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Divergent Regulatory Roles of NuRD Chromatin Remodeling Complex Subunits GATAD2 and CHD4 in Caenorhabditis elegans

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DIVERGENT REGULATORY ROLES OF NURD CHROMATIN REMODELING COMPLEX SUBUNITS GATAD2 AND CHD4 IN *CAENORHABDITIS ELEGANS*

by

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ABSTRACT

Divergent Regulatory Roles of NuRD Chromatin Remodeling Complex Subunits GATAD2 and CHD4 in *Caenorhabditis elegans*

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During stress, a protective cellular network known as the heat shock response (HSR) is induced to maintain protein-folding homeostasis, or proteostasis. While the HSR is essential for stress resistance, its misregulation is associated with neurodegenerative disease and cancer. Using the nematode model organism *Caenorhabditis elegans*, we have identified the chromatin remodeling complex NuRD (nucleosome remodeling and deacetylase) as a novel regulator of the HSR. Here, we begin with a brief introduction of the HSR and chromatin remodeling complexes in *C. elegans*, prior to presenting our findings in a series of two chapters. In chapter one, we outline a set of standardized protocols for facilitating accurate measurement of the HSR in *C. elegans*. In chapter two, we show that *dcp-66* and *let-418* subunits of the NuRD complex regulate the HSR in divergent ways. This paradigm extends to other stress responses and even to other pathways. Together, this work highlights the power of using *C. elegans* as a biological tool to discover novel genetic interactions important in physiology and disease.

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In dedication to my grandmother, Bette J. Aia.

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INTRODUCTION: THE HEAT SHOCK RESPONSE AND ATP-DEPENDENT REMODELING COMPLEXES IN *CAENORHABDITIS ELEGANS*

All organisms must be able to sense and respond to stress for survival. The heat shock response (HSR) is one of the most well-studied cellular stress response pathways and it is found in essentially all organisms, ranging from bacteria to humans. The HSR is activated by stressors including but not limited to: changes in temperature, starvation, water deprivation, infection, or inflammation. Ferruccio Ritossa first characterized the HSR in *Drosophila* through his discovery that polytene chromosomes inside salivary gland cells exposed to high temperatures made a distinct "puffing" pattern, one that could best be explained by an increase in transcription (Ritossa 1962). This transcriptional activation is caused by the heat shock factor 1 (HSF1) transcription factor, which binds to the promoters of heat shock (HS) genes, resulting in the production of heat-shock proteins (HSPs). Several HSPs are named based on their molecular weight (in kDa) with 5 common classes across species: HSP40, HSP60, HSP70, HSP90, and small HSPs. These HSPs act as chaperones that aid in the refolding of proteins misfolded by stress, thus maintaining protein homeostasis.

The HSR is required for stress resistance yet it is equally important for normal growth, development, aging, and disease. Much of what is known about the HSR in the context of aging and disease comes from studies of HSF1. While HSF1 inhibition accelerates aging, HSF1 overexpression delays aging in multiple model organisms, including the nematode *Caenorhabditis elegans* (Heydari *et al.* 1993; Heydari *et al.* 2000; Hsu *et al.* 2003; Morley and Morimoto 2004; Cohen *et al.* 2006). Similarly,

HSF1 inhibition exacerbates protein misfolding and toxicity in a Huntington's disease model, yet HSF1 overexpression suppresses this toxicity (Gomez-Pastor *et al*. 2017). Despite the benefits of increased HSF1 levels, high levels of HSF1 are associated with cancer (Santagata *et al.* 2011; Mendillo *et al.* 2012; Dai *et al.* 2012).

As the HSR is a universally conserved pathway, it can be studied in nearly any organism. *C. elegans* boasts several advantages for HSR research over other animal models in that the HSR can be studied at the cellular, molecular, and organismal levels with relative ease. For example, fluorescence reporters at the cellular level detect changes in gene activity, quantitative PCR (qPCR) at the molecular level measures endogenous gene expression, and a thermorecovery assay at the organismal level is dependent on whole-worm physiology. Additional advantages include the transparent nature of the worm, which allows for the detection of physiological or morphological effects, the ability to easily obtain genetic mutants, and its short development time (3 days), enabling experiments to be completed quickly.

However, one disadvantage of using *C. elegans* for HSR research is the disparity that exists amongst researchers over which experimental conditions should be used. For example, the *C. elegans* research community uses an array of different temperatures, times, and even sources of heat (*e.g.* water bath versus dry incubator). This can be particularly problematic when a novice researcher is looking to begin an experiment and may not know which conditions to select. While it is reported that temperatures

from 33°C to 37°C can be used, temperatures too high can elicit a severe response, distinct from the acute HSR that is typically studied. Despite these inconsistencies, the benefits of using *C. elegans* for HSR research far outweigh these disadvantages.

Our lab sought to identify new regulators of the HSR by completing a genome-wide RNAi screen using a heat shock (HS) inducible fluorescent reporter in *C. elegans* (Guisbert *et al.* 2013). From this screen, we identified 59 novel HSR regulators, of which 7 were positive activators and 52 were negative regulators. Whereas the negative regulators showed tissue-specific effects, the positive regulators were not specific to any one tissue but instead affected the HSR ubiquitously. To begin following up with the positive regulators, I selected deacetylase complex protein 66 (*dcp-66*), a subunit of the chromatin remodeling complex NuRD (nucleosome remodeling and deacetylase), as changes in chromatin were already shown to affect HSR regulation (Guertin and Lis 2010; Labbadia and Morimoto 2015).

Chromatin remodeling complexes are essential macromolecular machines that regulate gene expression through nucleosome repositioning. Remodeling complexes are abundant in the cell nucleus, with approximately 1 complex for every 10 nucleosomes (Rippe *et al*. 2007). In *C. elegans*, there are three well-characterized nucleosome-remodeling complexes: SWI/SNF, ISWI (NuRF), and CHD/Mi-2 (NuRD). The unique ATPase, core, and accessory subunits of each complex distinguish their diverse functions. Each complex acts on an exclusive set of target

genes and functions at precise stages during the cell cycle, throughout development, and in response to stress. Perturbations in nucleosome-remodeling activity can result in physical abnormalities, intellectual disabilities, neurodegenerative disorders, immunodeficiency, and cancer (Huang *et al.* 2003; Mirabella *et al.* 2016).

The ATPase subunits of all nucleosome-remodeling complexes are part of the Sucrose Non-Fermenting (Snf2) superfamily of helicase-related proteins (Flaus *et al.* 2006). This family is further divided into 24 subfamilies, including SWI/SNF, ISWI, and CHD/Mi-2; originally named after their discovery in yeast (SWI/SNF), *Drosophila* (ISWI), and human (Mi-2) (Côté *et al.* 1994; Tsukiyama *et al.* 1994; Zhang *et al.* 1998). Each complex contains a DNA-dependent ATPase (**Table 1**, highlighted in red) responsible for catalyzing the structural conformations of chromatin (Kornberg *et al.* 1999). In addition to nucleosome remodeling, the chromatin architecture is also altered by DNA modifications including methylation, as well as histone post-translational modifications (PTMs) (Mirabella *et al*. 2016).

Histone proteins contain highly conserved N-terminal tails that are exposed to PTMs such as acetylation, methylation, phosphorylation, ubiquitylation, ADP-ribosylation, glycosylation, and SUMOylation, which can change histone-DNA affinity and affect transcription (Kuo *et al.* 1998; Cheung *et al.* 2000; Shiio *et al*. 2003; Zhang *et al.* 2011). The core and accessory subunits of each complex can mediate these effects by providing secondary forms of contact between histones and DNA (**Table 1**,

highlighted in orange and blue). These subunits and their relevance to each remodeling complex in *C. elegans* are examined in further detail below.

TABLE 1

C. elegans gene	Human	Description
SWI/SNF complex (SWI/SNF family)		
swsn-4	BRG1/BRM	SWI/SNF nucleosome remodeling and ATPase
swsn-1	BAF155/170	SWIRM domain; core subunit
swsn-5	BAF47	RPT1 domain; core subunit
swsn-3	BAF57	matrix associated actin dependent regulator
swsn-2.1/ham-3, swsn-2.2	BAF60a/b/c	matrix associated actin dependent regulator
swsn-6	BAF53a/b	ACTL6A; actin-like 6a/6b
phf-10	BAF45a	PHD finger protein 10
dpff-1	BAF45b/c/d	double PHD fingers 2
let-526	BAF250a/b	SWI/SNF-A (BAF/BAP) subunit
swsn-7	BAF200	AT-rich interaction domain 2
pbrm-1	BAF180	poly bromodomain
swsn-9	BRD7/9	bromodomain containing 7/9
NuRF complex (ISWI family)		
isw-1	SNF2H	ISWI nucleosome remodeling and ATPase
nurf-1	BPTF	nucleosome remodeling factor
pyp-1	PPA1/2	inorganic pyrophosphatase
rba-1	RBBP7	RB binding protein 7
NuRD complex (CHD/Mi-2 family)		
chd-3	CHD3	Mi-2 nucleosome remodeling and ATPase
let-418	CHD4	Mi-2 nucleosome remodeling and ATPase
hda-1	HDAC1/2	histone deacetylase 1
lin-53	RBBP4	nucleosome remodeling factor
lin-40	MTA1	metastasis associated protein 1 ortholog
dcp-66	GATAD2A/B	deacetylase complex protein
mbd-2	MBD2/3	methyl DNA binding

Composition of SWI/SNF, NuRF, and NuRD Nucleosome Remodeling Complexes in *C. elegans*. ATPase subunits are highlighted in *red*. Core subunits are highlighted in *orange*. Accessory subunits are highlighted in *blue*. BAF (SWI/SNF-A) complex specific subunits are highlighted in *green*. PBAF (SWI/SNF-B) complex specific subunits are highlighted in *brown*. Unverified subunits are highlighted in *purple.* Kornberg *et al.* 1999; Large *et al.* 2014; Passannante *et al*. 2010.

