Nematode Hsp90: highly conserved but functionally diverse

VICTORIA GILLAN* *and* EILEEN DEVANEY

Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Garscube Estate, Bearsden Road, Glasgow, G61 1QH, UK

(*Received 13 December 2013; revised 14 February 2014; accepted 14 February 2014; first published online 10 April 2014*)

SUMMARY

Nematodes are amongst the most successful and abundant organisms on the planet with approximately 30000 species described, although the actual number of species is estimated to be one million or more. Despite sharing a relatively simple and invariant body plan, there is considerable diversity within the phylum. Nematodes have evolved to colonize most ecological niches, and can be free-living or can parasitize plants or animals to the detriment of the host organism. In this review we consider the role of heat shock protein 90 (Hsp90) in the nematode life cycle. We describe studies on Hsp90 in the free-living nematode *Caenorhabditis elegans* and comparative work on the parasitic species *Brugia pahangi*, and consider whether a dependence upon Hsp90 can be exploited for the control of parasitic species.

Key words: Nematodes, *Caenorhabditis elegans*, *Brugia pahangi*, Hsp90, heat shock protein, co-chaperone.

INTRODUCTION

The majority of nematodes are free-living, playing important roles in ecosystem health and diversity, but many are important and prolific parasites. For an idea of scale, approximately half the world's population, predominantly in tropical regions, harbour a nematode infection (www.nematode.net). These worms typically cause chronic infections that contribute significantly to morbidity, but rarely kill their hosts. The most abundant parasitic species are gastrointestinal nematodes such as *Ascaris lumbricoides*, which infects an estimated 807 million, closely followed by *Trichuris trichiura* (infecting an estimated 604 million) and the hookworms (infecting approximately 576 million) (http://www.cdc.gov/parasites/). These infections are often particularly prevalent and clinically important in children. Filarial nematodes are tissue-dwelling worms that account for an additional 157 million infections and are the causative agents of river blindness (*Onchocerca volvulus*) and elephantiasis (*Wuchereria bancrofti* and *Brugia* spp.). In addition to their importance as human pathogens, around 10% of global crop loss is due to plant parasitic nematodes (Nicol *et al*. 2011), while nematode parasites of livestock cause major economic production losses to grazing ruminants. Currently, infection is controlled by the use of anthelmintic drugs. However, anthelmintic resistance, particularly in nematodes of small ruminants such as sheep and goats, is endemic in most countries (Gilleard, 2006). Given that the same anthelmintic drugs are used in mass administration campaigns to treat human nematode infection, there is concern over the possible development of resistance in human nematodes (Vercruysse *et al*. 2012), underscoring the need for the development of new drugs to control these pathogens (Geary *et al*. 2010). In this review, we focus on the role of Hsp90 in both free-living and parasitic nematodes, specifically *Caenorhabditis elegans* and *Brugia* spp. (life cycles of which are outlined in Fig. 1) and discuss the potential of Hsp90 as a drug target for the treatment of some helminth infections.

* Corresponding author: Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Garscube Estate, Bearsden Road, Glasgow, G61 1QH, UK. E-mail: victoria.gillan@glasgow.ac.uk

NEMATODE GENOMES

Analysis of nematode phylogeny based on the small subunit ribosomal DNA sequences divided the phylum into five clades: Dorylaimia (Clade I), Enoplia (Clade II), Spurina (Clade III), Tylenchina (Clade IV) and Rhabditina (Clade V), each containing parasitic species (Blaxter *et al*. 1998). The free-living model organism *C. elegans* belongs to clade V, along with many important parasitic species of humans and animals, while filarial nematodes and *Ascaris* belong to clade III. *C. elegans* is commonly used to study the fundamental principles of biology and for understanding mechanisms of human disease, as well as acting as a model for parasitic nematodes (Kirienko *et al*. 2010; Hashmi *et al*. 2013; Li and Le, 2013). In 1998, the sequencing of the *C. elegans* genome heralded a new era of whole-organism research, and inspired other nematode genome sequencing projects. Draft genome sequences are now available for a range of parasitic species including *Brugia malayi* (Ghedin *et al*. 2007), *Ascaris suum* (Jex *et al*. 2011), *Trichinella spiralis*

Parasitology (2014), **141**, 1203–1215.
doi:10.1017/S0031182014000304

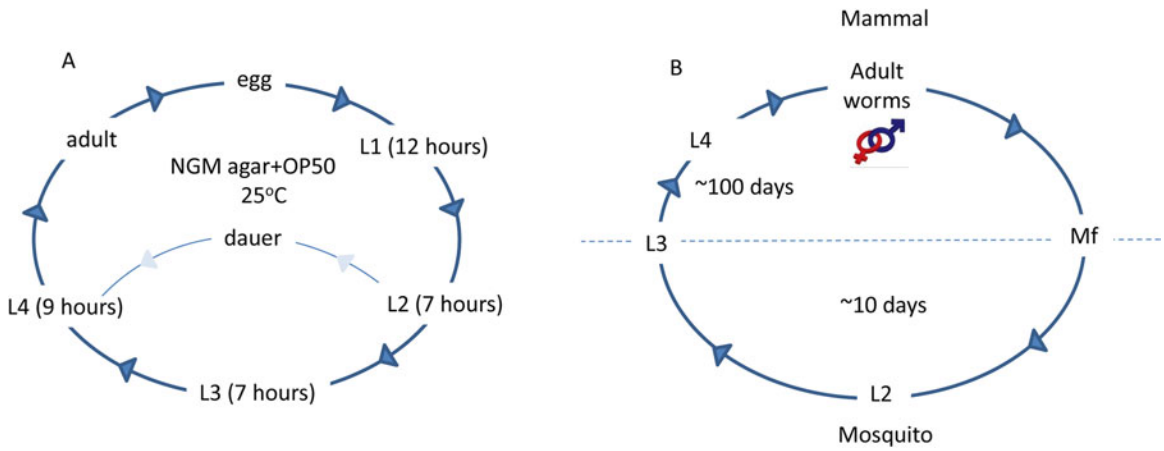

Fig. 1. Life cycle of the free-living nematode *C. elegans* and the parasitic lymphatic filarial nematodes. The normal hermaphroditic life cycle of *C. elegans* (A) takes approximately 3 days. Eggs are fertilized within the adult and after laying, hatch and worms proceed through four larval stages, each of which ends in a moult. Each adult will lay approximately 300 eggs and live for approximately 2 weeks. Under adverse conditions (i.e. lack of food, unfavourable temperatures or overcrowding), *C. elegans* can enter an alternative pathway called 'dauer'. In this state, worms can remain dormant for 3 months and can re-enter the life cycle when the environment becomes favourable. All the lymphatic filarial nematodes share an invariant life cycle (B). The full developmental cycle takes place in the mosquito (the intermediate host) or the human (the definitive host). Infection of the definitive host is initiated by the bite of a mosquito harbouring the infective L3. The L3 enters the body via the puncture site and migrates to the lymphatic system of the mammalian host. L3 moult through L4 to become sexually mature adult male and female worms, which have a lifespan of approximately 8 years. After sexual reproduction the female worms release microfilariae (Mf, first stage larvae), which migrate to the bloodstream and are available for ingestion by a mosquito taking a blood meal. Development from Mf to the L3 stage within the mosquito occurs in the thoracic muscles and is a temperature-dependent process (optimal 28 °C and 80% humidity). Mature L3 migrate to the feeding structures in the head of the mosquito, which facilitates their transmission to the definitive host. It should be noted that other species of parasitic nematode do not require an arthropod intermediate host and have 'free-living' stages, where they are present in the environment in an infective form. The schematic presented above refers to lymphatic filarial parasites only.

(Mitreva *et al*. 2011), *Dirofilaria immitis* (Godel *et al*. 2012), *Haemonchus contortus* (Laing *et al*. 2013; Schwarz *et al*. 2013), *Loa loa* (Desjardins *et al*. 2013), *Necator americanus* (Tang *et al*. 2014) and the plant parasitic nematodes *Melodoigyne incognita* (Abad *et al*. 2008), *Meloidogyne hapla* (Opperman *et al*. 2008), as well as the necromenic species *Pristionchus pacificus* (Dieterich *et al*. 2008; Rae *et al*. 2008) and additional *Caenorhabditis* species (Stein *et al*. 2003). In addition, the 50 helminth genomes project coordinated by the Wellcome Trust Sanger Institute aims to provide draft genomes for 50 helminths, including many important parasitic nematodes (see www.sanger.ac.uk/research/initiatives/globalhealth/research/helminthgenomes/).

Furthermore, there are databases of expressed sequence tags (ESTs), which have proved to be very useful in establishing a catalogue of the mRNA transcripts expressed in various species and in different life cycle stages. Access to ESTs can be found in various online databases, the most extensive of which is NEMBASE4 (http://www.nematodes.org/nembase4/) which reports on over 223 000 nematode genes across >60 species. Nematode transcriptomics (RNA sequencing) is set to blossom in the coming years with the advent of next generation sequencing with high throughput at significantly lower cost, thus enabling the production of large datasets. The comparative analysis of multiple nematode genomes should facilitate the identification of candidate genes of interest and hopefully lead to new drug targets for many nematode pathogens.

