From the Department of Biosciences and Nutrition
Karolinska Institutet, Stockholm, Sweden

THE PLASTICITY OF AGING AND SURVIVAL: A ROLE FOR THE THIOREDOXIN SYSTEM IN *CAENORHABDITIS ELEGANS*

Juan Carlos Fierro-González

Karolinska Institutet

Stockholm 2011

juan.carlos.fierro.gonzalez@ki.se
fierrojc@yahoo.com

TO MY FAMILY

"Caminante, son tus huellas
el camino, y nada más;
caminante, no hay camino:
se hace camino al andar.
Al andar se hace camino,
y al volver la vista atrás
se ve la senda que nunca
se ha de volver a pisar.
Caminante, no hay camino,
sino estelas en la mar."

"Wanderer, your footsteps are
the road, and nothing more;
wanderer, there is no road,
the road is made by walking.
By walking one makes the road,
and upon glancing behind
one sees the path
that never will be trod again.
Wanderer, there is no road,
only wakes upon the sea."

from Proverbios y Cantares
by Antonio Machado (1912)

trans. Betty Jean Craige in Selected
Poems of Antonio Machado (1979)

ABSTRACT

Thioredoxin and related systems regulate many biological processes in diverse species. In mammals, in addition to protecting against oxidative damage, they also play key roles as regulators of transcription factors, signaling cascades and immune responses. Many discoveries made in mammalian models have contributed to the description of numerous functions for the thioredoxin and related systems. However, studies performed in mammalian models offer limited information and versatility with respect to how the thioredoxin system dynamically interacts with the surrounding environment in living animals. For instance, *in vivo* examination of mammalian mutants is severely restricted since systemic mutations for thioredoxin and thioredoxin reductase result in embryonic lethality. In the invertebrate animal model *Caenorhabditis elegans*, survival programs during post-embryonic development and aging are plastic, and modifiable by the environment. Hence, *C. elegans* provides a framework for the use of effective cell-biological and genetic tools to investigate *in vivo* the biology of thioredoxins and related proteins in the context of a changing environment.

Here, we show that the *C. elegans* genome contains many putative homologs of the mammalian thioredoxin system and related molecules. Moreover, we report for the first time in any metazoan that a thioredoxin gene (*trx-1*) is expressed only in the nervous system and is involved in the regulation of aging (Paper I). In addition, we show that the selenoprotein, thioredoxin reductase (TRXR-1), instead of protecting against oxidative stress, is responsible, together with glutathione reductase (GSR-1), for the removal of old cuticle during molting in *C. elegans*. Our findings suggest that TRXR-1 and GSR-1 regulate molting likely by activating glutathione (GSH) function in the cuticle (Paper II). Next, we demonstrate that the thioredoxin TRX-1 is involved in ASJ neuron-dependent signaling pathways that regulate dauer formation in *C. elegans*. Our data suggest that redox-independent functions of TRX-1 in ASJ neurons are necessary to modulate neuropeptide expression, including that of the insulin-like neuropeptide gene *daf-28*, during dauer formation (Paper III). Lastly, we show for the first time in an *in vivo* animal model that a thioredoxin (TRX-1) is necessary for the metabolic changes triggered by dietary restriction (DR) to extend adult lifespan. We are also the first to show that DR upregulates thioredoxin (*trx-1*) expression in the nervous system. We propose that DR activates TRX-1 in ASJ neurons of aging adults to then stimulate the metabolic changes necessary to extend adult lifespan (Paper IV).

In conclusion, we show evidence for the crucial role of conserved members of the thioredoxin system in controlling aging and survival in *C. elegans*. Furthermore, the data presented suggest the plastic nature of molting, dauer formation and aging in *C. elegans* and how the thioredoxin system and related molecules assist to maintain such environmental sensitivity. Basic cell-biological processes and the thioredoxin and related systems possess a substantial degree of functional conservation between mammals and invertebrates. Hence, the novel roles discovered for thioredoxins and related molecules to regulate aging and survival in *C. elegans*, might lead the way in disclosing similar mechanisms in mammals.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which are referred to in the text by their roman numerals:

I. Miranda-Vizuete, A., **Fierro-González, J.C.**, Gahmon, G., Burghoorn, J., Navas, P., and Swoboda, P. (2006). Lifespan decrease in a *Caenorhabditis elegans* mutant lacking TRX-1, a thioredoxin expressed in ASJ sensory neurons. FEBS Lett. *580*, 484–490. doi:10.1016/j.febslet.2005.12.046.

Copyright © 2005 Federation of European Biochemical Societies. Reprinted with permission from Elsevier.

II. Stenvall, J., **Fierro-González, J.C.**, Swoboda, P., Saamarthy, K., Cheng, Q., Cacho-Valadez, B., Arnér, E.S.J., Persson, O.P., Miranda-Vizuete, A., and Tuck, S. (2011). Selenoprotein TRXR-1 and GSR-1 are essential for removal of old cuticle during molting in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. U.S.A. *108*, 1064–1069. doi:10.1073/pnas.1006328108

Copyright © 2011 National Academy of Sciences, USA.

III. **Fierro-González, J.C.**, Cornils, A., Alcedo, J., Miranda-Vizuete, A.*, and Swoboda, P.* (2011). The Thioredoxin TRX-1 Modulates the Function of the Insulin-Like Neuropeptide DAF-28 during Dauer Formation in *Caenorhabditis elegans*. PLoS ONE 6, e16561. doi:10.1371/journal.pone.0016561.

Copyright © 2011 Fierro-González et al.

IV. **Fierro-González, J.C.**, González-Barrios, M., Miranda-Vizuete, A.*, and Swoboda, P.* (2011). The thioredoxin TRX-1 regulates adult lifespan extension induced by dietary restriction in *Caenorhabditis elegans*. Biochem. Biophys. Res. Commun. *406*, 478–482. doi:10.1016/j.bbrc.2011.02.079.

Copyright © 2011 Elsevier Inc. Reprinted with permission from Elsevier.

* Equal contribution.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AF488CM	Alexa Fluor 488 C5 maleimide
AMPK	Adenosine monophosphate (AMP)-activated protein kinase
APE1/Ref-1	Apurinic/apyrimidinic endonuclease 1
ASJ	Amphid single-ciliated sensory neuron type J
ASK1	Apoptosis signal-regulating kinase 1
cGMP	Cyclic guanosine monophosphate
Daf	Dauer formation
DR	Dietary restriction
ER	Endoplasmic reticulum
ERdj5	DnaJ domain-containing ER-resident protein 5
ESR	Estrogen receptor
FLP	FMRFamide-related neuropeptide
FOXO	Forkhead box O
GFP	Green fluorescent protein
Grx	Glutaredoxin
GSH	Glutathione
GSR	Glutathione reductase
GSSG	Glutathione disulfide
Hif-1	Hypoxia-inducible factor 1
HSF-1	Heat shock factor family homolog 1
INS	Insulin-like neuropeptide
LXR	Liver X nuclear receptor
Msr	Methionine sulfoxide reductase
NADPH	Nicotinamide adenine dinucleotide phosphate
NEM	N-ethylmaleimide
NF-κB	Nuclear factor-κB
NLP	Neuropeptide-like protein
Nrf2	Nuclear factor erythroid-derived 2
Nrx	Nucleoredoxin
PDI	Protein disulfide isomerase
Prx	Peroxiredoxins
RACE	Rapid amplification of complementary DNA ends
RNAi	Ribonucleic acid interference
RNR	Ribonucleotide reductase
RT-PCR	Reverse transcriptase-polymerase chain reaction
SpTrx	Mammalian sperm-specific thioredoxin protein
TBP-2	Thioredoxin binding protein 2
TGF-β	Transforming growth factor β
TGR	Thioredoxin glutathione reductase
Trx	Thioredoxin
TrxR	Thioredoxin reductase
Txl	Thioredoxin-like protein
UTR	Untranslated region
Wnt	Wingless-type homolog

1. INTRODUCTION

1.1. The Thioredoxin System

1.1.1. General Aspects

Members of the thioredoxin (Trx) family of proteins are defined by two conserved structural motifs: (i) the Trx fold, and (ii) the -Cys-X-X-Cys- catalytic active site. The basic Trx fold consists of a three-layer α/β/α sandwich, with a 4–5 stranded β-sheet and 2–4 α-helices, depending on the protein (Holmgren et al., 1975; Qi and Grishin, 2005). The -Cys-X-X-Cys- motif contains a pair of amino acids flanked by two redox-active cysteines (Cys), which can be replaced by other amino acid residues depending on the redox protein (Fomenko and Gladyshev, 2003).

These two structural motifs are shared by many proteins essential for cellular thiol-redox pathways. These include reductants, like Trx and glutaredoxin (Grx), and oxidants, like protein disulfide isomerase (PDI). The Trx and Grx systems share many functions as antioxidants and signaling regulators to maintain the redox homeostasis inside the cell. However, each system has its own unique functions. For instance, it is known that Grxs are more flexible than Trxs in terms of target protein diversity and catalytic mechanism [reviewed in (Fernandes and Holmgren, 2004; Lillig et al., 2008)]. The Trx system consists of nicotinamide adenine dinucleotide phosphate (NADPH), thioredoxin reductase (TrxR) and Trx; whereas the Grx system is composed of NADPH, glutathione reductase (GSR), glutathione (GSH) and Grx.

Thioredoxins and related molecules catalyze oxidoreductase reactions by using the cysteinyl residues in the -Cys-X-X-Cys- motif to break disulfide groups into free thiols in oxidized target proteins (Figure 1). In the Trx system, oxidized Trx is reverted to the reduced state by TrxR, using electrons from NADPH (Figure 1A, top). In the Grx system, oxidized Grx is reduced by two molecules of GSH, which are oxidized to form glutathione disulfide (GSSG). Electrons are transferred from NADPH to GSSG via GSR (Figure 1A, bottom). In some organisms, the classical Trx and Grx systems described above have been shown to possess a remarkable catalytic flexibility [reviewed in (Arnér, 2009)]. For instance, the genome of *Drosophila melanogaster* encodes TrxR, Trx and Grx, but not GSR (Figure 1B). Interestingly, this organism completes the reduction of GSSG to form GSH by using Trx, which thus substitutes for GSR function. While GSSG fails to be a substrate for TrxR, Trx acts as an efficient electron carrier between TrxR and GSSG in this organism (Figure 1B) (Cheng et al., 2007; Kanzok et al., 2001).
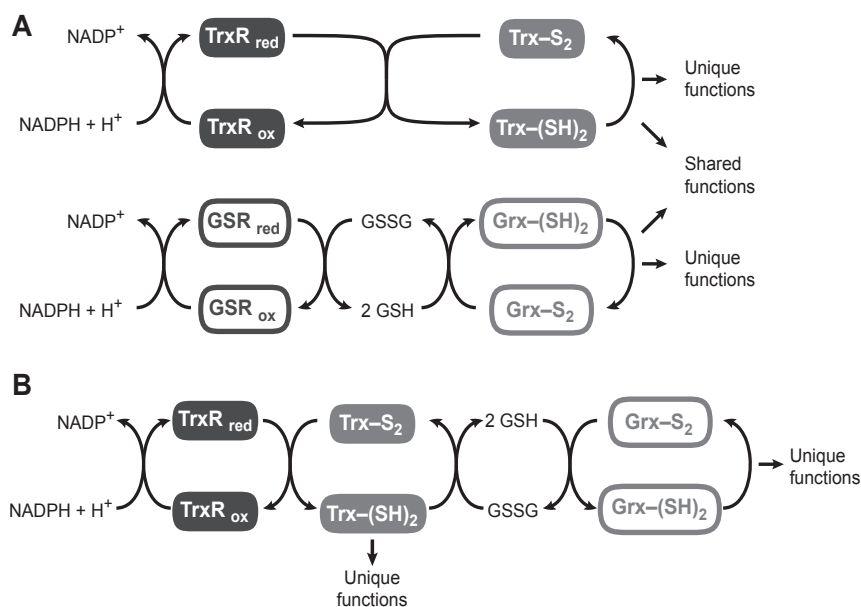
Figure 1. Redox cascades of the thioredoxin (Trx) and glutaredoxin (Grx) systems. (A) Classical Trx and Grx systems: the Trx system comprises nicotinamide adenine dinucleotide phosphate (NADPH), thioredoxin reductase (TrxR) and Trx. The Grx system consists of NADPH, glutathione reductase (GSR), glutathione (GSH) and Grx. (B) Catalytic mechanism in *D. melanogaster*. This organism lacks GSR; instead, Trx is responsible for reducing glutathione disulfide. See text for details. Figure adapted from (Arnér, 2009; Holmgren and Lu, 2010; Lillig and Holmgren, 2007).

In addition to the Trx and Grx systems introduced above, the mammalian genome also encodes other Trx homologs. Some of these Trx-related proteins are testis-specific, such as SpTrx1, SpTrx2, SpTrx3 and Txl-2 (Jiménez et al., 2004; Miranda-Vizuete et al., 2001; Sadek et al., 2001; Sadek et al., 2003). The rod-derived cone viability factor, RdCVF, is another tissue-specific mammalian Trx-like protein, which is localized to photoreceptors in the retina (Léveillard et al., 2004). In addition, there are members of the Trx family of proteins that specifically function in the endoplasmic reticulum (ER) as disulfide bond catalysts, such as PDI and ERdj5 [reviewed in (Benham, 2005; Kruusma et al., 2006)]. PDIs function as facilitators of protein folding in the ER and regulate target proteins via oxidation and isomerization reactions [reviewed in (Ellgaard and Ruddock, 2005; Wilkinson and Gilbert, 2004)]. The ER-resident chaperone ERdj5 is required for redox-dependent degradation of misfolded proteins and modulation of the unfolded protein response (UPR)

in the ER (Cunnea et al., 2003; Dong et al., 2008; Thomas and Spyrou, 2009; Ushioda et al., 2008). Other mammalian Trx-like proteins include Txl-1 and nucleoredoxin (Nrx). Txl-1 is an ubiquitously expressed cytosolic Trx-like protein (Lee et al., 1998), recently found to be functionally connected to the proteasome (Andersen et al., 2009; Wiseman et al., 2009). Nrx is expressed in all tissues and functions as a redox regulator of several transcription factors and the Wnt/β-catenin pathway (Funato et al., 2006; Hirota et al., 2000; Kurooka et al., 1997).

1.1.2. Mammalian Thioredoxin

In addition to their role as regulators of diverse transcription factors and signaling pathways, Trxs are generally regarded as essential components of the oxidative stress resistance apparatus and the immune system. The fold of Trxs is composed of five β-strands with two α-helices on each side (Holmgren et al., 1975). Trxs reduce target molecules with the two cysteinyl residues of the conserved -Trp-Cys-Gly-Pro-Cys- active site. The mammalian genome encodes two Trx proteins: the cytosolic Trx1 and the mitochondrial Trx2.

Mammalian Trx1 is probably one of the most thoroughly studied proteins in the Trx family. The gene encoding human Trx1 (termed *TXN*) has been shown to undergo alternative splicing. The alternatively spliced mRNA variants do not translate into functionally different proteins. Instead, they have been proposed to act in a regulatory mechanism, in which they could contribute to modulate the levels of *TXN* expression (Berggren and Powis, 2001; Hariharan et al., 1996; Jiménez and Miranda-Vizuete, 2003).

Trx1 acts as an electron donor for different metabolic enzymes, including ribonucleotide reductase (RNR), peroxiredoxin (Prx) and methionine sulfoxide reductase (Msr). RNR catalyzes the synthesis of deoxynucleotides by using nucleotides as substrates (Nordlund and Reichard, 2006). The fact that inactivation of Trx1 induces embryonic lethality in mice (Matsui et al., 1996), confirms that Trx1 is essential for cell proliferation and DNA synthesis. Prxs are H_2O_2-neutralizing enzymes involved in antioxidant defense and redox regulation of signaling pathways and cell differentiation (Rhee et al., 2005). The function of Trx1 as electron donor for Msr has been proposed to impact antioxidant defense and aging (Lillig and Holmgren, 2007; Stadtman et al., 2005).

In addition, Trx1 interacts with many transcription factors and signaling proteins to regulate multiple biological functions. The list of interacting factors includes apoptosis signal-regulating kinase 1 (ASK1), nuclear factor-κB (NF-κB), Trx binding protein 2 (TBP-2), hypoxia-inducible factor 1 (Hif-1), tumor suppres-

sor p53, apurinic/apyrimidinic endonuclease 1 (APE1/Ref-1), activator protein 1 (AP-1) and estrogen receptor (ESR), among others [reviewed in (Holmgren and Lu, 2010; Lillig and Holmgren, 2007; Meyer et al., 2009)].

Trx1 has also been linked to multiple pathologies, such as cancer, cardiovascular or degenerative diseases. In addition, Trx1 has been proposed to have a role in the aging process. The first report to demonstrate a direct effect of mammalian Trx1 on aging showed that overexpression of human Trx1 in mice extends lifespan (Mitsui et al., 2002). Subsequently, other studies were designed to understand how dietary restriction (DR) affects Trx1 function during aging. These studies found that DR modulates the expression of Trx1 in aged kidney and muscle cells (Cho et al., 2003; Jung et al., 2009; Rohrbach et al., 2006).