The SWI/SNF Complex

The SWI/SNF genes were initially discovered in *C. elegans* when the asymmetrical division of the most posterior seam cell, termed the T cell, was disrupted, resulting in cells of the incorrect fate (Sawa *et al.* 2000). In humans, there are two well-defined SWI/SNF complexes: BAF (SWI/SNF-A) and PBAF (SWI/SNF-B); each containing one of two ATPases (BRG1/BRM) that share core and accessory subunits, but feature additional subunits unique to each complex. These additional proteins have also been identified in *C. elegans* suggesting they also have two forms of the complex (**Table 1**, highlighted in green and brown).

The SWI/SNF complex is essential to *C. elegans* gonadogenesis. The ATPase SWSN-4 is required for the development of the somatic gonad (Lints *et al.* 2009; Large *et al*. 2014) and the core subunit SWSN-1 is also important in gonad formation, as a heterozygous deletion mutation results in animals sometimes missing one of the two gonadal arms (Large *et al.* 2014).

The accessory subunits of the SWI/SNF complex control precise stages of embryonic and larval development. Consistent with a role in gonadogenesis, BAF (*let-526*) and PBAF (*pbrm-1*) specific genes are required for distal tip cell (DTC) formation; a specialized cell found at the end of each gonadal arm that is responsible for extending the gonad and promoting germ cell mitosis during development. Despite the overlapping functions of BAF and PBAF specific SWI/SNF subunits, *swsn-7*

(PBAF) mutants arrest embryonically at the comma stage, whereas mutations in *let-526* (BAF) do not cause this arrest (Large *et al.* 2014). This implies that the PBAF (SWI/SNF-B) complex establishes the somatic gonad early on and its expression is required for embryonic development, whereas the BAF (SWI/SNF-A) complex functions in somatic gonad-derived tissues but is expendable to embryogenesis.

The accessory subunits SWSN-6, SWSN-9, PHF-10, and DPFF-1 are also required for gonadogenesis (Large *et al*. 2014). The additional accessory subunits SWSN-2.1 (HAM-3) and SWSN-2.2 have redundant roles in development of the somatic gonad, vulva, and germline, however each has an independent role during embryogenesis. For example, *ham-3* mutants exhibit hyperproliferation of intestinal cells, implying a role in cell cycle control, while *swsn-2.2* is specifically required for nuclear envelope assembly and chromosome segregation during cell division (Ertl *et al.* 2016). The final accessory subunit SWSN-3 is not required for gonadogenesis as *swsn-3* mutants appear phenotypically wild type (Large *et al.* 2014).

The NuRF Complex

Unlike the SWI/SNF complex whose major role seems restricted to early development, the NuRF complex is ubiquitously expressed throughout all stages of development and contributes to growth rate, dauer formation, lifespan, and reproductive timing (Andersen *et al.* 2006; Large *et al*. 2016). The *C. elegans* NuRF complex contains an ISWI family ATPase (ISW-1), three core subunits, and no additional accessory subunits (**Table 1**). In *C. elegans*, ISW-1 positively regulates gene expression by suppressing synthetic multivulva (synMuv) genes, or genes required to repress vulval differentiation. These genes are divided into three classes (A, B, and C) and any two genes from each of two separate classes must be mutated to produce the SynMuv phenotype (multiple vulvas) (Andersen *et al.* 2006). NURF-1 also acts to antagonize synMuv genes, providing evidence that ISW-1 and NURF-1 regulate vulval development together (Andersen *et al.* 2006). NURF-1 is unusually complicated, encoding at least 16 different isoforms (Large *et al.* 2016). This delivers additional evidence as to why the NuRF complex is so ubiquitous; each isoform could provide the complex with the flexibility required for quickly remodeling chromatin to adapt to the environment. If each isoform isn't beneficial, the isoforms themselves provide us with a history of alternative splicing events that might have had a function at one time. In support of this idea, a recent molecular model proposes that two *nurf-1* gene isoforms are required for gametogenesis, yet they function in opposite ways to promote either spermatogenesis or oogenesis (Xu *et al*. 2019).

The additional core NuRF subunit PYP-1 is responsible for hydrolyzing pyrophosphate into two molecules of phosphate, a highly exergonic reaction required in many biosynthetic reactions (Ko *et al.* 2007). It is expressed in nerve cords, accessory neurons, and coelomocytes from larval to adulthood (Ko *et al.* 2007). PYP-1 is also required for larval development and intestinal function, and has 9 isoforms. Most strikingly, *pyp-1* mutants show irregular and reduced numbers of cell nuclei in the intestine of L2 stage worms (Ko *et al.* 2007). This indicates that PYP-1 functions to maintain cell division, perhaps by positively regulating the cell cycle whereas SWI/SNF *ham-3* acts to repress it in the same cell type and at the same stage of development. As each complex has a unique ATPase and a distinct rate of nucleosome repositioning to allow for transcription, it could be possible that one remodeler 'outcompetes' the other; thereby denying access to its nucleosomal targets.

Lastly, the core subunit RBA-1 is an ortholog to the human histone binding protein RBBP7. *C. elegans* require *rba-1* for larval development (Andersen *et al.* 2006). RBA-1 contains several WD40 repeats that serve as platforms for the assembly of protein complexes. The domains lying outside the WD40 region determine the specificity of the protein; in this case a histone H4 binding site is present (Murzina *et al*. 2008). This leads to the conclusion that NuRF directly interacts with histones, representing a significant difference between NuRF and SWI/SNF (Georgel *et al*. 1997).

The NuRD Complex

Similarly to SWI/SNF and NuRF, the NuRD complex contains proteins defined by their helicase-like, DNA-binding, and subunit-linking domains. In contrast, the NuRD complex is the only complex to combine nucleosome remodeling with histone deacetylase activity (Xue *et al.* 1998). Several core genes of the NuRD complex are synMuv genes. The NuRD subunits *let-418*, *hda-1*, and *lin-53* are all Class B SynMuv genes (Fay *et al*. 2007), which is interesting because the NuRF complex targets this class (Andersen *et al.* 2006). This shows that the NuRF complex can indirectly regulate nucleosome remodeling and histone deacetylase activity of NuRD through directly increasing or decreasing gene expression of required NuRD subunits. Like the SWI/SNF complex there are two NuRD complexes in *C. elegans*; both include core subunits *hda-1*, *lin-53*, *lin-40*, *dcp-66*, and *mbd-2*, however each is defined by their catalytic ATPase: LET-418 or CHD-3 (**Table 1,** highlighted in red). CHD-3 and LET-418 are paralogs with 70% identity; nevertheless, the mutant phenotype of *let-418* results in mid-L1 arrest whereas *chd-3* mutants show no obvious phenotype (Passannante *et al*. 2010). *chd-3* mRNA is first expressed at the 16-cell stage embryo, but becomes highly expressed at the 28-cell stage (von Zelewsky *et al*. 2000). In contrast, *let-418* mRNA is highly expressed at the single cell and 2-cell stage, but remains expressed at the 28-cell stage (von Zelewsky *et al*. 2000). Additional work shows that *chd-3* most likely preforms a non-essential but redundant role in early development (Pebernard *et al.* 2016). Moreover, LET-418 acts as part of another complex, termed the MEC complex (composed of *let-418*,

hda-1, and *mep-1*). This complex represents the main LET-418 containing complex important in embryonic and larval development (Passannante *et al*. 2010). In early embryogenesis, LET-418 repression by PIE-1 is crucial to maintaining a germline vs. somatic cell fate (Unhavaithaya *et al.* 2002). The germline is already distinguished from the soma at the 16 to 24-cell stage (Strome 2005), indicating *let-418* and *chd-3* are expressed in both early germline and somatic cells.

A key difference between NuRD and the other complexes is its histone deacetylase activity. Histone deacetylase I (HDAC1) directly interacts with histone tails to remove acetyl groups from lysine, giving histones an increased positive charge which allows negatively-charged DNA to bind more tightly. HDAC1 is ubiquitous, plays an essential role in cell cycle progression, and does not always function as part of the complex (Denslow *et al*. 2007). In *C. elegans*, HDA-1 is required early in embryogenesis (as part of the MEC complex) and at the L1/L2 stage for the differentiation of the gonadal anchor cell (AC), the central organizer of vulval development (Ranawade *et al*. 2013).

LIN-53 contains a histone H4 binding site and WD40 domain similar to RBA-1 of the NuRF complex. LIN-53 is expressed during embryogenesis and newly hatched L1s, but becomes restricted to the head and tail, except at vulval development (L1/L2) where it is expressed in all vulval precursor cells (VPCs) that derive from the AC (Lu *et al*. 1998).

MTA1/LIN-40 is best defined in the context of cancer, where overexpression is broadly linked to tumor formation (Lai *et al*. 2011). MTA1 targets NuRD to different genomic locations by associating with certain transcription factors (Lai *et al*. 2011). LIN-40 is expressed in all somatic cells and functions in vulval cell division, though it is not a synMuv gene (Chen *et al*. 2001). In normal development, induced VPCs go through three rounds of cell division (Chen *et al*. 2001). In the last round of division cells divide along a specific divisional plane; however in *lin-40* mutants, cells that should orient themselves along a transverse plane end up longitudinal (Chen *et al*. 2001). If *lin-40* acts as part of the NuRD complex in this context, it is conceivable that NuRD represses longitudinal division during the final round of vulval cell divisions.

DCP-66 is the *C. elegans* ortholog of GATAD2A/B, a well-established subunit of the NuRD complex (Zhang 2011). Independently of NuRD, DCP-66 acts as a transcription factor in excretory cell differentiation (Zhao *et al*. 2005) and is highly expressed in the excretory cell of embryos and larva (Zhao *et al*. 2005). The role of DCP-66 in the NuRD complex has remained ambiguous until recently, where it was shown to act as a scaffold; required for connecting both catalytic activities of NuRD together into one complex (Spruijt *et al*. 2020).