HSP90 IN NEMATODES

Hsp90 is a highly conserved molecule across all species. For example, Hsp90 from *B. malayi* and *Brugia pahangi* are 99·9% identical. Hsp90 from humans and *Brugia* spp. share 77% identity, while Hsp90 from *C. elegans* and *Brugia* spp. are 84% identical. Despite this level of conservation, the function of Hsp90 seems to vary between different nematode species, as it does between normal and malignant mammalian cells (Kamal *et al*. 2003). The first indication that some nematodes may possess an atypical Hsp90 came from a study that demonstrated that *C. elegans* Hsp90 (DAF-21) was unable to bind to geldanamycin (GA), the prototype Hsp90 inhibitor (David *et al*. 2003). DAF-21 is clearly required in *C. elegans*, as demonstrated by the arrested phenotype of a loss of function mutant (Birnby *et al*. 2000). However, attempts to chemically inhibit DAF-21 by growth of the nematode on plates containing high levels of GA or by feeding worms on cultures of *Streptomyces hygroscopicus* (the actinomycete which synthesizes GA), produced no obvious phenotype

https://doi.org/10.1017/S0031182014000304 Published online by Cambridge University Press

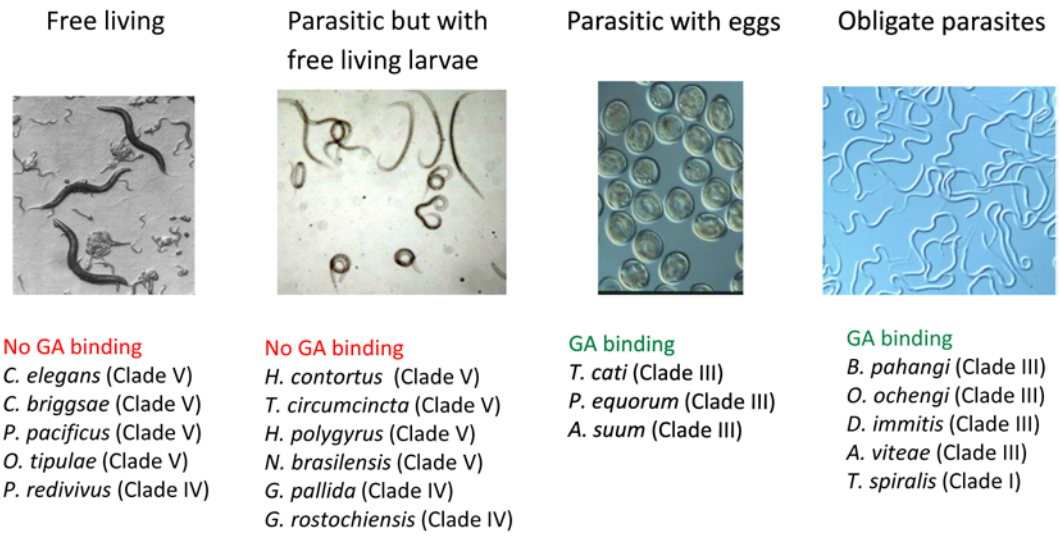

Fig. 2. Outline of nematode species and GA-binding properties. Extracts of nematodes were incubated with GA beads and pull-downs analysed by SDS-PAGE and immune-blotting. Details in Him *et al*. (2009).

(David *et al*. 2003). A subsequent survey of Hsp90 in 24 different nematode species demonstrated that *C. elegans* DAF-21 was not unique in this respect (Him *et al*. 2009). Using extracts of a variety of free-living and parasitic nematodes, it was shown that the ability to bind GA was associated with the life history of a particular species. Nematodes that are obligate parasites or those that live in the environment enclosed within an egg were able to bind GA; in contrast, free-living species and those with free-living larval stages did not bind GA (summarized in Fig. 2). This analysis is consistent with the adaptive evolution hypothesis proposed by David *et al*. in their original paper. *Streptomyces hygroscopicus* is a soil-dwelling species and thus nematodes with free-living stages in the environment may be exposed to GA or similar inhibitors during their life cycle, driving the evolution of a GA-resistant form of Hsp90. To further test this hypothesis, the amino acid sequences of Hsp90 from 15 species of nematodes were compared to determine if any of the GA-resistant strains showed evidence of adaptive Darwinian evolution. Although there was evidence of rapid diversifying evolution of *hsp90* along three separate nematode lineages, no evidence was found for amino acid changes that correlated with a change in GA-binding (Him *et al*. 2009). Thus the rapid evolution of the *hsp90* gene is presumably associated with some other important function, given its multifaceted role within the cell.

Studies using chimeric Hsp90 molecules demonstrated that the inability of *C. elegans* DAF-21 to bind GA was associated with the N-terminal region (David *et al*. 2003). GA is a natural product of *S. hygroscopicus* and studies on this organism and on other microbes that produce Hsp90 inhibitors, have cast some light upon the adaptive mechanisms by which these organisms avoid self-toxicity. A combination of elegant structural and mutational studies in *S. hygroscopicus* (Millson *et al*. 2011) and in the fungus, *Humicola fuscoatra*, which synthesizes the Hsp90 inhibitor Radicicol, demonstrated the importance of naturally occurring amino acid substitutions in the N-terminal domain of Hsp90, which alter the binding of the appropriate drug. For *H. fuscoatra*, a single leucine to isoleucine substitution (L34I) within the N-terminal binding pocket of Hsp90 significantly altered the binding of Radicicol, while retaining the ability to bind GA and ATP and its chaperone activity (Prodromou *et al*. 2009). These studies raise the question of whether such evolutionary pressures can result in similar mutations within *hsp90* of higher organisms. Despite its widespread use as a model organism, our understanding of the ecology of *C. elegans* in the wild remains patchy: does *C. elegans* live in the same ecological niche as inhibitor-producing microbes in nature? A recent study surveyed the bioavailability of over 1000 drug-like molecules in *C. elegans* and demonstrated that many molecules fail to accumulate in the worm and thus have no apparent activity (Burns *et al*. 2010), further suggesting that protection against toxins could be a credible selection pressure. However, while this phenomenon may contribute to the lack of activity of Hsp90 inhibitors on *C. elegans*, it does not explain the inability of DAF-21 to bind to Hsp90 inhibitors in soluble extracts.

FUNCTIONAL STUDIES ON HSP90 IN *C. ELEGANS*

Hsp90 has been well characterized in yeast and cultured mammalian cell lines, as an ATP-dependent, ubiquitous, molecular chaperone, which interacts with multiple 'client' proteins; these are proteins which require the activity of Hsp90 for folding or stability. However, much less is known about the cellular function of Hsp90 in the complete metazoan.

https://doi.org/10.1017/S0031182014000304 Published online by Cambridge University Press

Inevitably, much of our understanding of the role of Hsp90 in nematodes comes from work on *C. elegans*. This free-living nematode is easily maintained in the laboratory setting, unlike parasitic species, which often have complex life cycles involving intermediate and definitive hosts and/or stages that develop in the environment (summarized in Fig. 1). The life cycle of *C. elegans* takes only 3 days and, additionally, a range of powerful molecular techniques such as RNA interference (RNAi), mutagenesis and transgenesis, can be employed in *C. elegans*, whereas the application of such methods to most parasitic nematodes is in its infancy.

Mammals and yeast contain two isoforms of Hsp90 present in the cytosol, while in *C. elegans* there is a single *hsp90* orthologue, known as *daf-21*, located on chromosome V. Worms with a loss of function mutation in *daf-21* arrest at the L2–L3 stage (Birnby *et al*. 2000) confirming the requirement for wild type DAF-21 in life cycle progression in *C. elegans*. Early studies demonstrated that *C. elegans* underwent a classical heat shock response following exposure to elevated temperature, with the induction of a number of genes, particularly those encoding small Hsps (Candido *et al*. 1989; Jones *et al*. 1989). The expression of most Hsps in response to elevated temperature is regulated by heat shock factor (HSF-1), a transcription factor that binds to conserved heat shock elements in the upstream region of Hsp genes (Wu, 1995). However, in *C. elegans* knock-down of *hsf-1* by RNAi had minimal effect upon the levels of DAF-21 in nematodes exposed to heat shock, while significantly decreasing the expression of the small Hsp, Hsp-16 (Walker *et al*. 2003). These data suggest that *daf-21* expression may be regulated by factors other than the classic HSF-1. Interestingly, previous studies had shown that *daf-21* was up-regulated 15-fold in dauer larvae, an alternative developmental stage that *C. elegans* can enter upon encountering unfavourable conditions, such as overcrowding or lack of food. Dauer larvae are stress-resistant and long-lived. Entry into, and recovery from, the dauer stage is regulated by levels of a pheromone produced by the worms (Golden and Riddle, 1982). When worms were stimulated to exit the dauer stage, levels of *daf-21* mRNA declined rapidly (Dalley and Golomb, 1992). Similar findings were reported by Jones *et al*. (2001) who showed that the expression of *daf-21* was up-regulated in the dauer stage compared with mixed life cycle stages, but that this was not the case for *hsp-70* (Jones *et al*. 2001). However, comparative proteomic analysis of *C. elegans* mixed life cycle stages and dauer larvae showed no over-expression of DAF-21 protein in dauers (Jeong *et al*. 2009). Why *daf-21* mRNA should be so highly expressed in the dauer stage remains unclear. One possibility is that *daf-21* mRNA may be accumulated in dauer larvae in readiness for the transition to normal development, where elevated levels of DAF-21 could be required to chaperone proteins required for non-dauer development. Further studies would need to examine levels of DAF-21 over a time course, as the animal exits the dauer stage. Additional evidence for a role for DAF-21 in the dauer pathway comes from studies of a gain of function *daf-21* mutant. These worms bear a single amino acid substitution (E292K) in DAF-21 and are dauer-constitutive (Daf-c), i.e. enter the dauer state under favourable conditions of growth due to a defect in chemosensory ability. This Daf-c phenotype is shared with a second mutant, *daf-11*, which encodes a protein homologous to transmembrane guanylyl cyclases. Both the *daf-11* and the *daf-21* Daf-c phenotypes can be rescued using an analogue of cGMP. It was proposed that DAF-21 was required to stabilize DAF-11 or another component in the cGMP pathway (Birnby *et al*. 2000). A better understanding of the key client proteins of DAF-21 in *C. elegans* would help explain the requirement for Hsp90 in different life cycle stages of the nematode.

While studies from this laboratory have failed to demonstrate a significant induction of DAF-21 in *C. elegans* following heat shock (as quantified by immuno-blotting with a specific antibody) (Thompson *et al*. 2001; Devaney *et al*. 2005), other studies using *in situ* hybridization with *daf-21* specific probes have revealed differences in both the staining patterns and abundance of *daf-21* mRNA in *C. elegans* upon heat shock (Inoue *et al*. 2003). At normal growth temperatures, DAF-21 is predominantly localized to the germline of *C. elegans*, as detected by antibody staining (Gillan *et al*. 2009) as well as *in situ* hybridization (Inoue *et al*. 2003). A genome-wide RNAi screen reported that knock-down of *daf-21* leads to defects in oogenesis (Piano *et al*. 2000; Inoue *et al*. 2006). In more detailed experiments from this lab, it was shown that one of the most penetrant phenotypes obtained upon *daf-21* (*RNAi*) was a protruding vulva and sterility in the F_1 generation, due to a lack of gonad development (Gillan *et al*. 2009). Interestingly, *Drosophila* Hsp90 is expressed in the germline (Xiao and Lis, 1989) and in *Xenopus*, Hsp90 is expressed during oogenesis suggesting a conserved function between different species (Coumailleau *et al*. 1995). While the precise function of Hsp90 in the germline is still poorly understood, identification of DAF-21 client proteins can help explain mutant phenotypes, as exemplified by the interaction between the WEE-1.3 kinase and Hsp90. Here it was shown that DAF-21 indirectly regulates the meiotic prophase/metaphase transition during oocyte development through maintaining the activity of WEE-1.3 (Myt-1 orthologue in *C. elegans*), which is involved in cell cycle progression (Inoue *et al*. 2006).

