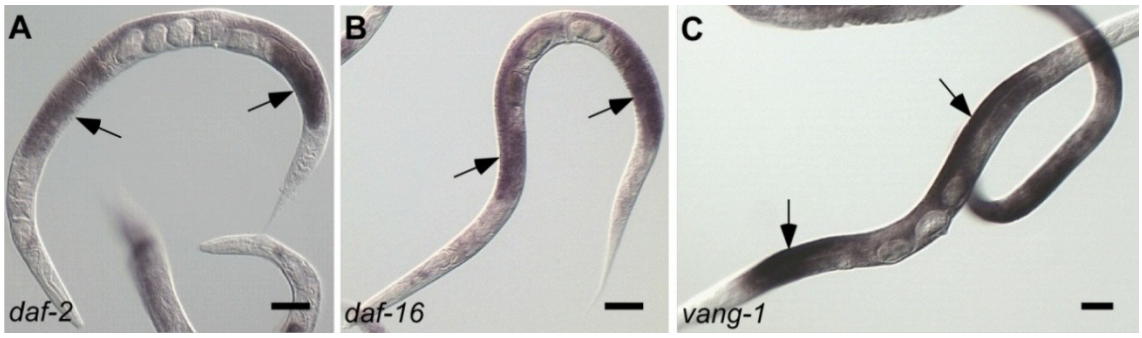

Fig. S2: Expression patterns in *C. elegans* adults of *daf-2* (A), *daf-16* (B) and *vang-1* (C) genes.

All images represent *in situ* hybridization to endogenous transcripts (enriched in the gonad, arrows) and are taken from the Nematode Expression Data Base (http://nematode.lab.nig.ac.jp/db2/index.php). Scale bars: 60 µm.

Sequences of VANG-1, VANG-1^{tm1422} and VANG-1^{ok1142} proteins:

MSYQDNRKLPKDTRSCVGGFRYEGHKKQLRPRYAQSEIGEPFIPRFSAIASEGQKIAPP
NEDWADNTTVLTGMTTDSFTMEEKVIYTPPIGRVIGRRCSRFVWLLASSLLCIISVVSA
PIMCSLPIIAPRFGFSMPAIQCDVDCEGLLFLMAIKTIFLVIAIGVLYWRKAMADMPRL
YFVRAALTFLVMFILFAFWLFYIVRIMFERYDNYKYIVSYSTSLLDALLWTHYLSVVL
LELRRLRAQFIVTIVRDPDGEMHTLNIGAGSIQEAATEILRFYTTRFSSFNIHLDNARQT
AVAKQSGMQGGTAGFKMYNIEQFGGQETVSEVNTRALMEAAARRRIGGYAEVMQE
ELDFEKRLKKRKYRLIAAAEDAFSHVQNTAESGTNQKPGINNQMDSLTAAQNVFTWI
VRPLTKYLKTTRLQSRHPSGEVTRHIERCLTLKLSHRTFLQRFFSDRIPQREIVGESKW
SVICDEAVSSGVQHGTYLVLKSHNPNTLTKNITNKIRKLDIDCGVQLVCTISSIPFFNL
TEQAKPGNEKFSLKISNESAV

Missing amino acids in *tm1422* and *ok1142* are shown in red and blue, respectively. Additional amino acids in *ok1142* are shown in yellow. For further details concerning VANG-1 see (Hoffmann et al., 2010).

Sequences of RNAi "feeding" clones

***vang-1* / (B0410.2)**
AGCCGACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCAACCTGGCTTATCG
AAATTAATACGACTCACTATAGGGAGACCGGCAGATCTGATATCATCGATGAATT
CGAGCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTGGATCCACCGGTTCCATG
GCTAGCCACGTGACGCGTGGATCCCCCGGGCTGCAGGAATTCGATATCAAGCTTA
TCGATACCGTCGACCTCGAGATGGTGCAAACAAGTTGAACTCCGCAGTCGATATC
TGGGTTGTGCGATTTGAGCACTAGATACGTTCCGTGTTGAACTCCAGAGCTCACC
GCCTCGTCGCAAATTACCGACCATTTCGATTCCCCGACAATTTCCCGTTGGGGAAT
ACGGTCACTGAAGAAGCGTTGGAGAAATGTTCGATTCTTTCAATGTGCCTCGTAA
CTTCTCCTGAAGGATGTCTTGACTGAAGACGAGTAGTTTTTAAATATTTGGTGAG
GGCCGGACAATCCATGTGAATACGTTTTGAGCAGCGGTCAGTGAGTCCATCTGGT
TGTTAATTCCAGGCTTTTGGTTCGTTCCGGACTCGGCAGTGTTCTGAACATGGGAG
AATGCGTCTTCGGCGGCGGCAATTAGGCGATACTTTCTCTTCTTTAACCGTTTTTC
AAAATCAAGTTCTTCCTGCATCACTTCAGCATATCCACCAATCCTTCTTCTCGCCG
CGGCTTCCATCAAAGCCCGCGTGTTGACTTCACTAACAGTTTCCTGCCCTCCAAAT
TGCTCGATATTGTACATCTTGAAGCCTGCGGTGCCTCCTTGCATTCCAGACTGCTT
TGCAACCGCCGTCTGTCTGGCGTTGTCAAGGTGAATGTTGAACGAGGAGAATCGA
GTTGTGTAGAAACGAAGAATCTCGGTGGCAGCTTCTTGAATTGAGCCAGCGCCAT
GTTCAA

***daf-16* / (R13H8.1)**
CGCGTATACGACTCACTATAGGGCGAATTGGGTACCGGGCCCCCCCTCGAGGTCG
ACGGTATCGATAAGCTTGATTTTGGATTTCGAAGAAGTGGATTCTGAGCACACGA
TCCACGGACACTGTTCAACTCGTGGTATGATGGTGGTGGAGCAATTGGTTCCGTC
TGAAAATTCTATATATTACTCAGTTTTTTCACTTAAAAAAAAAGAAAATTCTAATT
TTCGGTGTGAGAAAATATAAAATGATTTGCATGTTACTAATATTTTAGGGGACCT
TGAAACTAAATTCAGAATTAAAAATATTGAATTTAAGTAAAATTCAAATTTTTTA
ATTGTCTAAAAAGGAGCTCACTTGTACTAACCACAAGAAAATTTTCAAATTTTCC