Unlike the SWI/SNF and NuRF complexes, the NuRD complex can couple DNA methylation to nucleosome remodeling through the methyl-DNA binding domain of MBD2/3 (Wade *et al.* 1999). MBD-2 shares just 27% identity to human MBD2/3 and has not been found to associate with *let-418* or *chd-3 (*Passannante *et al.* 2010). Conversely, MBD-2 contains a coiled-coil (CC) domain that binds GATAD2A/B (Wade *et al.* 1999; Gnanapragasam *et al.* 2011; Cramer *et al.* 2017). Consequently, it is possible that MBD-2 associates with DCP-66 in *C. elegans*, though this has not been experimentally tested. In humans, DNA is commonly methylated at the 5th position of cytosine (5mC) and serves as a target for MBD2/3, which can recruit NuRD through GATAD2A/B leading to gene repression (Denslow *et al.* 2007). However, 5mC levels are undetectable in *C. elegans* (Greer *et al.* 2015) and MBD-2 lacks a methyl-binding domain. For these reasons, MBD-2 most likely associates with NuRD through DCP-66 and it may have additional functions outside of its role as a NuRD complex subunit, which remain to be identified in *C. elegans*.

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CHAPTER 1: STANDARDIZED METHODS FOR MEASURING INDUCTION OF THE HEAT SHOCK RESPONSE IN *CAENORHABDITIS ELEGANS*

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ABSTRACT

The heat shock response (HSR) is a cellular stress response induced by cytosolic protein misfolding that functions to restore protein folding homeostasis, or proteostasis. *Caenorhabditis elegans* occupies a unique and powerful niche for HSR research because the HSR can be assessed at the molecular, cellular, and organismal levels. Therefore, changes at the molecular level can be visualized at the cellular level and their impacts on physiology can be quantitated at the organismal level. While assays for measuring the HSR are straightforward, variations in the timing, temperature, and methodology described in the literature make it challenging to compare results across studies. Furthermore, these issues act as a barrier for anyone seeking to incorporate HSR analysis into their research. Here, a series of protocols is presented for measuring induction of the HSR in a robust and reproducible manner with RT-qPCR, fluorescent reporters, and an organismal thermorecovery assay. Additionally, we show that a widely used thermotolerance assay is not dependent on the well-established master regulator of the HSR, HSF-1, and therefore should not be used for HSR research. Finally, variations in these assays found in the literature are discussed and best practices are proposed to help standardize results across the field, ultimately facilitating neurodegenerative disease, aging, and HSR research.

INTRODUCTION

The heat shock response (HSR) is a universal cellular stress response induced by cytosolic protein misfolding caused by temperature increases and other proteotoxic stresses. Activation of the HSR in *Caenorhabditis elegans* leads to transcriptional upregulation of heat shock genes such as *hsp-70* and *hsp-16.2*. Many heat shock proteins (HSPs) function as molecular chaperones that restore protein folding homeostasis, or proteostasis, by directly interacting with misfolded or damaged proteins. The master regulator of the HSR is the transcription factor Heat Shock Factor 1 (HSF-1), whose activation is elegantly controlled via multiple mechanisms[1]. The role of HSF-1 is not restricted to stress. HSF-1 is required for normal growth and development, as deletion of *hsf-1* leads to larval arrest[2] . HSF-1 is also important during aging and age-related neurodegenerative diseases characterized by accumulation of protein aggregates and an inability to maintain proteostasis. Knockdown of *hsf-1* causes accumulation of protein aggregates and a shortened lifespan, while overexpression of *hsf-1* reduces protein aggregation and extends lifespan[3,4]. Therefore, regulation of HSF-1 at the molecular level has broad implications for organismal physiology and disease.

C. elegans is a powerful model organism for HSR research because the HSR can be measured at the molecular, cellular, and organismal levels[4-6]. Highlighting the power of this model, key advances in delineating the HSR pathway, such as tissue-specific differences in HSR regulation, have been discovered in *C. elegans*[7,8]. Furthermore,

C. elegans is widely used for aging research and is an emerging system for modeling diseases linked to proteostasis disruption.

Although heat shock experiments with *C. elegans* can be quick and reproducible, there are several questions to consider before beginning. For example, which temperature should be used for induction of the HSR and how long should the worms be exposed? Is it better to use a dry incubator or a water bath? Which developmental stage should be used? Unfortunately, the methodologies used to investigate the HSR vary widely from laboratory to laboratory, causing confusion when selecting the best methodologies and making it difficult to compare results across the field.

We present robust and standardized protocols for using RT-qPCR, fluorescent reporters, and thermorecovery to measure the HSR. While these three approaches are complementary, they each have unique advantages and disadvantages. For example, RT-qPCR is the most direct and quantitative measurement of the HSR, and this assay can be easily expanded to include many different heat shock-inducible genes. However, RT-qPCR is the most expensive, can be technically difficult, and requires the use of specialized equipment. In contrast, fluorescent reporters have the advantage of measuring tissue-specific differences in HSR induction. However, they are difficult to quantitate accurately, can only measure induction above a certain threshold, and require the use of a fluorescence microscope. Additionally, the reporter strains described here are developmentally delayed compared to the standard

N2 strain. Although newer reporter strains containing single-copy transgenes are available, they have not been tested here[9]. The third assay, thermorecovery, has the advantage of providing a physiologically relevant readout at the organismal level. However, this assay is arguably the least sensitive and most indirect. Finally, we discuss some common variations found in these assays and propose a set of best practices to facilitate research in this field.

PROTOCOL

1. Maintenance and synchronization of *C. elegans*

1. Maintain worms at 20 °C on Nematode Growth Medium (NGM) plates seeded with OP50 Escherichia coli bacteria by transferring several adults to fresh plates approximately 2x per week[10]. Care should be taken to prevent worms from running out of food, because this can affect their physiology[11].

1. Preparation of NGM plates.

1. Mix 3 g of NaCl, 2.5 g of Bacto-peptone, 20 g of agar, and deionized (DI) H_2O up to 1 L in a flask.

2. Autoclave the mixture for sterilization.

3. Allow mixture to cool to ~50 °C.

4. Add 25 mL of 1 M KH2PO4 (pH = 6), 1 mL of 1 M CaCl2, 1 mL of 1 M MgSO4, and 1 mL of cholesterol (5 mg/mL in 100% ethanol).

5. Using sterile technique, pour the mixture into 6 cm plates to yield approximately 100 plates. Pouring plates is easier if the mixture is first transferred to a 300 mL sterile beaker.

6. Allow 1 day to solidify at room temperature (RT) before seeding with bacteria or storing at 4 °C.

2. Seeding of OP50 bacteria onto NGM plates.

1. Grow a saturated overnight OP50 bacterial culture in LB at 30 °C or 37 °C.

2. Place approximately 300 µL of the culture onto the center of a 6 cm NGM plate.

3. Let plates dry at RT for 1-3 days as needed for the bacterial lawn to adhere to the plate. Plates can then be used or stored at 4 °C.

2. Grow the worms synchronously either by isolating freshly laid eggs (described here) or alternatively by collecting eggs after dissolving worms with bleach.

1. Transfer approximately 10 gravid adult worms to a fresh plate using a platinum wire pick. Egg-lay synchronization works best if the adults are in the first day of adulthood.

2. After approximately 1 h, remove the worms from the plate. This should result in 40-60 eggs per plate, depending on the conditions and the strain.

2. Fluorescent imaging of HSR reporters

1. Synchronize the worms (section 1.2) and maintain at 20 °C until the desired developmental stage. For the AM446 (*hsp-70p::gfp*) and CL2070 (*hsp-16.2p::gfp*) fluorescent reporter strains, young adult worms that have not yet reached reproductive maturity are generated 64 h after the egg-laying synchronization. NOTE: The developmental timing varies with each strain and the temperature at which the worms are raised. Both HSR reporter strains exhibit a slight developmental delay relative to N2. Importantly, the

magnitude of HSR induction declines approximately 2-4x after the onset of reproductive maturity (see Discussion).

2. Heat shock the worms by wrapping plates with paraffin film and submerging in a circulating water bath at 33 °C for 1 h. A thin strip of paraffin film should be wrapped 2x around the plate to seal the edges. Do not cover the bottom of the plate or it could interfere with heat transfer. Submerge the plates upside down using a test tube rack and a lead weight. Remember to include a negative control sample (no heat shock) if necessary. NOTE: If the paraffin film is not secure, then water will enter the plate and the plate should not be used for data collection.

3. Recover the worms by removing the plates from the water bath and drying with a paper towel. Remove the paraffin film and incubate the worms at 20 °C for 6-24 h. This recovery period allows sufficient time for GFP synthesis and folding before imaging.

4. Prepare slides for imaging. Slides should be prepared fresh for each use.

    1. Make a 3% agarose solution in water and heat using a microwave until the agarose is dissolved.

    2. Place a microscope slide for imaging between two other microscope slides that have a strip of laboratory tape on them to create a spacer for the agarose pad.

    3. Using a 1,000 µL pipette, place a drop (~150 µL) of the heated 3% agarose in the center of the microscope slide.

4. Immediately cover the microscope slide with a blank microscope slide perpendicular to the first slide so that the top slide rests on the laboratory tape on the adjacent slides. This spreads out the drop of agarose to create a pad of uniform width.