Similarly to Trx1, mutation of the gene encoding mitochondrial Trx2 in mice (termed *Txn2*) causes embryonic lethality (Nonn et al., 2003). Interestingly, the embryonic stage at which homozygous *Txn2* mutants die corresponds to the start of mitochondrial maturation. Although *Txn1* and *Txn2* homozygous mutant mice die during embryogenesis, their phenotypes do not completely overlap, suggesting that each Trx performs at least some functions independently of one another (Lillig and Holmgren, 2007).

In sum, the functions of Trxs are extensive and mostly depend on their disulfide oxidoreductase activity. However, in some cases, Trxs execute their functions in a redox-independent manner. For instance, human truncated Trx (Trx80) acts as a mitogenic cytokine (Pekkari et al., 2003) and human Trx1 binds and inhibits ASK1 (Liu and Min, 2002) by functions performed independently of their redox activity. Moreover, Trxs have been shown to facilitate the folding of proteins in a redox-independent fashion [reviewed in (Berndt et al., 2008)]. These observations manifest the potential of mammalian Trxs to adopt multiple functions in many diverse contexts.

1.1.3. Mammalian Thioredoxin Reductase

Mammalian TrxRs belong, together with GSR, to the pyridine nucleotide disulfide oxidoreductase family of proteins (Williams, 1992). TrxRs exhibit specific functions beyond serving as a mere electron donor for Trx [reviewed in (Arnér, 2009)]. The two subunits of the homodimeric mammalian TrxR arrange in a "head-to-tail" mode. Each monomer contains a flavin adenine dinucleotide (FAD)-binding domain, an NADPH-binding domain and an interface domain (Sandalova et al., 2001). The active site used to reduce Trx, as well as other specific target proteins, consists of a C-terminal -Gly-Cys-Sec-Gly-COOH motif (Cheng et al., 2009; Zhong

et al., 2000; Zhong et al., 1998). Sec in this motif represents the 21st amino acid selenocysteine, which is analogous to Cys and contains selenium in place of sulfur. TrxR has another FAD-associated -CVNVGC- active site, which is mainly used during the early steps of the catalytic mechanism as an intermediate step for electrons that flow from NADPH to the main Sec-containing active site (Arnér, 2009).

Selenoprotein translation requires that Sec be inserted at the recoded stop codon UGA by a specifically designed translation machinery (Berry, 2005). Incorporation requires among other factors a cis-acting RNA secondary structure, termed Sec incorporation sequence (SECIS); a unique selenocysteinyl-tRNA, referred to as tRNA[Ser]Sec; and a translation elongation factor, SelB/EFsec.

Three different mammalian genes encode the diverse gene products of TrxR1, TrxR2 and TGR. Both TrxR1 and TrxR2 are ubiquitously expressed, while TGR is mainly expressed in testis. Moreover, TrxR1 primarily constitutes the cytosolic form of TrxR, while TrxR2 is mainly mitochondrial (Arnér, 2009). Alternative transcript variants have been identified for the genes encoding mammalian TrxR1 and TrxR2 [(Miranda-Vizuete and Spyrou, 2002; Osborne and Tonissen, 2001; Rundlöf et al., 2004; Su and Gladyshev, 2004); and reviewed in (Arnér, 2009)]. The splice variants corresponding to the gene encoding mammalian TrxR1 (*TXNRD1*) are to date the most extensively studied. Each alternative *TXNRD1* transcript has been proposed to have a defined expression pattern in terms of cell, tissue or growth condition (Arnér, 2009).

TrxR1 and TrxR2 are essential for development, since inactivation of either of them results in embryonic lethality (Bondareva et al., 2007; Conrad et al., 2004; Jakupoglu et al., 2005). In order to ascertain the role of TrxR in different organs, tissues or cells, a number of research groups have used conditional knockout mice to target *Txnrd* deletions to the nervous system (Soerensen et al., 2008), heart (Conrad et al., 2004; Jakupoglu et al., 2005; Kiermayer et al., 2007) or lymphocytes (Geisberger et al., 2007). Only two out of eight tissue- or cell-specific knockout mice generated so far have been reported to cause obvious phenotypes [reviewed in (Conrad, 2009)]. In particular, heart-specific deletion of *Txnrd2* was identified to induce heart failure and postnatal death (Conrad et al., 2004). Furthermore, mice harboring a nervous system-deletion of *Txnrd1* develop to adulthood, but exhibit evident cerebellar defects (Soerensen et al., 2008). These findings suggest that TrxR1 and TrxR2 are differentially required for the development of specific organs, tissues or cells.

TrxRs have been associated to numerous human pathologies, ranging from cancer to male infertility, and including Alzheimer's disease [reviewed in (Arnér,

2009)]. In addition, TrxR function, and that of GSR, have been connected to the biological cause of aging in mammals. For instance, it is generally acknowledged that cataract formation and skin deterioration increase with age. Thus, different agents (e.g. UV light) that promote age-related skin deterioration have been shown to induce TrxR activity in mouse skin (Kumar and Holmgren, 1999; Schallreuter and Wood, 2001). Moreover, GSR activity in the crystalline lens decreases with aging and cataract formation and can be reactivated by the Trx system (Yan et al., 2007). In addition, aging induces a reduction of TrxR2 levels in rat muscle, which is reverted by the anti-aging effects of DR (Rohrbach et al., 2006). These findings clearly implicate TrxR, together with GSR, in mechanisms that regulate aging and age-related diseases.

1.2. Formulation of the Problem

A remarkable number of studies designed so far to understand the biology of the thioredoxin and related systems have been performed using mammalian models [reviewed in (Lillig and Holmgren, 2007)]. The main reason for using mammalian models, such as the mouse, has been that they share many similarities in terms of genome homology, anatomy, cell biology and physiology with humans. Most of these studies are based on *in vitro* and *ex vivo* experimentation, since genetic and cell-biological *in vivo* studies are time-consuming and expensive in these animal models. *In vitro* and *ex vivo* approaches in mammalian models are still expanding at a fast pace, and have contributed to the discovery of many of the functions described so far for the thioredoxin system. However, they offer limited information with regard to how cellular pathways interplay with environmental and internal cues in the context of a living animal. These limitations delay the advent of new discoveries at the genetic and cell-biological levels.

In addition, post-embryonic *in vivo* examination of mutants for the thioredoxin system cannot be performed in mammals because systemic mutation of thioredoxin and thioredoxin reductase results in embryonic lethality (Bondareva et al., 2007; Conrad et al., 2004; Jakupoglu et al., 2005; Matsui et al., 1996; Nonn et al., 2003). Furthermore, most members of the thioredoxin and related systems in mammals are expressed ubiquitously; only SpTrx1, SpTrx2, SpTrx3, Txl-2 and TGR are testis-specific [reviewed in (Miranda-Vizuete et al., 2004)] and RdCVF is retina-specific (Léveillard et al., 2004). This ubiquitous expression pattern in mammalian animal models hinders the effort to identify the *in vivo* function of thioredoxins and related molecules in a specific organ, tissue or cell (e.g. the nervous system). Such a goal would require a highly laborious experimental setup in

order to allocate the specific functions that pertain to, e.g., the nervous system, and discriminate from those that originate from other organs, tissues or cells [e.g. by generating nervous system-specific Cre-mediated deletions of the target mammalian thioredoxin system gene (Soerensen et al., 2008)]. As described in the previous section, six out of eight tissue- or cell-specific knockout mice generated so far lack any obvious phenotype [overviewed in (Conrad, 2009)], which argues against the efficiency of this approach.

Therefore, not only evolutionary proximity and anatomical similarity should be taken into account as the main factors when selecting an experimental animal model. In fact, many basic cell-biological processes and the way they respond to environmental and inter-cellular cues share a high degree of functional conservation across phyla, despite the inter-specific morphological variation [reviewed in (Fontana et al., 2010; Hariharan and Haber, 2003)]. Consequently, new insights into the biology of thioredoxins and related molecules *in vivo* could be gained by reducing the level of complexity of the experimental platform. Hence, the use of a simple, versatile and inexpensive *in vivo* model organism would further contribute to the discovery of novel mechanisms of action for the thioredoxin and related systems at a genetic and cell-biological level.

1.3. Caenorhabditis elegans: a Toolbox for in vivo Discovery

Over the years, the nematode *Caenorhabditis elegans* has emerged as an increasingly acknowledged and powerful invertebrate model organism, used to study important biological processes associated with human health *in vivo*. Since the original studies performed by Sydney Brenner on the genetics of behavior (Brenner, 1973), the list of biological processes studied by the *C. elegans* research community has expanded enormously. *C. elegans* is used today to study apoptosis, cell signaling, gene regulation, synaptic transmission, neural plasticity, metabolism and aging, in addition to many other biological processes (Kaletta and Hengartner, 2006; Riddle et al., 1997). Moreover, significant discoveries in the fields of biology and medicine were first made in *C. elegans*, including those concerning organ development and programmed cell death (Brenner, 1974; Ellis and Horvitz, 1986; Sulston, 1976), RNA interference (RNAi) (Fire et al., 1998) and the use of green fluorescent protein (GFP) for *in vivo* microscopy (Chalfie et al., 1994). Therefore, it is not surprising that the value of *C. elegans* as a model organism has been recognized by awarding the Nobel Prize to six *C. elegans* researchers on three occasions during the last decade (Nobel Prize web site, http://www.nobelprize.org).

C. elegans displays a number of qualities that make it a powerful tool for *in*

vivo discovery. First, many of the basic molecular and cellular pathways present in mammals are conserved in *C. elegans*, and its genome has been completely sequenced (The *C. elegans* Sequencing Consortium, 1998). In particular, *C. elegans* homologs have been determined for over 60% of the human proteins (Kuwabara and O'Neil, 2001; Lai et al., 2000; Sonnhammer and Durbin, 1997). Moreover, 12 out of 17 known signaling pathways are conserved between *C. elegans* and human (The National Research Council, 2000; Leung et al., 2008). Remarkably, it has been reported on many occasions that a specific human gene can functionally replace the endogenous putative homolog when expressed in *C. elegans* (Kao et al., 2007; Pierce et al., 2001).

Second, *C. elegans* is a sophisticated multicellular animal framed in an apparently simple body plan. The adult hermaphrodite consists of only 959 somatic cells (Riddle et al., 1997; Wood, 1988). However, cells assemble into many tissues and organs that ultimately form complex systems, including epithelial, nervous, muscular, excretory, alimentary and reproductive systems (Altun and Hall, 2008). In addition, the complete wiring diagram of the 302 neurons present in the hermaphrodite is known (White et al., 1986), which allows for better understanding of processes such as neural plasticity and synaptic transmission.

Third, *C. elegans* can easily be maintained in laboratory conditions by feeding on *Escherichia coli* (Stiernagle, 2006). Adult hermaphrodites of ~1 mm in length take ~3 days to develop from the egg stage (Figure 2) and have a large brood size of over 300 progeny. These attributes favor large-scale *in vivo* studies, which can be performed in 96-well microtiter plates because of the small size of adult *C. elegans* (Hope, 1999; Riddle et al., 1997). Moreover, hermaphrodites complete the process of development and senescence in over 2 weeks (Figure 2) (Riddle et al., 1997), which qualifies *C. elegans* as one of the preferred *in vivo* animal models for aging research.

Fourth, because *C. elegans* is transparent, fluorescent reporters can be used to visualize *in vivo* many cell-biological processes, such as axon growth or fat metabolism.

Last, the combination of powerful online and experimental tools with the predisposition of *C. elegans* to large-scale, genome-wide genetic screens [reviewed in (Antoshechkin and Sternberg, 2007)], highlight *C. elegans* as one of the models of choice for *in vivo* biomedical discovery.

Despite the advantages described above, *C. elegans* lacks many organs and tissues present in mammals, which imposes a limitation when attempting to model functions of the thioredoxin and related systems in such organs or tissues. How-
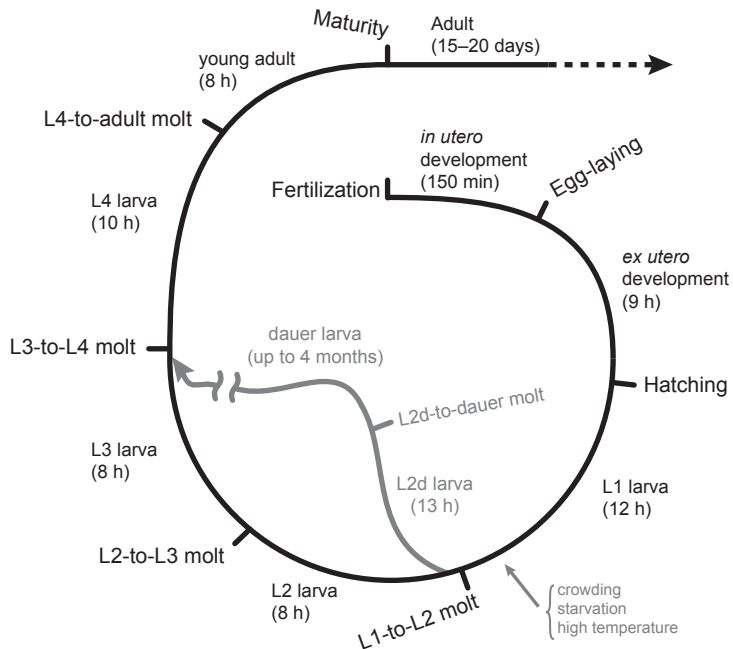
Figure 2. The life cycle of *C. elegans* at 22°C. Fertilization denotes time point zero. Numbers in parenthesis represent the time span of the indicated life stage. Figure adapted from (Altun and Hall, 2008; Braendle et al., 2008; Riddle et al., 1981)

ever, the strength of *C. elegans*, as highlighted above, resides in the fact that high-throughput cell-biological and genetic manipulations can be performed at better cost-effectiveness than in mammalian models. Hence, combining the knowledge acquired using *C. elegans* as an *in vivo* experimental platform with that achieved using mammalian models, can contribute to clarify complex aspects of the biology of thioredoxins and related proteins that would otherwise remain cryptic.

1.4. The plasticity of Aging and Survival: Lessons from C. elegans

1.4.1. General Aspects

The attributes of *Caenorhabditis elegans* as a model system for biomedical research, not only favor high-throughput, genome-wide examination of fundamental genetic and cell-biological processes. In addition, these advantageous resources can

also be applied to understand how the whole organism interacts with a changing environment from a cell-biological and genetic perspective. The relevance of understanding these questions are being progressively acknowledged in developmental and evolutionary biology, as well as in more applied fields of study like pharmaceutical and toxicological research [reviewed in (Braendle et al., 2008; Leung et al., 2008)].

Animals interact with their surrounding environment and consequently undergo a number of adaptive responses, which are modulated by the environment depending on the plasticity of the biological processes that regulate such dynamic responses. For instance, DR has been hypothesized to extend life in humans, based on recent studies conducted on a specific human cohort from Okinawa, Japan (Willcox et al., 2007; Willcox et al., 2006). In animal models, it has been shown that different nutrient regimens, which can even be administered at different time intervals, trigger an array of varying longevity outcomes (Piper and Bartke, 2008). In addition, a number of mammals can exhibit acute adaptive responses (e.g. metabolic rate depression, cell preservation or decrease in immune system function) to drastic changes in the environment by undergoing a state of natural torpor, termed hibernation (Bouma et al., 2010; Storey, 2010). Therefore, the environment stretches the potential capabilities of an organism's genotype to adapt and respond by exhibiting a dynamic range of genetic and cell-biological responses during development and aging, which can differentially affect survival outcomes throughout life.

However, very little is known about how the plasticity of biological processes upon environmental changes is regulated at the genetic and cell-biological levels during development and aging, and how it affects survival. In the next sections, I briefly overview the current knowledge on relevant aspects of development and aging in *C. elegans*, and integrate that knowledge in the context of other organisms.

1.4.2. Molting

Molting has evolved as a mechanism adopted by many species to provide the means for increased reproductive success, and consequently, for population survival. Nematodes and arthropods, together with other members of the animal clade Ecdysozoa (Aguinaldo et al., 1997), share the ability to undergo molting. Arthropods are among the most successful organisms on earth in ecological terms. This achievement is in part due to the advantage of having an external skeleton, termed cuticle or exoskeleton (Ewer, 2005; Page and Johnstone, 2007). The cuticle is a relatively rigid structure to which epidermis and muscles are attached. Therefore, these animals need to replace their old cuticle with a newly secreted one (i.e. molt) in or-

der to grow. Apart from contributing to progress in biological research, the study of molting in *C. elegans* can lead to advances in our understanding of dermatological processes at the cellular and genetic levels, with clear parallels in higher organisms.