TGAAAATTGCAAATTCTCATTTCTCACCTTAATCGGCTTCGACTCCTGCTTAATCT
GAACTCCACCAATATGAGTAGTTGCATCGATACGCATTTGATCAGTTCTATCAAC
AATATCACTTGGAATTGCTGGAACCGATTCGCCAACCCATGATGGGAATTCTAGA
TCATCATAGATATCACTTCCAATAGCTGGAGAAACACGAGACGACGATCCAGGA
ATCGAGAGGTTCGATTGAGTTCGGGGACTGAAAAAATTTGGAAATTTGGAAAATC
CAAAAAATAAAAATAAAACTAAATTACTAACCGGAAAGATGATGGAACGTTATC
AAATGCTCCTTGCATTGAATCATCATCATACAAATCGTGAGAAATCGTTTGAATC
GATCCGGCAATCGAATTTCCATTAAGTGTCGAGTGAAGGGAGCCCATCAATGCTC
TCTCCTTTATCCTCTTCTTGGCTCCGCGGCGAGATTTTTCGAGTTGAGCCTGAAAT
TTTAAGCGTATTTATAGTGAGGTGAAATTACCAAATATCATAGTAAAAATTTTCG
AAAAATTCTAGGAAAACTTTTAATTTGAGTCGAAAAGCTGAGAAATCCATTTTAG
CTCATTTAGCACTATAAAATTATTTCACTATCTCTACCT

***daf-2* / (Y55D5A.5)**

ACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACG
CAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGG
TTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCA
CTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGA
ATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCA
AGCGCGCAATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCTCGCGCGCGTAA
TACGACTCATATAGGGGCGAAGCACTAGTGGATCCCCCGGGCTGCAGGAATTCGC
TCGCGCGGTCCAATATCCATAAAAATGTCCGGGGTCGGTTCACCGTTCGACGTCT
CGTAGTACTGTTTGAAGACACTCTGCCACGAATCGACACACGCACTTCGATCCTC
TTCGATCGTCATGTTCTCATCGATTCGTGGGACTTCTTTGAAGAAGAGCTCGTAGC
CGAGAAACTTTCGCTGATCTATATCGGTAATGTTGAATGAGGGCCAACTAAAGAA
GACCGAGTCCGCGTTGACCGCTGTGATGCTCACGTTGATTGCCATATCCTCACAG
ATTGCCTTCTCACCATTTGTCCCTTCTGATTGATCTATCGGATCGAGTGGTATATTT
AACTTTGACATTAGCTGCTTGATATACTTGAAGCATAACATCTTGTTATTGGCAAT
TGACACAGTTCCACGATCAAGCGTCAAATCCGTCGTTGAATCGAATAGCTTTTTTT
AAATTC

***skn-1* / (T19E7.2)**

CGCGTATACGACTCACTATAGGGCGAATTGGGTACCGGGCCCCCCCTCGAGGTCG
ACGGTATCGATAAGCTTGATTGGGTACCCACTTGCCCTATTTGAGCAAGTGCACT
TTTACTAATAAGCTGAATGGGATCCCAAGAGACTTTCTAGACTTTCAGACTGAGC
AACTTACGTAGTGCAATCACTAAACGAGTGTCTCTGTGAGTGATATGGATCGAAC
AACGATTGAGTAGCTGTTGCAGTCACTGTGACGCTTCCTGGGCTCGTTTGAGTCTG
CTGTTGACGTCCTGAAGATCCAATGATGAGAGGAGACAGTGGAGTCTGACCAGT
GGATTGATAGGAATGATCATAGTCAATCTGTCCATTTGATACAACTGCTTGAAGA
CTGTCGTTTTGCATTCCAATGTAGGCGTAGTTGGATGTTGGGAACACTGGAAATTT
TTATAATTATAAGAAAACTGAAAGGTATGGGTTTCAAACTCTCTTATATCTTGAA
ATATTTTAAGCCGCTTCTAGGTTAACAACCCTCTGCCATTCCAAGATATGAGGGA
GCCCTGAAAGGAGGAAATGTTGGAATGTCTAGATAGGATGAAAGAGAGAGAGAG
AGAGAGAGAGAGATAGGAACCTCTGTCAAAAGTGCTCGAGACTCCTGAATAGGC
GTATGGATGTTGGTGATGATGGCCGTGTTGATCCACCTGTTGTTGATTGAACATTT
GAAGGTACATGTTCAGTATCTTCTTGGATTCTTCTTCTTGTTCGTCAAAAGAGTTG
ACAGCTGCTGGAGCGGTGTTGGTGGTGTTGGTGGGG

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MICS-1 interacts with mitochondrial ATAD-3 and modulates lifespan in *C. elegans*

Running Head:

MICS-1 regulates lifespan

Michael Hoffmann[1*]

Sebastian Honnen[2,3*]

Ertan Mayatepek[1]

Wim Wätjen[3]

Werner J. H. Koopman[4]

Olaf Bossinger[2]

And

Felix Distelmaier[1 #]

[1] Department of General Pediatrics, University Children's Hospital , Heinrich-Heine-University, D-40225 Düsseldorf, Germany

[2] Institute of Molecular and Cellular Anatomy, RWTH Aachen University, D-52074 Aachen, Germany

[3]Department of Toxicology, Heinrich-Heine-University, D-40225 Düsseldorf, Germany

[4] Department of Biochemistry, Nijmegen Center for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

[*] These two authors contributed equally

[#] **Correspondence should be addressed to**:

Felix Distelmaier,

Department of General Pediatrics, Heinrich-Heine-University, Moorenstr. 5, D-40225 Düsseldorf, Germany; Phone: +49-211-811-7640; Fax:+49-211-811-8757;

E-mail: felix.distelmaier@med.uni-duesseldorf.de

Abbreviations: MICS-1 = **mi**to**c**hondrial **s**caffolding protein-**1**; OMP25 = mitochondrial **o**uter **m**embrane **p**rotein **25**; ROS = **r**eactive **o**xygen **s**pecies