5. Carefully remove the top slide.

5. Immobilize the worms by using a 200 μL pipette to add a small drop (~5 μL) of 1 mM levamisole in M9 buffer to the center of the agarose pad. Then transfer 10 worms into the drop of levamisole using a platinum wire pick. Cover with a coverslip. Sealing the coverslip is not necessary for an upright microscope. Optionally, the worms can be aligned when they become paralyzed by spreading the levamisole off, to the outside of the agarose pad, and aligning the worms with a platinum wire pick. Alternatively, the levamisole can be soaked up using a laboratory wipe. NOTE: Image as soon as possible, because prolonged incubation in levamisole could alter fluorescence.

6. Image the worms using a fluorescence microscope. The details of image capture vary by microscope and software. NOTE: To directly compare image intensities, use identical microscope settings in one imaging session. Avoid oversaturating the image.

3. Measurement of HSR gene expression using RT-qPCR

1. Synchronize worms (section 1.2) and maintain at 20 °C until the desired developmental stage. For N2 worms, young adult worms that have not yet

reached reproductive maturity are generated 60 h after the egg laying synchronization. NOTE: The developmental timing varies with each strain and the temperature at which the worms are raised. Importantly, the magnitude of HSR induction declines approximately 2-4x after the onset of reproductive maturity (see Discussion).

2. Heat shock worms as described in step 2.2.

3. Take the plates out of the water bath, remove the paraffin film, and immediately collect the worms. The worms can be collected by washing the plates gently with 1 mL of M9, collecting the liquid in a microcentrifuge tube, and then removing the M9 after centrifugation at 400 x g for 1 min.

4. Lyse the worms and purify the RNA using organic extraction.

    1. Add 250 µL of RNA isolation reagent (see Table of Materials).

    2. Vortex tubes by hand for 30 s.

    3. Vortex tubes for 20 min at 4 °C using a microcentrifuge tube attachment (see Table of Materials).

    4. Add 50 µL of chloroform.

    5. Vortex for 30 s.

    6. Incubate the samples at RT for 3 min.

    7. Centrifuge at ≥14,000 x g for 15 min at 4 °C.

    8. Transfer the aqueous layer (i.e., top layer, ~125 µL) to a new microcentrifuge tube. NOTE: Avoid the organic layer and the material in the interface.

9. Add 50 μL of chloroform.

10. Vortex for 30 s.

11. Incubate the samples at RT for 3 min.

12. Centrifuge at ≥14,000 x g for 5 min at 4 °C.

13. Transfer the aqueous layer (~100 μL) to a new microcentrifuge tube. NOTE: Avoid the organic layer and the material in the interface.

14. Precipitate RNA with an equal volume (i.e., 100 μL) of isopropanol.

15. Incubate at -20 °C for at least 30 min, but preferably overnight. NOTE: The experiment can be paused here and the RNA can be stored at -20 °C.

16. Pellet the RNA by centrifugation at ≥14,000 x g for ≥30 min at 4 °C.

17. Remove as much of the supernatant as possible without disturbing the pellet. NOTE: The pellet will be small and may not be visible. The pellet may not adhere tightly to the side of the tube, so caution is necessary to avoid dislodging it.

18. Wash the pellet with 250 μL of 70% ice-cold ethanol made with RNase-free H_2O.

19. Centrifuge at ≥14,000 x g for ≥5 min at 4 °C.

20. Remove as much supernatant as possible without disturbing the pellet.

21. Perform a quick spin at RT to remove any remaining 70% ethanol.

22. Dry the pellet by leaving the tubes open at RT as long as needed; typically at least 20 min. Tubes can be covered with a lint-free tissue or aluminum foil to prevent contamination.

23. Resuspend the pellet in 20 µL of RNase-free H2O.

24. Determine the RNA concentration using a small volume spectrophotometer (2 µL). NOTE: The experiment can be paused here and the RNA can be temporarily stored at or below -20 °C.

5. Remove residual DNA by incubating with DNase I. It is recommended to use a commercially available kit (see Table of Materials) and to follow the manufacturer's instructions.

1. With this kit, prepare a 20 µL reaction with 500 ng of RNA and 1 µL of DNase I in a 37 °C water bath for 30 min.

2. Add 2.5 µL of DNase inactivation reagent (included in the kit) to each sample and incubate at RT for 5 min with occasional flicking/vortexing.

3. Spin down at 14,000 x g for 2 min.

4. Without disturbing the white pellet, transfer 15 µL of supernatant to a fresh microtube for cDNA synthesis.

6. Conduct cDNA synthesis. It is recommended to use a commercially available kit (see Table of Materials) and to follow the manufacturer's instructions.

1. With the kit, prepare a 20 µL reaction with 15 µL of DNase I-treated RNA from the previous step and 1 µL of reverse transcriptase.

2. Use the following program for cDNA synthesis: 25 °C for 5 min, 46 °C for 20 min, 95 °C for 1 min, 4 °C hold.

3. Dilute cDNA by adding 80 µL of RNase-free H2O directly to the sample.

4. Briefly vortex, then spin down and store at -20 °C until needed.

7. Perform qPCR. It is recommended to use a commercially available kit (see Table of Materials) and to follow the manufacturer's instructions.

1. With the kit, prepare a 25 µL reaction containing 2 µL of cDNA and 200 nM (each) of forward and reverse primers in one well of a 96-well plate.

2. Primer sequences for measuring the heat shock genes, *hsp-70* and *hsp-16.2*, and *18S* rRNA (for a normalization control) are listed in the Table of Materials. Multiple normalization controls can be used as desired.

3. Dilute cDNA samples 50x before measurement of *18S* to ensure that the assay is in the linear range. Appropriate qPCR conditions vary with the kit and primers used (see Representative Results).

4. Use a real-time PCR detection system (see Table of Materials) for qPCR with 40 cycles of 95 °C for 5 s denaturation, 58 °C for 30 s

annealing, and 72 °C for 30 s extension. NOTE: Optimal annealing temperatures can vary by primers and conditions.

5. Quantify using either the ΔΔCt or standard curve method[12].

4. Thermorecovery assay for measuring HSR at the organismal level

1. Synchronize the worms (section 1.2) and maintain at 20 °C until the desired developmental stage. For N2 worms, young adult worms that have not yet reached reproductive maturity are generated 60 h after the egg laying synchronization. NOTE: The developmental timing varies with each strain and the temperature at which the worms are raised. Importantly, the magnitude of HSR induction declines approximately 2-4x after the onset of reproductive maturity (see Discussion).

2. Heat shock the worms as described in step 2.2 for 6 h.

3. Remove the plates from the water bath, remove the paraffin film, and allow the worms to recover by incubation at 20 °C for 48 h.

4. Count the number of worms that can immediately crawl away after mechanical stimulation without jerky movement or paralysis. NOTE: The 6 h incubation is optimal for examining conditions that reduce thermorecovery, but longer exposure times may be needed to look for conditions that enhance thermorecovery.

TABLE OF MATERIALS

Name	Company	Catalog Number	Comments
18S-forward primer			TTGCGTCAACTGTGGTCGTG
18S-reverse primer			CCAACAAAAAGAACCGAAGT CCTG
AM446 rmIs223[phsp70::gfp; pRF4(rol-6(su1006))]	Morimoto lab	http:// groups.molbiosci.northwestern.edu/ morimoto/	
C12C8.1-forward primer			GTACTACGTACTCATGTGTCG GTATTT
C12C8.1-reverse primer			ACGGGCTTTCCTTGTTTTCC
CFX Connect Real-Time PCR Detection System	Bio Rad	1855200	
CL2070 dvIs70 [hsp-16.2p::GFP + rol-6(su1006)]	*Caenorhabditis* Genetics Center (CGC)	https://cgc.umn.edu/	
EasyLog Thermistor Probe Data Logger with LCD	Lascar	EL-USB-TP-LCD	
Greenough Stereo Microscope S9i Series	Leica		
Hard Shell 96 Well PCR Plates	Bio Rad	HSS9601	
hsp-16.2-forward primer			ACTTTACCACTATTTCCGTCC AGC
hsp-16.2-reverse primer			CCTTGAACCGCTTCTTTCTTTG
iScript cDNA Synthesis Kit	Bio Rad	1708891	
iTaq Universal Sybr Green Super Mix	Bio Rad	1725121	
Laser Scanning Confocal Microscope	Nikon	Eclipse 90i	
MultiGene OptiMax Thermo Cycler	Labnet	TC9610	
N2 (WT)	*Caenorhabditis* Genetics Center (CGC)	https://cgc.umn.edu/	
Nanodrop Lite Spectrophotometer	Thermo Scientific	ND-LITE	
Parafilm M Roll	Bemis	5259-04LC	
RapidOut DNA Removal Kit	Thermo Scientific	K2981	
Recirculating Heated Water Bath	Lauda Brinkmann	RE-206	
Traceable Platinum Ultra-Accurate Digital Thermometer	Fisher Scientific	15-081-103	
TRIzol Reagent	Invitrogen	15596026	RNA isolation reagent
TurboMix Attachment	Scientific Industries	SI-0564	
Vortex-Genie 2	Scientific Industries	SI-0236	

REPRESENTATIVE RESULTS

Using the protocols described in this manuscript, HSR induction was measured using fluorescent reporters, RT-qPCR, and thermorecovery assays. In each case, the procedure in section 1.2 was used to generate synchronized, young adult worms that had not reached reproductive maturity.

To visualize HSR induction at the cellular level, the AM446 (*hsp-70p::gfp*) and CL2070 (*hsp-16.2p::gfp*) fluorescent reporter strains were analyzed following section 2 of the protocol. Both reporters are constructed from the promoters of two well-established heat shock inducible genes that upon translation act as molecular chaperones to help refold misfolded proteins. In the negative control samples without heat shock, the *hsp 16.2* reporter only showed normal autofluorescence, but the *hsp-70* reporter had constitutive fluorescence in the anal depressor muscle as previously reported[4] (**Figure 1A**). After 1 h of heat shock at 33 °C, robust fluorescence was observed in both reporters; however, the pattern of expression was distinct depending on which reporter was used (**Figure 1B**). The *hsp-70* reporter was brightest in the intestine and spermatheca, whereas the *hsp-16.2* reporter was brightest in the pharynx. Additionally, the *hsp-16.2* reporter had a high degree of worm-to-worm variability in the amount of induction as previously described, but the *hsp-70* reporter did not[13].

