

The thioredoxin TRX-1 regulates adult lifespan extension induced by dietary restriction in *Caenorhabditis elegans*

Juan Carlos Fierro-González ^a, María González-Barrios ^b, Antonio Miranda-Vizuetes ^{b,c,†,*},
Peter Swoboda ^{a,†,*}

^a Karolinska Institute, Center for Biosciences at NOVUM, Department of Biosciences and Nutrition, S-141 83 Huddinge, Sweden.

^b Centro Andaluz de Biología del Desarrollo (CABD-CSIC), Dpto. de Fisiología, Anatomía y Biología Celular, Universidad Pablo de Olavide, E-41013 Sevilla, Spain.

^c Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, E-41013 Sevilla, Spain.

[†] These authors contributed equally as senior authors to this work.

* Corresponding authors:

Peter Swoboda, Karolinska Institute, Center for Biosciences at NOVUM, Department of Biosciences and Nutrition, Hälsovägen 7, S-141 83 Huddinge, Sweden.

Tel.: +46-8-5858 3734; Fax: +46-8-774 5538; **E-mail: peter.swoboda@ki.se.**

Antonio Miranda-Vizuetes, Centro Andaluz de Biología del Desarrollo (CABD-CSIC), Departamento de Fisiología, Anatomía y Biología Celular, Universidad Pablo de Olavide, Carretera de Utrera Km. 1, E-41013 Sevilla, Spain.

Tel.: +34 954 348558; Fax: +34 954 349376; **E-mail: amirviz@upo.es.**

Abstract

Dietary restriction (DR) is the only environmental intervention known to extend adult lifespan in a wide variety of animal models. However, the genetic and cellular events that mediate the anti-aging programs induced by DR remain elusive. Here, we used the nematode *Caenorhabditis elegans* to provide the first *in vivo* evidence that a thioredoxin (TRX-1) regulates adult lifespan extension induced by DR. We found that deletion of the gene *trx-1* completely suppressed the lifespan extension caused by mutation of *eat-2*, a genetic surrogate of DR in the worm. However, *trx-1* deletion only partially suppressed the long lifespan caused by mutation of the insulin-like receptor gene *daf-2* or by mutation of the sensory cilia gene *osm-5*. A *trx-1::GFP* translational fusion expressed from its own promoter in ASJ neurons (*P_{trx-1}::trx-1::GFP*) rescued the *trx-1* deletion-mediated suppression of the lifespan extension caused by mutation of *eat-2*. This rescue was not observed when *trx-1::GFP* was expressed from the *ges-1* promoter in the intestine. In addition, overexpression of *P_{trx-1}::trx-1::GFP* extended lifespan in wild type, but not in *eat-2* mutants. *trx-1* deletion almost completely suppressed the lifespan extension induced by dietary deprivation (DD), a non-genetic, nutrient-based model of DR in the worm. Moreover, DD upregulated the expression of a *trx-1* promoter-driven *GFP* reporter gene (*P_{trx-1}::GFP*) in ASJ neurons of aging adults, but not that of control *P_{gpa-9}::GFP* (which is also expressed in ASJ neurons). We propose that DR activates TRX-1 in ASJ neurons during aging, which in turn triggers TRX-1-dependent mechanisms to extend adult lifespan in the worm.

Keywords: *Caenorhabditis elegans*; thioredoxin; aging; lifespan; dietary restriction.

1. Introduction

Dietary restriction (DR) has been shown to extend adult lifespan in a wide range of organisms (reviewed in [1]). The anti-aging action of DR has been proposed to operate through activation of signaling cascades that subsequently increase stress resistance mechanisms to counteract organismal deterioration inflicted by long-term stress (reviewed in [2]). However, the signaling molecules that trigger the DR-induced pro-longevity machinery at the organismal level are still far from known.

Thioredoxins comprise a conserved family of proteins that mostly depend on their oxidoreductase activity to reduce disulfide bonds in many target proteins (reviewed in [3,4]). In addition to their role as antioxidants against oxidative stress, as electron donors for metabolic enzymes or as redox regulators of signaling molecules and transcription factors, thioredoxins have also been shown to prevent cytosolic proteins from aggregating in the cell (reviewed in [3,5]). Furthermore, thioredoxins have also been implicated in the regulation of aging. The first *in vivo* studies reporting the effects of mammalian Trx1 during aging showed that overexpression of human Trx1 in mice extends lifespan [6].

However, to date, it has not been studied *in vivo* whether thioredoxins regulate adult lifespan extension induced by DR. To our knowledge, the only studies designed to understand the relationship between thioredoxins and DR during aging have been performed *in vitro* using rat kidney [7,8] or combining *in vitro* and *ex vivo* methods using rat muscle and mouse myoblast cell lines, respectively [9]. Therefore, the *in vivo* mechanisms by which thioredoxins regulate lifespan extension induced by DR still remain unknown.

For that purpose, we used *Caenorhabditis elegans*, an excellent animal model that provides valuable *in vivo* genetic and cell biological tools. The *C. elegans* gene *trx-1* encodes a thioredoxin that is expressed in one pair of neurons in the nervous system: the ASJ sensory neurons. Previously we and others have shown that *trx-1* deletion shortens adult lifespan and increases the sensitivity to paraquat-induced oxidative stress [10,11]. In addition, transgenic *C. elegans* overexpressing *trx-1* in ASJ neurons of wild-type animals was shown to have extended adult lifespan [11].

Since *trx-1* regulates aging and stress resistance in the worm, we investigated whether it also regulates adult lifespan extension induced by DR. In our present study, we used genetic and cell biological tools to understand *in vivo* the relationship between the thioredoxin TRX-1 and adult lifespan extension through DR in *C. elegans*.

2. Materials and methods

2.1. Nematode strains and culture conditions

The standard methods used for culturing *C. elegans* were described previously ([12]; reviewed in [13]). Strains and transgenes used in this work are summarized in Supplementary Table S1. All strains were maintained at 20°C.