ABSTRACT

Caenorhabditis elegans open reading frame T21C9.1 encodes an uncharacterized protein, which is here named MICS-1 (mitochondrial scaffolding protein-1). It is predicted to be the homolog of human outer mitochondrial membrane protein 25 (OMP25 or synaptojanin-2-binding protein), which is a PDZ domain containing protein with a putative role in cellular stress response pathways. Here, we provide evidence that MICS-1 is an interacting partner of the mitochondrial protein ATAD-3 (homologue of human ATAD3), which is essential for *C. elegans* development. We demonstrate that *mics-1(RNAi)* animals or *mics-1* mutants display enhanced longevity with an increased mean lifespan of up to 54% compared to control animals. Of note, also *atad-3(RNAi)* promoted longevity, although to a lesser extend (29% compared to controls). In addition, thermal stress of *mics-1* mutants induced low reactive oxygen species (ROS) production, whereas *atad-3(RNAi)* animals were highly sensitive to this assay, displaying drastically increased ROS levels. Further studies revealed that MICS-1 and ATAD-3 associated longevity was partially dependent on the presence of DAF-16. However, for both conditions, we also observed a DAF-16 independent extension of lifespan. Finally, we observed an additional lifespan extension in *mics-1* mutants when subjected to *atad-3(RNAi)* whereas heat induced ROS production was even aggravated under this condition. This suggests (partially) independent effects of MICS-1 and ATAD-3 on lifespan and ROS production *in vivo*.

KEYWORDS: aging; ATAD3a; *C. elegans*; DAF-16; mitochondria; OXPHOS

INTRODUCTION

Mammalian AAA domain-containing protein 3 (ATAD3) is a member of the AAA-ATPase family. ATAD3 is highly conserved among different species. It exists in *Caenorhabditis elegans* (*C. elegans*), *Arabidopsis thaliana* and *Drosophila melanogaster* as a single gene. In humans, three forms of ATAD3 have been identified and are documented in the NCBI database (http://www. ncbi.nlm.gov): a 66-kDa ATAD3A (BC033109; the ancestral form of the ATAD3 proteins), a 72.6-kDa ATAD3B (NM_031921) and a 46-kDa ATAD3C (NM_001039211) (Fang et al., 2010). Mitochondrial research identified ATAD3 in a proteomic analysis of mouse mitochondrial inner membrane proteins (e.g. TOB3; Da Cruz et al., 2003; Mootha et al., 2003). Later, an apparent role of ATAD3A in mitochondrial nucleoid organization was suggested and it was claimed to have the ability to bind mitochondrial DNA D-loop structures (He et al., 2007). However, follow-up studies demonstrated that a direct interaction of the protein with D-loop structures is unlikely (Bogenhagen et al., 2008). Recent studies on the topology of ATAD3A in mitochondrial membranes indicate that the N-terminal part of ATAD3A is outside the inner membrane and that the C-terminal part is inside the matrix. It was suggested that ATAD3A regulates dynamic interactions between the mitochondrial outer and inner membrane sensed by the cell fission machinery (Gilquin et al., 2010b). Moreover, an interaction between S100B, a calcium sensor protein, and ATAD3A was demonstrated, which is important for the cytoplasmic processing and subcellular localization of ATAD3A (Gilquin et al., 2010a).

Previously, we identified the *C. elegans* protein ATAD-3 as a homologue of mammalian ATAD3 (Hoffmann et al., 2009). We demonstrated that ATAD-3 is a mitochondrial protein, which is essential for *C. elegans* development. However, the mechanism behind these observations remained unresolved. Of note, ATAD-3 exhibits a class I PDZ binding motif at its C-terminus (-ETAV), which might be important for the interaction with other proteins.

Here, we provide evidence that ATAD-3 interacts with an uncharacterized PDZ domain containing protein, which is here named MICS-1 (mitochondrial scaffolding protein-1). We demonstrate that depletion of MICS-1 drastically extends lifespan and lowers thermal stress induced reactive oxygen species (ROS) production. Also ATAD-3 depletion extended *C. elegans* lifespan, however heat stress induced ROS production was clearly increased in these animals. Further studies revealed that MICS-1 and ATAD-3 associated longevity was partially dependent on the presence of DAF-16. However, for both conditions, we also observed a DAF-16 independent extension of lifespan. Finally, *atad-3(RNAi)* in *mics-1* mutants additionally extended lifespan whereas stress induced ROS production was even aggravated in this condition.

METHODS

C. elegans strains and culture

Maintenance and handling of *C. elegans* were carried out as described previously (Brenner, 1974). Bristol N2 was used as the wild type strain. Single mutant strains were as follows: RB1335: *mics-1(ok1451)*, CB1370: *daf-2(e1370)*, CF1038: *daf-16(mu86)*. Transgenic strains were as follows:
TJ356: integrated DAF-16::GFP roller strain (Henderson and Johnson, 2001)

Measurement of reactive oxygen species (ROS)

Measurements of heat stress induced ROS production was essentially performed as described by others (Kampkötter et al., 2007). In brief, 8-16 adult worms per trial were washed in TBST for 30 min and than individually transferred to the wells of a 384-well microtiter plate containing a final concentration of 12,5 µM 2,7-dichlorohydrofluorescein diacetate (H2DCF-DA; Molecular Probes Inc., Leiden, The Netherlands) in TBST. The measurement was performed in a Wallac 1420 Victor^2 multilabel counter (Perkin-Elmer, Monza, Italy) for 240 min at designated temperatures. Absolute fluorescence intensity values at 240 min were analyzed. In addition, data was validated by performing a linear fit for representative traces during a time span of linear increase of the fluorescence signal (according to Koopman *et al*., 2006). The slope of the fit was analyzed. Essentially, the same results were obtained as for the 240 min endpoint analysis.

RNA-mediated interference (RNAi)

RNAi by "feeding" was performed essentially as described by Kamath *et al*., (2001). The RNAi feeding clones for *atad-3* and *mics-1* were obtained from the Ahringer RNAi library (Geneservice Limited, UK). Clones were verified by sequencing. HT115(DE3) bacteria carrying the empty L4440 vector were used as controls.

Lifespan assay

Lifespan was determined at 25 °C according to a modified protocol of Wilson *et al*., (2006). Worms were transferred and grown on plates (three plates per trial) seeded with HT115(DE3) bacteria harboring the empty L4440 "feeding"-vector or L4440 with a fragment of the gene of

interest (Brenner, 1974; Kamath et al., 2001; Timmons et al., 2001). Synchronized worms were transferred to fresh plates containing 25 µM FUDR (5-Fluoro-2′-deoxyuridine; Sigma-Aldrich) after reaching the young Adult stage for 48 h. Subsequently, worms were transferred to new plates containing FUDR for additional 48 h (until the reproductive period was over). Next, worms were taken to plates without FUDR (Hosono, 1978; Tissenbaum and Guarente, 2001). Individuals were investigated at least every second day and considered as dead if no pharyngeal pumping was evident and they failed to respond to repeated gentle prodding. Individuals were counted and censored when dying upon "rupture" or "bag of worms" phenotypes or were untraceable. The resulting data sets of adult lifespan were analyzed using Kaplan-Meier survival test and weighted log-rank tests (Woolson and Clarke, 2002).