Molting provides clear evolutionary and biological advantages in *C. elegans* in the context of survival. For instance, the ability to form the long-lived, stress-resistant dauer larva in unfavorable environments requires that molting be tightly coordinated (see Dauer Formation section below). Similarly, when the environment is favorable, four molts occur at the end of each larval stage (L1–L4) between hatching and adulthood (Figure 2) (Singh and Sulston, 1978). Before each molt, animals experience a gradual decrease in activity and feeding (lethargus) (Raizen et al., 2008). Then, the old cuticle separates from the epidermis (apolysis), while *de novo* synthesized components are deposited to form the new cuticle. Next, the worm completes apolysis by performing fast rotations around its longitudinal axis. Finally, the worm sheds and emerges from the old cuticle (ecdysis), and completes the molting process (Page and Johnstone, 2007).

The molecular control of molting is best known in insects. In brief, secretion of the neuropeptide prothoracicotropic hormone from the brain stimulates synthesis and secretion of the steroid hormone ecdysone, which is converted into the active hormone 20-hydroxyecdysone. This active hormone is the key regulator of molting in insects. 20-hydroxyecdysone stimulates transcriptional pathways that regulate molting by forming a complex with the ecdysone receptor (EcR) and ultraspiracle (USP) (Dubrovsky, 2005; Ewer, 2005). This signaling cascade involves the timely activation of members of the conserved nuclear receptor family of proteins (Ashburner, 1974; Huet et al., 1995; Sullivan and Thummel, 2003).

Comparatively less is known about the molecular control of molting in *C. elegans*. The *C. elegans* genome does not encode a homolog of EcR or of USP (Ewer, 2005; Magner and Antebi, 2008). Among the genes that affect the process of molting identified by genome-wide RNAi screens (Frand et al., 2005), no obvious equivalent to 20-hydroxyecdysone has been found. However, it is known that both cholesterol and steroid hormones are required to trigger molting (Entchev and Kurzchalia, 2005; Kuervers et al., 2003; Yochem et al., 1999), by a mechanism that likely operates through regulation of nuclear hormone receptors (Gissendanner and Sluder, 2000; Kostrouchova et al., 2001; Magner and Antebi, 2008). The process of ecdysis involves considerable tissue remodeling, which is facilitated by metalloproteases and other proteases (Brooks et al., 2003; Davis et al., 2004). In addition, different tissues have been shown to regulate molting (Frand et al., 2005). For instance, lethargus is regulated by epidermal growth factor signaling in neurons

(Van Buskirk and Sternberg, 2007), while proteins in the muscle dense bodies were recently shown to regulate apolysis (Zaidel-Bar et al., 2010).

The degree of environmental sensitivity of the molting process in *C. elegans* has not been examined in detail. *A priori*, the fact that many different tissues are involved in the control of molting (Frand et al., 2005), suggests that the process is robustly regulated and probably insensitive to environmental changes. Moreover, complete removal of exogenously supplied cholesterol can stop growth at early larval stages (Merris et al., 2003), indicating that the plasticity of this essential process might be limited.

1.4.3. Dauer Formation

Many vertebrate and invertebrate species have acquired the ability to endure periods of reduced metabolism and enhanced stress tolerance, which favor long-term survival when environmental conditions worsen (MacRae, 2010; Storey, 2010). This strategy of reversibly arresting into a hypometabolic state for survival is a common characteristic of hibernating and diapausing organisms (Storey and Storey, 2004). Hibernation has been reported in different groups of mammals (Geiser, 2004), while mostly invertebrates such as insects and nematodes undergo diapause (MacRae, 2010). The molecular mechanisms that regulate the dauer diapause of nematodes, including that of *C. elegans*, have been proposed to be conserved across phyla. Hence, knowledge obtained from studies on hypometabolism and cell preservation of the *C. elegans* dauer diapause, could potentially aid in developing new strategies for medical routines, such as organ transplantation or surgery (Storey, 2010).

The dauer diapause has evolved as an alternative developmental stage intended for increased survival in adverse environmental conditions. The advantages of this survival mechanism have proven successful, since it has been adopted by diverse nematode species (Cassada and Russell, 1975; Yarwood and Hansen, 1969). Under favorable conditions, the worm develops from embryo to adult through four larval stages (L1–L4) (Figure 2) (Singh and Sulston, 1978). If exposed to adverse conditions early during development (i.e. around the late L1 stage), animals divert development to form the dauer larva (Cassada and Russell, 1975). After the L1-to-L2 molt, worms already undergoing morphological and metabolic changes toward becoming dauers, develop to a distinct pre-dauer L2 stage (L2d) (Figure 2). Although L2d larvae are programmed to develop into dauer larvae, L2d larvae can develop into normal L3 larvae if environmental conditions improve (Riddle et al., 1997; Wood, 1988). Once formed, dauers can remain developmentally arrested

for several months, until favorable conditions are encountered. Upon return to favorable environments, commitment to dauer recovery occurs, the animal resumes growth and molts into a post-dauer L4 larva (Figure 2) (Cassada and Russell, 1975; Golden and Riddle, 1984).

Dauer formation causes broad, tightly regulated changes in the whole body (Riddle et al., 1997). Morphologically, dauers appear thinner than their L3 larva counterparts, their pharynxes are constricted (i.e. feeding is suppressed), and posses a specialized cuticle with longitudinal ridges called alae adapted for fast movement. With regard to their metabolic changes, a shift towards anaerobic fermentation occurs during dauer formation (Holt and Riddle, 2003; Vanfleteren and De Vreese, 1996) and dauers accumulate fat and carbohydrate reserves (Riddle et al., 1997). In general, dauer larvae are resistant to many forms of stress, such as starvation, heat, oxidative stress or desiccation.

Genetic studies and laser-mediated cell ablations have clarified the function of specific sensory neurons and neuroendocrine signaling pathways in the regulation of dauer formation (reviewed in (Braendle et al., 2008; Fielenbach and Antebi, 2008; Hu, 2007). During dauer formation, genes characteristic of reproductive growth are inactivated, while other genes important for survival are upregulated (Burnell et al., 2005; Wang and Kim, 2003). Initial genetic screens identified two opposite phenotypes with respect to dauer formation (Gottlieb and Ruvkun, 1994; Riddle et al., 1981; Thomas et al., 1993; Vowels and Thomas, 1992). Dauer formation defective mutants (Daf-d) show decreased sensitivity toward adverse environmental conditions and bypass dauer formation. On the other hand, dauer formation constitutive mutants (Daf-c) commit to dauer formation even under favorable conditions.

Different neuroendocrine signaling pathways that regulate dauer formation have been identified. The early steps of the signaling cascade take place in specific sets of amphid sensory neurons (Figure 3) (Bargmann and Horvitz, 1991). These neurons respond and process environmental stimuli that govern dauer formation, such as food, temperature and pheromone (Golden and Riddle, 1982; Golden and Riddle, 1984). The sensory neurons ADF, ASI and ASG inhibit dauer entry in favorable environments, while ASJ neurons are required for dauer recovery upon return to favorable external conditions. In addition, ASJ neurons, and mildly ASK neurons, promote dauer entry in unfavorable environments (Schackwitz et al., 1996). The signaling pathways participating in early sensory transduction in these sets of neurons include cilia components and associated regulatory factors (Haycraft et al., 2001; Shakir et al., 1993; Swoboda et al., 2000), G-protein-coupled receptor (GPCR) signaling (Kim et al., 2009; Zwaal et al., 1997) and cyclic guanosine
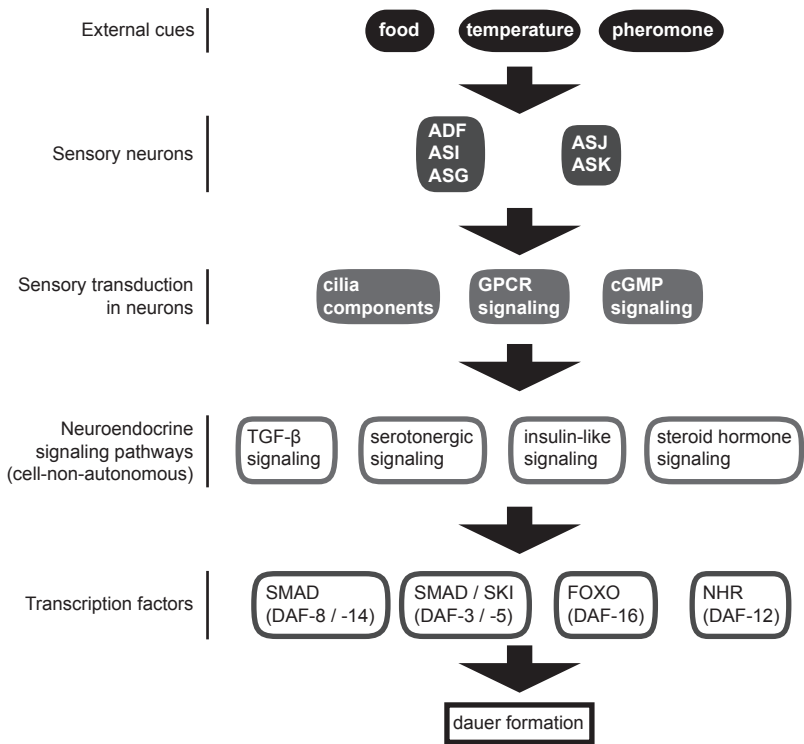
Figure 3. Simplified overview of a model for the regulatory pathways involved in dauer formation. NHR denotes nuclear hormone receptor. See text for details. Figure adapted from (Braendle et al., 2008; Fielenbach and Antebi, 2008).

monophosphate (cGMP) signaling (Figure 3) (Birnby et al., 2000; Schackwitz et al., 1996). These early components of the signaling cascade couple the integrated environmental inputs onto the transforming growth factor β (TGF-β) (Murakami et al., 2001; Ren et al., 1996), insulin-like (Cornils et al., 2011; Kimura et al., 1997; Li et al., 2003), serotonergic (Sze et al., 2000) and steroid hormone (Gerisch and Antebi, 2004; Mak and Ruvkun, 2004) signaling pathways (Figure 3). Next, these neuroendocrine signaling pathways rely on downstream transcriptional regulators, which are responsible for implementing the dauer/non-dauer switch. Important transcription factors that regulate dauer formation include the *C. elegans* homologs of SMAD (DAF-3, -8, -14), (Inoue and Thomas, 2000; Park et al., 2010a; Patterson et al., 1997), SKI (DAF-5) (da Graca et al., 2004), FOXO (DAF-16) (Gottlieb and Ruvkun, 1994; Ogg et al., 1997; Vowels and Thomas, 1992) and nuclear hor-

mone receptors LXR or VDR (DAF-12) (Figure 3) (Antebi et al., 2000; Ludewig et al., 2004; Magner and Antebi, 2008; Snow and Larsen, 2000). Consistent with a neuroendocrine regulatory mechanism, both insulin-like signaling and nuclear hormone receptor pathways have been shown to operate, at least in part, in a cell-non-autonomous fashion (Apfeld and Kenyon, 1998; Gerisch and Antebi, 2004; Mak and Ruvkun, 2004). In addition, the neuroendocrine signaling pathways and transcription factors that regulate dauer formation likely do not function in a simple hierarchical fashion. In fact, they have been proposed to work through feedback mechanisms and molecular cross talk (Gerisch and Antebi, 2004; Lee et al., 2001; Liu et al., 2004; Mak and Ruvkun, 2004; Vowels and Thomas, 1992). However, the mechanisms by which these complex signaling pathways communicate with each other remain largely unknown.

The signaling pathways that regulate the dauer/non-dauer switch during development in response to changes in the environment represent a well-recognized form of plasticity (Braendle et al., 2008; Fielenbach and Antebi, 2008). For instance, temperature and other environmental parameters (e.g. cholesterol or dauer pheromone levels) modulate the penetrance of the Daf phenotypes. Thus, a number of Daf-c mutants only form dauers at moderately elevated temperatures (25°C) (Gems et al., 1998), while certain Daf-d mutants, and even wild type worms, have been shown to form dauers at 27°C (Ailion and Thomas, 2000). Furthermore, although the dauer/non-dauer switch is classically depicted as a decision between two discrete morphs, intermediate morphs, commonly referred to as partial dauers or dauer-like larvae, have also been reported (Ohkura et al., 2003; Vowels and Thomas, 1992). In addition, diapause stages other than dauer, termed L1 diapause and L2 diapause, have also been described (Baugh and Sternberg, 2006; Ruaud and Bessereau, 2006). These few examples reveal the powerful developmental plasticity exhibited by *C. elegans* to cope with adverse environments. Still, many of the regulatory mechanisms that contribute to this developmental plasticity are ripe for discovery.

1.4.4. Aging

Humans have long been interested in extending their lives, although not at any cost: such life extension must also guarantee a delay in age-associated diseases. This basic idea has inspired major research efforts over the last decades, which in turn have provided important new perspectives on how we understand aging as a biological process today. Paradoxically, aging was for many years thought to be a passive, casual event driven by arbitrary deterioration. However, discoveries in lower organisms (including yeast, flies and worms) have identified signaling path-

ways and transcription factors that are involved in the regulation of aging [reviewed in (Haigis and Yankner, 2010; Kenyon, 2005; Kenyon, 2010)]. One could argue that these lifespan pathways shown to act in lower organisms might not regulate aging in more complex animals, such as mammals or humans. Interestingly, the effect of many of these signaling pathways on adult lifespan has been shown to be evolutionarily conserved (Harrison et al., 2009; Jia et al., 2004; Kaeberlein et al., 2005; Kapahi et al., 2004). Hence, new findings obtained from aging studies in *C. elegans*, could provide the knowledge necessary to develop drugs that may extend youthfulness and adult lifespan (Kenyon, 2010).

DR is an environmental intervention that extends lifespan in many species, from yeast to mammals (Klass, 1977; Lin et al., 2002; Loeb and Northrop, 1917; McCay et al., 1935), and corresponds to a reduction in food intake without malnutrition. Sensory neurons of *C. elegans* (Bishop and Guarente, 2007b; Park et al., 2010b) and *D. melanogaster* (Libert et al., 2007) have been proposed to play important roles in regulating DR-mediated lifespan extension. In particular, nutritional deficit cues are sensed by sensory neurons, which trigger a global induction of mitochondrial respiration through activation of neuroendocrine signals (Bishop and Guarente, 2007a).

During the process of aging, a number of nutrient-sensing pathways regulate the function of stress response genes. These nutrient-sensing pathways include sirtuins, AMP-activated protein kinase (AMPK), the insulin-like signaling pathway and the target of rapamycin (TOR) signaling pathway (Haigis and Yankner, 2010; Kenyon, 2010). The molecules involved in these pathways operate by sensing physiological changes, such as energy status, hypoxia or DNA and protein damage. DR has been shown to induce differential up- or downregulation of each of these nutrient-sensing pathways. This effect ultimately increases global stress resistance against subsequent stress, or nutritional deficit, and results in extended lifespan (Haigis and Yankner, 2010; Kenyon, 2010; Ristow and Zarse, 2010). Interestingly, studies performed in *C. elegans* suggest that the requirement for each nutrient-sensing pathway to extend lifespan varies depending on the DR protocol used (Figure 4) (Greer and Brunet, 2009; Kenyon, 2010). In particular, extreme, or moderate, life-long DR extends lifespan through TOR inhibition, or sirtuin activation, respectively (Hansen et al., 2007; Wang and Tissenbaum, 2006). Moreover, mid-life onset of DR extends lifespan through AMPK activation (Greer et al., 2007), while mid-life onset of intermittent fasting extends lifespan through inhibition of insulin-like signaling (Honjoh et al., 2009). The hormetic response elicited by DR induces global stress resistance and repair pathways, which include mitochondrial respiratory
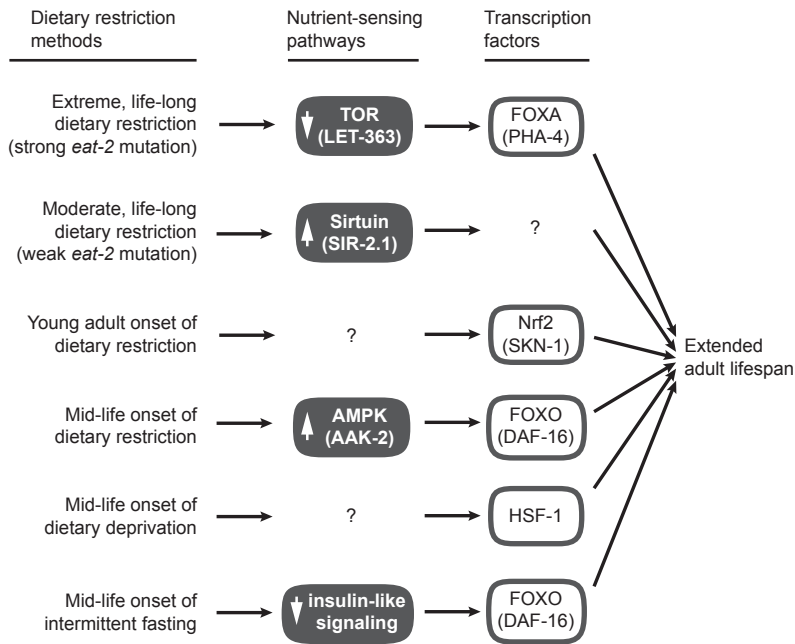
Figure 4. Different dietary restriction methods extend lifespan through specific nutrient-sensing pathways and transcription factors in *Caenorhabditis elegans*. *eat-2* mutants represent a classical genetic dietary restriction method in the worm (Lakowski and Hekimi, 1998). These mutants exhibit reduced food intake throughout life because of a pharyngeal pumping defect (Avery, 1993; Raizen et al., 1995). Different *eat-2* mutant alleles display the feeding-defective phenotype at varying strengths, resulting in either strong or weak (Avery, 1993). See text for details. Figure adapted from (Greer and Brunet, 2009; Kenyon, 2010).

metabolism, protein homeostasis, DNA damage repair and autophagy (Haigis and Yankner, 2010; Kenyon, 2010; Ristow and Zarse, 2010). In *C. elegans*, the changes in gene expression triggered by DR have been found to be mediated by a number of transcription factors (Figure 4), including the FOXO homolog DAF-16 (Greer et al., 2007; Honjoh et al., 2009), the FOXA homolog PHA-4 (Hansen et al., 2008; Panowski et al., 2007), the nuclear factor erythroid-derived (Nrf2) homolog SKN-1 (Bishop and Guarente, 2007b) and the heat shock factor family homolog HSF-1 (Steinkraus et al., 2008). DAF-16, HSF-1 and SKN-1 have been found to regulate aging cell-non-autonomously, suggesting that their effect likely involves neuroendocrine signaling (Bishop and Guarente, 2007b; Libina et al., 2003; Morley and Morimoto, 2004). Thus, cell protection and maintenance mechanisms can be closely coordinated by coupling DR to neuroendocrine signaling during aging (Park et al.,

2010b). In addition, feedback communication among tissues and molecular cross-talk likely favor this level of coordinated action (Libina et al., 2003; Murphy et al., 2003; Tullet et al., 2008). However, the mechanisms by which these nutrient-sensing pathways and transcription factors interplay and communicate in various tissues to regulate DR-mediated lifespan extension remain largely unknown.