2.2. Transgene injection constructs and germline transformation

The translational fusion constructs *Ptrx-1::trx-1::GFP* and *Pges-1::trx-1::GFP*, and the transcriptional fusion constructs *Ptrx-1::GFP* and *Pgpa-9::GFP* were previously reported [11,14,15]. For rescue experiments, 40 ng/μl of *Ptrx-1::trx-1::GFP* or *Pges-1::trx-1::GFP* were injected into *trx-1(ok1449) eat-2(ad1116)* animals. For overexpression experiments, 100 ng/μl of *Ptrx-1::trx-1::GFP* were injected into wild-type animals; the extrachromosomal arrays generated were then crossed into *eat-2(ad1116)* animals. For fluorescence intensity measurement experiments, 40 ng/μl of *Ptrx-1::GFP* or *Pgpa-9::GFP* were injected into wild-type animals. Each of the aforementioned constructs was coinjected with 30 ng/μl of the injection marker *Punc-122::DsRed* [16]. Germline transformation was performed as described [17].

2.3. Construction of double mutants

The construction of *trx-1(ok1449); daf-2(e1370)* was previously described [15]. To construct the *trx-1 eat-2* double mutant, *trx-1* homozygous males were crossed to *eat-2(ad1116)*

hermaphrodites, and F1 cross progeny hermaphrodites were grown singly. We then singled F2 progeny animals manifesting reduced pharyngeal pumping rates; these animals were considered to be homozygous for the *eat-2* mutation [18]. Of note, pharyngeal pumping rates of wild-type and *trx-1(ok1449)* animals were very similar. To construct the *trx-1; osm-5* double mutant, *trx-1* homozygous males were crossed to *osm-5(p813)* hermaphrodites, and F1 cross progeny males were again crossed to *osm-5(p813)* hermaphrodites. Since *osm-5* maps on to chromosome X, F1 cross progeny hermaphrodites generated from the second cross were expected to be homozygous for the *osm-5* mutation. To confirm the presence of the *osm-5* mutation, we performed a fluorescent dye-filling assay using DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate), as described [19]. In all cases, the presence of the *trx-1* deletion was demonstrated by performing PCR on single-worm lysates, based on methods previously described ([20]; reviewed in [21]).

2.4. Adult lifespan assays

Animals for lifespan analysis were raised at 20°C and then transferred to 25°C on day 0 of adulthood. We defined day 0 of adulthood as the day in which the L4-to-adult molt occurs. To avoid overcrowding, 12–20 adults were used per plate. During the reproductive period, animals were transferred every 1–2 days to fresh plates and thereafter approximately every 4–7 days. We scored animals as being dead when they had ceased to respond to gentle prodding. Animals that crawled off the plate, exploded (i.e. died by bursting through the vulva) or bagged (i.e. died by internal hatching of retained embryos) were censored at the time of the event. For each experiment, animals were analyzed in parallel. Two independent trials were performed for each strain. For rescue and overexpression experiments, one trial per each of two independent transgenic lines was performed. For the dietary deprivation

(DD) assay, we based our protocol on [22]: lifespan assay procedures were performed as described above, except that animals were transferred on day 3 of adulthood to plates containing 100 µg/ml ampicillin, either with a lawn of *E. coli* OP50 bacteria (*ad libitum*, AL) or without this source of food (DD). Statistical analyses and survival curves were performed using the JMP 7 software (SAS). *p* values were determined by the Wilcoxon and logrank tests. The Wilcoxon test puts more weight on early deaths compared to the logrank, while the logrank test is more appropriate for survival distributions whose hazard functions are proportional over time (i.e. the two survival curves do not cross). If the survival curves do not conform with the logrank test assumption of proportional hazard functions, the Wilcoxon test is recommended (reviewed in [23]). We considered that the Wilcoxon test is more appropriate to estimate lifespan differences between transgenic and non-transgenic animals for the rescue and overexpression experiments, because the transgene *Ptrx-1::trx-1::GFP* seems to affect mean lifespan more than maximum lifespan, which is not in agreement with the aforementioned logrank test assumption. We show both Wilcoxon and logrank tests in Supplementary Data for comparison.

2.5. Microscopy and fluorescence imaging

For fluorescence intensity measurement experiments, mean relative intensity of a transcriptional *Ptrx-1::GFP* or *Pgpa-9::GFP* reporter was assessed by quantifying GFP intensity in ASJ neurons of 5-day-old wild-type adults subjected to 2 days of DD as compared to 5-day-old wild-type adults fed AL. Animals were raised at 20°C, and then transferred to 25°C on day 0 of adulthood. DD and AL plates contained 100 µg/ml ampicillin. Animals were visualized on a Zeiss Axioplan fluorescence microscope at an optical magnification of x500. Worms were put into M9 buffer on a very thin 2% agarose pad

containing an anesthetic (NaN_3). All animals assayed were exposed to 5 mM NaN_3 for a maximum of 15 min prior to image acquisition, thereby avoiding the induction of evident physiological changes (cf. [15]). A Hamamatsu CCD camera and Openlab software (Improvision) were used for image acquisition at the brightest focal plane and a fixed exposure time. Pixel intensity in the entire ASJ cell body was determined from captured images in the form of maximum grey values by using NIH ImageJ software. Fold differences with respect to 5-day-old wild-type adults fed AL were calculated to show the mean relative intensity of 5-day-old wild-type adults subjected to 2 days of DD. For each experiment, animals were analyzed in parallel.