Protein-protein interactions

Co-Immunoprecipitation (Co-IP) and yeast 2-hybrid analysis was performed as described previously (Hoffmann et al., 2010). For Co-IP, 1 mg of protein lysate from either wild type or *atad-3(RNAi)* treated worms was preincubated with protein A agarose beads for 1 h at 4 °C on a rotating platform. Following centrifugation (12,000 rpm, 4 °C, 2 min), the supernatant was incubated with antibodies against ATAD-3 (rat, 1:1000; Hoffmann et al., 2009) for 2 h at 4 °C on a rotating platform. After addition and incubation with protein A agarose beads, beads were washed with TNT buffer three times. SDS–PAGE and western blot analyses were carried out as described above, using a cross reacting polyclonal against human omp25 (rabbit, 1:1000; Nemoto and De Camilli, 1999) and anti-rabbit secondary antibody (HRP conjugated, 1:5000). For yeast 2-hybrid analysis, a 280 bp fragment encoding for the PDZ domain of MICS-1 was cloned into GAL4 DNA-binding domain vector pGBKT7 vector (Clontech), and a 650 bp c-terminal fragment of ATAD-3 was cloned into pACT2 vector (Clontech), either with or without the -ETAV motif. Empty pGBKT7 and pACT2 vectors were used as controls, respectively. As an additional negative control we used *Drosophila* Bazooka PDZ domain construct (Hoffmann et al., 2010).

DAF-16 translocation and dauer assays

For analysis of subcellular DAF-16 localization, synchronized L4 stage TJ356 were placed on HT115 or RNAi plates for 48 h. The strain TJ356 expresses a DAF-16::GFP fusion protein and can be used to monitor DAF-16 subcellular localization after heat stress (Henderson and Johnson, 2001; Kampkötter et al., 2008)After treatment at 37°C for 30 min, worms were placed

on microscope slides in 1 mM levamisole and capped with cover slips. DAF-16 subcellular distribution was analyzed on an Axiolab fluorescence microscope (Zeiss, Göttingen, Germany) at 100-fold magnification. Distribution of DAF-16::GFP was classified into two categories (cytosolic and translocated, i.e. nuclear/intermediate).

Dauer assay was performed as described elsewhere (Hu, 2007). In brief, gravid adults were put on individual 60 mm NGM-plates where we allowed egg-laying for 4-6 h at 20 °C before worms were removed again. Plates were shifted to the assay temperature of 27 °C. After 48 - 60 h the stages were scored for L1 diapause and dauers. Individuals larger than L2 larvae but not predauer/dauer stages were pooled as "other".

RESULTS AND DISCUSSION

Bioinformatic prediction (Chen et al., 2008) identified an uncharacterized protein, which is encoded by the predicted open reading frame T21C9.1, as a putative interaction partner of ATAD-3. The protein is homologous to the human outer mitochondrial membrane protein 25 (OMP25 or synaptojanin-2-binding protein), which is a PDZ domain containing protein with roles in cellular stress response pathways (Court et al., 2005; Nemoto and De Camilli, 1999).

To verify this prediction, we performed a yeast 2-hybrid analysis, using the ATAD-3 C-terminal part, which confirmed the interaction of ATAD-3 with the above-mentioned protein (Fig.1A; see also Hoffmann et al., 2010). Interaction is abolished if the PDZ domain-binding motif "-ETAV" in the ATAD-3 C-terminal part is depleted. We will further refer to this protein as MICS-1 (mitochondrial scaffolding protein-1), which was named according to general properties of PDZ domain proteins to promote clustering of target proteins at plasma membranes (Hughes and Fields, 1999).

Up to now, there exists very few information about MICS-1. Derry et al. (2007) included MICS-1 in a study about CEP-1 regulated genes in response to UV radiation. Severance et al. (2010) identified MICS-1 in a screen for genes essential for metazoan heme homeostasis. However, no further details about the proteins function were provided. So far, MICS-1 was not investigated in RNAi lifespan screens in *C. elegans*.

To further support our yeast 2-hybrid analysis, we performed co-immunoprecipitations using ATAD-3 and MICS-1 antibodies (cross reacting antibody against OMP25; Nemoto and De Camilli, 1999). Incubation of protein lysates with ATAD-3 antibody and subsequent immunoblotting of the ATAD-3 bound proteins with MICS-1 antibody revealed a band at the predicted size of ~30 kD (Fig.1B). To further support the specificity of this result, we

performed the experiment also in protein lysate of *atad-3(RNAi*) animals. Here, the 30 kD band was absent (Suppl. Fig.1).

To gain more information about the role of MICS-1 in *C. elegans* development, we performed *mics-1(RNAi)* by "feeding" (see also Hoffmann *et al*., 2009; Brenner, 1974). Moreover, we analysed *mics-1* mutant worms (strain RB1335 carrying the allele ok1451; provided by the Caenorhabditis Genetics Center).

In contrast to *atad-3(RNAi)* animals, *mics-1* mutants and RNAi animals developed normally and showed no growths or fertility defects. Moreover, these animals appeared healthy and active over a considerably longer time period than wild type worms. Accordingly, we performed a systematic lifespan analysis in *mics-1* mutants and RNAi animals. Assays were performed at 25 °C as described in the literature (modified according to Wilson *et al*. (2006)). As depicted in Fig. 1C, we observed an increase in lifespan when MICS-1 expression was reduced. In *mics-1* mutants, mean adult lifespan was increased by 54%, which is one of the most profound effects of a single gene on *C. elegans* longevity reported so far. Of note, our current systematic analysis also revealed increased longevity in *atad-3(RNAi)* worms, although to a lesser extend (29% compared to control; see also Table 1).

