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REVIEW ARTICLE

Unique structure and regulation of the nematode detoxification gene regulator, SKN-1: implications to understanding and controlling drug resistance

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Abstract

Nematodes parasitize an alarming number of people and agricultural animals globally and cause debilitating morbidity and mortality. Anthelmintics have been the primary tools used to control parasitic nematodes for the past several decades, but drug resistance is becoming a major obstacle. Xenobiotic detoxification pathways defend against drugs and other foreign chemicals in diverse organisms, and evidence is accumulating that they play a role in mediating resistance to anthelmintics in nematodes. Related antioxidation pathways may also provide filarial parasites with protection against host free-radical–mediated immune responses. Upstream regulatory pathways have received almost no attention in nematode parasites, despite their potential to coregulate multiple detoxification and antioxidation genes. The nuclear eurythroid 2–related factor 2 (NRF2) transcription factor mediates inducible detoxification and antioxidation defenses in mammals, and recent studies have demonstrated that it promotes multidrug resistance in some human tumors. Recent studies in the free-living model nematode, *Caenorhabditis elegans*, have defined the homologous transcription factor, SKN-1, as a master regulator of detoxification and antioxidation genes. Despite similar functions, SKN-1 and NRF2 have important differences in structure and regulatory pathways. Protein alignment and phylogenetic analyses indicate that these differences are shared among many nematodes, making SKN-1 a candidate for specifically targeting nematode detoxification and antioxidation.

Keywords: xenobiotic detoxification, antioxidation, anthelminthic, SKN-1, NRF2, gene regulation, drug target, *Caenorhabditis elegans*

Introduction

Parasitic nematodes are a major cause of human mortality and morbidity in tropical and subtropical climates (Chan, 1997; Mathers et al., 2007) and infect millions of people in the United States and Europe who live in poverty (Hotez, 2009). The World Health Organization (WHO) estimates that 2 billion people are infected with parasitic nematodes worldwide (http://www.who.int/wormcontrol/statistics/). Despite being common, helminth-induced diseases receive less than 1% of research funds globally and are regarded as neglected tropical diseases (Hotez et al., 2008). Parasitic nematodes also burden human health and nutrition by parasitizing livestock and cause an estimated $80 billion loss of worldwide crop production each year (Jasmer et al., 2003). Programs to control parasitic nematodes in humans and animals rely heavily on the widespread administration of three classes of helminth-targeting drugs, or anthelmintics: benzimidazoles, which target the cytoskeleton through β-tubulin; levamisole and related compounds, which are nicotinic receptor agonists; and macrocyclic lactones, which have a high affinity for glutamate-gated chloride channels (Holden-Dye and Walker, 2007). Unfortunately, resistance is a widespread, growing problem (Kaplan, 2004; Kaplan and Vidyashankar, 2011). Multidrug resistance (MDR) is especially problematic because it reduces the efficacy of many drugs and is poorly understood (Gilleard, 2006; James et al., 2009).

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Drug resistance is a fundamental response in populations of cells and organisms when repeatedly exposed to sublethal doses of a toxic compound (Persidis, 1999; Kaplan, 2004; Prasad and Kapoor, 2004; Gilleard, 2006; James and Davey, 2008). Nematodes have large population sizes and high genetic diversity, which facilitates the evolution of resistance (Kaplan, 2004). This potential for drug resistance is best exemplified in livestock that are routinely treated with anthelmintics; resistance to every anthelmintic class of drugs has been reported for parasites of every major livestock host (Kaplan, 2004; Gilleard, 2006; Kaplan and Vidyashankar, 2011). Studies of human parasites have also suggested the potential for resistance to emerge (Geerts and Gryseels, 2001; Awadzi et al., 2004; Osei-Atweneboana et al., 2007).

It has been over 25 years since a new class of broadly applicable anthelmintic has been adopted for widespread use (Prichard and Geary, 2008). Two new classes of anthelmintics are being implemented for use in livestock (Kaminsky et al., 2008; Little et al., 2010), but resistance is likely to occur once new drugs are used widely. Therefore, new strategies, drug targets, and tools are urgently needed. Knowledge of the underlying molecular and genetic mechanisms is essential to the proper design and implementation of strategies to monitor the development of anthelmintic resistance and to maintain and extend the useful life of currently used drugs (Prichard, 1994; Gilleard, 2006; Lespine et al., 2008). Unfortunately, these processes are poorly understood, and tools for their analysis are sparse for parasitic nematodes that have limited genetic tractability (Geerts and Gryseels, 2001; von Samson-Himmelstjerna and Blackhall, 2005; Gilleard, 2006; Holden-Dye and Walker, 2007; Mitreva et al., 2007; James and Davey, 2008).

Nematode parasites of major concern

A full description of parasitic nematodes is beyond the scope of this review. Here, we briefly describe a few groups of concern based on existing resistance, medical or agricultural effects, and risk for resistance (listed in Table 1). Readers are directed to other reviews for details on livestock (Kaplan, 2004; Kaplan and Vidyashankar, 2011) and human (Albonico et al., 2008; Keiser and Utzinger, 2008; Bockarie and Deb, 2010) parasites.

Gastrointestinal (GI) parasites of livestock animals are common and diverse. GI nematodes attach to the stomach or intestinal mucosa of the host and feed on blood, causing anemia, malnutrition, dehydration, lethargy, and sometimes death. Important species include *Haemonchus contortus* and *Teladorsagia circumcincta* in sheep and goats, *Cooperia* spp. and *Ostertagia* spp. in cattle, and cyathostomins and *Parascaris equorum* in horses (Kaplan and Vidyashankar, 2011). Livestock farming has relied on anthelmintics for over 30 years. In the past 10–15 years, resistance has grown into a problem that threatens the viability of entire industries (Kaplan, 2004; Kaplan and Vidyashankar, 2011). Resistance to all three classes of anthelmintics has now been reported for parasites of all common livestock hosts. The widespread, rapid growth of resistance in multiple parasite and host livestock combinations suggests that resistance is an inevitable outcome of prolonged anthelmintic use (Kaplan and Vidyashankar, 2011).

A group of human GI parasites transmitted by the soil (i.e., soil-transmitted helminths) are often considered and treated together. They include roundworm (*Ascaris lumbricoides*), hookworms (*Ancylostoma duodenale* and *Necator americanus*), and whipworm (*Trichuris trichiura*), which, in combination, infect as many as 2 billion people in tropical and developing regions (Bethony et al., 2006; Albonico et al., 2008). Heavy infections cause a range of symptoms, including anemia, malnutrition, diarrhea, colitis, and impaired growth and cognitive development, and may also increase

Table 1. Nematode parasites of major concern.