3. Results

3.1. TRX-1 regulates adult lifespan extension induced by a genetic model of dietary restriction

To determine whether TRX-1 regulates the extension of adult lifespan mediated by dietary restriction (DR), we first conducted epistasis analysis using a mutation in *eat-2*. *eat-2(ad1116)* mutant animals represent a classical genetic model of DR in the nematode [24] and exhibit reduced food intake throughout life because of a pharyngeal pumping defect [18,25]. As would be expected for a gene essential for DR-mediated lifespan extension, loss of *trx-1* completely suppressed the long lifespan of *eat-2(ad1116)* mutant animals (Fig. 1A and Supplementary Table S2). The suppression of DR-extended lifespan by loss of *trx-1* is unlikely to be due to increased food intake, because pharyngeal pumping rates of *eat-2(ad1116)* single mutants and *trx-1(ok1449) eat-2(ad1116)* double mutants were very similar (data not shown). To confirm that the suppression of the DR-extended lifespan was caused by disruption of *trx-1*, we used the transgene *P_{trx-1}::trx-1::GFP* (which expresses a *trx-1::GFP* translational fusion from its own promoter in ASJ neurons). The suppression of the extended lifespan of *eat-2(ad1116)* by *trx-1(ok1449)* was rescued by *P_{trx-1}::trx-1::GFP* (Fig. 1B and Supplementary Table S2). In contrast, a transgene driving expression of *trx-1::GFP* from the *ges-1* promoter in the intestine (*P_{ges-1}::trx-1::GFP*) [15,26], did not rescue the *trx-1(ok1449)*-mediated suppression of *eat-2(ad1116)* lifespan extension (Fig. 1B and Supplementary Table S2). These data indicate that loss of *trx-1* is responsible for the suppression of the DR-mediated lifespan extension. Mutations in the insulin-like signaling receptor gene *daf-2*, extend lifespan in an *eat-2*-independent manner [24]. If loss of *trx-1* was fully epistatic to any mutation that extends lifespan, we would expect that *trx-1(ok1449)*

completely suppresses the lifespan extension caused by mutation of *daf-2* as well. However, *trx-1(ok1449)* only partially suppressed the lifespan extension caused by *daf-2(e1370)* (Fig. 1C and Supplementary Table S2). A similar result was obtained with a mutant in the gene *osm-5* (Supplementary Fig. S1 and Supplementary Table S3), encoding a homolog of the sensory cilia protein Tg737 [27], which has been proposed to regulate lifespan partially through DAF-2 signaling [28]. Although we cannot exclude that TRX-1 might also regulate lifespan partially through DAF-2 signaling, our findings suggest that loss of *trx-1* is fully epistatic only to mutation in *eat-2*. Therefore, TRX-1 is required for the response to DR that results in extended adult lifespan.

*3.2. Overexpression of *trx-1* extends adult lifespan under normal feeding conditions, but not under dietary restriction*

To further examine whether *trx-1* regulates DR-mediated adult lifespan extension, we overexpressed *trx-1* in wild type and *eat-2(ad1116)* single mutants. If TRX-1 was required for the extended lifespan caused by mutation of *eat-2*, one would predict that overexpression of *trx-1* would not further prolong the extended lifespan of *eat-2(ad1116)*, while it would extend wild-type lifespan. To validate this premise, the same transgene was used to overexpress *trx-1::GFP* in wild type and *eat-2(ad1116)*. While overexpression of *P_{trx-1}::trx-1::GFP* in wild type resulted in moderate but reproducible lifespan extension, the same overexpression transgene failed to further extend the long lifespan of *eat-2(ad1116)* (Fig. 1D and Supplementary Table S2). Our findings confirm that TRX-1 is required for the extended adult lifespan caused by DR.

3.3. TRX-1 regulates adult lifespan extension induced by a non-genetic, nutrient-based model

of dietary restriction

We asked whether *trx-1* is involved in other, non-genetic DR models in *C. elegans*. Several kinds of nutrient-based DR interventions can extend adult lifespan in the worm. These consist of either bacterial food dilution or substituting bacteria with specified growth media [29]. It has been proposed that *eat-2* mutants and nutrient-based DR extend lifespan through the same pathway because lifespan extension by mutation of *eat-2* is not additive with that caused by bacterial deprivation on plates [22,30]. Therefore, we used dietary deprivation (DD), since this protocol has been suggested to extend lifespan through the same pathway as mutation of *eat-2* [22]. Loss of *trx-1* almost completely suppressed the lifespan extension caused by DD (Fig. 2A and Supplementary Table S2). Thus, TRX-1 is also required for the adult lifespan extension induced by a non-genetic, nutrient-based model of DR.

3.4. trx-1 expression in ASJ neurons of aging adults is increased in response to dietary restriction

Because overexpression of *trx-1* extends adult lifespan under normal feeding conditions (i.e. *ad libitum*, AL), but not under DR, we asked whether *trx-1* expression in ASJ neurons of aging adults is increased under DR. To determine whether DR increases the expression levels of *trx-1* in ASJ neurons of aging adults, we quantified the expression of a *trx-1* promoter-driven *GFP* reporter gene (*P_{trx-1}::GFP*) in ASJ neurons of five-day-old adults grown under DD conditions for two days. DD significantly increased *P_{trx-1}::GFP* fluorescence in ASJ neurons of aging adults (Fig. 2B and Supplementary Fig. S2). The fluorescence increase was specific to the *trx-1* promoter, because if the *GFP* gene was expressed in ASJ neurons from

the *gpa-9* promoter [14], we observed instead a modest, but not statistically significant, decrease in expression under DD (Fig. 2B and Supplementary Fig. S2). These data suggest that *trx-1* may be transcriptionally upregulated in ASJ neurons of aging adults in response to DR.