To further address the consequences of MICS-1 depletion *in vivo* and to test, whether the observed effects on lifespan were associated with an altered oxidative balance, we analysed reactive oxygen species (ROS) production under resting and heat stress conditions. Of note, increased ROS levels or oxidative stress are well known modulators of lifespan in *C. elegans(Schulz et al., 2007)*. Assays were performed as described by others (Kampkötter et al., 2007; Koopman et al., 2006), using the fluorescent dye 2,7-dichlorohydrofluorescein diacetate (final concentration of 12,5 µM). The measurements were carried out for 240 min at 22°C or 37°C. At 22°C, no enhanced ROS levels were measured (data not shown). However, under thermal stress at 37°C, *mics-1* mutants and *mics-1(RNAi)* animals displayed considerably lower ROS levels compared to their wild type counterparts (Fig. 1D). In contrast, *atad-3(RNAi)* animals showed a drastic increase in heat induced ROS production, suggesting accelerated heat stress sensitivity.

Interestingly, in addition to this observation, we found that *atad-3(RNAi)* also increases ROS production under thermal stress conditions in *mics-1* mutants, suggesting that the ROS inducing effect of ATAD-3 depletion is independent of the presence of MICS-1 or even aggravated by MICS-1 depletion. Further evidence for the influence of ATAD-3 depletion on ROS production was delivered by experiments using *daf-2* mutant worms (Fig. 1D). DAF-2 is the insulin

receptor homolog in *C. elegans*, which is capable to inactivate DAF-16 (Ogg et al., 1997). For *daf-2* mutants, in which DAF-16 displays higher activity, enhanced resistance to stresses has been documented (Honda et al., 2008). In our assay, ROS production under heat stress conditions was generally low in these animals but under *atad-3*(*RNAi*) conditions, ROS levels were slightly but also significantly increased (Fig. 1D). Taken together, results obtained in our heat stress assay indicated a general role of ATAD-3 in the regulation of heat stress induced ROS production.

Importantly, the transcription factor DAF-16 (homologue of Forkhead box O Transcription factors in mammals) is strongly involved in determining the rate of aging and average lifespan in *C. elegans*. DAF-16 selectively up-regulates genes that contribute to specific protective mechanisms, while simultaneously down-regulating potentially deleterious genes (Murphy, 2006). In view of the effects of MICS-1 deficiency on lifespan and ROS production, we tested in how far this effect was depended on the presence of DAF-16. We found that *daf-16(RNAi)* in *mics-1* mutants reduces lifespan extension from 54% down to 37% compared to *daf-16(RNAi)* animals (Fig. 2A, see also Table 1). This means, that the *mics-1* mutant longevity effect partially depends on the activity of DAF-16.

Comparably, lifespan modulating effects of *atad-3(RNAi)* were also partially dependent on DAF-16 (Fig. 2A).). Nevertheless, in *daf-16(mu86)* submitted to *atad-3(RNAi)*, lifespan was still enhanced by about 13% if compared to *daf-16(mu86)* HT115 animals, suggesting also here a DAF-16 independent effect on *C. elegans* lifespan. At the current stage, it remains unclear, which additional transcription factors are involved in this phenomenon.

To complete our analysis of ATAD-3 and MICS-1 on aging, we performed *atad-3(RNAi)* in *mics-1* mutants. To our surprise, lifespan was enhanced to 83% if compared to wild type controls, and 19% if compared to the *mics-1* mutants (Fig. 2A, Table 1). This finding appeared unexpected in view of the increased heat stress-induced ROS production in this condition and suggests that the altered ROS levels might not be responsible for the modulation of lifespan, at least in MICS-1 depleted worms. This idea is further supported by the fact that ROS levels under resting conditions were not altered in *mics-1* mutants or *atad-3(RNAi)* animals.

Based on the observation that enhanced lifespan in MICS-1 depleted animals considerably depends on the presence of DAF-16, a higher DAF-16 activity might be suspected in theses worms. To address this question, we analyzed the subcellular localization of DAF-16 by performing *mics-1(RNAi)* in TJ356 worms and subsequent microscopic analysis. This strain carries a transgene encoding for a fusionprotein consisting of functional DAF-16 and the green

fluorescent protein (GFP). It was widely used to study the localization of DAF-16 transcription factor under various conditions to give a hint about its activation status (Henderson and Johnson, 2001; Kampkötter et al., 2008). After subjecting MICS-1 depleted TJ356 worms to thermal stress, we found that the intermediate and nuclear translocation of DAF-16 was higher in *mics-1(RNAi)* than in control animals (Fig. 2B). In combination with our DCF-DA data, these results suggest a more efficient heat stress induced translocation of DAF-16 and consequent activation of ROS protective pathways in MICS-1 depleted animals.

To further support this finding, we performed dauer assays. DAF-16 activity is important for the efficient transition to dauer arrest at higher temperatures (Fielenbach and Antebi, 2008). In keeping with the above findings, our experiments revealed that a significantly lower amount of *mics-1* mutant animals did not arrest as L1 diapause or in dauer stage under heat stress conditions, further suggesting a higher activity of DAF-16 in MICS-1 depleted worms.

In parallel to the above experiments, we also tested DAF-16 activity in *atad-3(RNAi)* treated animals. We found that under similar conditions, nuclear localization of DAF-16 was not significantly altered in these worms (Fig. 2B), and also the rate of L1 diapause/dauer formation was comparable to controls (Fig. 2C). These results suggest that *atad-3(RNAi)* effects are not directly modulated by DAF-16.

In conclusion, our *in vitro* data suggest that MICS-1 is a novel interacting partner of mitochondrial ATAD-3. Depletion of MICS-1 clearly promotes *C. elegans* longevity and lowers heat stress induced ROS production. Lifespan prolonging effects of MICS-1 were partially dependent on DAF-16. In line with this observation, DAF-16 displayed a higher degree of nuclear translocation in *mics-1(RNAi)* worms or *mics-1* mutants and animals showed an increased capability of entering the L1 diapause/dauer stage. On the other hand, ATAD-3 depletion also extended *C. elegans* lifespan and clearly increased heat induced ROS production. Also here, lifespan prolonging effects were partially dependent on DAF-16. Nevertheless, for MICS-1 and ATAD-3 deficiency, a considerable DAF-16-independent effect on lifespan was observed. This is certainly interesting and it will be an important next step to further investigate, which transcription factors and signalling pathways are responsible for this phenomenon. Surprisingly, we observed an additional lifespan extension in *mics-1* mutants when subjected to *atad-3(RNAi)* whereas heat induced ROS production was even aggravated under this condition. This suggests at least partially independent effects of MICS-1 and ATAD-3 on lifespan and ROS production.