Group of parasites	Examples	Common name	Clade	Host	Disease symptoms
GI/livestock	*Haemonchus contortus*	Barber pole worm	V	Sheep and goat	Anemia, diarrhea, lethargy, death
	Teladorsagia circumcincta	Brown stomach worm	V	Sheep and goat	
	Cooperia spp.		V	Cattle	
	Ostertagia spp.		V	Cattle	
	cyathostomins		V	Horse	
	Parascaris equorum		III	Horse	
GI/human	*Ascaris lumbricoides*	Roundworm	III	Human	Anemia, malnutrition, developmental and cognitive delay, intestinal obstruction, and increased risk of other infections
	Ancylostoma duodenale	Human hookworm	V	Human	
	Necator americanus	Human hookworm	V	Human	
	Trichuris trichiura	Whipworm	I	Human	
Filarial/human	*Wuchereria bancrofti*		III	Human	Lymphoedema, hydrocoeles, elephantiasis, immune suppression, blindness, and skin disease
	Brugia malayi	Filarial nematode	III	Human	
	Brugia timori		III	Human	
	Loa loa	Eye worm	III	Human	
	Onchocerca volvulus		III	Human	

Symptoms are listed by group (Kaplan, 2004; Albonico et al., 2008; Keiser and Utzinger, 2008; Bockarie and Deb, 2010; Kaplan and Vidyashankar, 2011). "Clade" refers to the five major phylogenetic groups within nematodes (Blaxter, 1998; Blaxter et al., 1998). See text for details.

susceptibility to malaria, tuberculosis, and human immunodeficiency virus (Bethony et al., 2006; Albonico et al., 2008). The socioeconomic benefits of deworming children are recognized, and global programs based on mass drug treatment with benzimidazole, levamisole, and nicotinic agonist class drugs are in place to reduce infection rates (Albonico et al., 2008).

There are eight nematode species known to live in the lymphatic vessels of humans, and these species are thought to infect 120 million people worldwide, with a total of 1.2 billion at risk (Bockarie and Deb, 2010; Taylor et al., 2010). The most-common species are *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*. Another important species is *Onchocerca volvulus*, which is thought to infect 37 million people, mostly in Africa; adults live in subcutaneous and deep tissues, and larvae migrate to the skin and eyes (Taylor et al., 2010). The diseases caused by these species are classified as filariasis (referring to small, thin larvae), with symptoms including lymphoedema, hydrocoeles, elephantiasis, immune suppression, blindness, and skin disease (Taylor et al., 2010). Transmission of filarial nematodes is by biting insects. The WHO initiated a global program to eliminate filarial diseases in 1999, and over 2 billion treatments with benzimidazole and macrocyclic lactone class drugs have been administered (Gustavsen et al., 2009; Hooper et al., 2009).

Although mass drug administration programs have been effective in interrupting or decreasing human nematode infections (Hooper et al., 2009), reports are beginning to emerge that at least support the potential for resistance (Geerts and Gryseels, 2000, 2001; Albonico et al., 2004; Keiser and Utzinger, 2008); resistance in humans is difficult to verify because controlled experiments (such as those conducted in livestock) cannot be performed (Kaplan, 2004). The unfortunate history of resistance in ruminant GI nematodes serves as a warning that widespread resistance in human parasites may be inevitable with prolonged drug selection (Geerts and Gryseels, 2001).

Xenobiotic detoxification promotes MDR

MDR is well studied in pathogenic microbes, fungi, and cancer cells and is often caused by the increased expression and activity of xenobiotic detoxification enzymes (Persidis, 1999; Prasad et al., 2002; Moye-Rowley, 2003; Prasad and Kapoor, 2004; Sipos and Kuchler, 2006; Lubelski et al., 2007). Despite the fundamental, conserved role of xenobiotic detoxification in drug resistance, these processes are poorly understood in nematodes. The limited knowledge of these processes in helminths has been reviewed in detail recently (Cvilink et al., 2009), and we only summarize it here briefly.

The general strategy for cellular detoxification is conserved. In animal cells, xenobiotic detoxification is modeled as occurring in three sequential, interdependent phases. In phase I, enzymes such as cytochrome P450s (CYPs), short-chain dehydrogenases, and reductases uncover or insert reactive and hydrophilic groups onto xenobiotics, leaving them more accessible for further processing (Iyanagi, 2007; Cvilink et al., 2009). The genome of *Caenorhabditis elegans*, the free-living genetic model, nematode, is predicted to contain 86 CYPs and 68 short-chain dehydrogenases (Lindblom and Dodd, 2006); the genome of *Pristionchus pacificus*, a beetle-associated nematode, is predicted to contain 198 CYPs (Dieterich et al., 2008).

In phase II, conjugating enzymes catalyze the addition of glutathione, glucuronic acid, amino acids, and sulphates to xenobiotics or their phase I metabolites, resulting in less-toxic, more water-soluble and excretable products (O'Brien and Tew, 1996; McLellan and Wolf, 1999; Strange et al., 2001; Townsend and Tew, 2003; Iyanagi, 2007; Torres-Rivera and Landa, 2008). Glutathione and glutathione *S*-tranferases (GSTs) also reduce electrophilic xenobiotics and oxygen free radicals generated by some xenobiotics and host immune systems (O'Brien and Tew, 1996; Strange et al., 2001; Torres-Rivera and Landa, 2008). Nematode GSTs have also been shown to possess prostaglandin isomerase activity and have been hypothesized to play a role in developmental signaling and modification of prostaglandin-regulated mammalian immune responses (Perbandt et al., 2008; Joachim et al., 2011; Joachim and Ruttkowski, 2011). Similar to phase I, there is a tremendous diversity of phase II genes, with 72 and 139 predicted glucuronosyltransferases in *C. elegans* and *P. pacificus*, respectively (Lindblom and Dodd, 2006; Dieterich et al., 2008); there are 48 and 54 predicted GSTs in *C. elegans* and *P. pacificus*, respectively. Increased activity and/or expression of phase I and/or II genes is associated with cambenzadole (related to benzimidazole) resistance in *H. contortus* (a major parasite of ruminants, commonly drug resistant, and an important model for experimental parasitology) (Kwalek et al., 1984) and ivermectin (a macrocyclic lactone) resistance in *C. elegans* (James et al., 2009) and *H. contortus* (Sotirchos et al., 2008).

A recent study analyzed the transcriptome of *C. elegans* 4 hours after exposure to the widely used benzimidole, albendazole, and found six phase I and 10 phase II enzymes among the 42 genes upregulated (Laing et al., 2010). This study also demonstrated that albendazole metabolites produced in *C. elegans* were similar to those in *H. contortus* and distinct from metabolites in mammals, suggesting that these two nematodes share common biochemical properties of detoxification that are distinct from vertebrates.

In phase III, transmembrane transporters export drugs and their metabolites out of cells. The major family of xenobiotic transporters is the adenosine triphosphate (ATP)-binding cassette (ABC) protein family, which includes three subgroups thought to be involved in drug resistance: ABCB [P-glycoprotein (P-gp) and MDR proteins], ABCC (multidrug resistance related protein; MRP), and ABCG (includes breast cancer resistance

protein). ABC proteins are found in all cellular forms of life (Cvilink et al., 2009) and are diverse in nematodes, with at least 60 family members in *C. elegans* (Zhao et al., 2007), 129 in *P. pacificus* (Dieterich et al., 2008), and 33 in *B. malayi* (filarial parasite of humans) (Ardelli et al., 2010). Binding of xenobiotics to these integral membrane proteins is followed by ATP binding and conformational changes that cause an export of compounds; ATP hydrolysis allows the regeneration of the initial state of the protein (Schinkel and Jonker, 2003). Comprehensive information on xenobiotic transporter diversity and function is well reviewed (Kerboeuf et al., 2003; Sheps et al., 2004; Alvarez et al., 2006; Lindblom and Dodd, 2006). ABC transporters have a well-established role in promoting chemotherapeutic resistance in some cancer cells and have been an important target for reversing resistance in humans (Kerboeuf et al., 2003). As a result, this phase of detoxification has received the most attention in parasites (Alvarez et al., 2006; Lespine et al., 2008).