4. Discussion

This study reports the first *in vivo* evidence that a thioredoxin (TRX-1) is involved in the regulation of adult lifespan extension induced by dietary restriction (DR). We have identified TRX-1 as a novel regulator of DR-mediated lifespan extension in *C. elegans*. We found that loss of *trx-1* completely suppressed the extended lifespan caused by mutation of *eat-2*, a genetic surrogate of DR in the worm [24]. Expression of *Ptrx-1::trx-1::GFP* in ASJ neurons rescued the *trx-1(ok1449)*-mediated suppression of *eat-2(ad1116)* lifespan extension. In addition, overexpression of *Ptrx-1::trx-1::GFP* extended lifespan in wild type but not in *eat-2(ad1116)*. Interestingly, loss of *trx-1* almost completely suppressed the lifespan extension produced by dietary deprivation (DD), a non-genetic model of DR in the worm [29]. Moreover, DD upregulated *Ptrx-1::GFP* expression in ASJ neurons of aging adults. Taken together, our results suggest a model whereby DR activates TRX-1 in ASJ neurons during aging, which in turn triggers TRX-1-dependent mechanisms directed at extending adult lifespan (Fig. 3).

Our study is the first report to show upregulation of a thioredoxin in the nervous system as a response to DR during aging (Fig 2B and Supplementary Fig. S2). Previously, mammalian thioredoxin has been shown to be modulated in aged kidney and muscle cells in dietary restricted rats [7,8,9]. In *C. elegans*, DR has been shown to activate expression of the transcription factor gene *skn-1* in ASI sensory neurons [31]. In addition, expression of the neuropeptide gene *nlp-7* [32] has been found to be upregulated under DR [33]. Thus, together with previous observations by others, our findings suggest that the concerted activation of neuronal regulators, including TRX-1, is likely an essential early step in the chain of events elicited by DR to promote adult lifespan extension.

The physiological changes induced by DR during aging do not only affect the nervous system, but also affect multiple other tissues in the worm, including the intestine [34, 35]. Given that *trx-1* is expressed only in ASJ neurons [11], and the fact that lifespan extension caused by DR was rescued when *trx-1::GFP* was expressed from its own promoter in ASJ neurons but not when expressed in the intestine (Fig. 1B and Supplementary Table S2), we favor a model in which TRX-1 regulates the physiological changes induced by DR during aging cell-non-autonomously in the whole organism (Fig. 3). We propose that DR causes upregulation of TRX-1 in ASJ neurons during aging, which then triggers a signaling cascade at the organismal level to elicit the metabolic changes necessary to extend adult lifespan (Fig. 3). Interestingly, it has been recently proposed that the neuropeptide NLP-7 similarly responds to DR-mediated signals triggered by the transcription factor SKN-1 in ASI neurons, which results in lifespan extension in the worm [33]. In addition, we have recently proposed that TRX-1 acts in ASJ neurons to adjust neuropeptide expression, including that of the insulin-like neuropeptide gene *daf-28*, during formation of stress-resistant, long-lived dauer larvae [15]. Therefore, the TRX-1-derived signals emanating from ASJ neurons involved in promoting DR-mediated adult lifespan extension are likely neuroendocrine signals. Current efforts in our labs are directed at identifying the potential neuroendocrine signals responding to TRX-1, which in turn may mediate adult lifespan extension through DR in *C. elegans*.

In summary, our findings represent the first study performed *in vivo* to show that a thioredoxin is required for the adaptive response that DR elicits to promote adult lifespan extension. We also show for the first time the upregulation of a thioredoxin in neurons in response to DR during aging. These novel findings will help elucidate the role of thioredoxin in neurons in connection with the pro-longevity events induced by DR during aging in *C.*

elegans, and most likely also in higher organisms.

Acknowledgements

We thank Gert Jansen for the *Pgpa-9::GFP* plasmid; the *Caenorhabditis* Genetics Center (which is funded by the NIH National Center for Research Resources, NCRR) and the *C. elegans* Gene Knockout Consortium for strains; Maria Trieb, Ninwa Youssef, Karin Fürtenbach and Johan Dethlefsen for excellent technical assistance; Joy Alcedo for helpful discussions; and Simon Tuck, Gautam Kao and Joy Alcedo for their insightful comments on the manuscript. This study was supported by grants from The Swedish Foundation for Strategic Research, The Swedish Research Council and The NordForsk Nordic *C. elegans* Network to PS; from Instituto de Salud Carlos III (Projects PI050065 and PI080557, co-financed by Fondo Social Europeo, FEDER) and Junta de Andalucía (Projects CVI-3629 and CVI-2697) to AMV.

References

- [1] M.D. Piper, A. Bartke, Diet and aging, *Cell Metab.* 8 (2008) 99–104.
- [2] M.C. Haigis, B.A. Yankner, The aging stress response, *Mol. Cell* 40 (2010) 333–344.
- [3] C.H. Lillig, A. Holmgren, Thioredoxin and related molecules--from biology to health and disease, *Antioxid. Redox Signal.* 9 (2007) 25–47.
- [4] Y. Meyer, B.B. Buchanan, F. Vignols, et al., Thioredoxins and glutaredoxins: unifying elements in redox biology, *Annu. Rev. Genet.* 43 (2009) 335–367.
- [5] C. Berndt, C.H. Lillig, A. Holmgren, Thioredoxins and glutaredoxins as facilitators of protein folding, *Biochim. Biophys. Acta* 1783 (2008) 641–650.
- [6] A. Mitsui, J. Hamuro, H. Nakamura, et al., Overexpression of human thioredoxin in transgenic mice controls oxidative stress and life span, *Antioxid. Redox Signal.* 4 (2002)

693–696.