The plasticity exhibited by the processes that regulate aging, is not unique to DR-mediated lifespan extension (cf. above). In addition, other external stimuli, such as chemical agents and temperature, can modify the rate of aging in a co-ordinated and plastic manner (Kenyon, 2005). For instance, some DR mimetics have been reported to extend lifespan in *C. elegans* and other species (Ingram et al., 2006). One of these DR mimetics, the anti-diabetic drug metformin, has recently been studied in *C. elegans* (Onken and Driscoll, 2010). In this report, increasing concentrations of metformin have been shown to extend lifespan, and this lifespan extension requires AMPK and SKN-1 function. Moreover, it has been recently shown that adult lifespan is very sensitive to changes in temperature, and that this process is orchestrated in *C. elegans* thermosensory neurons (Lee and Kenyon, 2009). In summary, the importance of these plastic responses to nutritional deficit, DR mimetics and temperature is sustained by the fact that these responses are not mere passive consequences of environmental changes, but, instead, are influenced by regulatory processes that contribute to such plasticity.

These examples show that lifespan, and consequently aging, retain a remark-able level of plasticity in *C. elegans*. The genetic and cell-biological processes that control adult lifespan and aging are considerably conserved across species. Thus, the plastic attributes of *C. elegans* favor the use of this animal model as a powerful *in vivo* platform to provide innovative insights into the biology of the thioredoxin and related systems and their impact on the aging process.

2. AIMS OF THIS THESIS

The main aspiration of this thesis has been to establish the invertebrate model organism *Caenorhabditis elegans* as a novel *in vivo* experimental platform to elucidate the role of the thioredoxin system in general physiology, survival and aging.

Specifically, the aims have been to:

Paper I: First, undertake a systematic survey of the *C. elegans* genome to identify putative homologs of the mammalian thioredoxin and related systems. Subsequently, accomplish at the molecular, cellular and genetic levels the initial characterization of the *C. elegans* thioredoxin gene *trx-1*, whose encoded protein has the highest amino acid identity compared to human Trx1.

Paper II: First, investigate whether the thioredoxin reductase TRXR-1, the only selenoprotein found in *C. elegans*, is involved in general protection against oxidative stress. Subsequently, understand how TRXR-1 functions together with the single glutathione reductase protein found in the worm, GSR-1, to regulate molting.

Paper III: Test whether the *C. elegans* thioredoxin protein TRX-1 participates in survival mechanisms associated with the ASJ sensory neurons. Therefore, the initial goal has been to determine whether it plays a role in formation of the stress resistant, long-lived dauer larva, a developmental stage triggered during unfavorable conditions.

Paper IV: Understand the mechanisms by which the thioredoxin protein TRX-1 regulates aging in *C. elegans*. More specifically, the goal has been to test whether TRX-1 regulates adult lifespan extension induced by dietary restriction, an environmental intervention known to extend lifespan in diverse model systems.

3. RESULTS

3.1. Paper I

Lifespan decrease in a *Caenorhabditis elegans* mutant lacking TRX-1, a thioredoxin expressed in ASJ sensory neurons.

Growing evidence shows that the thioredoxin system is implicated in the regulation of multiple aspects of normal physiology, pathology and aging in higher organisms. However, the *in vivo* mechanisms underlying these processes remain unclear, since knocking out components of the thioredoxin system in mammals results in lethality during embryogenesis, as mentioned above. In this paper, we make use of the invertebrate animal model *C. elegans* to perform the initial description of the thioredoxin system, and in particular of the thioredoxin gene *trx-1*, at the biochemical, cell biological and genetic levels.

First, using multiple sequence alignment methods we show that the *C. elegans* genome contains many putative homologs of the mammalian thioredoxin and related systems (summarized in Table 1). These include Trx1, Trx2, Txl-1 and ERdj5, together with the thioredoxin reductases TrxR1 and TrxR2. However, a number of other thioredoxins and related molecules in mammals are not present in the *C. elegans* genome. Examples of these are the testis-specific thioredoxin proteins SpTrx1, SpTrx2, SpTrx3 and Txl-2, and the testis-specific thioredoxin glutathione reductase TGR.

Following this initial overview of the *C. elegans* thioredoxin and related systems, we have focused on the *C. elegans* gene *trx-1*, since the protein it encodes has the highest amino acid identity compared to human Trx1. It had previously been reported in WormBase (http://www.wormbase.org), that *trx-1* consists of two splice variants: *trx-1a* and *trx-1b*. However, no experimental proof had been reported to demonstrate that the two splice variants are transcribed into mRNA. Thus, we decided to analyze the 5' and 3' UTRs of the two *trx-1* splice variants, by using RT-PCR and 5' RACE. We found that the two splice variants are indeed transcribed.

To further examine whether both splice variants are translated into proteins, we performed Western blots on worm extracts using specific antibodies for the proteins TRX-1a and TRX-1b. Detection of only TRX-1b, but not of TRX-1a, in worm extracts of transgenic worms expressing the *trx-1::GFP* translational fusion, suggests that the TRX-1b protein is the main product translated in worms. We next performed a classical enzymatic activity assay (Luthman and Holmgren, 1982), to investigate whether *C. elegans* TRX-1b can reduce disulfide bonds *in vitro*. As

Table 1. Thioredoxins and related proteins in humans, with their corresponding *C. elegans* homologs.

Human protein	*C. elegans* homolog
Trx1	TRX-1
Trx2	TRX-2
Grx1	GLRX-10
Grx2	GLRX-21, -22
Grx3	D2063.3
Grx5	GLRX-5
SpTrx1	n.f.
SpTrx2	n.f.
SpTrx3	n.f.
PDIA1	PDI-1, -2
PDIA3	PDI-3
ERdj5	DNJ-27
Txl-1	Y54E10A.3
Txl-2	n.f.
RdCVF	n.f.
Nrx*	C32D5.8
TrxR1	TRXR-1
TrxR2	TRXR-2
GSR	GSR-1
TGR	n.f.

Not all *C. elegans* Trx-like proteins are shown. Source: WormBase (http://www.wormbase.org) and NCBI (http://www.ncbi.nlm.nih.gov) web sites; except for *, (Funato and Miki, 2007). n.f., not found. See text for further details.

expected, this assay showed that TRX-1b retains its disulfide-reducing enzymatic activity.

We then asked whether *C. elegans trx-1* is expressed in all tissues, as is its human counterpart (Lillig and Holmgren, 2007). For that purpose, we analyzed the expression pattern of transgenic lines expressing a *trx-1::GFP* translational fusion. Interestingly, the expression pattern of *trx-1* is limited to a pair of neurons in the head of the worm: the ASJ sensory neurons. This expression pattern is consistent throughout life, from embryo to adult.

ASJ neurons participate in the regulation of dauer larva formation and in the control of aging (Alcedo and Kenyon, 2004; Bargmann and Horvitz, 1991; Schackwitz et al., 1996). To understand the role of TRX-1 in these functions associated with ASJ neurons, we analyzed *trx-1* mutants carrying the *ok1449* allele. Using Western

blot analyses we determined that the *ok1449* allele is a null mutation. While *trx-1(ok1449)* animals are similar to wild type with regard to dauer formation phenotypes, their adult lifespan is shorter than that of wild type. Moreover, wild-type animals overexpressing *trx-1::GFP* in ASJ neurons show extended adult lifespan.

In conclusion, TRX-1 arises as the first thioredoxin reported in animals that is expressed solely in neurons. In addition, the shortened lifespan phenotype exhibited by *trx-1(ok1449)* mutants endorses the use of *C. elegans* as a model organism to further investigate the *in vivo* functions of thioredoxins during stress and aging.

3.2. Paper II

Selenoprotein TRXR-1 and GSR-1 are essential for removal of old cuticle during molting in *Caenorhabditis elegans*

Although mammalian thioredoxin reductase TrxR1 has been proposed to protect against oxidative damage accumulated during aging (Arnér, 2009), its *in vivo* function is still unknown. Exposure of mammalian epidermis to different tumorigenic agents induces thioredoxin reductase activity (Kumar and Holmgren, 1999; Schallreuter and Wood, 2001). However, its role in the epidermis remains unclear. In this paper, we show how the *C. elegans* thioredoxin reductase TRXR-1 functions, in combination with the glutathione reductase GSR-1, to regulate both apolysis (i.e. separation of old and new cuticle) and ecdysis (i.e. shedding and emergence from the old cuticle) during molting.

We investigated the possibility that molting requires reduction of disulfide bonds in cuticle components to proceed. To understand this question, we made use of four different externally applied reagents during molting and at intermolt (i.e. the period between two consecutive molts). Using the thiol-reactive fluorescent reporter Alexa Fluor 488 C5 maleimide (AF488CM) (Sahaf et al., 2003), the thiol-reducing agent dithiothreitol (DTT) (Cleland, 1964), the thiol-blocking agent N-ethylmaleimide (NEM) (Cadenas et al., 1961), and the thiol-oxidizing agent diamide (Kosower et al., 1969), we find that reduction of disulfide bonds in cuticle components is required for molting to succeed.

We then tested whether the thioredoxin reductase TRXR-1, the sole selenoprotein in *C. elegans* (Buettner et al., 1999; Gladyshev et al., 1999; Taskov et al., 2005), is required for the reduction of disulfide bonds in cuticle components during molting. Since oxidative stress sensitivity and molting appeared to be normal for *trxr-1(sv47)* null mutants, we investigated whether TRXR-1 acts together with other redox proteins to regulate molting. We only observed growth arrest specifically at molt when *trxr-1(sv47)* animals were subjected to RNAi of the single glutathione reductase gene *gsr-1*. Further examination of *trxr-1(sv47); gsr-1(RNAi)* animals by using DTT and AF488CM supports that TRXR-1, together with GSR-1, are required for the reduction of cuticle components during molting. In addition, using GFP reporters, we observed that both *trxr-1* and *gsr-1* are expressed in the hypodermis and in the pharynx. Moreover, *trxr-1* is also expressed in the nervous system, while *gsr-1* is not. Hypodermis and pharynx are involved in the secretion of cuticle components during molting (Frand et al., 2005; Page and Johnstone, 2007). Using tissue-specific promoters to genetically rescue the associated molting arrest

phenotypes, we arrived at the conclusion that both TRXR-1 and GSR-1 are required in pharynx and hypodermis (but not in the nervous system) for molting to succeed.

One could argue that the growth arrest observed in *trxr-1(sv47); gsr-1(RNAi)* is due to synthesis defects of cuticle components during molting. To investigate this possibility, we performed a comparative analysis of the phenotypes exhibited by mutants for the *C. elegans* protein disulfide isomerase genes *pdi-2* and *pdi-3*, which manifest synthesis defects of cuticle components (Winter et al., 2007), and those observed in *trxr-1(sv47); gsr-1(RNAi)* animals. Together with electron microscopy studies of the cuticle, the comparative analysis suggests that cuticle synthesis is not disrupted in *trxr-1(sv47); gsr-1(RNAi)* animals. Similarly, expression of a GFP reporter for the cuticle components QUA-1 (Hao et al., 2006) and LON-3 (Nyström et al., 2002) in *trxr-1(sv47); gsr-1(RNAi)* animals appear to be normal. These findings suggest that TRXR-1 and GSR-1 regulate molting by means other than affecting synthesis of cuticle components during molting.

Since glutathione (GSH) is an important target molecule for the thioredoxin system (Kanzok et al., 2001; Muller, 1996), we next tested on the assumption that GSH might be one of the reducing agents acting downstream of both TRXR-1 and GSR-1 to promote molting success. Exposing wild-type and *trxr-1(sv47); gsr-1(RNAi)* worms to exogenously administered GSH, we could observe that this small molecule not only is essential for ecdysis, but also for apolysis. These findings were supported by staining with AF488CM and by inactivating the gene *gcs-1*, which is implicated in glutathione synthesis (An and Blackwell, 2003).

Using ^{75}Se-labeled *Escherichia coli* bacteria to grow *C. elegans trxr-1* deletion mutants and wild-type animals, we could confirm that the *C. elegans* gene *trxr-1* encodes a selenoprotein (Buettner et al., 1999; Gladyshev et al., 1999; Taskov et al., 2005). Next, we wanted to understand how crucial is selenocysteine for TRXR-1 function in the worm. For this purpose, we designed a strategy to indirectly block incorporation of selenocysteine into *C. elegans* TRXR-1. To do this, we fed wild-type and *gsr-1(RNAi)* worms with an *E. coli* strain unable to incorporate selenocysteine itself (*selD*⁻) (Leinfelder et al., 1990). Furthermore, we performed phenotypic and biochemical studies on a deletion mutant of *selb-1*, the *C. elegans* homolog of the selenoprotein-specific elongation factor SelB/EFsec (Fagegaltier et al., 2000). We also analyzed the consequences of replacing selenocysteine with cysteine, both in living *C. elegans* and in the classical insulin reduction assay (Holmgren and Bjornstedt, 1995). Together, these studies show that selenocysteine incorporation is essential for TRXR-1 function *in vivo* and *in vitro*.

C. elegans provides a unique toolbox to test whether molting is less efficient

in aged animals. This is represented by the postdauer molt: long-lived dauer larvae undergo the postdauer molt when good environmental conditions are restored. Analyzing the effect of silencing *gsr-1* by RNAi in wild-type and *trxr-1* (or *selb-1*) mutant young dauers, we could anticipate that TRXR-1 and GSR-1 are also required for the postdauer molt. Comparing the ability of young and old wild-type dauers to proceed through molt and resume growth upon food availability, we could deduce that age and molting efficiency are inversely correlated. Similarly comparing the levels of AF488CM staining in young and old dauers, we could see that age correlates with a decrease in sulfhydryl groups in the cuticle. We observed a similar inverse correlation between age and the expression levels of a *gsr-1::GFP* reporter in the hypodermis. Together, these findings suggest that molting is less efficient in aged animals and that this reduced efficiency correlates with a decrease in TRXR-1 and GSR-1 function.

In conclusion, our findings suggest a novel regulatory role for the selenoprotein TRXR-1 and GSR-1 in apolysis and ecdysis during molting in *C. elegans*, and that their contribution to the removal of old cuticle strongly decreases with age.

3.3. Paper III

The thioredoxin TRX-1 modulates the function of the insulin-like neuropeptide DAF-28 during dauer formation in *Caenorhabditis elegans*

For Paper I, we had performed the initial characterization of the *C. elegans* thioredoxin TRX-1. We decided to explore the possibility that TRX-1 participates in regulatory functions associated with ASJ neurons. In particular, we examined whether TRX-1 regulates formation of the stress-resistant, long-lived dauer larva in the worm.