The accumulation of DAF-21 in the germline of *C. elegans* is interesting in the context of recent studies, which have described a role for Hsp90 in

https://doi.org/10.1017/S0031182014000304 Published online by Cambridge University Press

various silencing pathways, including the piRNA and microRNA (miRNA) pathways (Izumi *et al*. 2013; Martinez and Gregory, 2013). These studies link the original observations of Lindquist and colleagues on the capacity of Hsp90 to buffer environmental change with a possible molecular mechanism. Altering the function of Hsp90 in *Drosophila* by mutation or by treatment with GA resulted in flies with various abnormalities of wings and eyes (Rutherford and Lindquist, 1998). It was proposed that high levels of cellular Hsp90 buffered pre-existing cryptic variation, which was then expressed when Hsp90 activity was compromised. However, the molecular mechanisms underlying these observations have remained elusive. Recent studies have shown that Hsp90 interacts in the Piwi pathway in *Drosophila*. piRNAs are a germline-specific class of small RNAs that silence transposons. Inhibition of Hsp90 function is proposed to inhibit the loading of RNA onto PIWI proteins (Izumi *et al*. 2013), resulting in the activation of transposons in *Drosophila* and the appearance of *de novo* mutations (Specchia *et al*. 2010; Gangaraju *et al*. 2011), suggesting one mechanism by which Hsp90 may regulate phenotypic change. Additional studies have implicated Hsp90 in epigenetic regulation of gene expression via its interaction with Trithorax (Trx), a chromatin remodelling factor (Ruden and Lu, 2008; Tariq *et al*. 2009). Trx is associated with active transcription and Hsp90 inhibition degraded Trx and resulted in a down-regulation in the expression of a number of genes. Genome-wide studies in *Drosophila* cells using chromatin immunoprecipitation showed that Hsp90 associated with multiple genes and that these were transcriptionally paused. Inhibition of Hsp90 released the pause, activating gene expression (Sawarkar *et al*. 2012).

Hsp90 is also involved in the loading of small interfering RNA duplexes (siRNA) and miRNA onto argonaute proteins in *Drosophila* and in human cells; argonaute proteins are the main components of the RNA Induced Silencing Complex (RISC). siRNA and microRNAs can only fulfil their function as post-transcriptional repressors of gene function (often referred to as the 'fine-tuners' of the genome) if they are successfully loaded onto these complexes (Iwasaki *et al*. 2010; Miyoshi *et al*. 2010). Whether Hsp90 plays similar roles in nematodes remains to be seen, but given the phenotypes of *daf-21*(*RNAi*) (F_1 sterility, protruding vulva phenotype, lack of gonad development, reduced brood size) and larval arrest in a homozygous *daf-21* mutant strain, it is an area which warrants further investigation.

HSP90 IN PARASITIC NEMATODES

In comparison to the information available on DAF-21 in *C. elegans*, relatively little is known about Hsp90 in parasitic species. *hsp90* has been cloned and partially characterized from the filarial worm *B. pahangi* (*Bp-hsp90*) (Devaney *et al*. 2005) and from the clade I parasite *T. spiralis* (Yang *et al*. 2013). In common with most other parasitic nematodes, lymphatic filarial worms undergo a heat shock as part of their life cycle. The infection is transmitted to humans by the bite of a mosquito carrying the third stage larvae (L3) in the mouthparts and head. The L3 enter the lymphatics where they develop through two moults to adult male and female adult worms, which can live for approximately 8 years. Following sexual reproduction, the adult female releases microfilariae into the lymph which then migrate to the circulatory system, where they provide a reservoir of infection for the vector (summarized in Fig. 1B). The transfer of the L3 between vector and mammalian host is associated with an elevation in temperature from the ambient (mosquito) to 37 °C (mammalian host). Using ^{35}S methionine labelling, an increase in expression of a range of heat shock proteins, including Hsp90, was observed as L3 were shifted from 28 to 37 °C (Jecock and Devaney, 1992). However, there is no evidence to suggest that the expression of *Bp-hsp90* (either mRNA or protein) is significantly increased following exposure of adult worms to heat shock conditions (Thompson *et al*. 2001; Devaney *et al*. 2005), suggesting that most *Bp-hsp90* is constitutively expressed. Parasitic nematodes secrete a range of molecules when cultured in serum-free medium (the so called excretory-secretory products or ES), and analysis of the ES of adult *B. malayi* demonstrated that *B. malayi* Hsp90 (*Bm*-Hsp90) was present (Kumari *et al*. 1994), a finding which has been confirmed in this laboratory using *B. pahangi* (Devaney *et al*. unpublished observations). Hsp90 is best characterized as a cytosolic chaperone, but studies on some cancer cells have shown that it can be expressed at the cell surface and secreted into medium (Eustace and Jay, 2004; Eustace *et al*. 2004). Extracellular Hsp90 was shown to be required for tumour cell invasion via the chaperoning of matrix metalloproteinases. It is difficult to envisage an extracellular function for *Bp*-Hsp90 in adult *B. pahangi*, but it could conceivably be important for chaperoning and folding other secreted molecules.

While there are many similarities in the molecular architecture of DAF-21 and *Bp*-Hsp90 there are also significant differences in the function of the molecule in the respective species. As referred to above, DAF-21 does not bind GA, while *Bp*-Hsp90 does. The subtle complexities of the Hsp90 machinery in different nematodes was demonstrated in a study which attempted to rescue the *C. elegans daf-21* mutant phenotype by heterologous expression of parasite *hsp90* genes (Gillan *et al*. 2009). Previous studies had demonstrated the efficacy of inter-species complementation experiments in transgenic *C. elegans*, where a parasite gene can rescue the *C. elegans*

https://doi.org/10.1017/S0031182014000304 Published online by Cambridge University Press

Table 1. Summary of *hsp90* inter-species rescue experiments in *C. elegans*. Extracted from Gillan *et al*. (2009)

	C. elegans	*H. contortus*	*B. pahangi*
Complementation of mutant	Yes	Partial rescues L2/L3 arrest	No rescue
RNAi	n/a	No rescue	No rescue
% id with *C. elegans*	100%	88%	84%

Table 2. Summary of *hsp90* inter-species rescue experiments in *S. cerevisiae*. Extracted from Palmer *et al*. (1995); Piper *et al*. (2003); Wider *et al*. (2009)

Species	% id with *S. cerevisiae*	Complementation
Escherichia coli	36%	No
Homo sapiens	59%	Yes
Trypanosoma cruzi	63%	Yes
Plasmodium falciparum	63%	Yes

mutant phenotype and restore wild type traits (Kwa *et al*. 1995; Britton and Murray, 2002; Couthier *et al*. 2004; Massey *et al*. 2006). However, despite a high level of amino acid homology (84% identical, 91% similar), expression of a *Bp-hsp90* transgene could not rescue a *C. elegans daf-21* mutant. Similarly, no rescue was observed by expression of the *Bp-hsp90* construct in wild type *C. elegans*, in which endogenous *daf-21* levels were reduced by RNAi. In these experiments, *Bp-hsp90* was introduced into *C. elegans* by microinjection of a plasmid construct containing the *Bp-hsp90* coding sequence under the control of the *C. elegans daf-21* promoter and 3′UTR regions, in an attempt to mimic the expression of the endogenous gene as closely as possible. To confirm that the parasite gene was efficiently transcribed in *C. elegans*, injection of a *Bp-hsp90 LacZ* translational reporter construct demonstrated that the parasite Hsp90 was expressed in most tissues of the transformed worms and, importantly, that the *B. pahangi* protein expressed in *C. elegans* bound to GA beads in a pull-down experiment (Gillan *et al*. 2009). Despite these findings, complementation of the *daf-21* mutant or *daf-21*(*RNAi*) worms was never observed. A more successful inter-species rescue was obtained using *hsp90* from *H. contortus* (*Hc-hsp90*), a trichostrongyloid nematode of sheep, which belongs to the same clade as *C. elegans*. *Hc*-Hsp90 is 88% identical and 93% similar to DAF-21 and experiments with an *Hc-hsp90* construct provided partial rescue of the *daf-21* mutant phenotypes. As control experiments had demonstrated that complementation with the wild-type *daf-21* in the same injection cassette restored the wild type phenotype of the *daf-21* mutant worms, a 'gradient' of rescue was proposed, i.e. despite high sequence homology the *B. pahangi* transgene was unable to rescue the mutant, *Hc-hsp90* was able to confer partial rescue but it required complementation with the wild-type *C. elegans* gene to completely rescue the mutant phenotypes and restore fertility (see Table 1 for summary). These results suggest that in metazoans, the ability to complement Hsp90 function may depend on factors other than sequence homology; successful rescue may require specific co-chaperones for full function of the transgene, or alternatively these data may reflect difference in the ability of the respective Hsp90 proteins to chaperone key client proteins. In contrast to the studies on nematode Hsp90, other inter-species complementation experiments using mutant *Saccharomyces cerevisiae* have been successful even when the degree of homology between the respective *hsp90* genes was relatively limited (see Table 2). Indeed, both *C. elegans* wild-type *daf-21* and human *hsp90b* were able to rescue an *S. cerevisiae hsp90* mutant despite sharing only 60·5 and 60·3% sequence homology, respectively. However, in this case complementation required the presence of STI-1/Hop (Hsp organizing protein) indicating that the expression of co-chaperone proteins is of primary importance to the functionality of Hsp90 (Piper *et al*. 2003).