- [7] C.G. Cho, H.J. Kim, S.W. Chung, et al., Modulation of glutathione and thioredoxin systems by calorie restriction during the aging process, *Exp. Gerontol.* 38 (2003) 539–548.
- [8] K.J. Jung, E.K. Lee, J.Y. Kim, et al., Effect of short term calorie restriction on pro-inflammatory NF- κ B and AP-1 in aged rat kidney, *Inflamm. Res.* 58 (2009) 143–150.
- [9] S. Rohrbach, S. Gruenler, M. Teschner, et al., The thioredoxin system in aging muscle: key role of mitochondrial thioredoxin reductase in the protective effects of caloric restriction?, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 291 (2006) R927–R935.
- [10] C. Jee, L. Vanoaica, J. Lee, et al., Thioredoxin is related to life span regulation and oxidative stress response in *Caenorhabditis elegans*, *Genes Cells* 10 (2005) 1203–1210.
- [11] A. Miranda-Vizuite, J.C. Fierro-González, G. Gahmon, et al., Lifespan decrease in a *Caenorhabditis elegans* mutant lacking TRX-1, a thioredoxin expressed in ASJ sensory neurons, *FEBS Lett.* 580 (2006) 484–490.
- [12] S. Brenner, The genetics of *Caenorhabditis elegans*, *Genetics* 77 (1974) 71–94.
- [13] T. Stiernagle, Maintenance of *C. elegans*, in: *The C. elegans Research Community* (Eds.), WormBook, 2006, doi/10.1895/wormbook.1.101.1, <http://www.wormbook.org>.
- [14] G. Jansen, K.L. Thijssen, P. Werner, et al., The complete family of genes encoding G proteins of *Caenorhabditis elegans*, *Nat. Genet.* 21 (1999) 414–419.
- [15] J.C. Fierro-González, A. Cornils, J. Alcedo, et al., The thioredoxin TRX-1 modulates the function of the insulin-like neuropeptide DAF-28 during dauer formation in *Caenorhabditis elegans*, *PLoS ONE* 6 (2011) e16561.
- [16] P.M. Loria, J. Hodgkin, O. Hobert, A conserved postsynaptic transmembrane protein affecting neuromuscular signaling in *Caenorhabditis elegans*, *J. Neurosci.* 24 (2004) 2191–2201.

- [17] C.C. Mello, J.M. Kramer, D. Stinchcomb, et al., Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences, *EMBO J.* 10 (1991) 3959–3970.
- [18] L. Avery, The genetics of feeding in *Caenorhabditis elegans*, *Genetics* 133 (1993) 897–917.
- [19] E.M. Hedgecock, J.G. Culotti, J.N. Thomson, et al., Axonal guidance mutants of *Caenorhabditis elegans* identified by filling sensory neurons with fluorescein dyes, *Dev. Biol.* 111 (1985) 158–170.
- [20] M. Edgley, A. D'Souza, G. Moulder, et al., Improved detection of small deletions in complex pools of DNA, *Nucleic Acids Res.* 30 (2002) e52.
- [21] J. Ahringer, Reverse genetics, in: The *C. elegans* Research Community (Eds.), WormBook, 2006, doi/10.1895/wormbook.1.47.1, <http://www.wormbook.org>.
- [22] G.D. Lee, M.A. Wilson, M. Zhu, et al., Dietary deprivation extends lifespan in *Caenorhabditis elegans*, *Aging Cell* 5 (2006) 515–524.
- [23] E.T. Lee, O.T. Go, Survival analysis in public health research, *Annu. Rev. Public Health* 18 (1997) 105–134.
- [24] B. Lakowski, S. Hekimi, The genetics of caloric restriction in *Caenorhabditis elegans*, *Proc. Natl. Acad. Sci. USA* 95 (1998) 13091–13096.
- [25] D.M. Raizen, R.Y. Lee, L. Avery, Interacting genes required for pharyngeal excitation by motor neuron MC in *Caenorhabditis elegans*, *Genetics* 141 (1995) 1365–1382.
- [26] E.J. Aamodt, M.A. Chung, J.D. McGhee, Spatial control of gut-specific gene expression during *Caenorhabditis elegans* development, *Science* 252 (1991) 579–582.
- [27] C.J. Haycraft, P. Swoboda, P.D. Taulman, et al., The *C. elegans* homolog of the murine cystic kidney disease gene Tg737 functions in a ciliogenic pathway and is disrupted in *osm-5* mutant worms, *Development* 128 (2001) 1493–1505.

- [28] J. Apfeld, C. Kenyon, Regulation of lifespan by sensory perception in *Caenorhabditis elegans*, *Nature* 402 (1999) 804–809.
- [29] E.L. Greer, A. Brunet, Different dietary restriction regimens extend lifespan by both independent and overlapping genetic pathways in *C. elegans*, *Aging Cell* 8 (2009) 113–127.
- [30] T.L. Kaeberlein, E.D. Smith, M. Tsuchiya, et al., Lifespan extension in *Caenorhabditis elegans* by complete removal of food, *Aging Cell* 5 (2006) 487–494.
- [31] N.A. Bishop, L. Guarente, Two neurons mediate diet-restriction-induced longevity in *C. elegans*, *Nature* 447 (2007) 545–549.
- [32] A.N. Nathoo, R.A. Moeller, B.A. Westlund, et al., Identification of neuropeptide-like protein gene families in *Caenorhabditis elegans* and other species, *Proc. Natl. Acad. Sci. USA* 98 (2001) 14000–14005.
- [33] S.K. Park, C.D. Link, T.E. Johnson, Life-span extension by dietary restriction is mediated by NLP-7 signaling and coelomocyte endocytosis in *C. elegans*, *FASEB J.* 24 (2010) 383–392.
- [34] W.B. Iser, C.A. Wolkow, DAF-2/insulin-like signaling in *C. elegans* modifies effects of dietary restriction and nutrient stress on aging, stress and growth, *PLoS ONE* 2 (2007) e1240.
- [35] M. Hansen, A. Chandra, L.L. Mitic, et al., A role for autophagy in the extension of lifespan by dietary restriction in *C. elegans*, *PLoS Genet.* 4 (2008) e24.

Figure legends

Fig. 1. TRX-1 regulates adult lifespan extension induced by a genetic model of dietary restriction (DR).

