C. elegans VANG-1 Modulates Life Span via Insulin/IGF-1-Like Signaling

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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. 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. 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 gerontogenes. 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 II detoxification transcription factor SKN-1/Nrf2 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.

Introduction

Wnt/plantar cell polarity (PCP) is one of three identified Wnt signaling pathways, along with Wnt/B-Catenin and Wnt/Calcium [1]. These signaling pathways are abundant in various developmental processes across the animal kingdom [2–6]. 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 [7,8]. 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 [9,10]. 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 [11–13].

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 [13]. VANG-1 was implicated in playing a major role in organ formation either by mediating correct intercalation of intestinal primordial cells during embryogenesis [13,15] or by establishing ground polarity in vulval development [12]. 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 aging. The corresponding genes of “Age” mutants are referred to as gerontogenes [16]. These mutants share a specific catalog of defects, e.g., a minimum of 20% life span increase and resistance against certain stress factors like reactive oxygen 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 [17–19]. DAF-2 is capable to phosphorylate target substrates, e.g., AGE-1/AAP-1, a PI3 kinase that generates P(3,4,5)P3 [20–22]. Via a phosphorylation cascade, downstream kinases PDK-1, AKT-1, AKT-2 and SGK-1 [23–25] are activated and in turn negatively regulate the forkhead transcription factor (FoxO), DAF-16 [26,27]. 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 in the nucleus [28]. 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 life span [29]. Besides DAF-16, inhibiting insulin/IGF-1-like signaling also activates heat-shock transcription factor HSF-1 and phase II detoxification transcription factor SKN-1, a Nrf1/2/3 protein ortholog [30,31].

In the present study, we identify VANG-1, the only C. elegans ortholog of the conserved CPBP 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 sfn-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/Itpa proteins identified in Drosophila, Xenopus, and mammals [32–34]. 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 supporting information S1) [13], 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 reflected by germline degeneration and a decline in oocyte quality [44,45]. Individual tm1422 mothers continue to produce viable progeny as they age (Fig. 2D). This phenotype also points to vang-1 being a typical gerontogene. 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 [46].

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 [47,48]. 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 [49], 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 [50]. 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 life span in a DAF-16 dependent manner [48,50].

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 [25,51]. 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 [52].

We performed our dauer assay in comparison to WT, daf-16(mu86) and daf-2(e1370) at 27°C [53]. Consistent with the literature, we found that daf-2(e1370), encoding the sole insulin receptor homologue in C. elegans [20], is dauer constitutive (~99% arrest), while daf-16(mu86) is dauer defective (0% arrest; Fig. 3B) [51,54]. 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 [51] and suggests higher activity of DAF-16 in tm1422 during early development.

We further investigated the role of the receptor tyrosine kinase DAF-2 [20], 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 [55]. 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 [29]. 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 [56,57].

We also tested the longevity promoting factor SKN-1/Nrf2, which orchestrates the phase II detoxification response including defense against oxidative stress [58]. 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 [58,59].

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 [60,61], second in the germline [62] and third, because of
its expression in ventral cord neurons [12,63], in strains showing enhanced neuronal RNAi [64].

The intestine is highly exposed to environmental toxins and pathogens and it has been speculated to be the major site of stress response [65]. 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, \textit{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 \textit{C. elegans} and mice, VANG-1 and Vangl2 Lp have been connected with correct uterine epithelium development in the reproductive tract [12,66], but its function in meiotic maturation and ovulation is still elusive. 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 [67–70]. During ovulation, sheath cells contract rapidly, the distal constriction of the spermatheca dilates, and sheath cells pull the distal spermatheca over the mature oocyte [71]. The decreased fertility/brood size, ovulation rate, and the increased reproduction span of \textit{tm1422} animals (Fig. 2 A,D–E) suggests VANG-1 being involved in the communication between germline and somatic gonad. To test if \textit{vang-1} also acts in the germline to control life span by insulin/IGF-1-like signaling, we performed germline-specific RNAi [62]. \textit{vang-1(RNAi)} in \textit{rrf-1} led to a significant increase in life span (13.5%, Fig. 4B; Table 1), which is about two third of whole life span extension observed in \textit{vang-1(RNAi)} animals (Fig. 1A; Table 1). In contrast, depletion of VANG-1 in the enhanced-neuronal RNAi strains TU3311 and TU3401 [64], has no effect on \textit{C. elegans} life span extension (Fig. 4C; Table 1).