Isolates of ivermectin-selected *H. contortus* have been shown to have an elevated expression of an ABCB gene, and verapamil, an inhibitor of P-gp, increased ivermectin efficacy in these resistant strains (Xu et al., 1998). Selection for MDR over multiple generations also resulted in the elevated expression of ABC transporter genes in *C. elegans*, and verapamil partially reversed resistance (James et al., 2009). Shorter term exposures of *B. malayi* and *H. contortus* to ivermectin and/or its analog, moxidectin, have also been shown to cause an increase in the expression of ABCB genes (Prichard and Roulet, 2007; Stitt et al., 2011).

Interestingly, detoxification gene numbers vary greatly between three nematode species in which they have been surveyed (e.g., *B. malayi*, *C. elegans*, and *P. pacificus*; see above) (Lindblom and Dodd, 2006; Dieterich et al., 2008; Ardelli et al., 2010). *B. malayi* resides in the controlled interstitial environments of human and insect host tissues and has the fewest number of detoxification genes, *C. elegans* feeds on bacteria on rotting plant tissues (Kiontke et al., 2011), and *P. pacificus* feeds on bacteria, fungi, and other nematodes that grow on beetle carcasses and has the highest number of detoxification genes (Herrmann et al., 2007). Thus, detoxification gene number may be highly flexible in nematodes and appears to evolve to match the chemical complexity of each species' environment (Dieterich et al., 2008). It will be interesting to learn whether GI parasites have a high number of detoxification genes to match their chemically diverse environments.

Given the tremendous diversity of detoxification genes and their fundamental and interdependent functions, many more detoxification genes in all three phases are likely to contribute to anthelmintic resistance in nematodes. Lowering costs of next-generation DNA and RNA sequencing and a growing number of sequenced parasite genomes (Ghedin et al., 2007; Mitreva et al., 2011; Sommer and Streit, 2011) will soon permit genome-wide identification of genes with altered expression in resistant strains and association of alleles with resistance. These approaches will provide a starting point for generating new hypotheses about the mechanisms of drug resistance, but ultimately target-specific pharmacological or genetic modulation will be needed to test the functional importance of individual genes and pathways.

Targeting detoxification

Lespine et al. (2008) recently proposed using inhibitors of xenobiotic detoxification and transport in combination with currently available anthelmintics as a strategy to treat infections by MDR parasitic nematodes. Inhibitors of glutathione synthesis and ABC transporters, such as buthionine sulfoxamine and verapamil (among others), are providing early evidence that this strategy might be effective (Xu et al., 1998; Molento and Prichard, 1999; James and Davey, 2008; Bartley et al., 2009; Stitt et al., 2011). However, currently available drugs that inhibit xenobiotic detoxification and transport have two important limitations for their use *in vivo*. The first limitation is that they only affect a single protein or class of proteins. The second major limitation of currently available detoxification-targeting drugs is that they also inhibit homologous pathways in mammalian hosts. Inhibition of host-detoxification mechanisms has the potential to be extremely harmful. For example, mice, cattle, and dogs with deficiencies in ABCB function suffer from neurotoxicity of ivermectin, which is normally very safe for mammals (Seaman et al., 1987; Schinkel et al., 1994; Roulet et al., 2003). Detoxification is also essential for normal cellular metabolism and redox homeostasis (Cvilink et al., 2009). Therefore, MDR reversal compounds would ideally target multiple detoxification proteins and would be specific for nematodes.

Transcription factors as master regulators of detoxification genes

In mammals, *Drosophila* (fruitfly), and *C. elegans*, detoxification pathways are tightly regulated, so that basal activity is low and exposure to toxic xenobiotics or oxidants simultaneously activates the expression of multiple genes through inducible transcription factors. The gene-regulatory pathways upstream from detoxification effectors have been largely ignored in parasitic helminths, despite their potential to control multiple genes in all phases of detoxification.

In mammals, two nuclear hormone receptors, pregnane X receptor (PXR; NR1I2) and constitutive androstane receptor (CAR; NR1I3), are activated by exogenous and endogenous ligands and control the expression of CYP genes (Pascussi et al., 2008). Although the *C. elegans* genome is predicted to encode over 270 nuclear hormone receptors, only nuclear hormone receptor (NHR)-8 is known to play a role in the tolerance of xenobiotics (Lindblom et al., 2001). The genes regulated by NHR-8 have not been identified, and far more work is needed to understand the contribution of

this and other NHRs to xenobiotic metabolism and drug resistance in nematodes. In mammals, CYP genes are also regulated by the aryl hydrocarbon receptor (AHR). *ahr-1* is the single AHR homolog in *C. elegans*, but this gene is not required for CYP gene expression and instead plays a role in development (Huang et al., 2004; Aarnio et al., 2010).

SKN-1 regulates multiple xenobiotic detoxification and antioxidation genes

The cap-n-collar family (CNC) of basic leucine zipper transcription factors regulates the expression of xenobiotic detoxification genes in *C. elegans*, *Drosophila*, and mammals (An and Blackwell, 2003; Kobayashi et al., 2008; Misra et al., 2011) and therefore may perform this function in all animals. Included in this family is the mammalian nuclear eurythroid 2–related factor 2 (NRF2), which was originally characterized as mediating cellular defense responses to oxidants (Kobayashi and Yamamoto, 2006). It is now clear that NRF2 also mediates cellular defenses to diverse xenobiotics by controlling the expression of genes in all three phases of detoxification (e.g., Thimmulappa et al., 2002; Hu et al., 2006). Recent studies have demonstrated that enhanced NRF2 activity mediates MDR in some cancer cells (Townsend and Tew, 2003; Ohta et al., 2008; Shibata et al., 2008; Singh et al., 2008; Wang et al., 2008; Hayes and McMahon, 2009; Hayes et al., 2010; Kensler and Wakabayashi, 2010). Genetic silencing of NRF2 was able to reverse resistance by decreasing the expression of several detoxification genes (Singh et al., 2008). These results highlight the potential for transcriptional regulators to orchestrate drug resistance through multiple genes and for targeting of a single transcription factor to reverse resistance.