A commonly used variation of section 2 is to perform the heat shock in a dry incubator instead of a circulating water bath. Therefore, the difference between the two methodologies was also tested. It was found that both protocols resulted in robust induction of the two fluorescent reporters using our conditions, although a circulating water bath is recommended as a best practice (see Discussion) (**Figure 1B**).

To test the dependence of the reporters on the transcription factor HSF-1, feeding RNAi was used to knockdown *hsf-1* before reporter induction was measured. It was found that fluorescence of both strains was severely reduced upon HSF-1 knockdown, indicating that these reporters are HSF-1-dependent as described in the literature[4] (**Figure 2**). However, it was also observed that pharyngeal fluorescence persisted in both reporters upon *hsf-1* knockdown, which is consistent with previous reports that the pharyngeal muscle is resistant to RNAi by feeding[14].

To quantitate whole worm induction of the HSR at the molecular level, two endogenous HSPs were measured with RT-qPCR using section 3 of the protocol. Samples were measured in triplicate, a standard curve was generated for each of the primers, and a melt curve was analyzed for each sample for quality control. It was found that a 33 °C heat shock for 1 h resulted in more than a 2,000x increase in relative expression for two heat shock genes, *hsp-70* and *hsp-16.2* (**Figure 3**). These results show that both endogenous genes are suitable for measuring HSR induction and that a 33 °C heat shock for 1 h is sufficient to generate a substantial response.

However, caution should be used in interpreting the absolute degree of heat shock induction, because the mRNA levels in the absence of heat shock are very low.

To analyze a physiological response to heat shock, an organismal thermorecovery assay was tested using section 4 of the protocol. It was found that exposure of worms to a 6 h heat shock at 33 °C led to a 20% decrease in worms with normal movement after a 48 h recovery (**Figure 4A**). The dependence of this assay on the HSF-1 transcription factor was tested using feeding RNAi to knockdown *hsf-1* before exposing worms to the stress. It was found that knockdown of *hsf-1* caused a dramatic decrease in normal movement, with >95% of worms showing jerky movement or paralysis after being prodded with a platinum wire pick.

We compared this thermorecovery assay to a widely used alternative organismal assay commonly referred to as thermotolerance. In the thermotolerance assay, worms are exposed to a continuous 35 °C temperature using a dry incubator, and the percentage of worms alive are measured at various timepoints. Using this assay, it was found that control worms continuously exposed to 35 °C died after approximately 8 h of exposure (**Figure 4B**). However, when the dependence of this assay on HSF-1 was tested using RNAi knockdown, it was found that inhibition of *hsf-1* did not cause a decrease in thermotolerance. Similar results have been previously shown using HSF-1 mutations (see Discussion). Therefore, the use of the thermotolerance assay to measure the HSR is not recommended, and thermorecovery is the preferred method for examining the HSR at the organismal level.

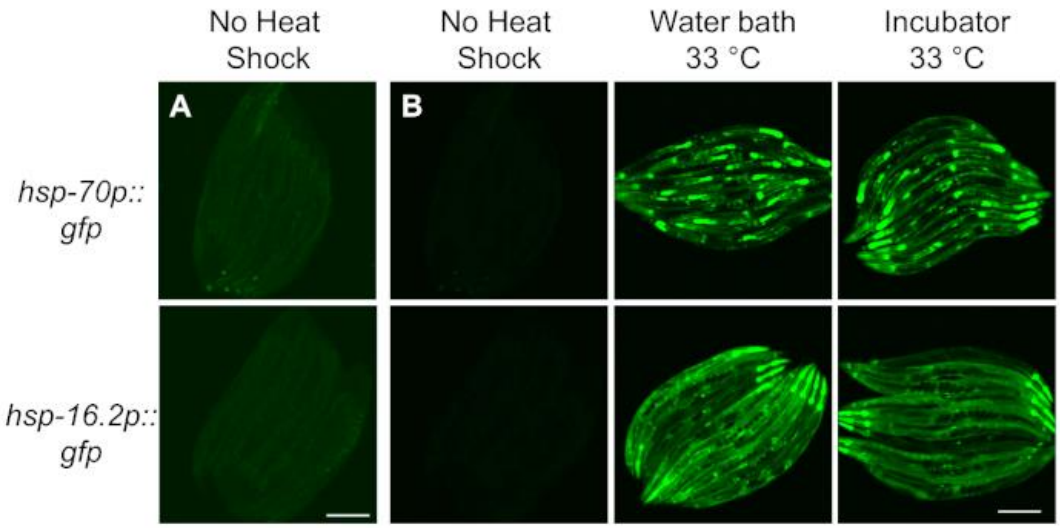

Figure 1. HSR induction measured with fluorescent reporters. (A) The basal and (B) heat-inducible expression of *hsp-70p::gfp* and *hsp-16.2p::gfp* reporter strains after 1 h of heat shock at 33 °C in a water bath or incubator. Worms were raised on OP50 bacteria for 64 h, heat shocked, and then recovered at 20 °C for 8 h before imaging. For reference, the no heat-shock worms in (A) were renormalized in (B) to match the range and saturation of the heat-shocked worms. Representative images of two experimental replicates are shown. Scale bar = 250 μm.

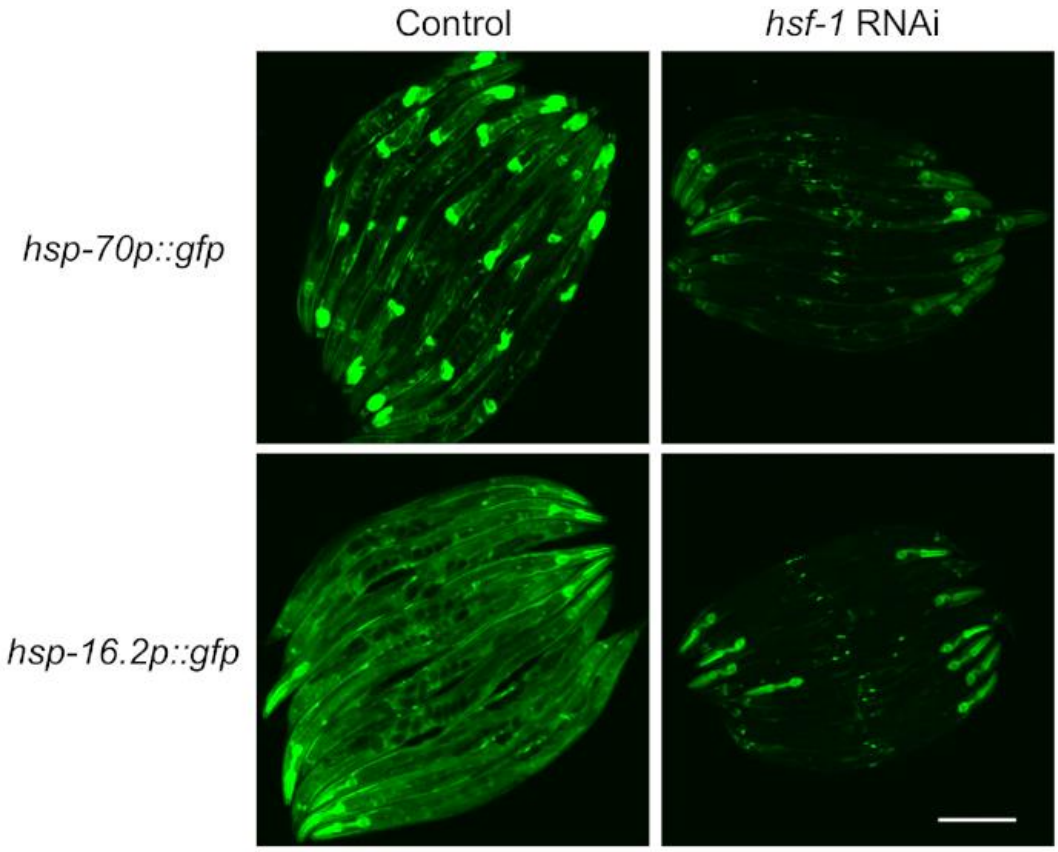

Figure 2. HSR induction measured with fluorescent reporters is dependent on HSF 1. Strains containing the *hsp-70p::gfp* and *hsp-16.2p::gfp* reporters were raised on control (L4440 empty vector) or *hsf-1* RNAi plates for 64 h, exposed to a 1 h heat shock at 33 °C in a water bath, and then recovered at 20 °C for 8 h before imaging. Representative images of two experimental replicates are shown. Scale bar = 250 μm