**Figure 2. vang-1 shows reproduction- and aging-related defects.** (A) \textit{vang-1(tm1422)} populations have a reduced brood size. The average brood size at 25°C in \textit{vang-1(tm1422)} (red, 111±41 progeny; n = 28*) is significantly reduced (p<0.0001**) in comparison to WT (blue, 194±50 progeny; n = 46†). Results are shown as mean±standard deviation. (B–C) \textit{ok1142} and \textit{tm1422} show decreased lipofuscin accumulation five and ten days after hatching. (B) Five days after hatching, \textit{ok1142} (green, RFU = 792.35±25, n = 31, p<0.001**) and \textit{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, \textit{ok1142} (green, RFU = 1083±32, n = 33, p<0.05**) and \textit{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\textsubscript{individual}−OD\textsubscript{background}/mm²). (D) In \textit{tm1422} the ovulation rate is reduced in comparison to WT. \textit{tm1422} has an ovulation rate 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) \textit{vang-1} populations have a prolonged reproductive span. The reproductive span in \textit{ok1142} (green, 6.6 d; n = 20**) and \textit{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). doi:10.1371/journal.pone.0032183.g002
As suggested by Calixto et al. [64] the neuronal expression of sid-1 in TU3311 might serve as a sink for double-stranded RNA used by non-neuronal RNAs 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 communication with N.J. Storm - it has been tested twice with up to 30 individuals per experiment for uptake of Dil [72]). Dye-fill defective (dyf-phenotype) mutants have previous been found long-lived [73]. 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) implicates the germline to be the primary site of vang-1 action concerning longevity in C. elegans. Components of the insulin/IGF-1-like signaling pathway have already been implicated to act in the germline, e.g., Michaelson et al. found that the effect of reducing daf-2 signaling on larval germline proliferation is dependent on daf-16 [74].

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 life span. 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 [75]. Bristol N2 was used as the WT strain. WT or mutant worms were synchronized as described previously [76].

Single mutants were as follows. TM1422: vang-1(tm1422) X (outcrossed x3); RB1125: vang-1(ok1142); C1370: daf-2(e1370) III; CF1038: daf-16(mu86) I. N2: ref-1(pk1417) I.

Transgenic strains were as follows. OLB11: sid-1(ne219)/[pOLB11(elt-2p::rde-1)+pRF4(rol-6[nu1006])]; TU3311: [unc-119:: YFP+unc-119::sid-1]; TU3401: sid-1(pk3321) I; [pCF790(nyro-2p::mCherry)+unc-119::sid-1]; TJ356: integrated DAF-16::GFP roller strain [50] (for further details see: http://cgcedb.msi.umn.edu/strain.php?id=13306).

RNA-mediated interference (RNAi)

RNAi by “feeding” was performed essentially as described by others [77]. In brief, after amplification of a single colony overnight (37°C, LBmedium, medium, HT115(DE3) bacteria (RNase III-deficient E. coli strain, carrying IPTG-inducible T7-polymerase) [77,78], were diluted to an OD600 of 0.9, and after addition of IPTG (1 mM) seeded on NGMplates (containing 1 mM IPTG). 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 polynkier) 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 [79] (see supporting information S2 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 [75] or HT115(DE3) [77,78] bacteria harboring the empty L4440 “feeding”-vector or L4440 with a fragment of the gene of interest [77]. 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 and weighted log-rank tests [80].

Determination of progeny

C. elegans populations were synchronized and hatched on NGM Agar plates at 25°C. On day three, single worms were transferred to fresh plates every day until adulthood (day 5-7), and progeny were counted daily. The bag of worms phenomenon is defined as vigorous movement (as seen in WT), but with an obvious swelling at the posterior of the animal, which is not responsive to gentle touch. Animals that demonstrate this phenotype are assumed to be sterile. For statistical analysis, the difference in number of progeny between groups was tested using the two-tailed Fisher’s exact test [81].

As Figure 1 shows, the majority of the progeny from the vang-1 RNAi treated worms did not show the bag of worms phenotype.

Table 1. Summary of life spans.