SKN-1 is the single CNC protein in *C. elegans*. Like NRF2, SKN-1 is activated by oxidants, electrophiles, and diverse xenobiotics and confers resistance by activating detoxification genes (An and Blackwell, 2003; An et al., 2005; Inoue et al., 2005; Kell et al., 2007; Hasegawa et al., 2008; Kahn et al., 2008; Tullet et al., 2008; Choe et al., 2009; Przybysz et al., 2009). Genome-wide transcriptional profiling of *C. elegans* with and without *skn-1* RNA interference (RNAi) has identified genes regulated by the transcription factor under basal conditions and during exposure to oxidative stress induced by hyperbaric hyperoxia, arsenite, and tert-butyl hydroxide (Oliveira et al., 2009; Park et al., 2009). Two hundred and thirty-three genes required SKN-1 for full expression under nonstressed conditions. Partially overlapping sets of 211, 118, and 64 genes required SKN-1 for increased expression with hyperbaric hyperoxia, arsenite, and organic peroxide, respectively. Sixty-five of the SKN-1-regulated genes identified in these studies are predicted to encode proteins that function in detoxification and/or antioxidation (Table 2). Functional categories include eight short-chain dehydrogenases, 20 GSTs, 10 glucuronosyltransferases, two CYPs, and two ABC superfamily genes. Importantly, the true number of genes regulated by SKN-1 is likely to be considerably higher, because the statistical requirements applied to transcriptome data are stringent and only four conditions have been tested. Our own microarray analysis of a *C. elegans* strain with constitutively active SKN-1 identifies another 44 detoxification genes under positive control of SKN-1, including six ABCB subfamily members (Choe et al., unpublished data).

Data are sparse, but two studies have provided evidence that SKN-1 target genes may play a role in anthelminthic resistance in *C. elegans*. *gcs-1*, a well-studied target of SKN-1 that encodes the rate-limiting enzyme for glutathione synthesis (e.g., gamma-glutamylcysteine synthetase; GCS), was 4-fold overexpressed in MDR strains of *C. elegans*, and the pharmacological inhibition of GCS with buthionine sulfoxamine reversed resistance (James and Davey, 2008). Five of the sixteen detoxification genes upregulated by albendazole are known to be regulated by SKN-1 (Laing et al., 2010). Taken together, the data available to date indicate that SKN-1 is a master regulator of multiple detoxification and antioxidation genes, some of which have been shown to be responsive to long-term selection or short-term exposure to anthelmintics.

SKN-1 is essential for embryonic development

Interestingly, *skn-1* was originally identified in forward genetic screens for *C. elegans* mutants with defective embryonic development (Bowerman et al., 1992). Loss-of-function *skn-1* mutant embryos do not develop intestinal or pharyngeal cells and thus do not survive. SKN-1 is maternally transcribed, but zygotically translated, to directly induce the expression of two paralogous GATA-factor-related zinc-finger proteins (e.g., MED-1/2) in four-cell–stage embryos to specify cell fates for the development of the posterior pharynx and intestine. In *skn-1* mutants (*skin* in excess), blastomeres that usually form the pharynx and intestine instead differentiate into excess body muscle and hypodermis (or, informally, skin). Therefore, targeting SKN-1 has the potential to inhibit development in embryos in addition to inhibiting xenobiotic detoxification and MDR in larvae and adults.

SKN-1 structure and regulation are distinct from NRF2

As described above, the inhibition of host-detoxification pathways can have dire consequences. Comparisons between CNC regulation and function in mammals and helminths can identify potential modes of action for pharmacological inhibitors that are specific to parasites. Studies to date have identified three distinctions between the regulation and function of SKN-1 in *C. elegans* and NRF2 in mammals: 1) DNA-binding domains; 2) mechanisms of transcriptional activation; and 3) regulation of protein levels. We discuss these here and present evidence that many nematodes share these distinctions.

Table 2. Antioxidation and deoxification genes regulated by SKN-1.

Sequence	Name	Description	Function	Control	Hyperoxia	Arsenite	tBOOH
Y54G11A.5	*ctl-2*	Catalase 2, peroxisomal	Antioxidation		X		
F26E4.12		Glutathione peroxidase	Antioxidation	X	X	X	X
R03G5.5		Glutathione peroxidase	Antioxidation	X	X		
C15F1.7	*sod-1*	Superoxide dismutase 1	Antioxidation		X		
C35B1.5		Thioredoxin, nucleoredoxin, and related proteins	Antioxidation	X	X	X	
Y52E8A.3		Thioredoxin, nucleoredoxin, and related proteins	Antioxidation	X		X	
K12G11.4	*sodh-2*	Alcohol dehydrogenase, class V	Phase I			X	
C54D1.4	*alh-10*	Aldehyde dehydrogenase	Phase I	X			
C07D8.6		Aldo/keto reductase family proteins	Phase I	X			
T08H10.1		Aldo/keto reductase family proteins	Phase I	X			
K09A11.2	*cyp-14A1*	Cytochrome P450 CYP2 subfamily	Phase I	X		X	
K09A11.3	*cyp-14A2*	Cytochrome P450 family 14A2	Phase I		X		
C46H11.2		Flavin-containing monooxygenase	Phase I	X			
F17A9.4		NADH:flavin oxidoreductase	Phase I		X		
F56D5.3		NADH:flavin oxidoreductase	Phase I		X		
C55A6.5	*sdz-8*	Predicted short chain-type dehydrogenase	Phase I			X	X
C55A6.6		Predicted short chain-type dehydrogenase	Phase I	X		X	X
C55A6.7		Predicted short chain-type dehydrogenase	Phase I	X			
F20G2.1		Predicted short chain-type dehydrogenase	Phase I	X		X	
F20G2.2		Predicted short chain-type dehydrogenase	Phase I	X		X	
F55E10.6		Predicted short chain-type dehydrogenase	Phase I			X	
K10H10.3	*dhs-8*	Predicted short chain-type dehydrogenase	Phase I	X		X	
R08H2.1	*dhs-23*	Predicted short chain-type dehydrogenase	Phase I	X		X	
F25D1.5		Reductases with broad range of substrate specificities	Phase I	X			
B0222.9		Xanthine dehydrogenase	Phase I		X		
F37B12.2	*gcs-1*	Gamma-glutamylcysteine synthetase	Phase II			X	
C53D5.5		Gamma-glutamyltransferase	Phase II	X			
E01A2.1		Glutamate-cysteine ligase regulatory subunit	Phase II			X	
Y34D9A.6	*glrx-10*	Glutaredoxin and related proteins	Phase II	X	X	X	
C02D5.3	*gsto-2*	Glutathione S-transferase	Phase II	X		X	X
D1053.1	*gst-42*	Glutathione S-transferase	Phase II	X			
F11G11.1	*gst-8*	Glutathione S-transferase	Phase II	X		X	X
F11G11.2	*gst-7*	Glutathione S-transferase	Phase II	X		X	X
F11G11.3	*gst-6*	Glutathione S-transferase	Phase II	X		X	
F35E8.8	*gst-38*	Glutathione S-transferase	Phase II	X	X	X	
F37B1.2	*gst-12*	Glutathione S-transferase	Phase II	X	X	X	
F37B1.3	*gst-14*	Glutathione S-transferase	Phase II	X	X	X	
F56A4.4		Glutathione S-transferase	Phase II	X		X	
K08F4.7	*gst-4*	Glutathione S-transferase	Phase II	X	X	X	X
R03D7.6	*gst-5*	Glutathione S-transferase	Phase II			X	X
R07B1.4	*gst-36*	Glutathione S-transferase	Phase II	X			
R107.7	*gst-1*	Glutathione S-transferase	Phase II	X		X	
T26C5.1	*gst-13*	Glutathione S-transferase	Phase II	X	X	X	
Y1H11.2	*gst-35*	Glutathione S-transferase	Phase II	X		X	
Y45G12C.2	*gst-10*	Glutathione S-transferase	Phase II	X	X	X	
Y53F4B.33	*gst-39*	Glutathione S-transferase	Phase II		X	X	
Y53F4B.37	*gst-32*	Glutathione S-transferase	Phase II		X		
Y53G8B.1		Glutathione S-transferase	Phase II	X			
F35E8.11	*cdr-1*	Glutathione S-transferase-like protein	Phase II	X		X	
M176.2		Glutathione synthetase	Phase II			X	
F25B4.8		Glutathione-dependent formaldehyde-activating enzyme	Phase II			X	
Y38F2AR.12		Hydantoinase B/oxoprolinase	Phase II			X	
C46F11.2		Orthologous to human mitochondrial glutathione reductase	Phase II			X	

(Continued)

Table 2. *(Continued)*.