First, we asked whether *trx-1* affects dauer formation. Since *trx-1(ok1449)* null mutants appear to be normal for dauer formation, we investigated whether TRX-1 affects the function of genes required for dauer formation. We analyzed the effects of *trx-1(ok1449)* on the dauer formation constitutive (Daf-c) phenotypes caused by mutation in members of the three classical pathways that regulate dauer formation in *C. elegans* (Fielenbach and Antebi, 2008; Hu, 2007). Combining the *trx-1(ok1449)* allele with mutations in the genes *daf-11* and *tax-4*, members of the cGMP signaling pathway, we could show that loss of *trx-1* causes a synthetic Daf-c phenotype. In addition, these observations also suggest that TRX-1 affects dauer formation mostly independently of the cGMP signaling pathway. We then analyzed genetic interactions of *trx-1(ok1449)* with *daf-c* mutations in the TGF-β signaling pathway, which suggests that TRX-1 affects dauer formation also independently of the TGF-β signaling pathway.

Next, we tested whether TRX-1 affects the insulin-like signaling pathway for dauer formation. We first analyzed double mutants of *trx-1* with the insulin-like neuropeptide gene *daf-28*. The latter was anticipated to be a good candidate for the genetic interaction with *trx-1* because both genes are expressed in ASJ neurons [Paper I; (Li et al., 2003)]. Our genetic interaction studies suggest that TRX-1 function is required for the Daf-c phenotype of *daf-28* mutants. Analysis of double and triple mutants of *trx-1* with the insulin-like receptor gene *daf-2* showed that TRX-1 requirement for the Daf-c phenotype of *daf-28* mutants depends on DAF-2 signaling. These results were confirmed by analyzing the genetic interaction of *trx-1(ok1449)* with mutations in the gene *pdk-1*. This gene encodes a homolog of the mammalian Akt/PKB kinase PDK1 (Paradis et. al., 1999), which regulates dauer formation by transducing DAF-2 signals onto DAF-16, a member of the FOXO family of transcription factors.

We next attempted to validate whether the genetic interactions observed

between *trx-1(ok1449)* and *daf-28* mutants were caused by loss of *trx-1*. For this purpose, we performed genetic rescue experiments by expressing the *trx-1::GFP* translational fusion from an array of tissue-specific promoters. Our findings suggest that loss of *trx-1* is indeed responsible for the genetic interactions observed between *trx-1(ok1449)* and *daf-28* mutants, and that TRX-1 functions specifically in ASJ neurons for dauer formation. We also analyzed the consequences of replacing *trx-1:GFP* active-site cysteines with serines for the genetic rescue experiments. Interestingly, these data suggest that TRX-1 does not require its redox activity for dauer formation.

We completed our genetic interaction studies with a cell-biological approach, in which we measured GFP fluorescence intensity in ASJ or ASI neurons for *trx-1* and *daf-28*. Comparing the GFP expression levels of *trx-1* and *daf-28* in wild-type and *daf-c* dauers, we could show that *trx-1* and *daf-28* have an opposing expression pattern in dauers, which is not manifested in normally growing L2/L3 larvae. Further analysis of their expression pattern in *trx-1* and *daf-28* mutant dauers, suggests a model in which TRX-1 contributes to the downregulation of *daf-28* expression during dauer formation, a process likely controlled by DAF-28-mediated feedback regulation.

In conclusion, we show for the first time that TRX-1 is implicated in the regulation of dauer formation in *C. elegans*. Our findings suggest that TRX-1 functions in ASJ neurons as a novel modulator of the insulin-like neuropeptide DAF-28 during dauer formation.

3.4. Paper IV

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

For Paper I, we had used *C. elegans* as a model organism to investigate the function of TRX-1 during aging. Despite the fact that thioredoxins have been implicated in the regulation of the pro-longevity effects of dietary restriction (DR), their *in vivo* function remains unknown. For paper IV, we use *C. elegans* to understand *in vivo* the mechanisms elicited by TRX-1 in the nervous system to promote DR-mediated adult lifespan extension.

First, we examined whether TRX-1 regulates DR-mediated adult lifespan extension. For this purpose, we performed epistasis analysis with a mutation in *eat-2*, which represents a classical genetic model of DR in *C. elegans* (Lakowski and Hekimi, 1998). We compared the results obtained with *eat-2*, with those seen with mutations in the insulin-like receptor gene *daf-2*, which regulates lifespan independently of *eat-2* (Lakowski and Hekimi, 1998), and the sensory cilia gene *osm-5*, which regulates lifespan partially through DAF-2 signaling (Apfeld and Kenyon, 1999). We completed this genetic analysis by performing rescue studies in which we expressed *trx-1* either in ASJ neurons or in the intestine. Together, our data suggest that TRX-1 regulates adult lifespan extension induced by a genetic model of DR. Our interpretation was confirmed by overexpressing *trx-1* in ASJ neurons of wild type and *eat-2* mutants.

Next, we tested whether TRX-1 is required for adult lifespan extension induced by other, non-genetic DR methods. Since dietary deprivation (DD) and mutation of *eat-2* extend lifespan by influencing the same pathway (Kaeberlein et al., 2006; Lee et al., 2006), we used DD as a representative non-genetic, nutrient-based model of DR. Our findings suggest that TRX-1 regulates adult lifespan extension induced by a non-genetic, nutrient-based model of DR.

We completed the studies described above by measuring fluorescence intensity of a GFP reporter for *trx-1* or the control *gpa-9* (Jansen et al., 1999). These measurements were performed in ASJ neurons of aging wild-type adults subjected to conditions of DD, and compared with siblings fed *ad libitum*. The results obtained suggest that *trx-1* is increased in ASJ neurons of aging adults in response to DR.

In conclusion, we report that TRX-1 is a novel regulator of DR-mediated adult lifespan extension in *C. elegans*. Our data suggest that DR activates TRX-1 in ASJ neurons during aging and consequently triggers TRX-1-dependent mechanisms that promote adult lifespan extension.

4. DISCUSSION

Multiple sequence alignment studies have revealed that the *C. elegans* genome contains many putative homologs of the mammalian thioredoxin system (Table 1 and Paper I; WormBase web site, http://www.wormbase.org). In recent years, we and others have begun to characterize these *C. elegans* thioredoxins and related molecules in order to gain insight into how their conserved biological functions are governed *in vivo*.

The first reports describing the *C. elegans* thioredoxin reductase TRXR-1 date back to 1999 (Buettner et al., 1999; Gladyshev et al., 1999). In these reports, it was demonstrated that the *C. elegans* thioredoxin reductase gene *trxr-1* encodes a selenoprotein. Later on, it was shown that TRXR-1 is the only selenoprotein found in the *C. elegans* genome (Taskov et al., 2005). In Paper II, we show that TRXR-1, together with the glutathione reductase GSR-1, are required for molting in the worm, a process that ensures reproductive success and survival. Subsequently, the biochemical characterization of the mitochondrial thioredoxin reductase TRXR-2 (Lacey and Hondal, 2006), revealed that its cysteine-containing active site is as catalytically active as it was previously reported for the cysteine-containing thioredoxin reductase of *Drosophila melanogaster* (Kanzok et al., 2001). However, apart from the fact that TRXR-2 appears to be dispensable for growth, development and molting in *C. elegans* (Paper II), its biological function is still unknown.

We (Paper I) and others (Jee et al., 2005) show that TRX-1 is implicated in the regulation of aging and oxidative stress resistance in *C. elegans*. The Morcos group recently proposed a linked role for the apurinic/apyrimidinic endonuclease 1 (APE1/Ref-1) homolog EXO-3, the tumor suppressor p53 homolog CEP-1 and thioredoxins TRX-1 and TRX-2 in the regulation of neuronal function and adult lifespan in *C. elegans* (Schlotterer et al., 2010). We propose that TRX-1 acts in ASJ neurons to adjust neuropeptide expression during formation of long-lived, stress-resistant dauer larvae (Paper III). Moreover, we also propose that activation of TRX-1 in ASJ neurons is required for the pro-longevity mechanisms triggered by DR during aging in *C. elegans* (Paper IV). These data together indicate that the thioredoxin TRX-1 likely acts in the nervous system to integrate multiple regulatory pathways devoted to countering the effects of aging and promoting survival in *C. elegans*.

The *C. elegans* genome contains putative homologs of the mammalian glutaredoxins Grx1, Grx2, Grx3 and Grx5 [Table 1; WormBase web site, http://www.wormbase.org; (Lillig et al., 2008)]. So far, only the *C. elegans* glutaredoxin gene

glrx-21, which encodes a homolog of mammalian Grx2, has been studied in detail (Morgan et al., 2010). The Estevez group has thus shown that GLRX-21 is involved in oxidative stress resistance mechanisms induced by selenium in *C. elegans*. On the other hand, we show that the *C. elegans* thioredoxin reductase TRXR-1 is not required for protection against acute oxidative damage (Paper II). In addition, we propose that the thioredoxin TRX-1 likely regulates neuroendocrine signaling locally in ASJ neurons to affect dauer formation and adult lifespan remotely in the whole organism (Papers III and IV). Therefore, thioredoxin reductases and thioredoxins likely play minor roles in protecting against acute oxidative stress. Since glutaredoxins and thioredoxins are genetically redundant for many functions in diverse species (Lillig et al., 2008), it might be possible that the redoxins involved in such global protective function against acute oxidative stress in *C. elegans* are likely glutaredoxins. Altogether, our findings described in Papers I through IV, and those generated in other laboratories (see above), qualify *C. elegans* as a versatile *in vivo* model organism to continue studying the function of thioredoxins and related molecules in general physiology.

Molting, dauer formation and aging are evolving through natural selection toward becoming tightly regulated and hard-wired biological processes. Still, they manage to retain the necessary plasticity to allow for adaptation to a changing environment (Braendle et al., 2008; Fielenbach and Antebi, 2008; Kenyon, 2005). The data presented in this thesis shows that molting, despite its regulatory robustness, retains modest levels of plasticity, which are modulated by TRXR-1 and GSR-1. In addition, our findings confirm the plastic nature of dauer formation and aging and how TRX-1 contributes to such plasticity.

Molting in *C. elegans* ensures that the animal grows and reaches adulthood to contribute to the gene pool. For that reason, it is subject to robust regulation, which occurs at regular intervals throughout development. However, we show in paper II that this regulatory robustness can be modified by the environment, and that modulators like TRXR-1 and GSR-1 participate in this adaptive process. Exogenously supplied GSH (the proposed target for TRXR-1 and GSR-1 during molting) generates a reducing environment, which can promote apolysis at intermolt and ecdysis in arrested worms in a dose-response manner. Moreover, the molting process can be modulated by externally adding the reducing agent DTT, the thiol-blocking agent NEM or the oxidizing agent diamide. Together, these results show that molting in *C. elegans* is a robust process regulated by TRXR-1 and GSR-1, which can become moderately plastic when challenged by changes in the environment.

The developmental switch between forming dauers or non-dauer, L3 larvae

in response to changing environmental conditions represents a classical example of plasticity in *C. elegans* (Braendle et al., 2008; Fielenbach and Antebi, 2008). Similarly, the rate of aging is highly sensitive to variation in the environment and can substantially deviate in response to temperature, chemical agents and dietary regimes (Kenyon, 2010). In papers III and IV, we observe a phenomenon that fulfills the principles of plasticity: *trx-1* expression in ASJ neurons fluctuates in response to signals of nutrient availability and/or overcrowding. This plastic response to changing environments occurs both during dauer formation and during aging (Papers III and IV). In paper IV, we also show that overexpression of *trx-1* has the ability to extend lifespan (cf. Paper I as well), a process that is modulated by the rate of food intake. Similarly plastic is the adult lifespan of wild-type *C. elegans* in response to distinct dietary regimes (Paper IV). Interestingly, this sensitivity shown by wild-type *C. elegans* toward food availability in the environment is abolished by mutation in *trx-1*, suggesting that TRX-1 is an essential contributor to the plasticity exhibited by the processes that regulate aging.

In summary, it is tempting to suggest that studies in lower organisms like *C. elegans* might provide important insights into how the situation is in mammals. The thioredoxin and related systems and the cellular processes they regulate are highly conserved across phyla. In this thesis, we show evidence for the crucial role of conserved members of the thioredoxin system in controlling aging and survival in *C. elegans*. Our work suggests that understanding how animals age and adapt to a changing environment *in vivo* can, at least in parts, be simplified to a cell-biological question. Therefore, deciphering the role of thioredoxins and related molecules in aging and survival in *C. elegans*, might bring us closer to solving that enigma in mammals than commonly thought.

5. CONCLUSIONS

With regard to the aims of this thesis, the main conclusions are summarized below:

Paper I: Multiple sequence alignment shows that the *C. elegans* genome contains many putative homologs of the mammalian thioredoxin system and related molecules. In addition, we show for the first time in any metazoan a thioredoxin gene (*trx-1*) that is expressed only in the nervous system and is involved in the regulation of aging.

Paper II: We show that the selenoprotein, thioredoxin reductase (TRXR-1) does not participate in protection against oxidative stress. Instead, it contributes, together with glutathione reductase (GSR-1), to the regulation of apolysis and ecdysis during molting in *C. elegans*. Our findings suggest a novel molecular mechanism of action for TRXR-1 and GSR-1 in the epidermis: both reductases regulate molting likely by activating GSH function in the cuticle.

Paper III: We demonstrate that the thioredoxin TRX-1 is involved in ASJ neuron-dependent signaling cascades that govern formation of long-lived, stress-resistant dauer larvae in *C. elegans*. Our findings suggest that redox-independent functions of TRX-1 in ASJ neurons are necessary to modulate neuropeptide expression, including that of the insulin-like neuropeptide gene *daf-28*, during dauer formation in response to a changing environment.

Paper IV: We provide the first *in vivo* observation that a thioredoxin (TRX-1) is necessary for the metabolic changes triggered by dietary restriction (DR) that extend adult lifespan. We are also the first to show that DR upregulates thioredoxin expression in the nervous system. We propose a model whereby TRX-1 activation in ASJ neurons of aging adults constitutes an essential early stimulus for the metabolic pathways that translate DR into adult lifespan extension.

6. FUTURE PERSPECTIVES

A continued effort for the characterization of the thioredoxin system in *C. elegans* will contribute to clarify important aspects of biology that need to be elucidated *in vivo*. In the following paragraphs, I provide suggestions that could constitute the foundation for new studies, based on ideas that derive from the work presented in this thesis.

In Papers I through IV, we have experimentally examined *in vivo* the biological functions of thioredoxin TRX-1, thioredoxin reductase TRXR-1 and glutathione reductase GSR-1 in *C. elegans*. Our contributions clearly provide new insights into the biology of thioredoxins and related molecules *in vivo* and their interactions with the surrounding environment. However, they might only constitute the tip of the iceberg. Meanwhile, other laboratories have mostly focused on the biochemical characterization of the different members of the thioredoxin system in *C. elegans*, as already discussed in a previous section. Therefore, future work is still needed to dissect the remaining putative homologs of the mammalian thioredoxin and related systems in *C. elegans* (Table 1). These include Trx2, TrxR2, Txl-1, ERdj5, and glutaredoxins Grx1, Grx3 and Grx5 among others.

In Paper I, we have performed an initial characterization of the two splice variants of thioredoxin *trx-1*. Analysis of the data presented in this thesis, prompted the following questions: Why a thioredoxin gene expressed in a sole pair of neurons has two splice variants? Could one of them be relevant during larval development and the other act during aging? Could it be that one of them acts locally in ASJ neurons, while the other is secreted [cf. Trx80 (Pekkari et al., 2000; Pekkari and Holmgren, 2004)] to affect distant tissues? The fact that only TRX-1b protein was detected in worm extracts (Paper I), suggests that the alternatively spliced *trx-1a* mRNA might not translate into a gene product. Instead, the *trx-1a* mRNA variant could participate in regulatory mechanisms devoted to control the fluctuations of *trx-1b* expression in response to nutrient availability and/or overcrowding (cf. Papers III and IV), in line with previously proposed mechanisms in humans (Berggren and Powis, 2001; Hariharan et al., 1996; Jiménez and Miranda-Vizuete, 2003). Therefore, future work is still needed to understand the differences in function between the two *trx-1* splice variants.

In Paper II, we show that *trxr-1* is expressed in hypodermis, pharynx and nervous system. Our genetic rescue experiments suggest that TRXR-1 function in the pharynx and hypodermis, but not in the nervous system, promotes molting in *C. elegans*. There are a number of cases in multicellular organisms in which a pro-

tein may perform distinct functions in multiple different tissues. For example, it has been shown that the *C. elegans* nuclear factor erythroid-derived (Nrf2) homolog SKN-1 functions in ASI neurons to regulate dietary restriction (DR)-mediated adult lifespan extension, while its role in the intestine is to protect against oxidative stress (Bishop and Guarente, 2007b). Thus, it will be of high interest to determine whether TRXR-1 has two distinct functions in *C. elegans* that are specified by tissue of expression: one in the hypodermis/pharynx and the other in the nervous system.

Although thioredoxins mostly depend on their oxidoreductase activity to perform their functions, they regulate certain biological processes in a redox-independent manner (Berndt et al., 2008; Lillig and Holmgren, 2007; Meyer et al., 2009). We show that *C. elegans* TRX-1 modulates the insulin-like neuropeptide DAF-28 in ASJ neurons during dauer formation through a mechanism independent of its redox activity (Paper III). We also demonstrate that TRX-1 is required for the extended adult lifespan exhibited by animals grown under DR (Paper IV). However, it is still not known whether TRX-1 requires its oxidoreductase activity to regulate adult lifespan in *C. elegans*. Further research will elucidate whether *C. elegans* TRX-1 regulates aging independently of its redox activity.