HSP90 CO-CHAPERONES IN NEMATODES

The interaction of Hsp90 with client and co-chaperone proteins is regulated by ATP-induced conformational changes in Hsp90, resulting in the folding and activation of substrate or 'client' proteins. Over the years, the number of Hsp90 client proteins has grown significantly and it has been shown to be essential in the maturation of many different types of protein in mammalian cells, including transcription factors, steroid receptors, serine/threonine and tyrosine kinases. A list of Hsp90 client proteins is curated by The Picard laboratory (http://www.picard.ch).

https://doi.org/10.1017/S0031182014000304 Published online by Cambridge University Press

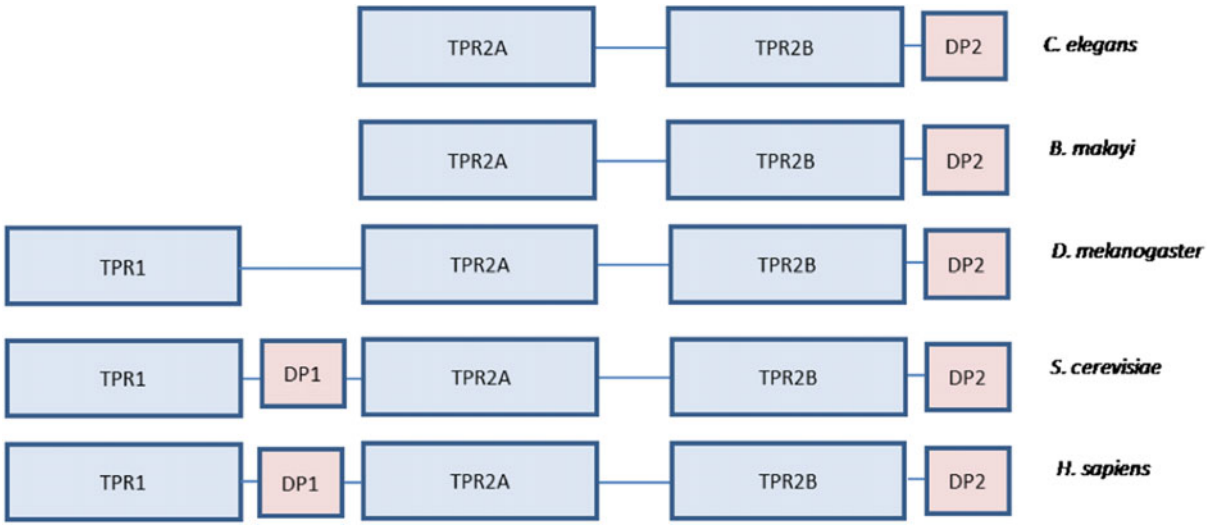

Fig. 3. Comparison of the domain structures of Hop from divergent species. TPR domains are shown in blue and DP domains in pink. Homologues of Hop show differences in domain organization. TPR1 and DP1 are absent in the nematode species *C. elegans* and *B. malayi* while only DP1 is lacking in *D. melanogaster*. All five domains are present in *H. sapiens* and *S. cerevisiae*. Adapted from Gaisier *et al*. (2009).

There are no published data from parasitic nematode species on the client proteins that require Hsp90 for function, but there is an extensive list of both predicted and confirmed clients for DAF-21 (http://www.wormbase.org). Many of these clients have homologues in the human system, such as small ribosomal proteins, serine/threonine kinases and cyclin-dependent kinases, in addition to some 'worm-specific' proteins, such as those essential for pharyngeal pumping, a process required for food intake. While it is likely that at least some *C. elegans* client proteins will be similar in other nematodes, the degree of conservation is currently unknown.

The identification and function of co-chaperones in different species is of interest given their importance in the outcome of client protein maturation. For example, some Hsp90 clients require a select group of co-chaperones and alternative co-chaperones may have differential effects on client proteins (Riggs *et al*. 2004; Felts *et al*. 2007; Smith and Toft, 2008). These findings are strengthened by evidence that many co-chaperones display mutually exclusive binding to Hsp90 (Pratt *et al*. 2004; Harst *et al*. 2005; Pearl and Prodromou, 2006). A study by Johnson and Brown illustrated the plasticity of the co-chaperone machinery in diverse eukaryotes and showed that the architecture of the Hsp90/co-chaperone machine varied in a species-specific manner. Their results suggested that with increasing complexity of the organism, additional co-factors may be required. While Hsp90 is universally required in metazoans, it is likely that the Hsp90 complex varies in different tissues/cells. *Caenorhabditis elegans* was shown to express orthologues of nine of the ten human Hsp90 co-chaperones used in the directed Psi-BLAST search against the complete *C. elegans* genome (Johnson and Brown, 2009). The only co-chaperone absent in *C. elegans* was Cyp40, which was first identified in mammalian steroid receptor complexes (Riggs *et al*. 2004). However, another striking difference in *C. elegans* was shown to occur within the sequence of the adaptor protein Hop (STI-1 in *C. elegans*), which has an essential role in mediating interactions between Hsp70 and Hsp90. In most eukaryotes, Hop consists of five domains: three tetratricopeptide repeat (TPR) domains and two dipeptide (DP) repeats, which act as linker domains, arranged TPR1–DP1–TPR2A–TPR2B–DP2 (Scheufler *et al*. 2000). However, *C. elegans* STI-1 lacks the TPR1 and DP1 domains (www.wormbase.org). This is an intriguing finding as, in other systems, the TPR1 domain has been shown to be essential for binding Hsp70, and for linking Hsp70 with Hsp90 (which binds Hop via TPR2A) (Scheufler *et al*. 2000). Hsp70 was observed to bind to *C. elegans* STI-1 despite the lack of the hypothetical binding site; however, in the presence of DAF-21, Hsp70 binding was completely abrogated suggesting that in *C. elegans* there may be no requirement for both chaperones to interact simultaneously, distinguishing *C. elegans* from other species (Gaiser *et al*. 2009). The lack of the TPR1 domain was also observed in the closely related *Caenorhabditis briggsae* and *Caenorhabditis remanei*. Interestingly, it appears this atypical Hop is not limited to the *Caenorhabditis* genus, as the *B. malayi* genome contains an orthologue of *C. elegans* STI-1, BMA-STI-1 (http://www.wormbase.org). This protein shares 65·5% amino acid identity with *C. elegans* STI-1 and similarly appears to lack TPR1 and DP1 domains suggesting that this could be a nematode-wide scenario (see Fig. 3).

https://doi.org/10.1017/S0031182014000304 Published online by Cambridge University Press

In 2007, a genome-wide promoter analysis addressed the tissue-specific expression of most proteins in *C. elegans* and showed that DAF-21 was expressed in many different cell types. This analysis allowed the correlation of DAF-21 expression with the expression profiles of its presumed co-chaperone proteins (Dupuy *et al*. 2007). Analysing the differential expression of DAF-21 and its co-chaperones allows for some speculation on the cellular activity of the Hsp90/co-chaperone complexes, as it is likely that tissue localization corresponds to function (data accessed through www.wormbase.org). For example, co-chaperones P23 (ZC395.1), Hop (STI-1) and FKB-6 were all shown to be ubiquitously expressed in *C. elegans*, whereas PP5 (PPH-5) and CDC-37 are expressed only in the intestine and embryo respectively, suggesting a more specific role. Perhaps the best example of cellular location correlating with function is illustrated by the expression pattern of the TPR-containing cofactor UNC-45 (uncoordinated), which is expressed in body wall muscle, intestinal muscle and vulval muscles. In *C. elegans*, the 95 rhomboid-shaped body wall muscle cells comprise one of the major tissues of the adult worm (www.wormatlas.org). Down-regulation of UNC-45 leads to paralysis via decreased myosin assembly, whereas an excess of UNC-45 results in myosin accumulation, both of which result in defective myofibril organization (Barral *et al*. 2002; Landsverk *et al*. 2007). The details of how UNC-45 and DAF-21 work together are still unclear, although it has been reported that UNC-45 interacts with Hsp90 via a TPR domain (Russell *et al*. 1999; Scheufler *et al*. 2000; Barral *et al*. 2002) and myosin motor domains through its COOH-terminal regions (Barral *et al*. 1998, 2002). Elegant studies by Ni *et al*. demonstrated that DAF-21 and UNC-45 interact in pull-down experiments using C. *elegans* lysates and proposed that this interaction might have an inhibitory effect on the myosin-chaperoning activity of UNC-45 (Ni *et al*. 2011).

Online sources (http://www.wormbase.org) demonstrate that *C. elegans* encodes 80 TPR proteins, so perhaps the fact that only nine are known homologues of human Hsp90 co-chaperones is surprising (for comparison, the proteome of *S. cerevisiae* contains about 25 proteins with TPR domains, seven of which are confirmed to interact with either Hsp90 or Hsp70). In a recent study, Haslbeck *et al*. analysed previously uncharacterized TPR proteins, known to be associated with the Hsp90/Hsp70 complex. The entire proteome of *C. elegans* was analysed and scores were given to each protein based on the level of homology to known co-chaperone TPR proteins of *Homo sapiens, S. cerevisiae* and *C. elegans*. The proteins with the highest score for potential binding to Hsc70/Hsp70 and Hsp90 were the ten *C. elegans* TPR proteins used in the sample set (SGT-1, UNC-45, FKB-6, PPH-5, STI-1, HIP-1, CHN-1, C56C10.10/AIP-1, C17G10.10/CNS-1). In addition, three uncharacterized *C. elegans* open-reading frames with homologues in *Drosophila* or humans emerged as possible Hsp90 interactors. Two of these, C34B2.5 and ZK370.8 were shown to bind both Hsc70 and DAF-21 with low micromolar affinities; mutation of amino acids in the Hsp90 binding site for TPR proteins (EEVD sequence) was shown to disrupt the interaction. Interestingly, this study demonstrates that the majority of TPR proteins in *C. elegans* have no binding affinity for DAF-21 (Haslbeck *et al*. 2013).

HSP90 AS A DRUG TARGET IN NEMATODES

The realization that Hsp90 acted as a chaperone for various oncogenic proteins led to a major effort to develop novel small molecule inhibitors of Hsp90 for use in various tumours (Neckers *et al*. 1999). More recently the potential of Hsp90 inhibitors as novel chemotherapeutic agents for various parasitic infections has been studied (see other articles in this special issue). As referred to previously, there is a need for novel drugs to treat humans and animals infected with helminth parasites and studies *in vitro* have shown a dependence on Hsp90 in parasitic nematodes (Devaney *et al*. 2005) and in the trematode parasite *Schistosoma japonicum* (Wenkert *et al*. 2010). The design of new anthelmintics generally centres on attempts to establish and exploit molecular targets that are exclusive to the parasite, with the aim of minimizing potential damage to the host. Although this approach can be successful, an alternative course is to target a common pathway shared by the infectious agent and the host, which has evolved over time to perform different functions unique to the respective species. The benefits of focusing drug discovery on such pathways are two-fold; the scope for identifying new targets is broadened and not confined to parasite-specific molecules, many of which are hypothetical proteins of unknown function, and the ubiquity of such molecules in other systems may allow for drugs designed in this way to be used in other parasitic infections. The repurposing of compounds developed to treat other conditions is an attractive proposition for drug development for neglected tropical diseases, where the potential to recoup drug development costs is limited. In this respect, Hsp90 inhibitors have been shown to be effective against a variety of tropical pathogens including *Plasmodium* (Kumar *et al*. 2003; Shahinas *et al*. 2010, 2013), *Trypanosoma* (Pallavi *et al*. 2010) and *Leishmania* (Petersen *et al*. 2012).