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FIGURE AND FIGURE LEGENS

Figure 1:

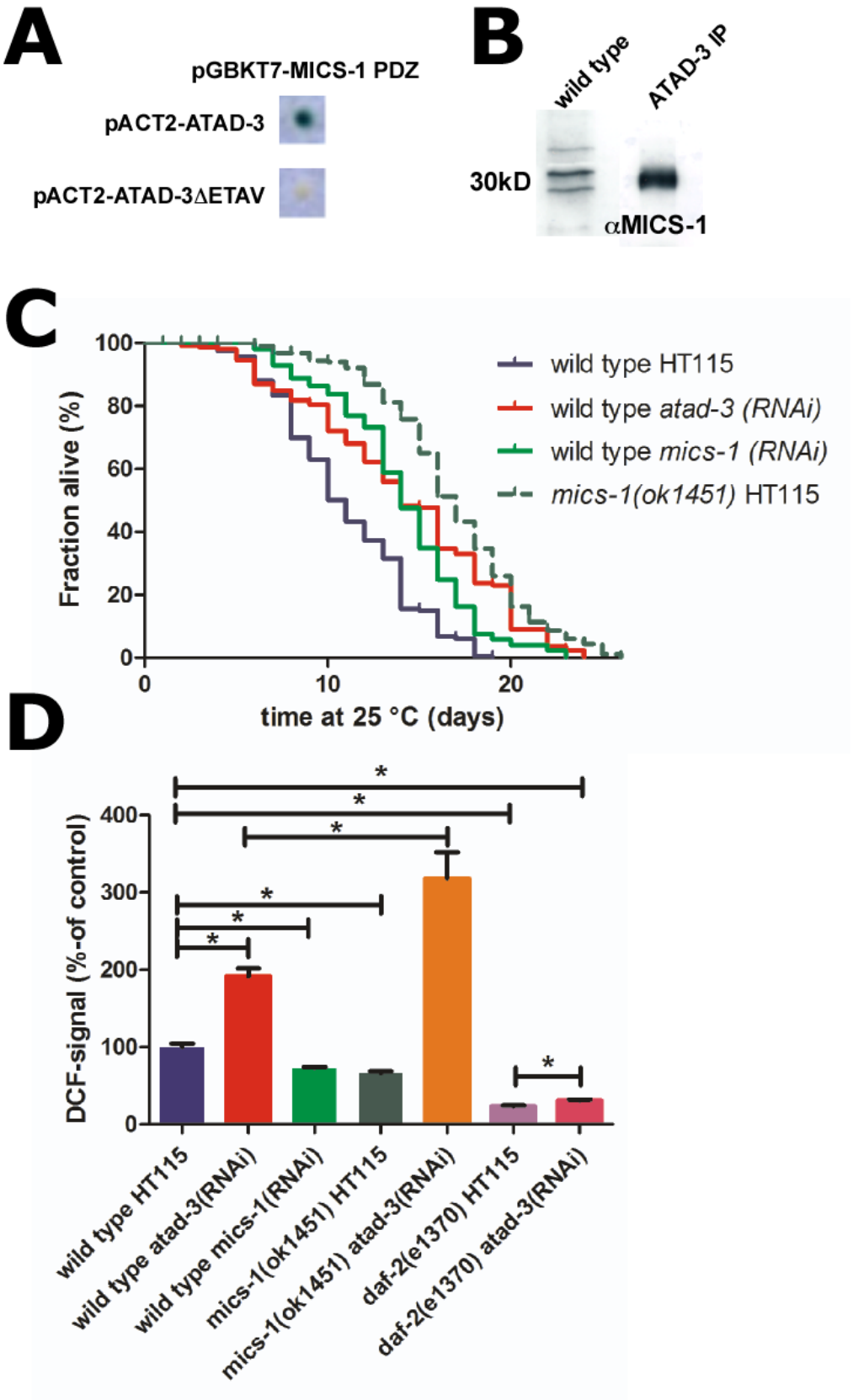

Fig. 1: MICS-1 interacts with ATAD-3 and its depletion promotes longevity and lowers heat stress-induced ROS production in *C. elegans*

A) Yeast 2-hybrid analysis identifies an interaction of MICS-1 via the PDZ domain binding motif -ETAV in the C-terminal part of ATAD-3. **B)** Co-immunoprecipitations of ATAD-3 IPs, probed with MICS-1 antibodies, revealed a signal of the predicted size of MICS-1 protein at ~30kD in wild type lysate and ATAD-3 IPs. **C)** Survival plot of wild type HT115, *mics-1* HT115, wild type *mics-1(RNAi)* and wild type *atad-3(RNAi)*, respectively. Mean lifespan for *mics-1* HT115 was 16.9 +/- 0.2 days (n=270) vs. 11 +/- 0.2 days (n=362) for wild type animals (p<0.01; Mantel-Cox log rank test). Mean lifespan for wild type *mics-1(RNAi)* was 14.1 +/- 0.3 days (n=233; p<0.01 compared to wild type). Mean lifespan for wild type *atad-3(RNAi)* was 14.2 +/- 0.4 days (n=274; p<0.01 compared to wild type). HT115 indicate the feeding RNAi *E. coli* strain carrying the empty feeding vector, used as control. **D)** Thermal induced reactive oxygen production (ROS) at 37°C (expressed as percent of control). Asterisks indicate significant differences (p<0.05) compared to control. In total, the following number of individual adult worms were analyzed in at least 2 independent trials: wild type HT115 (n=74), wild type *atad-3(RNAi)* (n=50), *mics-1* HT115 (n=35), wild type *mics-1(RNAi)* (n=21), *mics-1 atad-3(RNAi)* (n=16), *daf-2(e1370)* HT115 (n=16), *daf-2(e1370) atad-3(RNAi)* (n=19). Statistical significance was calculated using unpaired two-tailed student´s t-test.