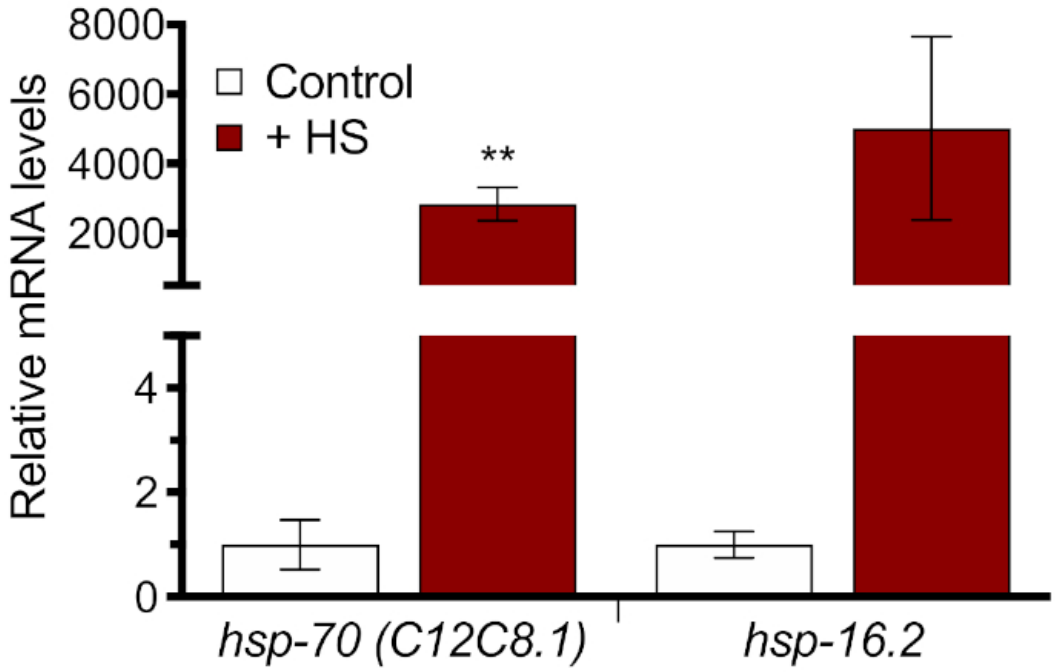

Figure 3. HSR induction measured with RT-qPCR. N2 worms were raised on HT115 bacteria for 60 h and then heat shocked for 1 h in a 33 °C water bath. The relative mRNA levels of *hsp-70 (C12C8.1)* and *hsp-16.2* are shown normalized to the no heat-shock control. Values plotted are the mean of four biological replicates and error bars represent ± SEM. Statistical significance was calculated using an unpaired Student's t-test. **$p < 0.01$.

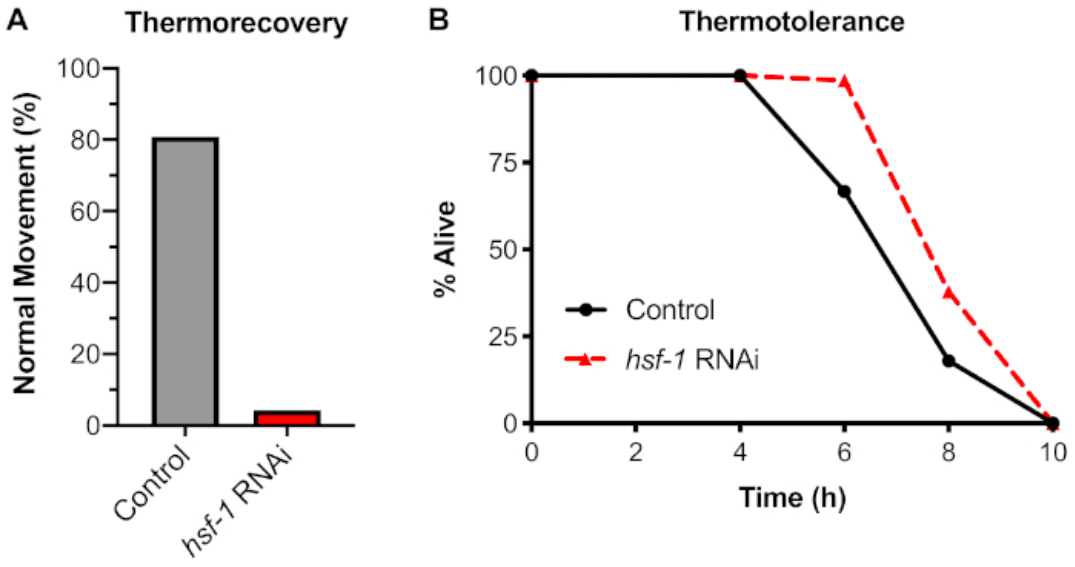

Figure 4. Thermorecovery, but not thermotolerance, is dependent on HSF-1. N2 worms were raised on control (L4440) or *hsf-1* RNAi plates for 60 h and then shifted to either: (A) A 33 °C water bath for 6 h and recovered at 20 °C for 48 h before scoring for normal movement (thermorecovery), or (B) A 35 °C dry incubator and removed every 2 h until dead (thermotolerance). Each assay was done with n ≥ 30 individuals on 2 independent days. The average is shown.

DISCUSSION

In the literature a wide variety of temperatures, times, and equipment have been used to assay the HSR, which has introduced unnecessary caveats and led to difficulty in comparing results between laboratories. For example, temperatures ranging anywhere from 32-37 °C and times from 15 min to several hours have been used to induce the HSR[15]. However, it is reported that lethality occurs as early as 3 h at 37 °C for all stages and 1.5 h for day 1 adults[15]. Furthermore, we show that exposure of worms to 35 °C causes lethality that is not HSF-1 dependent, making these conditions poorly suited for analysis of the HSR. In contrast, a heat shock of 33 °C for 1 h is robust enough to elicit strong induction of heat shock genes, yet mild enough to not affect worm viability. Indeed, exposure to 33 °C for as long as 6 h only causes 20% of worms to display abnormal movement. Therefore, we propose using a temperature of 33 °C and a time of 1 h as a standardized condition for RT-qPCR and fluorescent reporter assays. Recent experiments have revealed that developmental staging of worms for HSR experiments is particularly important. It was recently shown that in *C. elegans* the inducibility of the HSR declines (i.e., collapses) by >50% when hermaphrodites begin egg laying[5]. Staging the worms correctly is critical because there are often differences in developmental timing in strains carrying mutations. If temperature-sensitive mutants are used, this will also impact results if they are not synchronized by their reproductive age. Therefore, it is recommended to carefully measure the onset of egg laying for every strain to determine when the collapse occurs. The window of time after the L4 molt and before the initiation of reproductive

maturity is narrow; therefore, care must be taken so that the HSR collapse does not inadvertently cause variability in results. In addition to developmental timing, surprisingly small changes in temperature, as little as 1 °C, can have substantial effects on the HSR. For example, thermosensory neurons in *C. elegans* are sensitive to temperature changes as small as ±0.05 °C[16]. Thus, it is imperative to use a thermometer that can accurately measure the temperature. Therefore, we propose as best practice the use of a calibrated device for temperature measurement that is precise enough to measure temperatures within ±0.1 °C. Furthermore, a thermometer with a data-logging functionality should be used to measure temperature variations across time. Many incubators are specified to have thermal variations of more than 1 °C in different parts of the incubator and across time, which can have significant effects on HSR experiments. As a best practice, we suggest using incubators that have sufficient insulation and circulation to minimize temperature fluctuations. For conducting heat shock experiments, we propose a best practice of a circulating water bath. The time it takes for an agar plate to reach a desired temperature is approximately 6-7 min in a water bath but much longer in a dry incubator[15,17]. However, if a circulating water bath is not available, we have shown that robust HSR induction also occurs in a dry incubator using our conditions. If a dry incubator is used, opening of the incubator for the duration of the stress should be minimized. It is well-established that induction of heat shock genes is dependent on the master regulator of the HSR, HSF-1. Here, we present evidence that the two more indirect assays, fluorescent reporters and thermorecovery, are also dependent on HSF-1.

Significantly, we found that a commonly used alternative organismal assay, thermotolerance, is not HSF-1 dependent using *hsf-1* RNAi (**Figure 4**). Similar results have been previously reported using an *hsf-1* mutant or a *ttx-3* mutant, which blocks the HSR[18-20]. Together, these results indicate that the thermotolerance assay should not be used for HSR research. Furthermore, this suggests that a best practice is to test the HSF-1 dependence for any assay used to measure the HSR. Taken together, we present a series of standardized protocols and best practices for robust and reproducible measurement of HSR induction in *C. elegans*. We hope that these methodologies will decrease variability in HSR experiments and increase reproducibility. Facilitating direct comparisons of HSR research between laboratories will serve to accelerate research in the HSR field. Furthermore, standardization will benefit research into aging and neurodegenerative diseases with which the HSR is intimately associated.

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CHAPTER 2: DIVERGENT REGULATORY ROLES OF NURD CHROMATIN REMODELING COMPLEX SUBUNITS GATAD2 AND CHD4 IN *CAENORHABDITIS ELEGANS*

ABSTRACT

During stress, a protective cellular network known as the heat shock response (HSR) is induced to maintain protein-folding homeostasis, or proteostasis. Previously, we identified the *Caenorhabditis elegans* GATAD2 ortholog, *dcp-66*, as a novel regulator of the HSR. Here, we extend these findings to show that *dcp-66*, a subunit of the NuRD chromatin remodeling complex, positively regulates the HSR at the cellular, molecular, and organismal levels. We found that of the two nucleosome repositioning paralogs that define the NuRD complex, only *let-418* (CHD4) regulates the HSR. Surprisingly, in contrast to the positive regulation of the HSR by *dcp-66*, *let-418* negatively regulates the HSR. These divergent effects extend to the regulation of other stress responses including oxidative, genotoxic, and ER stress. Furthermore, we used a transcriptomic approach to reveal that other distinct pathways including innate immunity and embryogenesis are divergently regulated. We incorporate these findings into a simple molecular model whereby the mechanism of NuRD recruitment to promoters can result in the divergent effects of NuRD subunits. Overall, our work establishes new insights into the role of NuRD in cell physiology. Intriguingly, several NuRD subunits are misregulated and/or mutated during cancer; therefore, these findings could help explain how the HSR becomes misregulated during cancer.

INTRODUCTION

The heat shock response (HSR) is a cellular stress response pathway activated by elevated temperature and other stresses that perturb protein folding homeostasis, or proteostasis (Hipp *et al.* 2019). HSR activation induces binding of the Heat Shock Factor 1 (HSF1) transcription factor to the promoters of heat shock (HS) genes, resulting in upregulation of a set of HS proteins (HSPs). Many HSPs are molecular chaperones that function to restore proteostasis (Gomez-Pastor *et al.* 2018).