A) Loss of *trx-1* completely suppressed the lifespan extension produced by *eat-2(ad1116)*, a genetic surrogate of DR. B) The inability of *trx-1(ok1449) eat-2(ad1116)* double mutants to extend lifespan was rescued by expression of *P_{trx-1}::trx-1::GFP* in ASJ neurons ($p < 0.01$ relative to non-transgenic control by Wilcoxon test, black lines). This rescue was not achieved in the same genetic background when *trx-1::GFP* was expressed from the *ges-1* promoter in the intestine ($p = 0.1$ relative to non-transgenic control by Wilcoxon test, grey lines). C) Loss of *trx-1* partially reduced but did not completely eliminate the lifespan extension caused by mutation of the insulin-like receptor gene *daf-2*. D) Overexpression of *P_{trx-1}::trx-1::GFP* extended lifespan in wild type ($p = 0.01$ relative to non-transgenic control by Wilcoxon test, black lines), but not in *eat-2(ad1116)* ($p = 0.6$ relative to non-transgenic control by Wilcoxon test, grey lines). In panels B and D, the non-transgenic control represents the non-transgenic siblings that segregated from the same transgenic parents. Each panel shows the data derived from one experiment. All statistical data can be found in Supplementary Table S2. Note that the x-axis time scale is different between panels.

Fig. 2. TRX-1 regulates adult lifespan extension induced by a non-genetic, nutrient-

based model of dietary restriction (DR). A) Dietary deprivation (DD), a non-genetic model of DR, only moderately extended the lifespan of *trx-1(ok1449)* animals (mean lifespan change: +9.2% in *trx-1(ok1449)*, grey lines vs. +40.2% in wild type, black lines). AL, *ad libitum*. Statistical data can be found in Supplementary Table S2. B) Two days of DD increased the expression of *P_{trx-1}::GFP* in ASJ neurons of aging adults ($p < 0.01$ relative to

wild type, AL, by unpaired two-tailed *t*-test). However, the same intervention slightly, but not significantly, decreased the expression of control *Pgpa-9::GFP* in ASJ neurons of aging adults ($p = 0.3$ relative to wild type, AL, by unpaired two-tailed *t*-test). Two independent transgenic lines were examined for each of the two transcriptional *Ptrx-1::GFP* and *Pgpa-9::GFP* reporters, and the results were very similar (cf. Supplementary Fig. S2). The data derived from one transgenic line are presented. Each bar represents the mean relative fluorescence intensity of 48–54 animals \pm standard error of the mean. Each panel shows the data obtained from one experiment.

Fig. 3. A speculative model for the function of TRX-1 under dietary restriction (DR) during aging in *C. elegans*. In response to DR, TRX-1 is activated in ASJ neurons of adult *C. elegans*. This response then likely triggers a signaling cascade throughout the whole animal that results in metabolic changes necessary to extend adult lifespan.

Figure 1
[Click here to download high resolution image](#)

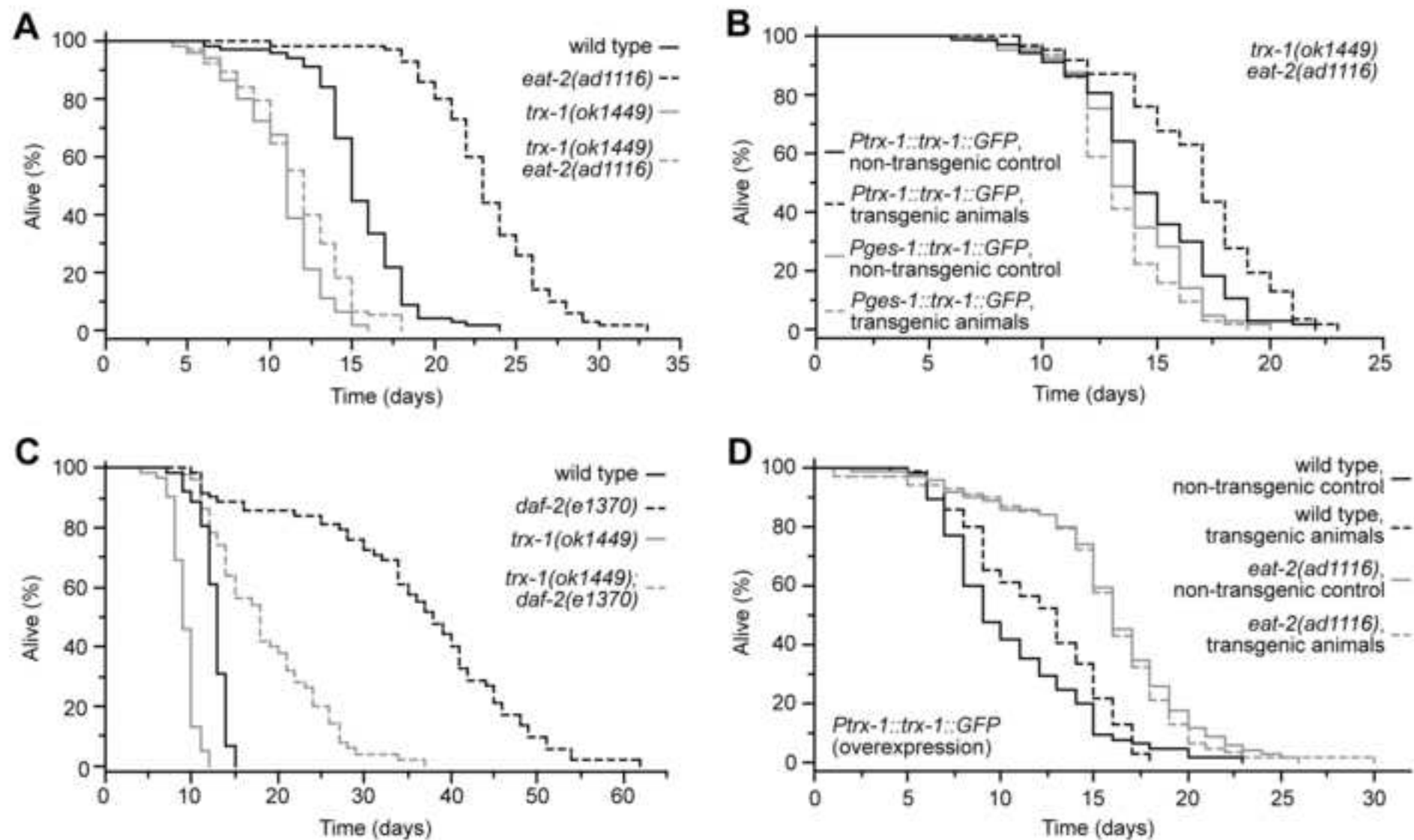


Figure 2
[Click here to download high resolution image](#)