Recent studies from this laboratory have focused on the potential of Hsp90 inhibitors as macrofilaricidal agents in filarial infection. Currently, the drugs used to control filarial nematodes in mass drug administration (MDA) programmes, such as diethylcarbamazine (DEC) and ivermectin, largely target

https://doi.org/10.1017/S0031182014000304 Published online by Cambridge University Press

the circulating microfilariae (Mf), resulting in reduced transmission rates. However, Mf repopulate the circulation necessitating continued treatment over the long reproductive lifespan of adult worms, incurring significant costs for control programmes and increasing the chances of resistance emerging (Prichard *et al*. 2012). It has long been a goal of WHO to identify a suitable drug that kills adult filarial worms but, to date, no such agent has been developed (Molyneux *et al*. 2003). At higher concentrations of drug, DEC can have macrofilaricidal activity, although in most long-term studies, Mf were shown to re-emerge in the blood of some patients, 1–5 years post-treatment. This suggests either that a proportion of adult worms survived DEC treatment and recover Mf production or may reflect re-infection (Terhell *et al*. 2003). Targeting the *Wolbachia* intracellular endosymbiont of many pathogenic filarial worms with doxycycline remains the most promising new therapy that affects adult worms. However, this antibiotic is contraindicated in pregnant women and children and prolonged dosing is not ideal for MDA, but the search continues for a more satisfactory antibiotic (Hoerauf, 2008). While the *B. malayi* genome may yet illuminate novel parasite-specific drug targets, we have previously identified a requirement for Hsp90 as a possible weak spot in filarial nematodes. The prototype Hsp90 inhibitor, GA, a naturally occurring benzoquinone ansamycin, binds the N-terminal ATP pocket of Hsp90 disrupting its function and resulting in the degradation and/or inhibition of client proteins. In initial studies, *in vitro* exposure of *B. pahangi* adult female worms to 1 μM GA (a concentration chosen as it inhibits mammalian Hsp90 activity) was shown to result in a significant reduction in Mf production from adult worms after 24 h and by 48 h, Mf release had ceased completely (Devaney *et al*. 2005). As well as inhibiting Mf output, GA was shown to kill adult worms, as after 7 days of exposure to drug, 100% of adult female worms were dead. GA probably has a specific effect on adult worms (as well as inhibition of Mf output), as further experiments demonstrated that adult males were also killed by exposure to 1·0 μM GA. As referred to above, most species of filarial worms are known to harbour endosymbiotic bacteria, and to ensure that these effects were not a result of GA targeting the *Wolbachia* of *B. pahangi*, the experiments were repeated using the *Wolbachia*-free species *Acanthocheilonema viteae* and similar results were obtained. Comparable data were produced by Wenkert *et al*. who tested GA and a number of GA-derivatives against adult *B. malayi in vitro*. In their study, all four derivatives tested were active at concentrations down to 500 nM. While these results show promise, GA suffers from limitations as a chemotherapeutic agent due to unacceptable levels of hepatoxicity and would not be suitable for use in filarial infection. Thus most recent studies on Hsp90 inhibitors have focused on novel small molecule inhibitors (Wenkert *et al*. 2010).

In subsequent studies, we investigated whether members of the purine-scaffold series of Hsp90 inhibitors, developed by the Chiosis laboratory (Chiosis and Tao, 2006; Taldone *et al*. 2011), would have activity against adult *B. pahangi*. In initial experiments, a fluorescence polarization (FP) assay, originally developed as a high-throughput screen for the detection of small molecule inhibitors of Hsp90 in tumour cells, was adapted to *Brugia* sp. (Taldone *et al*. 2010). The assay is based on the ability of small molecules to compete with the binding of fluorescently labelled GA to Hsp90 in cellular homogenates, thus negating the need for the production of purified recombinant protein as well as enabling investigation of Hsp90 in its native conformation. Previous studies had demonstrated that Hsp90 in tumour cells is present in multi-chaperone complexes with high ATPase activity and a higher affinity for N-terminal inhibitors compared with Hsp90 from normal cells, where it is present in a latent form with a reduced affinity for these inhibitors (Kamal *et al*. 2003). Subsequent studies confirmed this finding and showed that Hsp90-specific pull-downs using the purine scaffold compound, PU-H71 conjugated to a solid support, identified a complex of interacting proteins from tumour cells (Moulick *et al*. 2011). Interestingly, the affinity of *Bp*-Hsp90 for GA was shown to be similar to tumour cell Hsp90 in the FP assay, while extracts of *C. elegans* did not show significant binding, consistent with previous results. In addition, some selectivity was noted in the binding of the purine-scaffold compounds to *Bp*-Hsp90 (Taldone *et al*. 2010); a 3-fold change in selectivity ratio was observed between PU-H71 and PU-WS10, two compounds with almost identical structure, suggesting that the FP assay could identify molecules which may specifically target parasite Hsp90.

In more recent work from this laboratory, we examined whether Hsp90 inhibitors might affect adult worms *in vivo* (Gillan *et al*. 2014). For this purpose we selected an isoxazole inhibitor, NVP-AUY922, which had previously been demonstrated to exhibit anti-tumour activity in a mouse xenograft tumour model (Eccles *et al*. 2008; Garon *et al*. 2013). Experiments *in vitro* had shown NVP-AUY922 to be remarkably active against both adult worms and Mf at very low concentrations, killing Mf at concentrations down to 1·56 nM and 50% of adult worms at 25 nM. Using a model system in which adult *B. pahangi* worms are transplanted into the peritoneal cavity of mice (Devaney *et al*. 2002), we demonstrated that administration of three doses of NVP-AUY922 at 50 mg kg^{-1} effectively killed adult worms (Gillan *et al*. 2014). No weight loss or other deleterious effects were observed in treated animals over the time course. While these experiments provide proof of principle that inhibition of

https://doi.org/10.1017/S0031182014000304 Published online by Cambridge University Press

Hsp90 is lethal to adult filarial worms *in vivo*, further experiments are required with different routes of drug administration and different doses of drug.

CONCLUSIONS

Recent studies on the free-living model nematode *C. elegans* have shed new light on the mechanisms of Hsp90 function in metazoans, particularly in relation to the tissue-specific requirement for certain co-chaperones. Additional studies will be required to fully explain the apparent resistance of *C. elegans* to Hsp90 inhibitors and the molecular mechanisms underlying this phenomenon. However, given the studies reviewed above it is likely that the function of Hsp90 differs between free-living nematodes and their parasitic counterparts. It is possible that part of this discrepancy will be explained by differences in the Hsp90 interactome in free-living and parasitic species, such as filarial worms, perhaps equivalent to the differences in Hsp90 function in normal versus tumour cells. While aspects of their basic biology, such as moulting, are conserved amongst all nematodes, there are significant differences between free-living and parasitic species in life cycles, modes of transmission, reproduction and dependence on hosts. As momentum to eliminate neglected tropical diseases grows, the prospect of repositioning existing drugs is an appealing one. However, while preliminary results show that chemical inhibition of Hsp90 is lethal to adult filarial worms, further research is required to determine whether Hsp90 truly represents an 'Achilles heel' in filarial nematodes, suitable for exploitation as a chemotherapeutic target.

ACKNOWLEDGEMENTS

We acknowledge the support for our studies on Hsp90 from the Wellcome Trust (grant number 076734/Z/05/Z) and the BBSRC (grant number BB/E013473/1) and we would like to thank Dr Collette Britton and Dr Jane Kinnaird for critical reading of this manuscript.

REFERENCES

Abad, P., Gouzy, J., Aury, J. M., Castagnone-Sereno, P., Danchin, E. G., Deleury, E., Perfus-Barbeoch, L., Anthouard, V., Artiguenave, F., Blok, V. C., Caillaud, M. C. and Coutinho, P. M. (2008). Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. *Nature Biotechnology* **26**, 909–915.

Barral, J. M., Bauer, C. C., Ortiz, I. and Epstein, H. F. (1998). Unc-45 mutations in *Caenorhabditis elegans* implicate a CRO1/She4p-like domain in myosin assembly. *Journal of Cell Biology* **143**, 1215–1225.

Barral, J. M., Hutagalung, A. H., Brinker, A., Hartl, F. U. and Epstein, H. F. (2002). Role of the myosin assembly protein UNC-45 as a molecular chaperone for myosin. *Science* **295**, 669–671. doi: 10.1126/science.1066648.

Birnby, D. A., Link, E. M., Vowels, J. J., Tian, H., Colacurcio, P. L. and Thomas, J. H. (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.

Blaxter, M. L., De Ley, P., Garey, J. R., Liu, L. X., Scheldeman, P., Vierstraete, A., Vanfleteren, J. R., Mackey, L. Y., Dorris, M., Frisse, L. M., Vida, J. T. and Thomas, W. K. (1998). A molecular evolutionary framework for the phylum Nematoda. *Nature* **392**, 71–75. doi: 10.1038/32160.

Britton, C. and Murray, L. (2002). A cathepsin L protease essential for *Caenorhabditis elegans* embryogenesis is functionally conserved in parasitic nematodes. *Molecular and Biochemical Parasitology* **122**, 21–33.

Burns, A. R., Wallace, I. M., Wildenhain, J., Tyers, M., Giaever, G., Bader, G. D., Nislow, C., Cutler, S. R. and Roy, P. J. (2010). A predictive model for drug bioaccumulation and bioactivity in *Caenorhabditis elegans*. *Nature Chemical Biology* **6**, 549–557. doi: 10.1038/nchembio.380.

Candido, E. P., Jones, D., Dixon, D. K., Graham, R. W., Russnak, R. H. and Kay, R. J. (1989). Structure, organization, and expression of the 16-kDa heat shock gene family of *Caenorhabditis elegans*. *Genome* **31**, 690–697.

Chiosis, G. and Tao, H. (2006). Purine-scaffold Hsp90 inhibitors. *IDrugs* **9**, 778–782.

Coumailleau, P., Billoud, B., Sourrouille, P., Moreau, N. and Angelier, N. (1995). Evidence for a 90 kDa heat-shock protein gene expression in the amphibian oocyte. *Developmental Biology* **168**, 247–258. doi: 10.1006/dbio.1995.1077.

Couthier, A., Smith, J., Mcgarr, P., Craig, B. and Gilleard, J. S. (2004). Ectopic expression of a *Haemonchus contortus* GATA transcription factor in *Caenorhabditis elegans* reveals conserved function in spite of extensive sequence divergence. *Molecular and Biochemical Parasitology* **133**, 241–253.