Genetic rescue studies suggest that TRX-1 acts in ASJ neurons and modifies dauer formation and aging remotely by affecting distant tissues cell-non-autonomously. This cell-non-autonomous effect most likely occurs via a neuroendocrine response (Papers III and IV). Whether the neuroendocrine signal is TRX-1 itself (see above), an ASJ-derived molecule [e.g. DAF-28, INS-1, -9, NLP-3 or FLP-21 (Li and Kim, 2008; Nathoo et al., 2001; Pierce et al., 2001)], or both, still needs to be fully investigated.

We have shown that TRX-1 acts in ASJ neurons to adjust *daf-28* expression during dauer formation (Paper III). In addition, we have also demonstrated that *trx-1* regulates DR-mediated adult lifespan extension (Paper IV). Recent studies suggest that DAF-28 secretion from neurons is modulated by mitochondrial function (Billing et al., 2011). Mitochondrial function regulates aging in *C. elegans* (Feng et al., 2001; Hartman et al., 2001; Tsang et al., 2001), and it has recently been proposed to mediate the pro-longevity effects induced by DR (Bishop and Guarente, 2007b; Schulz et al., 2007). Therefore, integrating our results and those provided by others, it could be possible that nutritional deficit activates TRX-1 in ASJ neurons during dauer formation and aging, to then modify neuroendocrine signaling. Subsequently, these neuroendocrine signals [e.g. mitokines (Durieux et al., 2011)] could activate mitochondrial function in distant tissues (e.g. intestine, pharynx) (Bishop and Guarente, 2007b). Consequently, activation of stress pathways in the

whole organism could maintain long-term stress resistance and thus promote long life in dauers and adults. Further investigation will clarify whether TRX-1 couples DR to increased mitochondrial function in order to promote stress resistance and long life.

7. ACKNOWLEDGMENTS

8. REFERENCES

Aguinaldo, A.M., Turbeville, J.M., Linford, L.S., Rivera, M.C., Garey, J.R., et al. (1997). Evidence for a clade of nematodes, arthropods and other moulting animals. Nature *387*, 489-493.

Ailion, M., and Thomas, J.H. (2000). Dauer formation induced by high temperatures in *Caenorhabditis elegans*. Genetics *156*, 1047-1067.

Alcedo, J., and Kenyon, C. (2004). Regulation of *C. elegans* longevity by specific gustatory and olfactory neurons. Neuron *41*, 45-55.

Altun, Z.F., and Hall, D.H. (2008). Handbook of *C. elegans* Anatomy. In: WormAtlas. http://www.wormatlas.org/hermaphrodite/hermaphroditehomepage.htm

An, J.H., and Blackwell, T.K. (2003). SKN-1 links *C. elegans* mesendodermal specification to a conserved oxidative stress response. Genes Dev *17*, 1882-1893.

Andersen, K.M., Madsen, L., Prag, S., Johnsen, A.H., Semple, C.A., et al. (2009). Thioredoxin Txnl1/TRP32 is a redox-active cofactor of the 26 S proteasome. J Biol Chem *284*, 15246-15254.

Antebi, A., Yeh, W.H., Tait, D., Hedgecock, E.M., and Riddle, D.L. (2000). *daf-12* encodes a nuclear receptor that regulates the dauer diapause and developmental age in *C. elegans*. Genes Dev *14*, 1512-1527.

Antoshechkin, I., and Sternberg, P.W. (2007). The versatile worm: genetic and genomic resources for *Caenorhabditis elegans* research. Nat Rev Genet *8*, 518-532.

Apfeld, J., and Kenyon, C. (1998). Cell nonautonomy of *C. elegans daf-2* function in the regulation of diapause and life span. Cell *95*, 199-210.

Apfeld, J., and Kenyon, C. (1999). Regulation of lifespan by sensory perception in *Caenorhabditis elegans*. Nature *402*, 804-809.

Arnér, E.S. (2009). Focus on mammalian thioredoxin reductases--important selenoproteins with versatile functions. Biochim Biophys Acta *1790*, 495-526.

Ashburner, M. (1974). Sequential gene activation by ecdysone in polytene chromosomes of *Drosophila melanogaster*. II. The effects of inhibitors of protein synthesis. Dev Biol *39*, 141-157.

Avery, L. (1993). The genetics of feeding in *Caenorhabditis elegans*. Genetics *133*, 897-917.

Bargmann, C.I., and Horvitz, H.R. (1991). Control of larval development by chemosensory neurons in *Caenorhabditis elegans*. Science *251*, 1243-1246.

Baugh, L.R., and Sternberg, P.W. (2006). DAF-16/FOXO regulates transcription of *cki-1*/Cip/Kip and repression of *lin-4* during *C. elegans* L1 arrest. Curr Biol *16*, 780-785.

Benham, A.M. (2005). Oxidative protein folding: an update. Antioxid Redox Signal *7*, 835-838.

Berggren, M.M., and Powis, G. (2001). Alternative splicing is associated with decreased expression of the redox proto-oncogene thioredoxin-1 in human cancers. Arch Biochem Biophys *389*, 144-149.

Berndt, C., Lillig, C.H., and Holmgren, A. (2008). Thioredoxins and glutaredoxins as facilitators of protein folding. Biochim Biophys Acta *1783*, 641-650.

Berry, M.J. (2005). Knowing when not to stop. Nat Struct Mol Biol *12*, 389-390.

Billing, O., Kao, G., and Naredi, P. (2011). Mitochondrial function is required for secretion of DAF-28/insulin in *C. elegans*. PLoS ONE *6*, e14507.

Birnby, D.A., Link, E.M., Vowels, J.J., Tian, H., Colacurcio, P.L., et al. (2000). A transmembrane guanylyl cyclase (DAF-11) and Hsp90 (DAF-21) regulate a common set of chemosensory behaviors in *Caenorhabditis elegans*. Genetics *155*, 85-104.

Bishop, N.A., and Guarente, L. (2007a). Genetic links between diet and lifespan: shared mechanisms from yeast to humans. Nat Rev Genet *8*, 835-844.

Bishop, N.A., and Guarente, L. (2007b). Two neurons mediate diet-restriction-induced longevity in *C. elegans*. Nature *447*, 545-549.

Bondareva, A.A., Capecchi, M.R., Iverson, S.V., Li, Y., López, N.I., et al. (2007). Effects of thioredoxin reductase-1 deletion on embryogenesis and transcriptome. Free Radic Biol Med *43*, 911-923.

Bouma, H.R., Carey, H.V., and Kroese, F.G. (2010). Hibernation: the immune system at rest? J Leukoc Biol *88*, 619-624.

Braendle, C., Milloz, J., and Félix, M.A. (2008). Mechanisms and evolution of environmental responses in *Caenorhabditis elegans*. Curr Top Dev Biol *80*, 171-207.

Brenner, S. (1973). The genetics of behaviour. Br Med Bull *29*, 269-271.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. Genetics *77*, 71-94.

Brooks, D.R., Appleford, P.J., Murray, L., and Isaac, R.E. (2003). An essential role in molting and morphogenesis of *Caenorhabditis elegans* for ACN-1, a novel member of the angiotensin-converting enzyme family that lacks a metallopeptidase active site. J Biol Chem *278*, 52340-52346.

Buettner, C., Harney, J.W., and Berry, M.J. (1999). The *Caenorhabditis elegans* homologue of thioredoxin reductase contains a selenocysteine insertion sequence (SECIS) element that differs from mammalian SECIS elements but directs selenocysteine incorporation. J Biol Chem *274*, 21598-21602.

Burnell, A.M., Houthoofd, K., O'Hanlon, K., and Vanfleteren, J.R. (2005). Alternate metabolism during the dauer stage of the nematode *Caenorhabditis elegans*. Exp Gerontol *40*, 850-856.

Cadenas, E., Kaji, H., Park, C.R., and Rasmussen, H. (1961). Inhibition of the insulin effect on sugar transport by N-ethylmaleimide. J Biol Chem *236*, PC63-PC64.

Cassada, R.C., and Russell, R.L. (1975). The dauerlarva, a post-embryonic developmental variant of the nematode *Caenorhabditis elegans*. Dev Biol *46*, 326-342.

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W.W., and Prasher, D.C. (1994). Green fluorescent protein as a marker for gene expression. Science *263*, 802-805.

Cheng, Q., Sandalova, T., Lindqvist, Y., and Arnér, E.S. (2009). Crystal structure and catalysis of the selenoprotein thioredoxin reductase 1. J Biol Chem *284*, 3998-4008.

Cheng, Z., Arscott, L.D., Ballou, D.P., and Williams, C.H., Jr. (2007). The relationship of the redox potentials of thioredoxin and thioredoxin reductase from *Drosophila melanogaster* to the enzymatic mechanism: reduced thioredoxin is the reductant of glutathione in *Drosophila*. Biochemistry *46*, 7875-7885.

Cho, C.G., Kim, H.J., Chung, S.W., Jung, K.J., Shim, K.H., et al. (2003). Modulation of glutathione and thioredoxin systems by calorie restriction during the aging process. Exp

Gerontol 38, 539-548.

Cleland, W.W. (1964). Dithiothreitol, a New Protective Reagent for SH Groups. Biochemistry *3*, 480-482.

Conrad, M. (2009). Transgenic mouse models for the vital selenoenzymes cytosolic thioredoxin reductase, mitochondrial thioredoxin reductase and glutathione peroxidase 4. Biochim Biophys Acta *1790*, 1575-1585.

Conrad, M., Jakupoglu, C., Moreno, S.G., Lippl, S., Banjac, A., et al. (2004). Essential role for mitochondrial thioredoxin reductase in hematopoiesis, heart development, and heart function. Mol Cell Biol *24*, 9414-9423.

Cornils, A., Gloeck, M., Chen, Z., Zhang, Y., and Alcedo, J. (2011). Specific insulin-like peptides encode sensory information to regulate distinct developmental processes. Development *138*, 1183-1193.

Cunnea, P.M., Miranda-Vizuete, A., Bertoli, G., Simmen, T., Damdimopoulos, A.E., et al. (2003). ERdj5, an endoplasmic reticulum (ER)-resident protein containing DnaJ and thioredoxin domains, is expressed in secretory cells or following ER stress. J Biol Chem *278*, 1059-1066.

da Graca, L.S., Zimmerman, K.K., Mitchell, M.C., Kozhan-Gorodetska, M., Sekiewicz, K., et al. (2004). DAF-5 is a Ski oncoprotein homolog that functions in a neuronal TGF beta pathway to regulate *C. elegans* dauer development. Development *131*, 435-446.

Davis, M.W., Birnie, A.J., Chan, A.C., Page, A.P., and Jorgensen, E.M. (2004). A conserved metalloprotease mediates ecdysis in *Caenorhabditis elegans*. Development *131*, 6001-6008.

Dong, M., Bridges, J.P., Apsley, K., Xu, Y., and Weaver, T.E. (2008). ERdj4 and ERdj5 are required for endoplasmic reticulum-associated protein degradation of misfolded surfactant protein C. Mol Biol Cell *19*, 2620-2630.

Dubrovsky, E.B. (2005). Hormonal cross talk in insect development. Trends Endocrinol Metab *16*, 6-11.

Durieux, J., Wolff, S., and Dillin, A. (2011). The cell-non-autonomous nature of electron transport chain-mediated longevity. Cell *144*, 79-91.

Ellgaard, L., and Ruddock, L.W. (2005). The human protein disulphide isomerase family: substrate interactions and functional properties. EMBO Rep *6*, 28-32.

Ellis, H.M., and Horvitz, H.R. (1986). Genetic control of programmed cell death in the nematode *C. elegans*. Cell *44*, 817-829.

Entchev, E.V., and Kurzchalia, T.V. (2005). Requirement of sterols in the life cycle of the nematode *Caenorhabditis elegans*. Semin Cell Dev Biol *16*, 175-182.

Ewer, J. (2005). How the ecdysozoan changed its coat. PLoS Biol *3*, e349.

Fagegaltier, D., Hubert, N., Yamada, K., Mizutani, T., Carbon, P., et al. (2000). Characterization of mSelB, a novel mammalian elongation factor for selenoprotein translation. EMBO J *19*, 4796-4805.

Feng, J., Bussiere, F., and Hekimi, S. (2001). Mitochondrial electron transport is a key determinant of life span in *Caenorhabditis elegans*. Dev Cell *1*, 633-644.

Fernandes, A.P., and Holmgren, A. (2004). Glutaredoxins: glutathione-dependent redox enzymes with functions far beyond a simple thioredoxin backup system. Antioxid Redox

Signal *6*, 63-74.

Fielenbach, N., and Antebi, A. (2008). *C. elegans* dauer formation and the molecular basis of plasticity. Genes Dev *22*, 2149-2165.

Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., et al. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature *391*, 806-811.

Fomenko, D.E., and Gladyshev, V.N. (2003). Identity and functions of CxxC-derived motifs. Biochemistry *42*, 11214-11225.

Fontana, L., Partridge, L., and Longo, V.D. (2010). Extending healthy life span--from yeast to humans. Science *328*, 321-326.

Frand, A.R., Russel, S., and Ruvkun, G. (2005). Functional genomic analysis of *C. elegans* molting. PLoS Biol *3*, e312.

Funato, Y., Michiue, T., Asashima, M., and Miki, H. (2006). The thioredoxin-related redox-regulating protein nucleoredoxin inhibits Wnt-beta-catenin signalling through dishevelled. Nat Cell Biol *8*, 501-508.

Funato, Y., and Miki, H. (2007). Nucleoredoxin, a novel thioredoxin family member involved in cell growth and differentiation. Antioxid Redox Signal *9*, 1035-1057.

Geisberger, R., Kiermayer, C., Homig, C., Conrad, M., Schmidt, J., et al. (2007). B- and T-cell-specific inactivation of thioredoxin reductase 2 does not impair lymphocyte development and maintenance. Biol Chem *388*, 1083-1090.

Geiser, F. (2004). Metabolic rate and body temperature reduction during hibernation and daily torpor. Annu Rev Physiol *66*, 239-274.

Gems, D., Sutton, A.J., Sundermeyer, M.L., Albert, P.S., King, K.V., et al. (1998). Two pleiotropic classes of *daf-2* mutation affect larval arrest, adult behavior, reproduction and longevity in *Caenorhabditis elegans*. Genetics *150*, 129-155.

Gerisch, B., and Antebi, A. (2004). Hormonal signals produced by DAF-9/cytochrome P450 regulate *C. elegans* dauer diapause in response to environmental cues. Development *131*, 1765-1776.

Gissendanner, C.R., and Sluder, A.E. (2000). *nhr-25*, the *Caenorhabditis elegans* ortholog of *ftz-f1*, is required for epidermal and somatic gonad development. Dev Biol *221*, 259-272.

Gladyshev, V.N., Krause, M., Xu, X.M., Korotkov, K.V., Kryukov, G.V., et al. (1999). Selenocysteine-containing thioredoxin reductase in *C. elegans*. Biochem Biophys Res Commun *259*, 244-249.

Golden, J.W., and Riddle, D.L. (1982). A pheromone influences larval development in the nematode *Caenorhabditis elegans*. Science *218*, 578-580.

Golden, J.W., and Riddle, D.L. (1984). The *Caenorhabditis elegans* dauer larva: developmental effects of pheromone, food, and temperature. Dev Biol *102*, 368-378.

Gottlieb, S., and Ruvkun, G. (1994). *daf-2*, *daf-16* and *daf-23*: genetically interacting genes controlling Dauer formation in *Caenorhabditis elegans*. Genetics *137*, 107-120.

Greer, E.L., and Brunet, A. (2009). Different dietary restriction regimens extend lifespan by both independent and overlapping genetic pathways in *C. elegans*. Aging Cell *8*, 113-127.

Greer, E.L., Dowlatshahi, D., Banko, M.R., Villen, J., Hoang, K., et al. (2007). An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in *C.*

elegans. Curr Biol *17*, 1646-1656.

Haigis, M.C., and Yankner, B.A. (2010). The aging stress response. Mol Cell *40*, 333-344.

Hansen, M., Chandra, A., Mitic, L.L., Onken, B., Driscoll, M., et al. (2008). A role for autophagy in the extension of lifespan by dietary restriction in *C. elegans*. PLoS Genet *4*, e24.

Hansen, M., Taubert, S., Crawford, D., Libina, N., Lee, S.J., et al. (2007). Lifespan extension by conditions that inhibit translation in *Caenorhabditis elegans*. Aging Cell *6*, 95-110.

Hao, L., Mukherjee, K., Liegeois, S., Baillie, D., Labouesse, M., et al. (2006). The hedgehog-related gene *qua-1* is required for molting in *Caenorhabditis elegans*. Dev Dyn *235*, 1469-1481.