The HSR is extensively regulated, indicating a considerable cost for inappropriate activation. The core regulatory module that senses disruptions to proteostasis consists of at least three distinct negative feedback loops between HSF1 and the HSP70, HSP90, and TRiC/CCT molecular chaperones (Abravaya *et al.* 1992; Zou *et al.* 1998; Neef *et al.* 2014). HSF1 is also extensively modified post-translationally, including phosphorylation and acetylation that can affect its subcellular localization, DNA binding, and transcriptional activity (Vihervaara and Sistonen 2014). Furthermore, the ability of HSF1 to activate HS genes is influenced by chromatin accessibility at HS gene promoters (Guertin and Lis 2010; Labbadia *et al.* 2011).

In addition to its role during stress response, the HSR has a central role in development, aging, and disease (Anckar and Sistonen 2011). Inhibition of the HSR accelerates aging, while HSR activation delays aging in multiple model organisms, including the nematode *Caenorhabditis elegans* (Hsu *et al.* 2003; Morley and

Morimoto 2004). Similarly, HSR inhibition exacerbates disease progression in neurodegenerative disease models such as Huntington's disease, whereas HSR activation is beneficial (Gomez-Pastor *et al*. 2018). In contrast, HSR inhibition reduces tumorigenesis, while HSR activity is associated with poor outcomes in cancer (Santagata *et al*. 2011; Dai *et al.* 2012; Carpenter *et al*. 2017).

Recently, we discovered a novel set of HSR regulators through genetic screening (Guisbert *et al*. 2013) . Of these regulators, deacetylase complex protein 66 (*dcp-66*) was of interest as it is associated with chromatin remodeling and chromatin accessibility is a factor in HSR regulation (Guertin and Lis 2010; Labbadia *et al*. 2011). *dcp-66* is the *C. elegans* ortholog of GATAD2, a well-established subunit of the Nucleosome Remodeling and Deacetylase (NuRD) complex (Xue *et al.* 1998; Wade *et al*. 1998; Kon *et al*. 2005; Brackertz *et al*. 2006). This complex, also known as Mi-2/NuRD, is the only chromatin remodeler that combines both nucleosome repositioning and histone deacetylase catalytic activities (Clapier and Cairns 2009). Classically, the NuRD complex is depicted as a transcriptional repressor due to its role in histone deacetylation and its association with methylated DNA (Zhang *et al.* 1999; Ahringer 2000). However, new research shows that the complex can also activate transcription (Bornelöv *et al*. 2018; Zhang *et al*. 2018).

The NuRD complex has six unique protein subunits, many of which have multiple paralogs (Denslow and Wade 2007). The complex contains one of two ATP-

dependent chromatin remodeling subunits; either Mi-2a (CHD3) or Mi-2b (CHD4) in humans, which correspond to CHD-3 and LET-418 in worms (**Figure 1**) (Passannante *et al.* 2010; Hoffmeister *et al.* 2017). Structural studies have shown that this core is connected to the rest of the complex by the GATAD2 subunit (Spruijt *et al*. 2020; Low *et al*. 2020). Humans have two paralogs of GATAD2; GATAD2A and GATAD2B, whereas worms have only a single homolog, DCP-66 (Wade *et al*. 1999; Brackertz *et al*. 2002; Feng *et al*. 2002). The remaining NuRD subunits include a deacetylase (HDAC1/2), DNA and protein binding subunits (MBD2/3 and MTA1/2/3), and histone interacting proteins (RBBP4/7).

The NuRD complex plays an important role in several physiological and pathological processes (Ahringer 2000; Lai and Wade 2011; Hu and Wade 2012). In stem cells, NuRD acts as a guardian of pluripotency, facilitating transcriptional reprogramming and differentiation (Ramírez and Hagman 2009; Rais *et al*. 2013). NuRD has additional roles in development, where it is required for embryogenesis and larval morphogenesis (Unhavaithaya *et al*. 2002; Andersen *et al*. 2008; Erdelyi *et al*. 2017; Saudenova and Wicky 2018). In pathology, NuRD complex subunits are associated with disorders including dermatomyositis and intellectual impairment (Basta and Rauchman 2015). Additionally, NuRD subunits are frequently overexpressed or mutated in malignant tumors (Lai and Wade 2011; Wang *et al.* 2019). Importantly, the increased expression of NuRD subunits correlates with reduced overall survival in cancer patients (Shao *et al*. 2020a).

Our discovery that NuRD regulates the HSR is intriguing as it suggests a new role for NuRD in physiology, apart from its well-studied roles in development. Furthermore, this connection could be relevant to disease given their individual importance in cancer. Therefore, we investigated the relationship between these two pathways.

MATERIALS AND METHODS

Worm Maintenance & Strains

C. elegans were maintained using standardized methods at 20°C (Brenner 1974). Worms were synchronized by egg laying for 1 hour unless otherwise noted. The following strains were used: N2 wild-type (var. Bristol), AM446 *rmIs223[hsp70p::gfp; rol-6(su1006)]*, MT14390 *let-418*(*n3536*), and HX103 *chd-3*(*eh4*). To determine the timing of egg laying, singled worms were scored every 4 hours for the number of eggs laid using a Leica light microscope.

RNAi Treatment

RNAi was performed by feeding initiated from hatching, unless stated otherwise (Timmons *et al*. 2001). Saturated overnight cultures of HT115 bacteria were induced with 1 mM IPTG for 3.5 hours before seeding to nematode growth medium (NGM) agar plates containing 100 μg/ml ampicillin, 12.5 μg/ml tetracycline, and 1 mM IPTG. In all experiments, the L4440 empty vector was used as the control.

Microscopy

Worms were mounted for imaging onto 3% agarose pads in 1 mM levamisole. Brightfield images were taken with a Zeiss Axioskop 2 using the Plan Neofluar 40x/0.75 objective. Fluorescent images were captured with a Nikon Ti Eclipse laser-scanning confocal microscope using the Plan Apo 10x/0.45 DIC objective. Images

were analyzed with EZ-C1 FreeViewer. Acquisition and display parameters were kept the same within each experiment.

RT-qPCR: RNA was extracted with TRIzol (Invitrogen) and treated with DNase (Thermo Fisher Scientific) before completing cDNA synthesis as previously described (Golden *et al*. 2020). RT-qPCR was performed with the following conditions: (5 s at 95°C, 30 s at 58°C, 30 s at 72°C) x 40 cycles. Relative expression was calculated from cycle threshold values using standard curves and normalized to *18S* rRNA. All primer pairs used in this study are listed in **Table S1**.

Stress Assays: Heat shock for microscopy and RT-qPCR was performed for 1 hour at 33°C on plates wrapped in parafilm and submerged in a water bath (Golden *et al*. 2020). To assess thermorecovery, worms were incubated for 6 hours at 33°C and then returned for 48 hours to 20°C (Golden *et al*. 2020). Worms were scored as moving normally if they immediately crawled away after mechanical stimulation without signs of abnormal, jerky movement or paralysis. Paraquat and dithiothreitol (DTT) resistance was measured on plates supplemented with paraquat (30 mM) or DTT (10 mM). For ultraviolet (UV) resistance, plated worms were placed, uncovered, in a Stratalinker 1800 (Stratagene) and irradiated with 254 nm of light at 1500 J/m^2 before immediately moving to fresh plates. Survival was scored every 6 hours (paraquat and UV) or 24 hours (DTT) by absence of touch response.

Lifespan Assay

Worms were raised at 20°C until the L4 larval stage, at which point they were plated to RNAi and maintained at 25°C throughout the duration of the experiment. Animals were transferred to fresh plates daily for the first 5 days of adulthood to remove progeny. Worms were scored as dead in the absence of touch response and removed. Bagged, desiccated, or missing animals were censored from analysis.

RNA-Seq & Analysis

Approximately 500 worms on a single 10 cm plate were synchronized by bleaching and then raised to young adulthood (prior to the onset of egg laying) before harvesting RNA as described (Golden *et al*. 2020). RNA was polyA-selected using poly-T oligo-attached magnetic beads. Library preparation and sequencing was performed by Novogene. Sequencing (150 bp, paired end) was performed using an Illumina NovaSeq 6000 system. RNA-seq analysis was completed with the Galaxy web-based platform (Afgan *et al.* 2018). Raw reads were quality checked with FastQC (Andrews 2010) prior to adaptor removal and trimming with Trimmomatic (Bolger *et al.* 2014), resulting in an average base quality Phred score of 36. Reads were aligned to the WBcel235 *C. elegans* reference genome using HISAT2, where 92.2-93.5% of paired-end reads mapped uniquely (Kim *et al.* 2015). Mapped reads were then assembled using StringTie (Pertea *et al*. 2015). Gene read counts were calculated using featureCounts (Liao *et al.* 2014). DESeq2 was used to determine differentially expressed genes (Love *et al*. 2014). Heatmaps were generated through

the web-based tool ClustVis (Metsalu and Vilo 2015) and gene ontology (GO) enrichment was performed using the Database for Annotation, Visualization, Integration, and Discovery (DAVID) functional annotation tool (version 6.8) (Dennis *et al*. 2003). See http://dx.doi.org/10.17504/protocols.io.brgpm3vn for a detailed RNA-seq analysis protocol.

Statistical Analyses

Graph Pad Prism 9 was used to perform statistical analyses and graphing. All experiments were completed with at least three independent biological replicates. P values were calculated using Student's t-test, ANOVA, log-rank, or Fisher's exact test as indicated. Model figure was created with biorender.com.