Dalley, B. K. and Golomb, M. (1992). Gene expression in the *Caenorhabditis elegans* dauer larva: developmental regulation of Hsp90 and other genes. *Developmental Biology* **151**, 80–90.

David, C. L., Smith, H. E., Raynes, D. A., Pulcini, E. J. and Whitesell, L. (2003). Expression of a unique drug-resistant Hsp90 ortholog by the nematode *Caenorhabditis elegans*. *Cell Stress and Chaperones* **8**, 93–104.

Desjardins, C. A., Cerqueira, G. C., Goldberg, J. M., Dunning Hotopp, J. C., Haas, B. J., Zucker, J., Ribeiro, J. M., Saif, S., Levin, J. Z., Fan, L., Zeng, Q., Russ, C., Wortman, J. R., Fink, D. L., Birren, B. W. and Nutman, T. B. (2013). Genomics of *Loa loa*, a *Wolbachia*-free filarial parasite of humans. *Nature Genetics* **45**, 495–500. doi: 10.1038/ng.2585.

Devaney, E., Gillan, V., Wheatley, I., Jenson, J., O'connor, R. and Balmer, P. (2002). Interleukin-4 influences the production of microfilariae in a mouse model of *Brugia* infection. *Parasite Immunology* **24**, 29–37.

Devaney, E., O'Neill, K., Harnett, W., Whitesell, L. and Kinnaird, J. H. (2005). Hsp90 is essential in the filarial nematode *Brugia pahangi*. *International Journal for Parasitology* **35**, 627–636.

Dieterich, C., Clifton, S. W., Schuster, L. N., Chinwalla, A., Delehaunty, K., Dinkelacker, I., Fulton, L., Fulton, R., Godfrey, J., Minx, P., Mitreva, M., Roeseler, W., Tian, H., Witte, H., Yang, S. P., Wilson, R. K. and Sommer, R. J. (2008). The *Pristionchus pacificus* genome provides a unique perspective on nematode lifestyle and parasitism. *Nature Genetics* **40**, 1193–1198.

Dupuy, D., Bertin, N., Hidalgo, C. A., Venkatesan, K., Tu, D., Lee, D., Rosenberg, J., Svrzikapa, N., Blanc, A., Carnec, A., Carvunis, A. R., Pulak, R., Shingles, J., Reece-Hoyes, J., Hunt-Newbury, R., Viveiros, R., Mohler, W. A., Tasan, M., Roth, F. P., Le Peuch, C., Hope, I. A., Johnsen, R., Moerman, D. G., Barabasi, A. L., Baillie, D. and Vidal, M. (2007). Genome-scale analysis of *in vivo* spatiotemporal promoter activity in *Caenorhabditis elegans*. *Nature Biotechnology* **25**, 663–668. doi: 10.1038/nbt1305.

Eccles, S. A., Massey, A., Raynaud, F. I., Sharp, S. Y., Box, G., Valenti, M., Patterson, L., De Haven Brandon, A., Gowan, S., Boxall, F., Aherne, W., Rowlands, M., Hayes, A., Martins, V., Urban, F., Boxall, K., Prodromou, C., Pearl, L., James, K., Matthews, T. P., Cheung, K. M., Kalusa, A., Jones, K., McDonald, E., Barril, X., Brough, P. A., Cansfield, J. E., Dymock, B., Drysdale, M. J., Finch, H., Howes, R., Hubbard, R. E., Surgenor, A., Webb, P., Wood, M., Wright, L. and Workman, P. (2008). NVP-AUY922: a novel heat shock protein 90 inhibitor active against xenograft tumor growth, angiogenesis, and metastasis. *Cancer Research* **68**, 2850–2860. doi: 10.1158/0008-5472.can-07-5256.

Eustace, B. K. and Jay, D. G. (2004). Extracellular roles for the molecular chaperone, hsp90. *Cell Cycle* **3**, 1098–1100.

Eustace, B. K., Sakurai, T., Stewart, J. K., Yimlamai, D., Unger, C., Zehetmeier, C., Lain, B., Torella, C., Henning, S. W., Beste, G., Scroggins, B. T., Neckers, L., Ilag, L. L. and Jay, D. G. (2004).

Functional proteomic screens reveal an essential extracellular role for hsp90 alpha in cancer cell invasiveness. *Nature Cell Biology* **6**, 507–514. doi: 10.1038/ncb1131.

Felts, S. J., Karnitz, L. M. and Toft, D. O. (2007). Functioning of the Hsp90 machine in chaperoning checkpoint kinase I (Chk1) and the progesterone receptor (PR). *Cell Stress and Chaperones* **12**, 353–363.

Gaiser, A. M., Brandt, F. and Richter, K. (2009). The non-canonical Hop protein from *Caenorhabditis elegans* exerts essential functions and forms binary complexes with either Hsc70 or Hsp90. *Journal of Molecular Biology* **391**, 621–634. doi: 10.1016/j.jmb.2009.06.051.

Garon, E. B., Finn, R. S., Hamidi, H., Dering, J., Pitts, S., Kamranpour, N., Desai, A. J., Hosmer, W., Ide, S., Avsar, E., Jensen, M. R., Quadt, C., Liu, M., Dubinett, S. M. and Slamon, D. J. (2013). The HSP90 inhibitor NVP-AUY922 potently inhibits non-small cell lung cancer growth. *Molecular Cancer Therapy* **12**, 890–900. doi: 10.1158/1535-7163.mct-12-0998.

Gangaraju, V. K., Yin, H., Weiner, M. M., Wang, J., Huang, X. A. and Lin, H. (2011). *Drosophila* Piwi functions in Hsp90-mediated suppression of phenotypic variation. *Nature Genetics* **43**, 153–158. doi: 10.1038/ng.743.

Geary, T. G., Woo, K., McCarthy, J. S., Mackenzie, C. D., Horton, J., Prichard, R. K., De Silva, N. R., Olliaro, P. L., Lazdins-Helds, J. K., Engels, D. A. and Bundy, D. A. (2010). Unresolved issues in anthelmintic pharmacology for helminthiases of humans. *International Journal for Parasitology* **40**, 1–13. doi: 10.1016/j.ijpara.2009.11.001.

Ghedin, E., Wang, S., Spiro, D., Caler, E., Zhao, Q., Crabtree, J., Allen, J. E., Delcher, A. L., Guiliano, D. B., Miranda-Saavedra, D., Angiuoli, S. V. and Creasy, T. (2007). Draft genome of the filarial nematode parasite *Brugia malayi*. *Science* **317**, 1756–1760.

Gillan, V., Maitland, K., McCormack, G., Nik Him, N. A. and Devaney, E. (2009). Functional genomics of hsp-90 in parasitic and free-living nematodes. *International Journal for Parasitology* **39**, 1071–1081. doi: S0020-7519(09)00167-2 [pii]10.1016/j.ijpara.2009.02.024.

Gillan, V., O'Neill, K., Maitland, K., Sverdrup, F. M. and Devaney, E. (2014). A repurposing strategy for Hsp90 inhibitors demonstrates their potency against filarial nematodes. *PLoS Neglected Tropical Disease* **8**, e2699. doi: 10.1371/journal.pntd.0002699.

Gilleard, J. S. (2006). Understanding anthelmintic resistance: the need for genomics and genetics. *International Journal for Parasitology* **36**, 1227–1239. doi: 10.1016/j.ijpara.2006.06.010.

Godel, C., Kumar, S., Koutsovoulos, G., Ludin, P., Nilsson, D., Comandatore, F., Wrobel, N., Thompson, M., Schmid, C. D., Goto, S., Bringaud, F., Wolstenholme, A., Bandi, C., Epe, C., Kaminsky, R., Blaxter, M. and Maser, P. (2012). The genome of the heartworm, *Dirofilaria immitis*, reveals drug and vaccine targets. *Journal of the Federation of American Societies for Experimental Biology* **26**, 4650–4661. doi: 10.1096/fj.12-205096.

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

Harst, A., Lin, H. and Obermann, W. M. (2005). Aha1 competes with Hop, p50 and p23 for binding to the molecular chaperone Hsp90 and contributes to kinase and hormone receptor activation. *Biochemical Journal* **387**, 789–796. doi: 10.1042/BJ20041283.

Hashmi, S., Wang, Y., Parhar, R. S., Collison, K. S., Conca, W., Al-Mohanna, F. and Gaugler, R. (2013). A *C. elegans* model to study human metabolic regulation. *Nutrition and Metabolism* **10**, 31. doi: 10.1186/1743-7075-10-31.

Haslbeck, V., Eckl, J. M., Kaiser, C. J., Papsdorf, K., Hessling, M. and Richter, K. (2013). Chaperone-interacting TPR proteins in *Caenorhabditis elegans*. *Journal of Molecular Biology* **425**, 2922–2939. doi: 10.1016/j.jmb.2013.05.019.

Him, N. A., Gillan, V., Emes, R. D., Maitland, K. and Devaney, E. (2009). Hsp-90 and the biology of nematodes. *BMC Evolutionary Biology* **9**, 254. doi: 10.1186/1471-2148-9-254.

Hoerauf, A. (2008). Filariasis: new drugs and new opportunities for lymphatic filariasis and onchocerciasis. *Current Opinion in Infectious Disease* **21**, 673–681. doi: 10.1097/QCO.0b013e328315cde7.

Inoue, T., Takamura, K., Yamae, H., Ise, N., Kawakami, M., Tabuse, Y., Miwa, J. and Yamaguchi, Y. (2003). *Caenorhabditis elegans* DAF-21 (Hsp90) is characteristically and predominantly expressed in germline cells: spatial and temporal analysis. *Development Growth and Differentiation* **45**, 369–376.

Inoue, T., Hirata, K., Kuwana, Y., Fujita, M., Miwa, J., Roy, R. and Yamaguchi, Y. (2006). Cell cycle control by daf-21/Hsp90 at the first meiotic prophase/metaphase boundary during oogenesis in *Caenorhabditis elegans*. *Development Growth and Differentiation* **48**, 25–32. doi: 10.1111/j.1440-169X.2006.00841.x.

Iwasaki, S., Kobayashi, M., Yoda, M., Sakaguchi, Y., Katsuma, S., Suzuki, T. and Tomari, Y. (2010). Hsc70/Hsp90 chaperone machinery mediates ATP-dependent RISC loading of small RNA duplexes. *Molecular Cell* **39**, 292–299. doi: 10.1016/j.molcel.2010.05.015.

Izumi, N., Kawaoka, S., Yasuhara, S., Suzuki, Y., Sugano, S., Katsuma, S. and Tomari, Y. (2013). Hsp90 facilitates accurate loading of precursor piRNAs into Piwi proteins. *RNA* **19**, 896–901. doi: 10.1261/rna.037200.112.