Hariharan, I.K., and Haber, D.A. (2003). Yeast, flies, worms, and fish in the study of human disease. N Engl J Med *348*, 2457-2463.

Hariharan, J., Hebbar, P., Ranie, J., Philomena, Sinha, A.M., et al. (1996). Alternative forms of the human thioredoxin mRNA: identification and characterization. Gene *173*, 265-270.

Harrison, D.E., Strong, R., Sharp, Z.D., Nelson, J.F., Astle, C.M., et al. (2009). Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature *460*, 392-395.

Hartman, P.S., Ishii, N., Kayser, E.B., Morgan, P.G., and Sedensky, M.M. (2001). Mitochondrial mutations differentially affect aging, mutability and anesthetic sensitivity in *Caenorhabditis elegans*. Mech Ageing Dev *122*, 1187-1201.

Haycraft, C.J., Swoboda, P., Taulman, P.D., Thomas, J.H., and Yoder, B.K. (2001). 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*, 1493-1505.

Hirota, K., Matsui, M., Murata, M., Takashima, Y., Cheng, F.S., et al. (2000). Nucleoredoxin, glutaredoxin, and thioredoxin differentially regulate NF-kappaB, AP-1, and CREB activation in HEK293 cells. Biochem Biophys Res Commun *274*, 177-182.

Holmgren, A., and Bjornstedt, M. (1995). Thioredoxin and thioredoxin reductase. Meth Enzymol *252*, 199-208.

Holmgren, A., and Lu, J. (2010). Thioredoxin and thioredoxin reductase: current research with special reference to human disease. Biochem Biophys Res Commun *396*, 120-124.

Holmgren, A., Soderberg, B.O., Eklund, H., and Branden, C.I. (1975). Three-dimensional structure of Escherichia coli thioredoxin-S2 to 2.8 A resolution. Proc Natl Acad Sci USA *72*, 2305-2309.

Holt, S.J., and Riddle, D.L. (2003). SAGE surveys *C. elegans* carbohydrate metabolism: evidence for an anaerobic shift in the long-lived dauer larva. Mech Ageing Dev *124*, 779-800.

Honjoh, S., Yamamoto, T., Uno, M., and Nishida, E. (2009). Signalling through RHEB-1 mediates intermittent fasting-induced longevity in *C. elegans*. Nature *457*, 726-730.

Hope, I.A. ed. (1999). *C. elegans*: A Practical Approach. Oxford University Press, Oxford.

Hu, P.J. (2007). Dauer. In: The *C. elegans* Research Community, ed. WormBook. doi/10.1895/ wormbook.1.144.1, http://www.wormbook.org.

Huet, F., Ruiz, C., and Richards, G. (1995). Sequential gene activation by ecdysone in *Drosophila melanogaster*: the hierarchical equivalence of early and early late genes. Development *121*, 1195-1204.

Ingram, D.K., Zhu, M., Mamczarz, J., Zou, S., Lane, M.A., et al. (2006). Calorie restriction

mimetics: an emerging research field. Aging Cell *5*, 97-108.

Inoue, T., and Thomas, J.H. (2000). Targets of TGF-beta signaling in *Caenorhabditis elegans* dauer formation. Dev Biol *217*, 192-204.

Jakupoglu, C., Przemeck, G.K., Schneider, M., Moreno, S.G., Mayr, N., et al. (2005). Cytoplasmic thioredoxin reductase is essential for embryogenesis but dispensable for cardiac development. Mol Cell Biol *25*, 1980-1988.

Jansen, G., Thijssen, K.L., Werner, P., van der Horst, M., Hazendonk, E., et al. (1999). The complete family of genes encoding G proteins of *Caenorhabditis elegans*. Nat Genet *21*, 414-419.

Jee, C., Vanoaica, L., Lee, J., Park, B.J., and Ahnn, J. (2005). Thioredoxin is related to life span regulation and oxidative stress response in *Caenorhabditis elegans*. Genes Cells *10*, 1203-1210.

Jia, K., Chen, D., and Riddle, D.L. (2004). The TOR pathway interacts with the insulin signaling pathway to regulate *C. elegans* larval development, metabolism and life span. Development *131*, 3897-3906.

Jiménez, A., and Miranda-Vizuete, A. (2003). Purification and characterization of delta3Trx-1, a splicing variant of human thioredoxin-1 lacking exon 3. Protein Expr Purif *27*, 319-324.

Jiménez, A., Zu, W., Rawe, V.Y., Pelto-Huikko, M., Flickinger, C.J., et al. (2004). Spermatocyte/ spermatid-specific thioredoxin-3, a novel Golgi apparatus-associated thioredoxin, is a specific marker of aberrant spermatogenesis. J Biol Chem *279*, 34971-34982.

Jung, K.J., Lee, E.K., Kim, J.Y., Zou, Y., Sung, B., et al. (2009). Effect of short term calorie restriction on pro-inflammatory NF-kB and AP-1 in aged rat kidney. Inflamm Res *58*, 143-150.

Kaeberlein, M., Powers, R.W., 3rd, Steffen, K.K., Westman, E.A., Hu, D., et al. (2005). Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. Science *310*, 1193-1196.

Kaeberlein, T.L., Smith, E.D., Tsuchiya, M., Welton, K.L., Thomas, J.H., et al. (2006). Lifespan extension in *Caenorhabditis elegans* by complete removal of food. Aging Cell *5*, 487-494.

Kaletta, T., and Hengartner, M.O. (2006). Finding function in novel targets: *C. elegans* as a model organism. Nat Rev Drug Discov *5*, 387-398.

Kanzok, S.M., Fechner, A., Bauer, H., Ulschmid, J.K., Müller, H.M., et al. (2001). Substitution of the thioredoxin system for glutathione reductase in *Drosophila melanogaster*. Science *291*, 643-646.

Kao, G., Nordenson, C., Still, M., Rönnlund, A., Tuck, S., et al. (2007). ASNA-1 positively regulates insulin secretion in *C. elegans* and mammalian cells. Cell *128*, 577-587.

Kapahi, P., Zid, B.M., Harper, T., Koslover, D., Sapin, V., et al. (2004). Regulation of lifespan in *Drosophila* by modulation of genes in the TOR signaling pathway. Curr Biol *14*, 885-890.

Kenyon, C. (2005). The plasticity of aging: insights from long-lived mutants. Cell *120*, 449-460.

Kenyon, C.J. (2010). The genetics of ageing. Nature *464*, 504-512.

Kiermayer, C., Conrad, M., Schneider, M., Schmidt, J., and Brielmeier, M. (2007). Optimization of spatiotemporal gene inactivation in mouse heart by oral application of tamoxifen citrate. Genesis *45*, 11-16.

Kim, K., Sato, K., Shibuya, M., Zeiger, D.M., Butcher, R.A., et al. (2009). Two chemoreceptors

mediate developmental effects of dauer pheromone in *C. elegans*. Science *326*, 994-998.

Kimura, K.D., Tissenbaum, H.A., Liu, Y., and Ruvkun, G. (1997). *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. Science *277*, 942-946.

Klass, M.R. (1977). Aging in the nematode *Caenorhabditis elegans*: major biological and environmental factors influencing life span. Mech Ageing Dev *6*, 413-429.

Kosower, N.S., Kosower, E.M., Wertheim, B., and Correa, W.S. (1969). Diamide, a new reagent for the intracellular oxidation of glutathione to the disulfide. Biochem Biophys Res Commun *37*, 593-596.

Kostrouchova, M., Krause, M., Kostrouch, Z., and Rall, J.E. (2001). Nuclear hormone receptor CHR3 is a critical regulator of all four larval molts of the nematode *Caenorhabditis elegans*. Proc Natl Acad Sci USA *98*, 7360-7365.

Kruusma, J., Benham, A.M., Williams, J.A., and Kataky, R. (2006). An introduction to thiol redox proteins in the endoplasmic reticulum and a review of current electrochemical methods of detection of thiols. The Analyst *131*, 459-473.

Kuervers, L.M., Jones, C.L., O'Neil, N.J., and Baillie, D.L. (2003). The sterol modifying enzyme LET-767 is essential for growth, reproduction and development in *Caenorhabditis elegans*. Mol Genet Genomics *270*, 121-131.

Kumar, S., and Holmgren, A. (1999). Induction of thioredoxin, thioredoxin reductase and glutaredoxin activity in mouse skin by TPA, a calcium ionophore and other tumor promoters. Carcinogenesis *20*, 1761-1767.

Kurooka, H., Kato, K., Minoguchi, S., Takahashi, Y., Ikeda, J., et al. (1997). Cloning and characterization of the nucleoredoxin gene that encodes a novel nuclear protein related to thioredoxin. Genomics *39*, 331-339.

Kuwabara, P.E., and O'Neil, N. (2001). The use of functional genomics in *C. elegans* for studying human development and disease. J Inherit Metab Dis *24*, 127-138.

Lacey, B.M., and Hondal, R.J. (2006). Characterization of mitochondrial thioredoxin reductase from *C. elegans*. Biochem Biophys Res Commun *346*, 629-636.

Lai, C.H., Chou, C.Y., Ch'ang, L.Y., Liu, C.S., and Lin, W. (2000). Identification of novel human genes evolutionarily conserved in *Caenorhabditis elegans* by comparative proteomics. Genome Res *10*, 703-713.

Lakowski, B., and Hekimi, S. (1998). The genetics of caloric restriction in *Caenorhabditis elegans*. Proc Natl Acad Sci USA *95*, 13091-13096.

Lee, G.D., Wilson, M.A., Zhu, M., Wolkow, C.A., de Cabo, R., et al. (2006). Dietary deprivation extends lifespan in *Caenorhabditis elegans*. Aging Cell *5*, 515-524.

Lee, K.K., Murakawa, M., Takahashi, S., Tsubuki, S., Kawashima, S., et al. (1998). Purification, molecular cloning, and characterization of TRP32, a novel thioredoxin-related mammalian protein of 32 kDa. J Biol Chem *273*, 19160-19166.

Lee, R.Y., Hench, J., and Ruvkun, G. (2001). Regulation of *C. elegans* DAF-16 and its human ortholog FKHRL1 by the *daf-2* insulin-like signaling pathway. Curr Biol *11*, 1950-1957.

Lee, S.J., and Kenyon, C. (2009). Regulation of the longevity response to temperature by thermosensory neurons in *Caenorhabditis elegans*. Curr Biol 19, 715-722.

Leinfelder, W., Forchhammer, K., Veprek, B., Zehelein, E., and Bock, A. (1990). *In vitro*

synthesis of selenocysteinyl-tRNA(UCA) from seryl-tRNA(UCA): involvement and characterization of the *selD* gene product. Proc Natl Acad Sci USA *87*, 543-547.

Leung, M.C., Williams, P.L., Benedetto, A., Au, C., Helmcke, K.J., et al. (2008). *Caenorhabditis elegans*: an emerging model in biomedical and environmental toxicology. Toxicol Sci *106*, 5-28.

Léveillard, T., Mohand-Saïd, S., Lorentz, O., Hicks, D., Fintz, A.C., et al. (2004). Identification and characterization of rod-derived cone viability factor. Nat Genet *36*, 755-759.

Li, C., and Kim, K. (2008). Neuropeptides. In: The *C. elegans* Research Community, ed. WormBook. doi/10.1895/wormbook.1.142.1, http://www.wormbook.org.

Li, W., Kennedy, S.G., and Ruvkun, G. (2003). *daf-28* encodes a *C. elegans* insulin superfamily member that is regulated by environmental cues and acts in the DAF-2 signaling pathway. Genes Dev *17*, 844-858.

Libert, S., Zwiener, J., Chu, X., Vanvoorhies, W., Roman, G., et al. (2007). Regulation of *Drosophila* life span by olfaction and food-derived odors. Science *315*, 1133-1137.

Libina, N., Berman, J.R., and Kenyon, C. (2003). Tissue-specific activities of *C. elegans* DAF-16 in the regulation of lifespan. Cell *115*, 489-502.

Lillig, C.H., Berndt, C., and Holmgren, A. (2008). Glutaredoxin systems. Biochim Biophys Acta *1780*, 1304-1317.

Lillig, C.H., and Holmgren, A. (2007). Thioredoxin and related molecules – from biology to health and disease. Antioxid Redox Signal *9*, 25-47.

Lin, S.J., Kaeberlein, M., Andalis, A.A., Sturtz, L.A., Defossez, P.A., et al. (2002). Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration. Nature *418*, 344-348.

Liu, T., Zimmerman, K.K., and Patterson, G.I. (2004). Regulation of signaling genes by TGFbeta during entry into dauer diapause in *C. elegans*. BMC Dev Biol *4*, 11.

Liu, Y., and Min, W. (2002). Thioredoxin promotes ASK1 ubiquitination and degradation to inhibit ASK1-mediated apoptosis in a redox activity-independent manner. Circ Res *90*, 1259-1266.

Loeb, J., and Northrop, J.H. (1917). On the influence of food and temperature upon the duration of life. J Biol Chem *32*, 103–121.

Ludewig, A.H., Kober-Eisermann, C., Weitzel, C., Bethke, A., Neubert, K., et al. (2004). A novel nuclear receptor/coregulator complex controls *C. elegans* lipid metabolism, larval development, and aging. Genes Dev *18*, 2120-2133.

Luthman, M., and Holmgren, A. (1982). Rat liver thioredoxin and thioredoxin reductase: purification and characterization. Biochemistry *21*, 6628-6633.

MacRae, T.H. (2010). Gene expression, metabolic regulation and stress tolerance during diapause. Cell Mol Life Sci *67*, 2405-2424.

Magner, D.B., and Antebi, A. (2008). *Caenorhabditis elegans* nuclear receptors: insights into life traits. Trends Endocrinol Metab *19*, 153-160.

Mak, H.Y., and Ruvkun, G. (2004). Intercellular signaling of reproductive development by the *C. elegans* DAF-9 cytochrome P450. Development *131*, 1777-1786.

Matsui, M., Oshima, M., Oshima, H., Takaku, K., Maruyama, T., et al. (1996). Early embryonic lethality caused by targeted disruption of the mouse thioredoxin gene. Dev Biol *178*,

179-185.

McCay, C.M., Crowell, M.F., and Maynard, L.A. (1935). The effect of retarded growth upon the length of life span and upon the ultimate body size. J Nutr *10*, 63–79.

Merris, M., Wadsworth, W.G., Khamrai, U., Bittman, R., Chitwood, D.J., et al. (2003). Sterol effects and sites of sterol accumulation in *Caenorhabditis elegans*: developmental requirement for 4alpha-methyl sterols. J Lipid Res *44*, 172-181.

Meyer, Y., Buchanan, B.B., Vignols, F., and Reichheld, J.P. (2009). Thioredoxins and Glutaredoxins: Unifying Elements in Redox Biology. Annu Rev Genet *43*, 335-367.

Miranda-Vizuete, A., Ljung, J., Damdimopoulos, A.E., Gustafsson, J.A., Oko, R., et al. (2001). Characterization of Sptrx, a novel member of the thioredoxin family specifically expressed in human spermatozoa. J Biol Chem *276*, 31567-31574.

Miranda-Vizuete, A., Sadek, C.M., Jiménez, A., Krause, W.J., Sutovsky, P., et al. (2004). The mammalian testis-specific thioredoxin system. Antioxid Redox Signal *6*, 25-40.

Miranda-Vizuete, A., and Spyrou, G. (2002). Genomic organization and identification of a novel alternative splicing variant of mouse mitochondrial thioredoxin reductase (TrxR2) gene. Mol Cells *13*, 488-492.

Mitsui, A., Hamuro, J., Nakamura, H., Kondo, N., Hirabayashi, Y., et al. (2002). Overexpression of human thioredoxin in transgenic mice controls oxidative stress and life span. Antioxid Redox Signal *4*, 693-696.

Morgan, K.L., Estevez, A.O., Mueller, C.L., Cacho-Valadez, B., Miranda-Vizuete, A., et al. (2010). The glutaredoxin GLRX-21 functions to prevent selenium-induced oxidative stress in *Caenorhabditis elegans*. Toxicol Sci *118*, 530-543.

Morley, J.F., and Morimoto, R.I. (2004). Regulation of longevity in *Caenorhabditis elegans* by heat shock factor and molecular chaperones. Mol Biol Cell *15*, 657-664.

Muller, E.G. (1996). A glutathione reductase mutant of yeast accumulates high levels of oxidized glutathione and requires thioredoxin for growth. Mol Biol Cell *7*, 1805-1813.

Murakami, M., Koga, M., and Ohshima, Y. (2001). DAF-7/TGF-beta expression required for the normal larval development in *C. elegans* is controlled by a presumed guanylyl cyclase DAF-11. Mech Dev *109*, 27-35.

Murphy, C.T., McCarroll, S.A., Bargmann, C.I., Fraser, A., Kamath, R.S., et al. (2003). Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. Nature *424*, 277-283.

Nathoo, A.N., Moeller, R.A., Westlund, B.A., and Hart, A.C. (2001). Identification of neuropeptide-like protein gene families in *Caenorhabditis elegans* and other species. Proc Natl Acad Sci USA *98*, 14000-14005.