Sequence	Name	Description	Function	Control	Hyperoxia	Arsenite	tBOOH
AC3.7	*ugt-1*	UDP-glucuronosyl and UDP-glucosyl transferase	Phase II	X			
B0310.5	*ugt-46*	UDP-glucuronosyl and UDP-glucosyl transferase	Phase II	X			
C08F11.8	*ugt-22*	UDP-glucuronosyl and UDP-glucosyl transferase	Phase II	X			
C17G1.3	*ugt-23*	UDP-glucuronosyl and UDP-glucosyl transferase	Phase II	X			
C18C4.3	*ugt-48*	UDP-glucuronosyl and UDP-glucosyl transferase	Phase II	X		X	
C35A5.2	*ugt-33*	UDP-glucuronosyl and UDP-glucosyl transferase	Phase II				X
F35H8.6	*ugt-58*	UDP-glucuronosyl and UDP-glucosyl transferase	Phase II			X	
K04A8.10		UDP-glucuronosyl and UDP-glucosyl transferase	Phase II	X			
Y39G10AR.6	*ugt-31*	UDP-glucuronosyl and UDP-glucosyl transferase	Phase II	X			
ZC443.6	*ugt-16*	UDP-glucuronosyl and UDP-glucosyl transferase	Phase II	X			
W09D6.6	*hmt-1*	Heavy metal exporter HMT1, ABC superfamily	Phase III			X	
T02D1.5	*pmp-4*	Peroxisomal long-chain acyl-CoA transporter, ABC superfamily	Phase III				X

WormBase sequence and gene (when availible) names are listed. Genes are from Park et al. (2009) (hyperoxia) and Olivera et al. (2009) (control, arsenic, and tBOOH) using the authors' statistical stringencies. Genes in bold were identified as upregulated by albendazole in a transcriptomic analysis (Laing et al., 2010) and genes in bold and underlined were identified as upregulated in a candidate gene study in ivermectin resistance stains (James and Davey, 2008). tBOOH, tert-butyl hydroxide.

SKN-1 structure is unique in nematodes

Despite being in the basic region leucine zipper (bZIP) family of DNA-binding transcriptional regulators and sharing a conserved core basic region with other CNCs, SKN-1 is unique because it lacks a leucine zipper (Blackwell et al., 1994). Other known bZIP proteins have leucine-rich α-helix regions that mediate protein dimerization and stabilize core basic amino-acid regions so that they can bind to two adjacent major grooves in DNA. The interaction between SKN-1 and its target DNA bases [(A/T)(A/T)T(G/A)TCAT] was studied in detail with biochemical and crystal structure analyses (Blackwell et al., 1994; Carroll et al., 1997; Pal et al., 1997; Rupert et al., 1998; Kophengnavong et al., 1999). These studies found that SKN-1 forms a unique monomeric DNA-binding motif that uses a second and novel basic (positive) amino-acid region that stabilizes DNA binding by interacting with the negative phosphate backbone (Rupert et al., 1998) and contributes to target DNA sequence specificity (Kophengnavong et al., 1999). Relative to human NRF2, *C. elegans* SKN-1 is also missing a region at its carboxyl terminus named Neh3, which contributes transcriptional activity by interacting with another DNA-binding protein named CHD6 (Nioi et al., 2005).

To determine whether the novel SKN-1 basic region and loss of the leucine zipper and Neh3 features are shared by SKN-1 in other species, we performed BLAST searches using *C. elegans* SKN-1 and human NRF2 as the query protein sequences. Five major clades have been defined in the nematode phylum (I–V), with clades I and II the most basal and IV and V the most derived (Blaxter, 1998; Blaxter et al., 1998). We compiled predicted SKN-1/NRF2 homologs from one species in clade I, one in clade III, three in clade IV, four in clade V, and eight other species representing diverse animal phyla. A protein alignment of the carboxyl-terminal regions of these predicted proteins is shown in Figure 1A (see the Supplemental Information for details of the alignment method). The core basic region, which interacts with the major grove in target DNA (Rupert et al., 1998), is found in all species. Conversely, nematode clades III, IV, and V share a unique upstream feature that corresponds to the second basic region originally identified in SKN-1 of *C. elegans* (Blackwell et al., 1994; Rupert et al., 1998). Figure 1B highlights amino acids that align with the *C. elegans* SKN-1 basic region by functional properties and lists the predicted charge for this region from each protein at pH 7.0. This short element carries a net charge of 2.9–4.9 at pH 7.0 in nematode clades III–V, but a charge of –2.8–2.1 in all other groups, including *Xiphinema index* (a plant pathogen vector) in nematode clade I. All nematode proteins from clades III–V also have a glycine at position 4 that contributes to DNA binding (Kophengnavong et al., 1999). Because clades III–V form their own lineage within Nematoda (Blaxter, 1998), this SKN-1 basic region most likely originated in their common ancestor.

In turn, all predicted proteins from non-nematode species share a downstream leucine zipper and Neh3 domain (Figure 1A). Secondary structure prediction (PSIPRED) recovers an α-helix in human NRF2, which corresponds to its predicted leucine zipper (Moi et al., 1994), providing an important positive control for our other secondary structure predictions (Figure 1C). A well-defined α-helix is also predicted in all proteins from other animal phyla, except Nematoda. The presence of this α-helix/leucine zipper includes the phyla Porifera (sponge) and Cnidaria (sea anemone) that are well accepted as basal within Metazoa (Halanych, 2004; Ruiz-Trillo et al., 2008). Thus, this structural element extends at least as far back as to all animals. Conversely, the absence of this α-helix/leucine zipper includes clade I of nematodes, which is basally related to clades III–V (Blaxter, 1998). Thus, this structural element was most likely lost within the nematode common ancestor.