Jecock, R. M. and Devaney, E. (1992). Expression of small heat shock proteins by the third-stage larva of *Brugia pahangi*. *Molecular and Biochemical Parasitology* **56**, 219–226.

Jeong, P. Y., Na, K., Jeong, M. J., Chitwood, D., Shim, Y. H. and Paik, Y. K. (2009). Proteomic analysis of *Caenorhabditis elegans*. *Methods in Molecular Biology* **519**, 145–169. doi: 10.1007/978-1-59745-281-6_10.

Jex, A. R., Liu, S., Li, B., Young, N. D., Hall, R. S., Li, Y., Yang, L., Zeng, N., Xu, X., Xiong, Z., Chen, F., Wu, X., Zhang, G., Fang, X., Kang, Y., Anderson, G. A., Harris, T. W., Campbell, B. E., Vlaminck, J., Wang, T., Cantacessi, C., Schwarz, E. M., Ranganathan, S., Geldhof, P., Nejsum, P., Sternberg, P. W., Yang, H., Wang, J., Wang, J. and Gasser, R. B. (2011). *Ascaris suum* draft genome. *Nature* **479**, 529–533. doi: 10.1038/nature10553.

Johnson, J. L. and Brown, C. (2009). Plasticity of the Hsp90 chaperone machine in divergent eukaryotic organisms. *Cell Stress and Chaperones* **14**, 83–94. doi: 10.1007/s12192-008-0058-9.

Jones, D., Dixon, D. K., Graham, R. W. and Candido, E. P. (1989). Differential regulation of closely related members of the hsp16 gene family in *Caenorhabditis elegans*. *DNA* **8**, 481–490.

Jones, S. J., Riddle, D. L., Pouzyrev, A. T., Velculescu, V. E., Hillier, L., Eddy, S. R., Stricklin, S. L., Baillie, D. L., Waterston, R. and Marra, M. A. (2001). Changes in gene expression associated with developmental arrest and longevity in *Caenorhabditis elegans*. *Genome Research* **11**, 1346–1352. doi: 10.1101/gr.184401.

Kamal, A., Thao, L., Sensintaffar, J., Zhang, L., Boehm, M. F., Fritz, L. C. and Burrows, F. J. (2003). A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. *Nature* **425**, 407–410. doi: 10.1038/nature01913.

Kirienko, N. V., Mani, K. and Fay, D. S. (2010). Cancer models in *Caenorhabditis elegans*. *Development Dynamics* **239**, 1413–1448. doi: 10.1002/dvdy.22247.

Kumar, R., Musiyenko, A. and Barik, S. (2003). The heat shock protein 90 of *Plasmodium falciparum* and antimalarial activity of its inhibitor, geldanamycin. *Malaria Journal* **2**, 30. doi: 10.1186/1475-2875-2-30.

Kumari, S., Lillibridge, C. D., Bakeer, M., Lowrie, R. C., Jr., Jayaraman, K. and Philipp, M. T. (1994). *Brugia malayi*: the diagnostic potential of recombinant excretory/secretory antigens. *Experimental Parasitology* **79**, 489–505. doi: 10.1006/expr.1994.1110.

Kwa, M. S., Veenstra, J. G., Van Dijk, M. and Roos, M. H. (1995). Beta-tubulin genes from the parasitic nematode *Haemonchus contortus* modulate drug resistance in *Caenorhabditis elegans*. *Journal of Molecular Biology* **246**, 500–510. doi: 10.1006/jmbi.1994.0102.

Laing, R., Kikuchi, T., Martinelli, A., Tsai, I. J., Beech, R. N., Redman, E., Holroyd, N., Bartley, D. J., Beasley, H., Britton, C., Curran, D., Devaney, E., Gilabert, A., Hunt, M., Jackson, F., Johnston, S. L., Kryukov, I., Li, K., Morrison, A. A., Reid, A. J., Sargison, N., Saunders, G. I., Wasmuth, J. D., Wolstenholme, A., Berriman, M., Gilleard, J. S. and Cotton, J. A. (2013). The genome and transcriptome of *Haemonchus contortus*, a key model parasite for drug and vaccine discovery. *Genome Biology* **14**, R88. doi: 10.1186/gb-2013-14-8-r88.

Landsverk, M. L., Li, S., Hutagalung, A. H., Najafov, A., Hoppe, T., Barral, J. M. and Epstein, H. F. (2007). The UNC-45 chaperone mediates sarcomere assembly through myosin degradation in *Caenorhabditis elegans*. *Journal of Cell Biology* **177**, 205–210. doi: 10.1083/jcb.200607084.

Li, J. and Le, W. (2013). Modeling neurodegenerative diseases in *Caenorhabditis elegans*. *Experimental Neurology* **250C**, 94–103. doi: 10.1016/j.expneurol.2013.09.024.

Martinez, N. J. and Gregory, R. I. (2013). Argonaute2 expression is post-transcriptionally coupled to microRNA abundance. *RNA* **19**, 605–612. doi: 10.1261/rna.036434.112.

Massey, H. C., Jr., Bhopale, M. K., Li, X., Castelletto, M. and Lok, J. B. (2006). The fork head transcription factor FKTF-1b from *Strongyloides stercoralis* restores DAF-16 developmental function to mutant *Caenorhabditis elegans*. *International Journal for Parasitology* **36**, 347–352. doi: 10.1016/j.ijpara.2005.11.007.

Millson, S. H., Chua, C. S., Roe, S. M., Polier, S., Solovieva, S., Pearl, L. H., Sim, T. S., Prodromou, C. and Piper, P. W. (2011). Features of the *Streptomyces hygroscopicus* HtpG reveal how partial geldanamycin resistance can arise with mutation to the ATP binding pocket of a eukaryotic Hsp90. *Journal of the Federation of American Societies for Experimental Biology* **25**, 3828–3837. doi: 10.1096/fj.11-188821.

Mitreva, M., Jasmer, D. P., Zarlenga, D. S., Wang, Z., Abubucker, S., Martin, J., Taylor, C. M., Yin, Y., Fulton, L., Minx, P., Yang, S. P., Warren, W. C., Fulton, R. S., Bhonagiri, V., Zhang, X., Hallsworth-Pepin, K., Clifton, S. W., McCarter, J. P., Appleton, J., Mardis, E. R. and Wilson, R. K. (2011). The draft genome of the parasitic nematode *Trichinella spiralis*. *Nature Genetics* **43**, 228–235. doi: 10.1038/ng.769.

Miyoshi, T., Takeuchi, A., Siomi, H. and Siomi, M. C. (2010). A direct role for Hsp90 in pre-RISC formation in *Drosophila*. *Nature Structural and Molecular Biology* **17**, 1024–1026. doi: 10.1038/nsmb.1875.

Molyneux, D. H., Bradley, M., Hoerauf, A., Kyelem, D. and Taylor, M. J. (2003). Mass drug treatment for lymphatic filariasis and onchocerciasis. *Trends in Parasitology* **19**, 516–522.

Moulick, K., Ahn, J. H., Zong, H., Rodina, A., Cerchietti, L., Gomes Dagama, E. M., Caldas-Lopes, E., Beebe, K., Perna, F., Hatzi, K., Vu, L. P., Zhao, X., Zatorska, D., Taldone, T., Smith-Jones, P., Alpaugh, M., Gross, S. S., Pillarsetty, N., Ku, T., Lewis, J. S., Larson, S. M., Levine, R., Erdjument-Bromage, H., Guzman, M. L., Nimer, S. D., Melnick, A., Neckers, L. and Chiosis, G. (2011). Affinity-based proteomics reveal cancer-specific networks coordinated by Hsp90. *Nature Chemical Biology* **7**, 818–826. doi: 10.1038/nchembio.670.

Neckers, L., Mimnaugh, E. and Schulte, T. W. (1999). Hsp90 as an anti-cancer target. *Drug Resistance Update* **2**, 165–172. doi: 10.1054/drup.1999.0082.

Ni, W., Hutagalung, A. H., Li, S. and Epstein, H. F. (2011). The myosin-binding UCS domain but not the Hsp90-binding TPR domain of the UNC-45 chaperone is essential for function in *Caenorhabditis elegans*. *Journal of Cell Science* **124**, 3164–3173. doi: 10.1242/jcs.087320.

Nicol, J. M., Turner, S. J., Coyne, D. L., Den Nijs, L., Hockland, S. and Maafi, Z. T. (2011). Current nematode threats to world agriculture. In *Genomics and Molecular Genetics of Plant-Nematode Interactions* (ed. Jones, J., Gheysen, G. and Fenoll, C.), pp. 21–43. Springer, Dordrecht, the Netherlands.

Opperman, C. H., Bird, D. M., Williamson, V. M., Rokhsar, D. S., Burke, M., Cohn, J., Cromer, J., Diener, S., Gajan, J., Graham, S., Houfek, T. D., Liu, Q., Mitros, T., Schaff, J., Schaffer, R., Scholl, E., Sosinski, B. R., Thomas, V. P. and Windham, E. (2008). Sequence and genetic map of *Meloidogyne hapla*: a compact nematode genome for plant parasitism. *Proceedings of the National Academy of Sciences USA* **105**, 14802–14807.

Pallavi, R., Roy, N., Nageshan, R. K., Talukdar, P., Pavithra, S. R., Reddy, R., Venketesh, S., Kumar, R., Gupta, A. K., Singh, R. K., Yadav, S. C. and Tatu, U. (2010). Heat shock protein 90 as a drug target against protozoan infections: biochemical characterization of Hsp90 from *Plasmodium falciparum* and *Trypanosoma evansi* and evaluation of its inhibitor as a candidate drug. *Journal of Biological Chemistry* **285**, 37964–37975. doi: 10.1074/jbc.M110.155317.

Palmer, G., Louvion, J. F., Tibbetts, R. S., Engman, D. M. and Picard, D. (1995). *Trypanosoma cruzi* heat-shock protein 90 can functionally complement yeast. *Molecular and Biochemical Parasitology* **70**, 199–202.

Pearl, L. H. and Prodromou, C. (2006). Structure and mechanism of the Hsp90 molecular chaperone machinery. *Annual Review of Biochemistry* **75**, 271–294. doi: 10.1146/annurev.biochem.75.103004.142738.

Petersen, A. L., Guedes, C. E., Versoza, C. L., Lima, J. G., De Freitas, L. A., Borges, V. M. and Veras, P. S. (2012). 17-AAG kills intracellular *Leishmania amazonensis* while reducing inflammatory responses in infected macrophages. *PLoS One* **7**, e49496. doi: 10.1371/journal.pone.0049496.