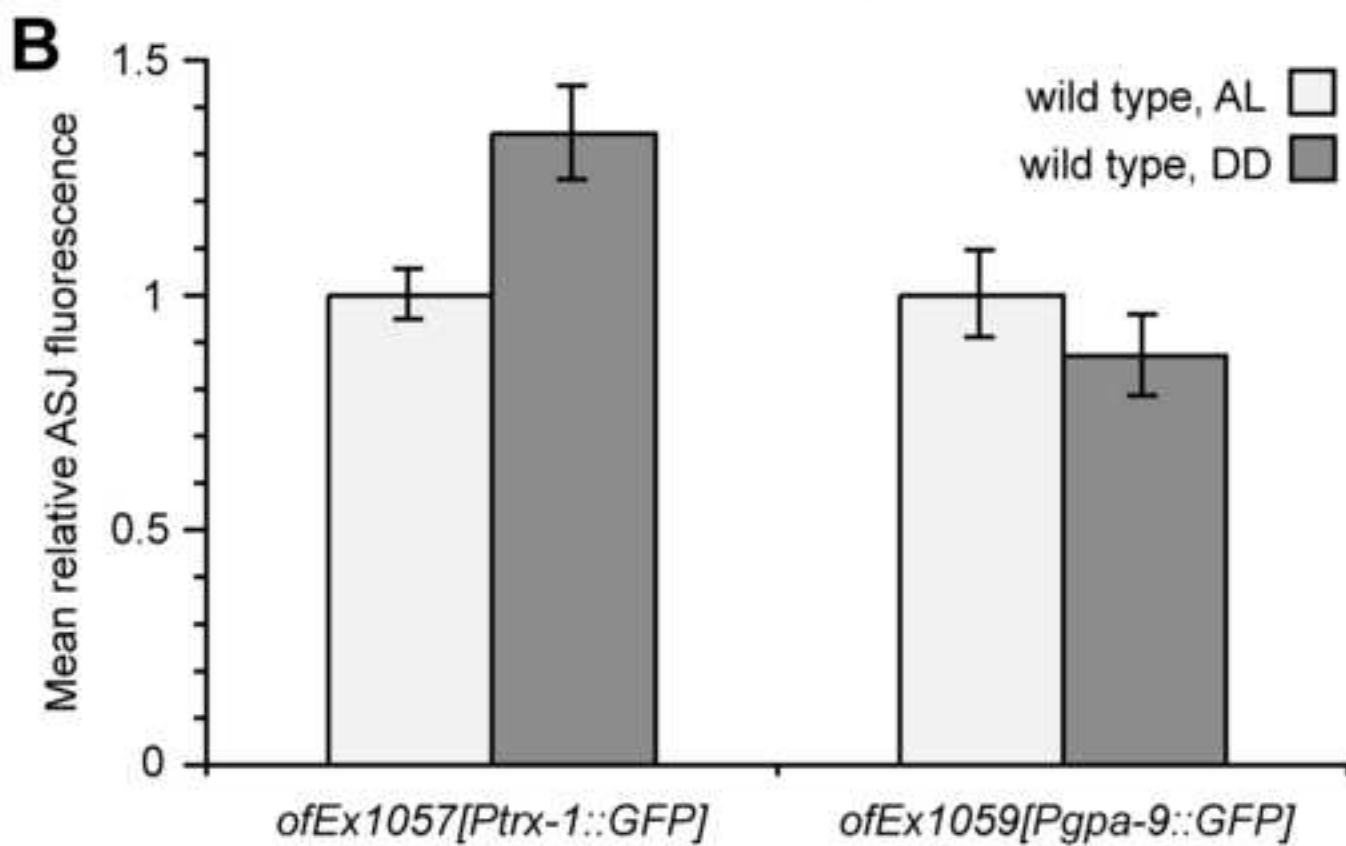
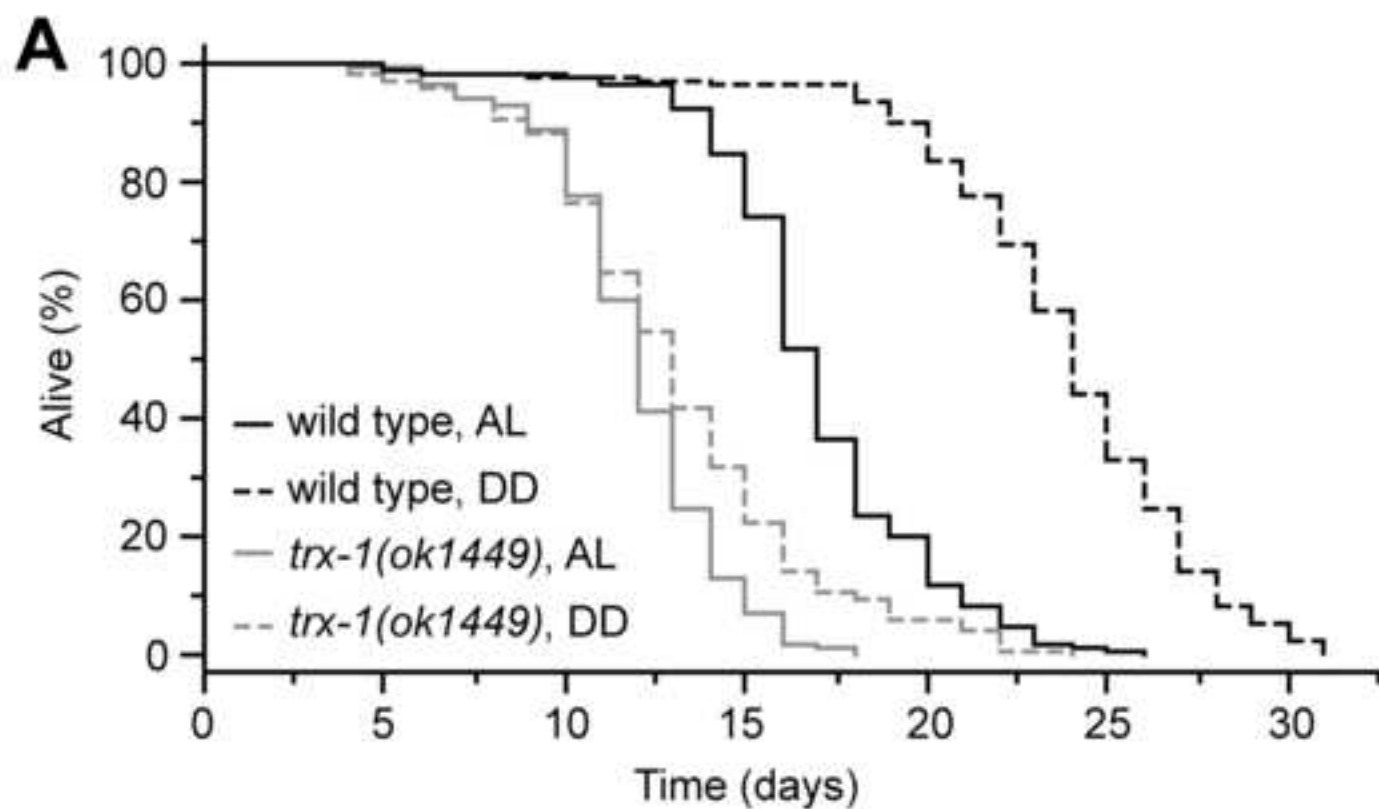
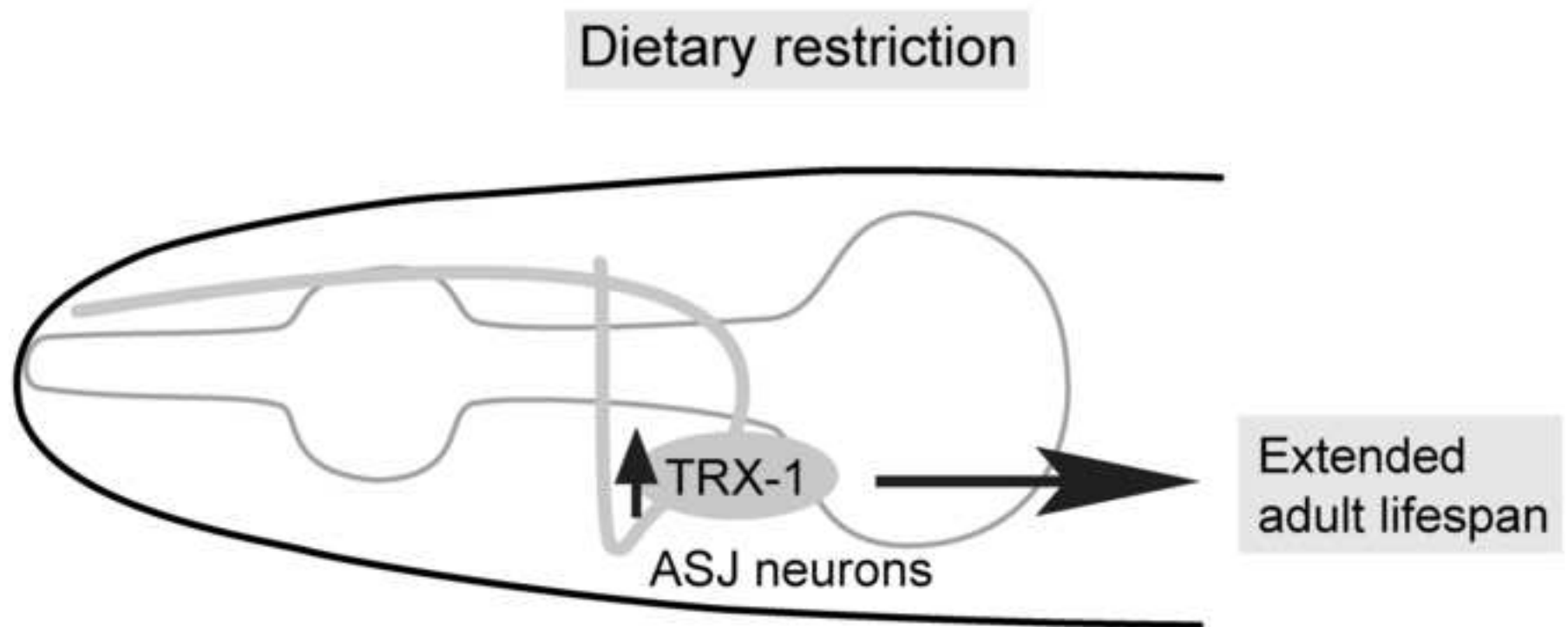


Figure 3
[Click here to download high resolution image](#)



Electronic Supplementary Data (online publication only)

[Click here to download Electronic Supplementary Material \(online publication only\): jcfg_supplem_data.pdf](#)