The C-termini of all SKN-1 proteins from nematodes (except for *Meloidogyne* and *Heterodera*) are either

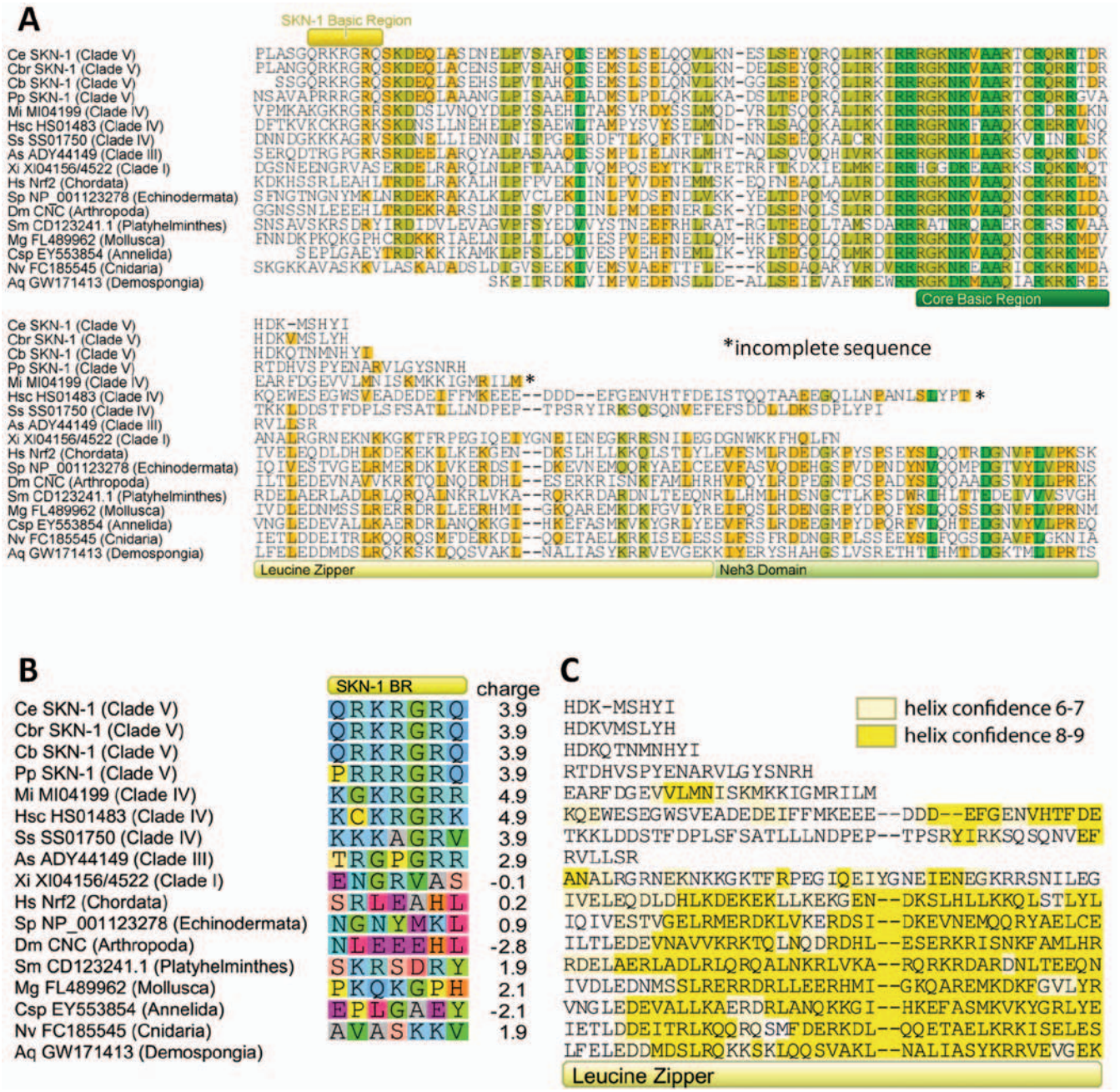

Figure 1. Alignment of carboxyl-terminal regions of SKN-1/NRF2 homologs from multiple nematodes and diverse animal phyla (**A**) Proteins are listed by species abbreviations, protein names or accession numbers, and phylum or nematode clade in parentheses. The alignment is color coded by degree of conservation. (**B**) Alignment of the SKN-1 basic region color coded by amino-acid properties and listing the charge of the region. Species are: Ce, *C. elegans*; Cb, *C. briggsae*; Cbr, C. *brenneri*; Pp, *P. pacificus*; Mi, *Meloidogyne incognita*; Hsc, *Heterodera schachtii*; Ss, *Strongyloides stercoralis*; As, *Ascaris suum*; Xi, *X. index*; Hs, *Homo sapiens*; Sp, *Strongylocentrotus purpuratus*; Dm, *Drosophila melanogaster*; Sm, *Schistosoma mansoni*; Mg, *Mytilus galloprovincialis*; Csp, *Capitella* spp.; Nv, *Nematostella vectensis*; and Aq, *Amphimedon queenslandica*. Sequences are from WormBase (Ce, Cb, Cbr, and Pp), Nematode.net (Xi, Mi, Hsc, and Ss), or the National Center for Biotechnology Information (all others). (**C**) Alignment of the leucine zipper color coded according to the confidence of an α-helix ("9" being highest). See Supplementary Information for details on the charge calculation and secondary structure predictions.

known or inferred to be complete on the basis of an in-frame stop codon. Conversely, the completeness of the C-termini for *Meloidogyne* and *Heterodera* SKN-1 remain less clear because they lack stop codons. Nevertheless, this uncertainty about C-terminal completeness is regarded as of minor concern to our conclusion of an absent leucine zipper, because only the *Heterodera* sequence ends short of its full alignment to this domain (Figure 1A). The absence of Neh3 is less certain, because both of these sequences end before their full alignment to this domain.

These patterns suggest that the SKN-1 basic region could contribute to DNA binding in nematodes of clades III–V. Evolution of this region may have permitted, or

compensated for, the loss or divergence of the leucine zipper. Loss of Neh3 by at least some nematodes suggests that SKN-1 may activate the transcription of target genes differently than in other animals, including mammals (Nioi et al., 2005). Sequence data for SKN-1/NRF2 from more species within basal nematode clades and other Ecdysozoa (the superfamily that includes nematodes, arthropods, and several smaller phyla) are needed to refine the phylogenetic origin of these changes, and functional studies are needed to verify the role of the SKN-1 basic region in nematodes other than *C. elegans*.

SKN-1 regulation is distinct from NRF2 in mammals

SKN-1 activity is low under basal conditions, but is highly induced by exposure to xenobiotics and oxidants. To identify regulators of SKN-1, we recently performed a genome-wide RNAi screen for genes that repress transcription of the SKN-1 target gene, *gst-4*. Proteasome subunits, a ubiquitin ligase, and a WD40 repeat protein named *wdr-23* were among the genes identified (Choe et al., 2009). Subsequent genetic and biochemical studies support a model (Figure 2A) in which WDR-23 recruits SKN-1 to the CUL4/DDB1 ubiquitin ligase in the cell nucleus and targets the transcription factor for degradation by the proteasome. The principal role of WDR-23 in repressing SKN-1 was independently confirmed by a forward genetic screen (Hasegawa and Miwa, 2010). Although the physiological function of NRF2 is very similar to SKN-1, it is regulated by a distinct ubiquitin ligase. In the cytoplasm of mammalian cells, NRF2 is recruited to the CUL3 ubiquitin ligase by a kelch repeat protein named KEAP1 (McMahon et al., 2003, 2004, 2010; Kobayashi et al., 2006, 2008; Zhang, 2010) (Figure 2B). A protein orthologous to KEAP1 (see our phylogenetic results below) also represses the *Drosophila* CNC, indicating that this pathway is conserved (Sykiotis and Bohmann, 2008). Although WD40 repeat and kelch repeat proteins both fold into β-propellers, they share no sequence homology (Chaudhuri et al., 2008; Hudson and Cooley, 2008). These ubiquitin ligases directly repress their respective CNC proteins, and exposure to oxidants or electrophiles is thought to activate the transcription factors by releasing them from repression (Choe et al., 2009; Giudice et al., 2010; Zhang, 2010; Keum, 2011).