Nonn, L., Williams, R.R., Erickson, R.P., and Powis, G. (2003). The absence of mitochondrial thioredoxin 2 causes massive apoptosis, exencephaly, and early embryonic lethality in homozygous mice. Mol Cell Biol *23*, 916-922.

Nordlund, P., and Reichard, P. (2006). Ribonucleotide reductases. Annu Rev Biochem *75*, 681-706.

Nyström, J., Shen, Z.Z., Aili, M., Flemming, A.J., Leroi, A., et al. (2002). Increased or decreased levels of *Caenorhabditis elegans lon-3*, a gene encoding a collagen, cause reciprocal changes in body length. Genetics *161*, 83-97.

Ogg, S., Paradis, S., Gottlieb, S., Patterson, G.I., Lee, L., et al. (1997). The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. Nature *389*, 994-999.

Ohkura, K., Suzuki, N., Ishihara, T., and Katsura, I. (2003). SDF-9, a protein tyrosine phosphatase-like molecule, regulates the L3/dauer developmental decision through hormonal signaling in *C. elegans*. Development *130*, 3237-3248.

Onken, B., and Driscoll, M. (2010). Metformin induces a dietary restriction-like state and the oxidative stress response to extend *C. elegans* Healthspan via AMPK, LKB1, and SKN-1. PLoS ONE *5*, e8758.

Osborne, S.A., and Tonissen, K.F. (2001). Genomic organisation and alternative splicing of mouse and human thioredoxin reductase 1 genes. BMC genomics *2*, 10.

Page, A.P., and Johnstone, I.L. (2007). The cuticle. In: The *C. elegans* Research Community, ed. WormBook. doi/10.1895/wormbook.1.138.1, http://www.wormbook.org.

Panowski, S.H., Wolff, S., Aguilaniu, H., Durieux, J., and Dillin, A. (2007). PHA-4/Foxa mediates diet-restriction-induced longevity of *C. elegans*. Nature *447*, 550-555.

Paradis, S., Ailion, M., Toker, A., Thomas, J.H., and Ruvkun, G. (1999) A PDK1 homolog is necessary and sufficient to transduce AGE-1 PI3 kinase signals that regulate diapause in *Caenorhabditis elegans*. Genes Dev *13*, 1438-1452.

Park, D., Estevez, A., and Riddle, D.L. (2010a). Antagonistic Smad transcription factors control the dauer/non-dauer switch in *C. elegans*. Development *137*, 477-485.

Park, S.K., Link, C.D., and Johnson, T.E. (2010b). Life-span extension by dietary restriction is mediated by NLP-7 signaling and coelomocyte endocytosis in *C. elegans*. FASEB J *24*, 383-392.

Patterson, G.I., Koweek, A., Wong, A., Liu, Y., and Ruvkun, G. (1997). The DAF-3 Smad protein antagonizes TGF-beta-related receptor signaling in the *Caenorhabditis elegans* dauer pathway. Genes Dev *11*, 2679-2690.

Pekkari, K., Avila-Cariño, J., Gurunath, R., Bengtsson, A., Scheynius, A., et al. (2003). Truncated thioredoxin (Trx80) exerts unique mitogenic cytokine effects via a mechanism independent of thiol oxido-reductase activity. FEBS Lett *539*, 143-148.

Pekkari, K., Gurunath, R., Arnér, E.S., and Holmgren, A. (2000). Truncated thioredoxin is a mitogenic cytokine for resting human peripheral blood mononuclear cells and is present in human plasma. J Biol Chem *275*, 37474-37480.

Pekkari, K., and Holmgren, A. (2004). Truncated thioredoxin: physiological functions and mechanism. Antioxid Redox Signal *6*, 53-61.

Pierce, S.B., Costa, M., Wisotzkey, R., Devadhar, S., Homburger, S.A., et al. (2001). Regulation of DAF-2 receptor signaling by human insulin and *ins-1*, a member of the unusually large and diverse *C. elegans* insulin gene family. Genes Dev *15*, 672-686.

Piper, M.D., and Bartke, A. (2008). Diet and aging. Cell Metab *8*, 99-104.

Qi, Y., and Grishin, N.V. (2005). Structural classification of thioredoxin-like fold proteins. Proteins *58*, 376-388.

Raizen, D.M., Lee, R.Y., and Avery, L. (1995). Interacting genes required for pharyngeal excitation by motor neuron MC in *Caenorhabditis elegans*. Genetics *141*, 1365-1382.

Raizen, D.M., Zimmerman, J.E., Maycock, M.H., Ta, U.D., You, Y.J., et al. (2008). Lethargus is

a *Caenorhabditis elegans* sleep-like state. Nature *451*, 569-572.

Ren, P., Lim, C.S., Johnsen, R., Albert, P.S., Pilgrim, D., et al. (1996). Control of *C. elegans* larval development by neuronal expression of a TGF-beta homolog. Science *274*, 1389-1391.

Rhee, S.G., Chae, H.Z., and Kim, K. (2005). Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. Free Radic Biol Med *38*, 1543-1552.

Riddle, D.L., Blumenthal, T., Meyer, B.J., and Priess, J.R. eds. (1997). *C. elegans* II. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Riddle, D.L., Swanson, M.M., and Albert, P.S. (1981). Interacting genes in nematode dauer larva formation. Nature *290*, 668-671.

Ristow, M., and Zarse, K. (2010). How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis). Exp Gerontol *45*, 410-418.

Rohrbach, S., Gruenler, S., Teschner, M., and Holtz, J. (2006). 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*, R927-935.

Ruaud, A.F., and Bessereau, J.L. (2006). Activation of nicotinic receptors uncouples a developmental timer from the molting timer in *C. elegans*. Development *133*, 2211-2222.

Rundlöf, A.K., Janard, M., Miranda-Vizuete, A., and Arnér, E.S. (2004). Evidence for intriguingly complex transcription of human thioredoxin reductase 1. Free Radic Biol Med *36*, 641-656.

Sadek, C.M., Damdimopoulos, A.E., Pelto-Huikko, M., Gustafsson, J.A., Spyrou, G., et al. (2001). Sptrx-2, a fusion protein composed of one thioredoxin and three tandemly repeated NDP-kinase domains is expressed in human testis germ cells. Genes Cells *6*, 1077-1090.

Sadek, C.M., Jiménez, A., Damdimopoulos, A.E., Kieselbach, T., Nord, M., et al. (2003). Characterization of human thioredoxin-like 2. A novel microtubule-binding thioredoxin expressed predominantly in the cilia of lung airway epithelium and spermatid manchette and axoneme. J Biol Chem *278*, 13133-13142.

Sahaf, B., Heydari, K., and Herzenberg, L.A. (2003). Lymphocyte surface thiol levels. Proc Natl Acad Sci USA *100*, 4001-4005.

Sandalova, T., Zhong, L., Lindqvist, Y., Holmgren, A., and Schneider, G. (2001). Three-dimensional structure of a mammalian thioredoxin reductase: implications for mechanism and evolution of a selenocysteine-dependent enzyme. Proc Natl Acad Sci USA *98*, 9533-9538.

Schackwitz, W.S., Inoue, T., and Thomas, J.H. (1996). Chemosensory neurons function in parallel to mediate a pheromone response in *C. elegans*. Neuron *17*, 719-728.

Schallreuter, K.U., and Wood, J.M. (2001). Thioredoxin reductase - its role in epidermal redox status. J Photochem Photobiol B, Biol *64*, 179-184.

Schlotterer, A., Hamann, A., Kukudov, G., Ibrahim, Y., Heckmann, B., et al. (2010). Apurinic/apyrimidinic endonuclease 1, p53, and thioredoxin are linked in control of aging in *C. elegans*. Aging Cell *9*, 420-432.

Schulz, T.J., Zarse, K., Voigt, A., Urban, N., Birringer, M., et al. (2007). Glucose restriction

extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. Cell Metab *6*, 280-293.

Shakir, M.A., Fukushige, T., Yasuda, H., Miwa, J., and Siddiqui, S.S. (1993). *C. elegans osm-3* gene mediating osmotic avoidance behaviour encodes a kinesin-like protein. Neuroreport *4*, 891-894.

Singh, R., and Sulston, J. (1978). Some observations on moulting in *Caenorhabditis elegans*. Nematologica *24*, 63–71.

Snow, M.I., and Larsen, P.L. (2000). Structure and expression of *daf-12*: a nuclear hormone receptor with three isoforms that are involved in development and aging in *Caenorhabditis elegans*. Biochim Biophys Acta *1494*, 104-116.

Soerensen, J., Jakupoglu, C., Beck, H., Forster, H., Schmidt, J., et al. (2008). The role of thioredoxin reductases in brain development. PLoS ONE *3*, e1813.

Sonnhammer, E.L., and Durbin, R. (1997). Analysis of protein domain families in *Caenorhabditis elegans*. Genomics *46*, 200-216.

Stadtman, E.R., Van Remmen, H., Richardson, A., Wehr, N.B., and Levine, R.L. (2005). Methionine oxidation and aging. Biochim Biophys Acta *1703*, 135-140.

Steinkraus, K.A., Smith, E.D., Davis, C., Carr, D., Pendergrass, W.R., et al. (2008). Dietary restriction suppresses proteotoxicity and enhances longevity by an *hsf-1*-dependent mechanism in *Caenorhabditis elegans*. Aging Cell *7*, 394-404.

Stiernagle, T. (2006). Maintenance of *C. elegans*. In: The *C. elegans* Research Community, ed. WormBook. doi/10.1895/wormbook.1.101.1, http://www.wormbook.org.

Storey, K.B. (2010). Out cold: biochemical regulation of mammalian hibernation - a mini-review. Gerontology *56*, 220-230.

Storey, K.B., and Storey, J.M. (2004). Metabolic rate depression in animals: transcriptional and translational controls. Biol Rev Camb Philos Soc *79*, 207-233.

Su, D., and Gladyshev, V.N. (2004). Alternative splicing involving the thioredoxin reductase module in mammals: a glutaredoxin-containing thioredoxin reductase 1. Biochemistry *43*, 12177-12188.

Sullivan, A.A., and Thummel, C.S. (2003). Temporal profiles of nuclear receptor gene expression reveal coordinate transcriptional responses during *Drosophila* development. Mol Endocrinol *17*, 2125-2137.

Sulston, J.E. (1976). Post-embryonic development in the ventral cord of *Caenorhabditis elegans*. Philos Trans R Soc Lond, B, Biol Sci *275*, 287-297.

Swoboda, P., Adler, H.T., and Thomas, J.H. (2000). The RFX-type transcription factor DAF-19 regulates sensory neuron cilium formation in *C. elegans*. Mol Cell *5*, 411-421.

Sze, J.Y., Victor, M., Loer, C., Shi, Y., and Ruvkun, G. (2000). Food and metabolic signalling defects in a *Caenorhabditis elegans* serotonin-synthesis mutant. Nature *403*, 560-564.

Taskov, K., Chapple, C., Kryukov, G.V., Castellano, S., Lobanov, A.V., et al. (2005). Nematode selenoproteome: the use of the selenocysteine insertion system to decode one codon in an animal genome? Nucleic Acids Res *33*, 2227-2238.

The *C. elegans* Sequencing Consortium (1998). Genome sequence of the nematode *C. elegans*: a platform for investigating biology. Science *282*, 2012-2018.

The National Research Council (2000). Scientific Frontiers in Developmental Toxicology and

Risk Assessment. The National Academies Press, Washington, DC.

Thomas, C.G., and Spyrou, G. (2009). ERdj5 sensitizes neuroblastoma cells to endoplasmic reticulum stress-induced apoptosis. J Biol Chem *284*, 6282-6290.

Thomas, J.H., Birnby, D.A., and Vowels, J.J. (1993). Evidence for parallel processing of sensory information controlling dauer formation in *Caenorhabditis elegans*. Genetics *134*, 1105-1117.

Tsang, W.Y., Sayles, L.C., Grad, L.I., Pilgrim, D.B., and Lemire, B.D. (2001). Mitochondrial respiratory chain deficiency in *Caenorhabditis elegans* results in developmental arrest and increased life span. J Biol Chem *276*, 32240-32246.

Tullet, J.M., Hertweck, M., An, J.H., Baker, J., Hwang, J.Y., et al. (2008). Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in *C. elegans*. Cell *132*, 1025-1038.

Ushioda, R., Hoseki, J., Araki, K., Jansen, G., Thomas, D.Y., et al. (2008). ERdj5 is required as a disulfide reductase for degradation of misfolded proteins in the ER. Science *321*, 569-572.

Van Buskirk, C., and Sternberg, P.W. (2007). Epidermal growth factor signaling induces behavioral quiescence in *Caenorhabditis elegans*. Nat Neurosci *10*, 1300-1307.

Vanfleteren, J.R., and De Vreese, A. (1996). Rate of aerobic metabolism and superoxide production rate potential in the nematode *Caenorhabditis elegans*. J Exp Zool *274*, 93-100.

Vowels, J.J., and Thomas, J.H. (1992). Genetic analysis of chemosensory control of dauer formation in *Caenorhabditis elegans*. Genetics *130*, 105-123.

Wang, J., and Kim, S.K. (2003). Global analysis of dauer gene expression in *Caenorhabditis elegans*. Development *130*, 1621-1634.

Wang, Y., and Tissenbaum, H.A. (2006). Overlapping and distinct functions for a *Caenorhabditis elegans* SIR2 and DAF-16/FOXO. Mech Ageing Dev *127*, 48-56.

White, J.G., Southgate, E., Thomson, J.N., and Brenner, S. (1986). The structure of the nervous system of *Caenorhabditis elegans*. Philos Trans R Soc Lond, B, Biol Sci 314, 1–340.

Wilkinson, B., and Gilbert, H.F. (2004). Protein disulfide isomerase. Biochim Biophys Acta *1699*, 35-44.

Willcox, B.J., Willcox, D.C., Todoriki, H., Fujiyoshi, A., Yano, K., et al. (2007). Caloric restriction, the traditional Okinawan diet, and healthy aging: the diet of the world's longest-lived people and its potential impact on morbidity and life span. Ann N Y Acad Sci *1114*, 434-455.

Willcox, D.C., Willcox, B.J., Todoriki, H., Curb, J.D., and Suzuki, M. (2006). Caloric restriction and human longevity: what can we learn from the Okinawans? Biogerontology *7*, 173-177.

Williams, C.H., Jr. (1992). Lipoamide dehydrogenase, glutathione reductase, thioredoxin reductase, and mercuric ion reductase — a family of flavoenzyme transhydrogenases. In: Müller F., ed. Chemistry and Biochemistry of Flavoenzymes. CRC Press, Boca Raton, FL.

Winter, A.D., McCormack, G., and Page, A.P. (2007). Protein disulfide isomerase activity is essential for viability and extracellular matrix formation in the nematode *Caenorhabditis elegans*. Dev Biol *308*, 449-461.

Wiseman, R.L., Chin, K.T., Haynes, C.M., Stanhill, A., Xu, C.F., et al. (2009). Thioredoxin-related Protein 32 is an arsenite-regulated Thiol Reductase of the proteasome 19 S particle. J Biol Chem *284*, 15233-15245.

Wood, W.B. ed. (1988). The Nematode *Caenorhabditis elegans*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Yan, H., Harding, J.J., Xing, K., and Lou, M.F. (2007). Revival of glutathione reductase in human cataractous and clear lens extracts by thioredoxin and thioredoxin reductase, in conjunction with alpha-crystallin or thioltransferase. Curr Eye Res *32*, 455-463.

Yarwood, E.A., and Hansen, E.L. (1969). Dauer Larvae of *Caenorhabditis briggsae* in Axenic Culture. J Nematol *1*, 184-189.

Yochem, J., Tuck, S., Greenwald, I., and Han, M. (1999). A gp330/megalin-related protein is required in the major epidermis of *Caenorhabditis elegans* for completion of molting. Development *126*, 597-606.

Zaidel-Bar, R., Miller, S., Kaminsky, R., and Broday, L. (2010). Molting-specific downregulation of *C. elegans* body-wall muscle attachment sites: the role of RNF-5 E3 ligase. Biochem Biophys Res Commun *395*, 509-514.

Zhong, L., Arnér, E.S., and Holmgren, A. (2000). Structure and mechanism of mammalian thioredoxin reductase: the active site is a redox-active selenolthiol/selenenylsulfide formed from the conserved cysteine-selenocysteine sequence. Proc Natl Acad Sci USA *97*, 5854-5859.

Zhong, L., Arnér, E.S., Ljung, J., Aslund, F., and Holmgren, A. (1998). Rat and calf thioredoxin reductase are homologous to glutathione reductase with a carboxyl-terminal elongation containing a conserved catalytically active penultimate selenocysteine residue. J Biol Chem *273*, 8581-8591.

Zwaal, R.R., Mendel, J.E., Sternberg, P.W., and Plasterk, R.H. (1997). Two neuronal G proteins are involved in chemosensation of the *Caenorhabditis elegans* Dauer-inducing pheromone. Genetics *145*, 715-727.