Figure 2:

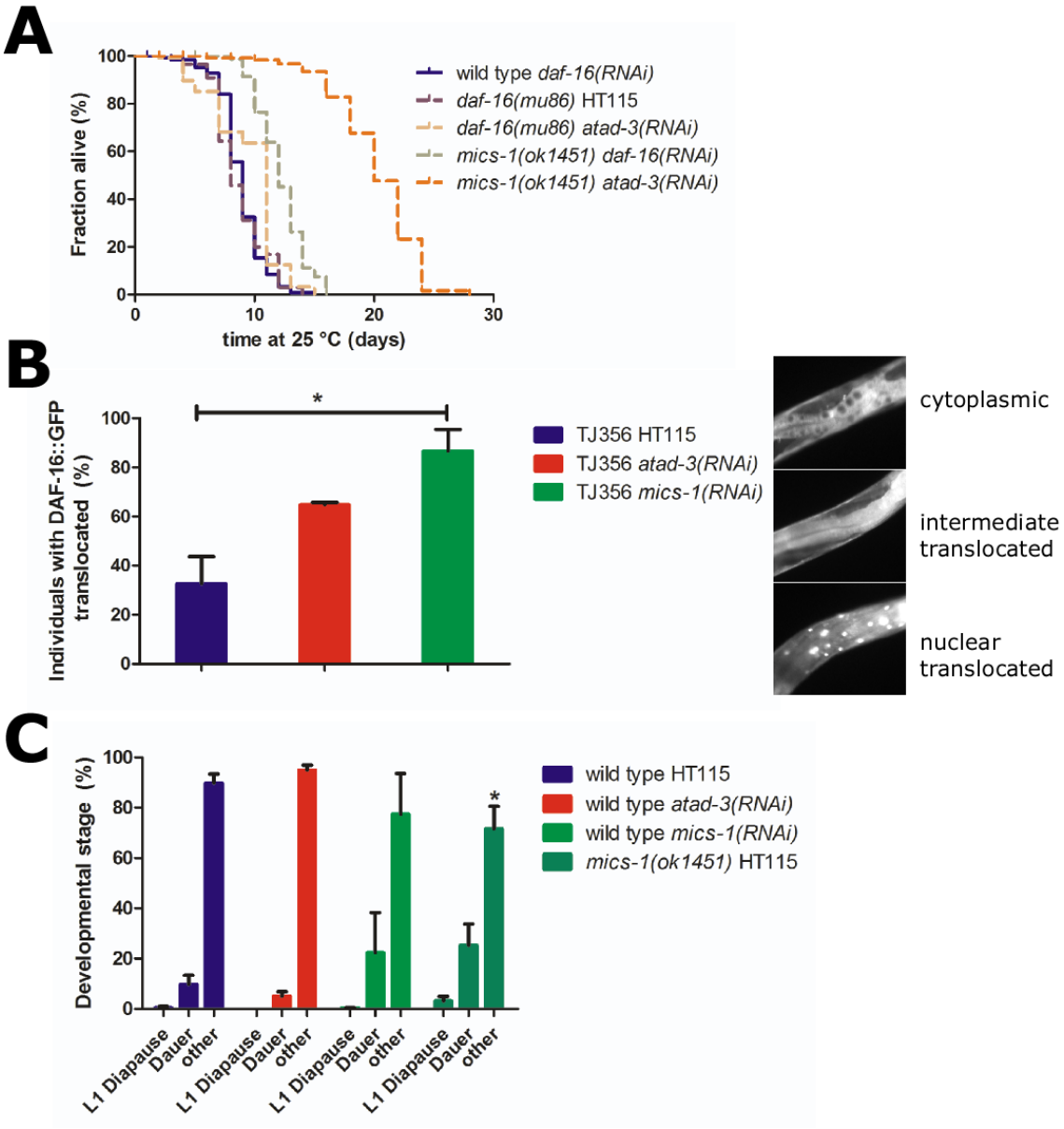
A
Fraction alive (%)
time at 25 °C (days)
wild type daf-16(RNAi)
daf-16(mu86) HT115
daf-16(mu86) atad-3(RNAi)
mics-1(ok1451) daf-16(RNAi)
mics-1(ok1451) atad-3(RNAi)
B
Individuals with DAF-16::GFP translocated (%)
TJ356 HT115
TJ356 atad-3(RNAi)
TJ356 mics-1(RNAi)
cytoplasmic
intermediate translocated
nuclear translocated
C
Developmental stage (%)
wild type HT115
wild type atad-3(RNAi)
wild type mics-1(RNAi)
mics-1(ok1451) HT115
L1 Diapause
Dauer
other

Fig. 2: Lifespan extension in *mics-1* mutants is partially independent of the presence of DAF-16 and is additionally enhanced by *atad-3(RNAi)*

A) Survival plot of wild type *daf-16(RNAi)*, *daf-16(mu86)* HT115, *daf-16(mu86) atad-3(RNAi)*, *mics-1 daf-16(RNAi)* and *mics-1 atad-3*(RNAi). Mean lifespan for wild type *daf-16(RNAi)* was 8.9 +/- 0.2 days (n=142), for *daf-16(mu86)* HT115 was 8.4 +/- 0.2 days (n=221), for *daf-16(mu86) atad-3(RNAi)* was 9.5 +/- 0.3 days (n=136), for *mics-1 daf-16(RNAi)* was 12.2 +/- 0.2 (n=91) and for *mics-1 atad-3(RNAi)* was 20.2 +/-0.2 (n=130). **B)** Strain TJ356 was used to demonstrate subcellular DAF-16 localization after heat stress in *atad-3(RNAi)* and *mics-1(RNAi)*. Typical images for cytoplasmic, intermediate and nuclear localization of DAF-16::GFP are depicted on the right. **C)** Dauer assays of wild type HT115, *mics-1* HT115, wild type *atad-3(RNAi)* and wild type *mics-1(RNAi)*. Asterisks indicate significant differences ($p< 0.05$) compared to control. Statistical significance was calculated using unpaired two-tailed student´s t-test.

Figure S1:

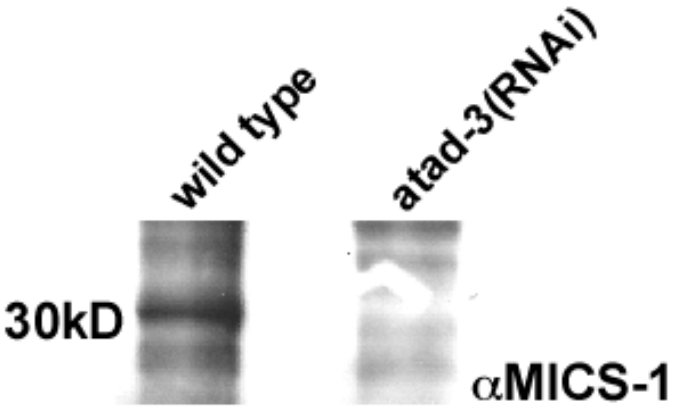

Fig. S1: Co-immunoprecipitations of MICS-1 with ATAD-3 in wild type and *atad-3(RNAi)* lysates

Immunoblotting of MICS-1 antibody after Co-IPs is shown (left lane = wild type lysate; right lane = *atad-3(RNAi)* lysate). The characteristic signal of MICS-1 antibody at 30kD is present in wild type but absent in *atad-3(RNAi)* lysate.