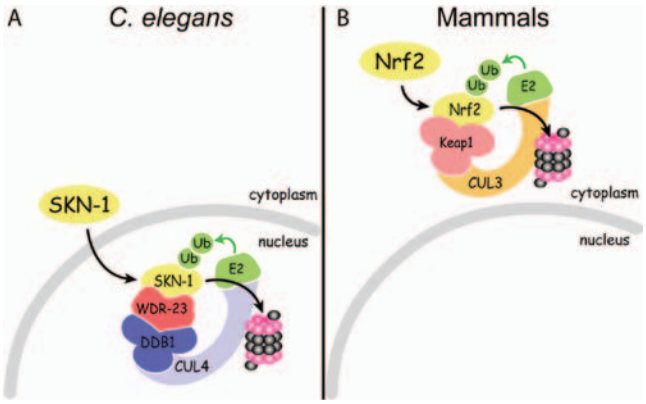

Figure 2. SKN-1 in *C. elegans* and NRF2 in mammals are regulated by distinct ubiquitin ligases. CUL3 and CUL4 are distinct ubiquitin ligases. WDR-23 and KEAP1 are distinct proteins that are thought to function in different cellular compartments (i.e., nucleus versus cytosol). Both pathways are thought to label their target with ubiquitin for proteasomal degradation.

Although the precise mechanisms of NRF2 activation by oxidants and electrophiles are still being defined and appear to vary by the chemical nature of the inducer, there are some well-accepted models. In general, activation is thought to occur by direct modifications of KEAP1 or phosphorylation of NRF2 that, in turn, promote the stabilization of NRF2 (Kaspar et al., 2009). KEAP1 contains multiple redox-reactive cysteines that are directly modified by NRF2-activating electrophiles or endogenous stress-related chemicals (Holtzclaw et al., 2004; Kobayashi et al., 2009; Giudice et al., 2010; McMahon et al., 2010). Modification of KEAP1 stabilizes NRF2 either by preventing interaction (Dinkova-Kostova et al., 2002; Wakabayashi et al., 2004) or causing ubiquitinylation and degradation of KEAP1 (Eggler et al., 2005; Hong et al., 2005; Zhang et al., 2005).

A growing body of evidence indicates that multiple protein kinases also contribute to the regulation of NRF2 abundance and activity (Giudice et al., 2010; Keum, 2011). These include protein kinase C (Huang et al., 2002; Bloom and Jaiswal, 2003), mitogen-activated protein kinases (MAPKs) (Zipper and Mulcahy, 2000; Buckley et al., 2003), PKR-like endoplasmic reticulum kinase (PERK) (Cullinan et al., 2004), and glycogen synthase kinase 3 (Salazar et al., 2006; Rojo et al., 2008; Rada et al., 2011). The molecular mechanisms linking all these kinases to NRF2 activation are still being defined, but some have been reported to modify interactions with KEAP1, modulate degradation by additional degradation pathways, or regulate nuclear export of the transcription factor (Huang et al., 2002; Bloom and Jaiswal, 2003; Kaspar et al., 2009; Giudice et al., 2010; Keum, 2011; Rada et al., 2011). Phosphorylation of a KEAP1 tyrosine residue has also been shown to play a role in NRF2 regulation (Jain et al., 2008).

Protein kinase pathways also regulate SKN-1 during stress. p38 MAPK (PMK-1) and extracellular-regulated kinase (e.g., MPK-1) pathways directly phosphorylate SKN-1 at residues (e.g., S74 and S340) that promote nuclear accumulation and activation (Inoue et al., 2005; Okuyama et al., 2010). Four other kinases have been implicated in the regulation of SKN-1 during stress, but it is not known whether they phosphorylate the factor directly (Kell et al., 2007). GSK-3 and insulin-like receptor-regulated kinases (e.g., SGK-1 and AKT-1/2) inhibit SKN-1 nuclear accumulation by direct phosphorylation (An et al., 2005; Tullet et al., 2008). Using genetics, we provided evidence that WDR-23 acts downstream from at least three of these protein kinase pathways to regulate SKN-1, suggesting that phosphorylation may modify regulation by WDR-23 (Choe et al., 2009). Like KEAP1, WDR-23 has several cysteine residues (i.e., 17). It is not yet known whether electrophiles modify these cysteines and contribute to SKN-1 activation.

Given the importance of ubiquitin ligases to the regulation of CNC proteins, it will be important to determine whether WDR-23 or KEAP1 regulates SKN-1 in nematodes other than just *C. elegans*. WDR-23 is an evolutionarily ancient protein that is present in plants, fungi, and animals, including, probably, all nematodes (Choe et al., 2009). The human and *C. elegans* WDR-23 orthologs interact with DDB1 through a specific "DWD" domain (Angers et al., 2006; Jin et al., 2006; Lee and Zhou, 2007; Choe et al., 2009) that is also conserved in plant WDR-23 orthologs (Lee et al., 2008), suggesting that the ubiquitin ligase function of the protein may be conserved in all eukaryotes. Alternatively, the CNC protein family is not present in plants, and WDR23 does not appear to regulate or interact with NRF2 in human cells (Choe et al., unpublished observations), suggesting that WDR-23 has switched substrates during evolution.

The kelch repeat protein family is found in bacteria, plants, fungi, and animals (Prag and Adams, 2003). Surprisingly, a putative KEAP1 ortholog has not been described in *C. elegans*, raising the critical question of whether KEAP1 exists in nematodes and other related protostomes. Therefore, we performed maximum likelihood and Bayesian phylogenetic analyses of protein sequences found by BLAST searching with human KEAP1 in representatives of nematode clades I, III, and V and diverse animal phyla (Figures 3 and Supplementary Figure 1; see Supplementary Information for details of the analyses). The final alignment of kelch repeat proteins covers 988 amino acids in 114 different protein sequences from four nematodes [*Trichenella spiralis* (tissue-dwelling parasite of humans), *Loa loa* (filarial parasite of humans), *B. malayi,* and *C. elegans*)], six other invertebrate phyla, and human. The bootstrap maximum likelihood tree, which is more conservative than the Bayesian tree in terms of its group support, provides clear evidence for the recognition of a well-defined subfamily that includes human KEAP1 (Figure 3). Human KEAP1 first strongly groups (99% bootstrap score) with a single sequence from each of the major bilaterian phyla of Annelida, Echinodermata, Mollusca, and Platyhelminths