| Background | Conditions | LS +/- SEM | N | Significance |
|------------|------------|------------|---|--------------|
| 1          | WT         | 10.2 +/- 0.2 | 214 |
| 2          | tm1422     | 14.3 +/- 0.4 | 174 | *(1)         |
| 3          | ok1142     | 12.9 +/- 0.5 | 114 | *(1)         |
| 4          | WT         | 18 C/OP50    | 96.6 +/- 0.8 | 61 |
| 5          | tm1422     | 27.1 +/- 0.9 | 43  | *(4)         |
| 6          | WT         | 20 C/HIT15   | 21.4 +/- 0.4 | 70 |
| 7          | tm1422     | 25.6 +/- 0.4 | 71  | *(25)        |
| 8          | WT         | 20 C/daf-16(RNAi) | 19.5 +/- 0.5 | 75 | *(25)        |
| 9          | tm1422     | 22.5 +/- 0.5 | 71  | *(26) 0.72(25) |
| 10         | WT         | 19.8 +/- 0.2 | 936 | *(1)         |
| 11         | tm1422     | 15.8 +/- 0.2 | 480 | *(10)        |
| 12         | WT         | 19.3 +/- 0.2 | 242 | 0.37(11)     |
| 13         | tm1422     | 14.9 +/- 0.2 | 133  | 0.76(100)    |
| 14         | WT         | 13.0 +/- 0.2 | 138 | *(11)        |
| 15         | tm1422     | 13.2 +/- 0.2 | 138 | *(11)        |
| 16         | WT         | 11.9 +/- 0.2 | 142 | *(100.2717)  |
| 17         | tm1422     | 12.4 +/- 0.2 | 247 | *(11)        |
| 18         | WT         | 25.8 +/- 1.1 | 126 | *(100.6219)  |
| 19         | tm1422     | 25.6 +/- 1.3 | 135 | *(11)        |
| 20         | OLB11      | 14 +/- 0.3   | 250 | *(10)        |
| 21         | OLB11      | 22.6 +/- 0.7 | 85  | *(20)        |
| 22         | OLB11      | 14.4 +/- 0.3 | 195 | 0.24(20)     |
| 23         | NL2098(ref-1) | 12.9 +/- 0.3 | 309 |
| 24         | NL2098(ref-1) | 14.6 +/- 0.3 | 274 | *(23)        |
| 25         | TU3401     | 20 C/OP50    | 17.0 +/- 0.3 | 380 |
| 26         | TU3401     | 20 C/vang-1(RNAi) | 17.7 +/- 0.3 | 284 0.8625 |
| 27         | TU3311     | 19.5 +/- 0.5 | 212 |
| 28         | TU3311     | 20 C/vang-1(RNAi) | 21.1 +/- 0.4 | 280 | *(27)        |

Life spans (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 [60,61], germline-specific RNAi strain NL2098 [62] and the neuron-enhanced and neuron-specific strains TU3311 and TU3401 [64]. OP50 [75] and RNAi HT115 [77,78] indicate standard RNAi 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 parentheses (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. doi:10.1371/journal.pone.0032183.t001
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 [53]. 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 [46])**

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 [71])

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) [50] 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, anesthetized with 20 mM sodium azide in M9 buffer [76] and coated with a cover slip. Epi-fluorescence (excitation, 365 nm; emission, 420 nm) images were taken with Image ProPlus software (Version 4.5, Media Cybernetics, Silver Spring, MD, USA) using a CoolSnap CF Digital Monochrome Camera (Intas, Göttingen, Germany) mounted on an AXIO Lab fluorescence microscope (Zeiss, Göttingen, Germany) and dedicated to three categories concerning VANG-1::GFP translocation into the nuclei.

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 [81] with some modifications described in [82]. After synchronization [76] both strains were cultured on NGM plates with *OP50* bacteria [75] 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 1 × 10^7 *OP50* bacteria/ml [82]. 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 (see above). 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 [81]. For the application of thermal stress the fluorescence reader (Wallace Victor® 1420 multilabel counter, Perkin Elmer, Wellesley, USA) was preheated to 37°C. The measurement of each well 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).

For the determination of the intracellular amount of ROS synthesized 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 [86]. After the complete transfer of the individual worms 2 µl 250 µM 2,7-dichlorodihydrofluorescein diacetate (H2DCF-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 H2DCF-DA is intracellular converted to membrane-impermeable, non-fluorescent H2DCF, 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 [82,83,86]. 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).

Supporting Information

**Figure S1** DAF-16::GFP translocation into the nucleus.

In TJ356 (DAF-16::GFP) worms [50], 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.

(TIF)

**Figure 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.

(TIF)

**Supporting Information S1** Sequences of VANG-1, VANG-1*tm1422* and VANG-1*ko1142* proteins. Missing amino acids in *tm1422* and *ko1142* are shown in red and blue, respectively. Additional amino acids in *ko1142* are shown in yellow. For further details concerning VANG-1 see [13].

(DOCX)

**Supporting Information S2** Sequences of RNAi “feeding”-clones.

(DOCX)

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Author Contributions

Conceived and designed the experiments: SH AK OB. Performed the experiments: SH CB VS. Analyzed the data: SH OB. Contributed reagents/materials/analysis tools: MH YK. Wrote the paper: SH OB.
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