Piano, F., Schetter, A. J., Mangone, M., Stein, L. and Kemphues, K. J. (2000). RNAi analysis of genes expressed in the ovary of *Caenorhabditis elegans*. *Current Biology* **10**, 1619–1622.

Piper, P. W., Panaretou, B., Millson, S. H., Trumana, A., Mollapour, M., Pearl, L. H. and Prodromou, C. (2003). Yeast is selectively hypersensitised to heat shock protein 90 (Hsp90)-targeting drugs with heterologous expression of the human Hsp90beta, a property that can be exploited in screens for new Hsp90 chaperone inhibitors. *Gene* **302**, 165–170.

Pratt, W. B., Galigniana, M. D., Harrell, J. M. and Defranco, D. B. (2004). Role of Hsp90 and the Hsp90-binding immunophilins in signalling protein movement. *Cell Signalling* **16**, 857–872. doi: 10.1016/j.cellsig.2004.02.004.

Prichard, R. K., Basanez, M. G., Boatin, B. A., McCarthy, J. S., Garcia, H. H., Yang, G. J., Sripa, B. and Lustigman, S. (2012). A research agenda for helminth diseases of humans: intervention for control and elimination. *PLoS Neglected Tropical Diseases* **6**, e1549. doi: 10.1371/journal.pntd.0001549.

Prodromou, C., Nuttall, J. M., Millson, S. H., Roe, S. M., Sim, T. S., Tan, D., Workman, P., Pearl, L. H. and Piper, P. W. (2009). Structural basis of the radicicol resistance displayed by a fungal Hsp90. *ACS Chemical Biology* **4**, 289–297. doi: 10.1021/cb9000316.

Rae, R., Riebesell, M., Dinkelacker, I., Wang, Q., Herrmann, M., Weller, A. M., Dieterich, C. and Sommer, R. J. (2008). Isolation of naturally associated bacteria of necromenic *Pristionchus* nematodes and fitness consequences. *Journal of Experimental Biology* **211**, 1927–1936. doi: 10.1242/jeb.014944.

Riggs, D. L., Cox, M. B., Cheung-Flynn, J., Prapapanich, V., Carrigan, P. E. and Smith, D. F. (2004). Functional specificity of co-chaperone interactions with Hsp90 client proteins. *Critical Reviews in Biochemistry and Molecular Biology* **39**, 279–295. doi: 10.1080/10409230490892513.

Ruden, D. M. and Lu, X. Y. (2008). Hsp90 affecting chromatin remodeling might explain transgenerational epigenetic inheritance in *Drosophila*. *Current Genomics* **9**, 500–508. doi: 10.2174/138920208786241207.

Russell, L. C., Whitt, S. R., Chen, M. S. and Chinkers, M. (1999). Identification of conserved residues required for the binding of a tetratricopeptide repeat domain to heat shock protein 90. *Journal of Biological Chemistry* **274**, 20060–20063.

Rutherford, S. L. and Lindquist, S. (1998). Hsp90 as a capacitor for morphological evolution. *Nature* **396**, 336–342. doi: 10.1038/24550.

Sawarkar, R., Sievers, C. and Paro, R. (2012). Hsp90 globally targets paused RNA polymerase to regulate gene expression in response to environmental stimuli. *Cell* **149**, 807–818.

Scheufler, C., Brinker, A., Bourenkov, G., Pegoraro, S., Moroder, L., Bartunik, H., Hartl, F. U. and Moarefi, I. (2000). Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine. *Cell* **101**, 199–210. doi: 10.1016/S0092-8674(00)80830-2.

Schwarz, E. M., Korhonen, P. K., Campbell, B. E., Young, N. D., Jex, A. R., Jabbar, A., Hall, R. S., Mondal, A., Howe, A. C., Pell, J., Hofmann, A., Boag, P. R., Zhu, X. Q., Gregory, T. R., Loukas, A., Williams, B. A., Antoshechkin, I., Brown, C. T., Sternberg, P. W. and Gasser, R. B. (2013). The genome and developmental transcriptome of the strongylid nematode *Haemonchus contortus*. *Genome Biology* **14**, R89. doi: 10.1186/gb-2013-14-8-r89.

Shahinas, D., Liang, M., Datti, A. and Pillai, D. R. (2010). A repurposing strategy identifies novel synergistic inhibitors of *Plasmodium falciparum* heat shock protein 90. *Journal of Medicinal Chemistry* **53**, 3552–3557. doi: 10.1021/jm901796s.

Shahinas, D., Folefoc, A., Taldone, T., Chiosis, G., Crandall, I. and Pillai, D. R. (2013). A purine analog synergizes with chloroquine (CQ) by targeting *Plasmodium falciparum* Hsp90 (PfHsp90). *PLoS One* **8**, e75446. doi: 10.1371/journal.pone.0075446.

Smith, D. F. and Toft, D. O. (2008). Minireview: the intersection of steroid receptors with molecular chaperones: observations and questions. *Molecular Endocrinology* **22**, 2229–2240. doi: 10.1210/me.2008-0089.

Specchia, V., Piacentini, L., Tritto, P., Fanti, L., D'Alessandro, R., Palumbo, G., Pimpinelli, S. and Bozzetti, M. P. (2010). Hsp90 prevents phenotypic variation by suppressing the mutagenic activity of transposons. *Nature* **463**, 662–665. doi: 10.1038/nature08739.

Stein, L. D., Bao, Z., Blasiar, D., Blumenthal, T., Brent, M. R., Chen, N., Chinwalla, A., Clarke, L., Clee, C., Coghlan, A., Coulson, A., D'Eustachio, P., Fitch, D. H., Fulton, L. A., Fulton, R. E., Griffiths-Jones, S., Harris, T. W., Hillier, L. W., Kamath, R., Kuwabara, P. E., Mardis, E. R., Marra, M. A., Miner, T. L., Minx, P., Mullikin, J. C., Plumb, R. W., Rogers, J., Schein, J. E., Sohrmann, M., Spieth, J., Stajich, J. E., Wei, C., Willey, D., Wilson, R. K., Durbin, R. and Waterston, R. H. (2003). The genome sequence of *Caenorhabditis briggsae*: a platform for comparative genomics. *PLoS Biology* **1**, E45. doi: 10.1371/journal.pbio.0000045.

Taldone, T., Gillan, V., Sun, W., Rodina, A., Patel, P., Maitland, K., O'Neill, K., Chiosis, G. and Devaney, E. (2010). Assay strategies for the discovery and validation of therapeutics targeting *Brugia pahangi* Hsp90. *PLoS Neglected Tropical Diseases* **4**, e714. doi: 10.1371/journal.pntd.0000714.

Taldone, T., Zatorska, D., Patel, P. D., Zong, H., Rodina, A., Ahn, J. H., Moulick, K., Guzman, M. L. and Chiosis, G. (2011). Design, synthesis, and evaluation of small molecule Hsp90 probes. *Bioorganic and Medicinal Chemistry* **19**, 2603–2614. doi: 10.1016/j.bmc.2011.03.013.

Tang, Y. T., Gao, X., Rosa, B. A., Abubucker, S., Hallsworth-Pepin, K., Martin, J., Tyagi, R., Heizer, E., Zhang, X., Bhonagiri-Palsikar, V., Minx, P., Warren, W. C., Wang, Q., Zhan, B., Hotez, P. J.,

Sternberg, P. W., Dougall, A., Gaze, S. T., Mulvenna, J., Sotillo, J., Ranganathan, S., Rabelo, E. M., Wilson, R. K., Felgner, P. L., Bethony, J., Hawdon, J. M., Gasser, R. B., Loukas, A. and Mitreva, M. (2014). Genome of the human hookworm *Necator americanus*. *Nature Genetics* **46**, 261–269. doi: 10.1038/ng.2875.

Tariq, M., Nussbaumer, U., Chen, Y., Beisel, C. and Paro, R. (2009). Trithorax requires Hsp90 for maintenance of active chromatin at sites of gene expression. *Proceedings of the National Academy of Sciences USA* **106**, 1157–1162. doi: 10.1073/pnas.0809669106.

Terhell, A. J., Haarbrink, M., Van Den Biggelaar, A., Mangali, A., Sartono, E. and Yazdanbakhsh, M. (2003). Long-term follow-up of treatment with diethylcarbamazine on anti-filarial IgG4: dosage, compliance, and differential patterns in adults and children. *American Journal of Tropical Medicine and Hygiene* **68**, 33–39.

Thompson, F. J., Cockroft, A. C., Wheatley, I., Britton, C. and Devaney, E. (2001). Heat shock and developmental expression of hsp83 in the filarial nematode *Brugia pahangi*. *European Journal of Biochemistry* **268**, 5808–5815.

Vercruysse, J., Levecke, B. and Prichard, R. (2012). Human soil-transmitted helminths: implications of mass drug administration. *Current Opinion in Infectious Disease* **25**, 703–708. doi: 10.1097/QCO.0b013e328358993a.

Walker, G. A., Thompson, F. J., Brawley, A., Scanlon, T. and Devaney, E. (2003). Heat shock factor functions at the convergence of the stress response and developmental pathways in *Caenorhabditis elegans*. *Journal of the Federation of American Societies for Experimental Biology* **17**, 1960–1962. doi: 10.1096/fj.03-0164fje.

Wenkert, D., Ramirez, B., Shen, Y. and Kron, M. A. (2010). *In vitro* activity of geldanamycin derivatives against *Schistosoma japonicum* and *Brugia malayi*. *Journal of Parasitology Research* **2010**, 716498. doi: 10.1155/2010/716498.

Wider, D., Peli-Gulli, M. P., Briand, P. A., Tatu, U. and Picard, D. (2009). The complementation of yeast with human or *Plasmodium falciparum* Hsp90 confers differential inhibitor sensitivities. *Molecular and Biochemical Parasitology* **164**, 147–152.

Wu, C. (1995). Heat shock transcription factors: structure and regulation. *Annual Review of Cell Developmental Biology* **11**, 441–469. doi: 10.1146/annurev.cb.11.110195.002301.

Xiao, H. and Lis, J. T. (1989). Heat shock and developmental regulation of the *Drosophila melanogaster* hsp83 gene. *Molecular Cell Biology* **9**, 1746–1753.

Yang, Y., Qin, W., Zarlenga, D., Cao, L. and Tian, G. (2013). TsDAF-21/Hsp90 is expressed in all examined stages of *Trichinella spiralis*. *Veterinary Parasitology* **194**, 171–174. doi: 10.1016/j.vetpar. 2013.01.048.