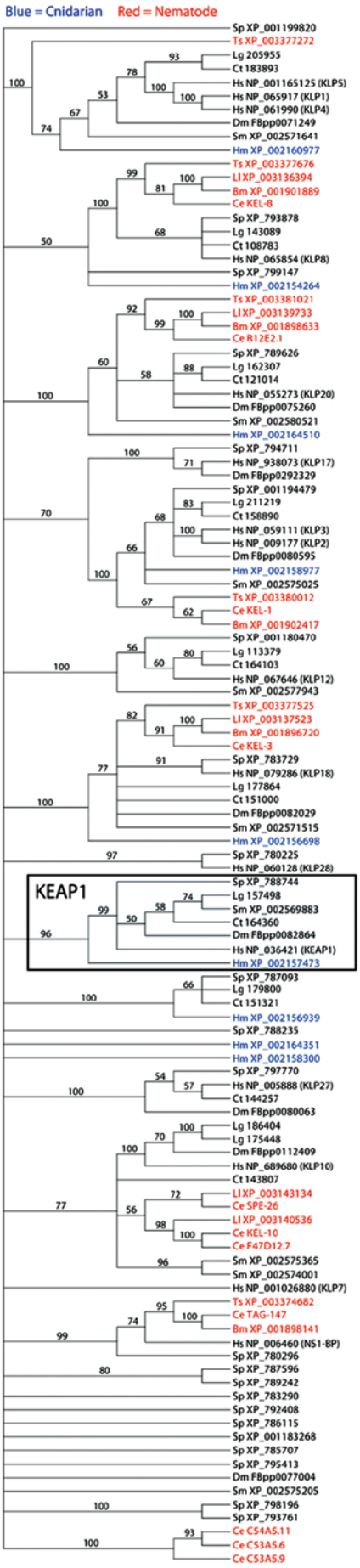

Figure 3. Majority-rule consensus tree summarizing the 1,000 phylogenies from bootstrap maximum likelihood analysis. Bootstrap scores are presented next to those internal branches with >50% support. Each sequence name consists of a two-letter abbreviation for its genus/species, followed by its database accession number in FlyBase (Dm), Joint Genome Institute (Ct and Lg), WormBase (Ce), and the National Center for Biotechnology Information (all others). The names for the human sequences also include specific kelch protein designations (in parentheses) as recognized in the literature. Nematode and hydra sequences are highlighted in red and blue, respectively. The box highlights those sequences that are inferred as orthologs of the KEAP1 subfamily according to these results. Abbreviations: Hm, *Hydra magnipapillata* (Cnidaria); Sm, *Schistosoma mansoni* (Platyhelminths); Ts, *Trichinella spiralis* (Nematoda, clade I); Bm, *B. malayi* (Nematoda, clade III); Ll, *L. loa* (Nematoda, clade III); Ce, *C. elegans* (Nematoda, clade V); Lg, *Lottia gigantean* (Mollusca); Ct, *Capitella teleta* (Annelida); Dm, *Drosophila melanogaster* (Arthropoda); Sp, *Strongylocentrotus purpuratus* (Echinodermata); and Hs, *Homo sapiens* (Chordata).

and includes the previously described KEAP1-like protein from *Drosophila* (Sykiotis and Bohmann, 2008). This group then joins (96% score) with a single sequence from Cnidaria. Because Cnidaria is phylogenetically basal within Eumetazoa (Halanych, 2004; Dunn et al., 2008), these strong bootstrap results allow for the recognition of a KEAP1 subfamily that spans all multicellular animals more recent than sponges and some other less-known groups. The Bayesian phylogeny provides further support for the same KEAP1 subfamily (100% posterior probability), but with better resolution of relationships within the subfamily (Supplementary Figure 1).

Nematodes are well accepted as members of the Ecdyzoa protostome clade (Halanych, 2004; Giribet et al., 2009). Therefore, it is surprising that there are no nematode sequences that join with *Drosophila* (arthropod) KEAP1. Indeed, no nematode sequence is found anywhere within the entire KEAP1 subfamily (Figure 3 and Supplementary Figure 1). Thus, our trees strongly agree that the Kelch repeat proteins of the four represented nematodes are all paralogs of KEAP1 and that none are orthologs. By definition, orthologs are more closely related to each other than are paralogs (Goodman et al., 1979). Thus, our successful recovery of more distantly related paralogs from the genomic databases of these nematodes serves as a critical positive control that these four species are indeed missing a KEAP1 ortholog (Hyndman et al., 2009). Given that our four species of nematodes belong to distantly related clades of Nematoda, I, III, and V (Blaxter et al., 1998), the lack of a KEAP1 ortholog may be phylum wide. If so, the absence of KEAP1 would be attributed to the loss and/or silencing of the gene in the nematode common ancestor or in an even older ancestor that nematodes share with other ecdyzoan phyla. Gene loss has been shown to be particularly high for nematodes (Mitreva et al., 2011). Further work is needed to determine whether WDR-23 functionally compensates for the loss of KEAP1 in nematodes other than *C. elegans*.

Summary

Drug resistance is a major obstacle to control of parasitic nematodes, and mechanisms of resistance are poorly understood in this group of helminths. In diverse organisms, MDR is often associated with or caused by changes to xenobiotic detoxification mechanisms. Transcription factors, such as SKN-1, regulate multiple detoxification genes and are promising, but largely unexplored, candidates for understanding and reversing resistance. SKN-1 is also essential for embryonic development. Despite very similar functions, many aspects of SKN-1 structure and regulation in *C. elegans* are distinct from NRF2 in mammals. Comparative analyses predict that SKN-1 structure and regulation could be similar among many nematodes, but unique relative to other animal phyla (see Table 3 for a summary), providing promising targets for specificity. Given that many parasitic nematodes have limited genetic tractability, the development of small-molecule modulators of SKN-1 would provide useful tools for studying the function of this transcriptional pathway. Transgenic *C. elegans* reporter strains for SKN-1 activity have been developed and perform well in whole-animal, high-throughput assays, making screening for small-molecule inhibitors feasible (Leung et al., 2011).

Table 3. Summary of evidence for the presence or absence of SKN-1/NRF2 domains, KEAP1, and WDR-23.

	SKN-1				
Taxononmic group	LeuZ	BR	Neh3	KEAP1	WDR-23
Demospongia	+	?	+	?	+
Cnidaria	+	–	+	+	+
Platyhelminthes	+	–	+	+	+
Annelida	+	–	+	+	+
Mollusca	+	–	+	+	+
Nematoda clade I	–	–	–	–	+
Nematoda clade II	?	?	?	?	?
Nematoda clade III	–	+	–	–	+
Nematoda clade IV	–	+	?	?	+
Nematoda clade V	–	+	–	–	+
Arthropoda	+	–	+	+	+
Echinodermata	+	–	+	+	+
Chordata	+	–	+	+	+

+, present; –, absent; ?, not tested or tested but conclusion is not clear; LeuZ, leucine zipper; BR, basic region.

Important questions concerning SKN-1 function and regulation include

- Are mutations in *wdr-23* or *skn-1* associated with MDR in parasitic nematodes?
- What genes does SKN-1 regulate in parasitic species and are these associated with MDR?
- Does WDR-23 regulate SKN-1 in parasitic nematodes?
- Is SKN-1 activated by any of the common anthelmintics?
- Does SKN-1 defend parasites from host-immune responses?
- Is the function of SKN-1 in embryonic development conserved in other nematodes?
- How do WDR-23 and SKN-1 interact in *C. elegans* and how conserved are the interaction motifs among other nematodes?

Declaration of interest

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