TABLES

Table 1

	Background	Conditions	LS +/- SEM	N	Significance
1	wild type	*HT115*	11 +/- 0.2	362	
2	wild type	*atad-3(RNAi)*	14.2 +/- 0.4	274	*(1)
3	wild type	*mics-1(RNAi)*	14.1 +/- 0.3	233	*(1)
4	wild type	*daf-16(RNAi)*	8.9 +/- 0.2	142	*(1)
5	*mics-1*	*HT115*	16.9 +/- 0.2	270	*(1)
6	*mics-1*	*daf-16(RNAi)*	12.2 +/- 0.2	91	*(4)
7	*mics-1*	*atad-3(RNAi)*	20.2 +/- 0.2	130	*(5)
8	*daf-16(mu86)*	*HT115*	8.4 +/- 0.2	221	*(1)
9	*daf-16(mu86)*	*atad-3(RNAi)*	9.5 +/- 0.3	136	*(8)

Table 1: Summary of lifespan data

Summary of life span (LS) measurements with standard error of the mean (SEM) for the different experimental conditions. HT115 indicate the feeding RNAi *E. coli* strain, carrying the empty feeding vector (Kamath et al., 2001; Timmons et al., 2001). Significant results are indicated by *(p<0.01; Mantel-Cox log rank test) with corresponding experiments in parenthesis.

4. Ausblick

Im Rahmen der vorliegenden Dissertation konnten die Gene *vang-1*, *atad-3* und *mics-1* als neue Modulatoren bezüglich der Lebensspanne und Stressresistenz in *C. elegans* identifiziert werden. Weiterführende Untersuchungen müssen nun klären auf welcher Ebene sie in die entsprechenden Signalwege eingreifen.

Im Falle von *vang-1*, ein Schlüsselgen aus dem planaren Zellpolaritäts (PCP)-Signalweg in anderen Systemen, wäre die Rolle im Insulin/IGF-1 Signalweg (IIS) zu prüfen. So könnte VANG-1 beispielweise über sein PDZ-Bindemotiv mit dem multi-PDZ Domänen Protein MPZ-1/MUPP-1 interagieren. Von diesem ist bereits bekannt, dass es in einen Komplex mit DAF-18/Pten und ARR-1/Arrestin vorliegt, welcher die PI3-Kinase AGE-1 im IIS negativ reguliert. Unterstützt wird diese Hypothese durch vorläufige Ergebnisse, die zeigen, dass der Funktionsverlust von ARR-1 zwar zu einer Verlängerung der Lebensspanne im WT jedoch nicht in *vang-1* Mutanten führt. Da *vang-1* bezüglich der Lebensspanne in der Keimbahn von *C. elegans* wirkt, wären weiterhin die dort bereits bekannten DAF-16-abhängigen Mechanismen sowohl über KRI-1 als auch die sogenannten „***d**afachronic **a**cid**s**“ (DAs), näher zu untersuchen. In vorläufigen RNAi Experimenten (*„feeding“*) gegen KRI-1 konnte kein Effekt auf die Langlebigkeit von *vang-1* Mutanten gezeigt werden. DAs bilden eine Klasse endokrin wirkender Stoffe, die effizient über HPLC nachgewiesen werden können. Für Mutanten im DAs-Syntheseweg (z.B. *daf-36*, Rieske Oxygenase) wurden bereits Wachstumdefekte der Gonade beschrieben, wie sie auch bei *vang-1* Mutanten auftreten. Durch einen Vergleich der über HPLC ermittelten DAs Stoffmengen-Profile in *vang-1* Mutanten und im WT könnte ihre Rolle bezüglich der Langlebigkeit in diesem Fall näher spezifiziert werden. Ein weiterer wichtiger Punkt wäre die Aufklärung der subzellulären Lokalisation von VANG-1 in der Gonade. Dazu existieren bereits generierte anti-Peptid Antikörper. Da es sich bei *vang-1* um ein homologes Schlüsselgen aus dem PCP-Signalweg anderer Systeme handelt, wäre zu überprüfen inwieweit weitere in *C. elegans* konservierte PCP-Komponenten ebenfalls Lebensspanne und Stressresistenz modulieren.

Aufgrund der Interaktion von ATAD-3 mit MICS-1 wird eine Gruppe langlebiger Mutanten zunehmend interessanter (z.B. *nuo-6*, *cco-1* oder *isp-1*), da sie sich bezüglich des ROS-Levels und der Langlebigkeit ähnlich wie *atad-3(RNAi)* Tiere verhalten. Diese sogenannten „Mit-Mutanten“ kodieren für Komponenten der Elektronentransportkette, für die bisher weder in *C. elegans* noch im humanen System eine Verbindung zu ATAD-3/ATAD3 gezeigt werden konnte. Im Falle des hier beschriebenen MICS-1 Proteins, dem Homolog des humanen

Synaptojanin bindenden Proteins OMP25, ist bereits bekannt, dass der Funktionsverlust von *C. elegans* Synaptojanin (UNC-26) ebenfalls zu Langlebigkeit führt (Lakowski und Hekimi, 1996). Es wäre also zu prüfen, ob die für das humane System beschriebene Interaktion beider Proteine in *C. elegans* ebenfalls konserviert ist. Da die Lebensspanne in *mics-1* Mutanten anscheinend unabhängig von DAF-16/FoxO moduliert wird, könnte nach Erzeugung einer *mics-1;daf-16* Doppelmutante weitere Modulatoren der Langlebigkeit, wie SKN-1/Nrf und kalorische Restriktion, auf genetische Interaktionen mit *mics-1* getestet werden.

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Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, dass ich die hier vorliegende Dissertation selbst angefertigt und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe. Alle Stellen, die dem Wortlaut oder dem Sinn nach anderen Werken entnommen wurden, habe ich unter Angabe der Quelle als Entlehnung deutlich gemacht.
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Sebastian Honnen