RESULTS

dcp-66 is a positive regulator of the HSR in *C. elegans*

We identified *dcp-66* as a regulator of the heat shock response (HSR) in a genome-wide RNAi screen (Guisbert *et al*. 2013). To investigate the role of *dcp-66* in HSR regulation, we tested the effects of *dcp-66* RNAi knockdown on an established HSR fluorescent reporter containing the promoter of the *hsp-70* gene fused to GFP. Upon heat shock (HS), worms containing this reporter have increased fluorescence including prominent fluorescence in the spermatheca and intestine (**Figure 2A**). RNAi knockdown of *dcp-66* was found to substantially reduce this fluorescence across multiple tissues (**Figure 2A**). To test whether *dcp-66* similarly affected endogenous *hsp-70*, mRNA levels were measured using RT-qPCR (**Figure 2B**). Consistent with the reporter assay, *dcp-66* RNAi decreased *hsp-70* mRNA levels by 46% ($p < 0.05$). These effects were not restricted to *hsp-70* as two other HSR-dependent genes, *F44E5.5* and *hsp-16.11*, were also decreased 74% ($p < 0.01$) and 72% ($p < 0.05$), respectively, by *dcp-66* inhibition (**Figure 2B**). Together, these results establish *dcp-66* as a positive regulator of the HSR.

Induction of the HSR is required for organismal recovery from thermal stress (Labbadia and Morimoto 2015). Therefore, we tested whether the decrease in the HSR upon *dcp-66* knockdown was sufficient to affect thermorecovery. We used an established assay, where N2 wild-type worms have a 17% decrease in normal movement after recovery from a 6 hour, 33ºC HS **(Figure 2C)**. In contrast, RNAi

knockdown of *dcp-66* decreased the recovery of normal movement to 52% ($p < 0.0001$). These results establish that *dcp-66* acts as a positive regulator of the HSR in a manner that is physiologically relevant to organismal health.

let-418, a core subunit of the NuRD complex, negatively regulates the HSR

The mechanism for regulation of the HSR by *dcp-66* could involve either the known role of *dcp-66* as a subunit of the NuRD chromatin remodeling complex or represent a novel, independent pathway. To distinguish between these hypotheses, we interrogated the role of other NuRD components on the HSR. In *C. elegans*, there are at least two distinct NuRD complexes, defined by their ATP-dependent nucleosome repositioning subunit, either CHD-3 or LET-418 (**Figure 3A**) (Passannante *et al*. 2010). Therefore, we measured the HSR in worms with mutations in these two subunits. The *chd-3(eh4)* mutation contains a 2kb deletion that removes most of the helicase domain, yet animals appear phenotypically wild-type (von Zelewsky *et al*. 2000). The *let-418(n3536)* mutation is a temperature sensitive point mutation (P675L) in the helicase domain that results in a partial loss-of-function at standard growth conditions of 20°C (Käser-Pébernard *et al*. 2016; Kubota *et al*. 2021).

Worms containing the *chd-3(eh4)* mutation mounted a similar HSR as wild-type controls as evidenced by RT-qPCR quantitation of *hsp-70* expression (**Figure 3B**). Unexpectedly, worms containing the *let-418(n3536)* mutation showed a

hyperactivation of the HSR with a 1.9-fold increase in *hsp-70* mRNA levels relative to control worms ($p < 0.0001$) (**Figure 3B**).

We next tested whether activation of the HSR by *let-418* was sufficient to affect thermorecovery. Importantly, *let418(n3536)* worms were more resistant to heat stress, with 94% of worms displaying normal movement ($p < 0.05$) (**Figure 3C**). Together, these results indicate that *let-418* functions as a physiologically relevant negative HSR regulator, in contrast to the positive regulation by *dcp-66*.

To explore the genetic interaction between *dcp-66* and *let-418*, the effects of *dcp-66* knockdown in the context of the *let-418* mutation were assessed. In contrast to the effects of *dcp-66* in wild-type worms, RNAi inhibition of *dcp-66* did not reduce the HSR in the *let-418(n3536)* background (**Figure 3B**). However, in the thermorecovery assay, there was a stronger decrease in movement upon *dcp-66* knockdown in the *let-418* background compared to its effects on wild-type worms ($p < 0.0001$) (**Figure 3C**). This discrepancy between the molecular and physiological effects of NuRD in thermal stress could arise from the altered physiology that is apparent in the double *let-418(n3536); dcp-66* RNAi worms, where there is a complete lack of embryos **(Figure 3D)**. While the exact nature of this genetic interaction is unclear, in both the molecular and physiological assays there is a strong genetic interaction between *dcp-66* and *let-418*, consistent with the two genes functioning in the same complex.

dcp-66 and *let-418* divergently regulate multiple stress responses

Similar to its negative regulatory role in the HSR, recent work has shown that *let-418* negatively regulates the oxidative stress response and the DNA damage response (De Vaux *et al*. 2013; Turcotte *et al*. 2018). Therefore, we tested whether the divergent relationship between *dcp-66* and *let-418* would extend to other stress responses.

We first tested the effects of NuRD subunits on the survival of worms during oxidative stress by exposing them to 30 mM paraquat, a small molecule that produces superoxides. In these conditions, 56% of wild-type worms survived beyond 24 hours (**Figure 4A**). We found that the *let-418(n3536)* mutant had improved survival relative to control worms ($p < 0.01$) with 67% of worms surviving beyond 24 hours, in agreement with previously reported results (**Figure 4A**) (De Vaux *et al*. 2013). In contrast, *dcp-66* knockdown significantly decreased survival ($p < 0.0001$) with 5% of worms alive after 24 hours of oxidative stress (**Figure 4A**). Therefore, similar to their roles in the HSR, *dcp-66* and *let-418* regulate the response to oxidative stress in a divergent manner.

We next tested the effects of NuRD subunits on the response of worms to genotoxic stress induced by exposure to 254 nm UVC light. In these conditions, half of the control worms died 48 hours after exposure (**Figure 4B**). We found that the *let-418(n3536)* mutant had an enhanced median survival ($p < 0.05$) of 54 hours

compared to the control (**Figure 4B**). Conversely, loss of *dcp-66* decreased median survival to 30 hours relative to control worms ($p < 0.0001$) (**Figure 4B**). Therefore, these results broaden the divergent effects of NuRD subunits to genotoxic stress.

To test whether the role of NuRD subunits in stress responses would extend to other subcellular compartments, we next tested their response to DTT, a reducing agent that causes protein misfolding in the endoplasmic reticulum (ER). Upon exposure to DTT, half of the control worms died after 13 days (**Figure 4C**). In these conditions, the *let-418(n3536)* mutation did not significantly affect survival compared to control (**Figure 4C**). However, RNAi knockdown of *dcp-66* substantially reduced survival to 2 days ($p < 0.0001$) (**Figure 4C**). Therefore, these results extend the divergent effects of *dcp-66* and *let-418* to ER stress.

Stress responses are strongly associated with longevity even in the absence of stress (Shore and Ruvkun 2013). Therefore, we tested whether regulation of stress responses by *dcp-66* and *let-418* would be sufficient to affect lifespan. We found that there was no significant increase in lifespan for the *let-418(n3536)* mutant relative to control (**Figure 4D**). This suggests that stress response induction by *let-418* is insufficient to extend lifespan, however previous work reported a small lifespan extension of this mutant using different conditions (De Vaux *et al*. 2013). Nevertheless, in both cases *let-418* does not decrease lifespan. In contrast, knockdown of *dcp-66* decreased median lifespan from 13 to 11 days compared to

control ($p < 0.0001$) (**Figure 4D)**. For this assay, *dcp-66* knockdown was initiated at the L4 larval stage to avoid previously documented developmental effects (Zhao *et al*. 2005). The effects of *dcp-66* in lifespan are consistent with its role in stress responses and in agreement with previously published work (Shore *et al*. 2012).

NuRD affects lifespan but not HSR attenuation during the onset of egg laying

In *C. elegans*, the HSR and other stress responses are coordinately repressed (or 'collapse') at the onset of egg laying, and this effect is mediated by chromatin accessibility (Labbadia and Morimoto 2015). Therefore, we tested the role of NuRD in this repression. Since stress response repression occurs at the onset of egg laying, we first established the relevant time courses. Both control and *let-418(n3536)* worms initiated egg laying by 64 hours post synchronization (**Figure 5A**). The average number of eggs laid by *let-418(n3536)* worms was reduced compared to control worms, indicating a reduced brood size consistent with previous reports (McMurchy *et al*. 2017; Turcotte *et al*. 2018). Having established the appropriate timing, we measured induction of *hsp-70* both before and after egg laying. As expected, control worms had a 68% repression of the HSR, concurrent with the onset of reproduction ($p < 0.0001$) (**Figure 5B**). Similarly, the *let-418(n3536)* strain showed a decrease in HS gene expression with the onset of reproduction. Interestingly, the *let-418* mutants had hyperstimulation of *hsp-70* mRNA levels relative to control both before and after the onset of egg laying. Together, these data indicate that regulation of the HSR by *let-418* is unaffected by the changes in

chromatin that occur at the onset of reproduction and *let-418* is not required for the collapse.

Additionally, worms containing the *chd-3(eh4)* mutation were found to have no effect on *hsp-70* mRNA levels, neither before nor after reproductive maturity relative to control (**Figure 5B**). Therefore, the NuRD complex containing *chd-3* does not regulate the HSR or the collapse.

We found that worms exposed to *dcp-66* RNAi laid few if any eggs over the entire time course, likely from a previously described vulva defect (**Figure 5C**) (Poulin *et al.* 2005; Roy *et al*. 2014). At timepoints corresponding to the onset of egg laying in control worms, knockdown of *dcp-66* prevented attenuation of the HSR, suggesting that *dcp-66* has a role in the HSR collapse (**Figure 5D**). However, other aspects of reproduction including germline stem cells and egg shell formation influence HSR attenuation (Shemesh *et al*. 2013; Sala *et al*. 2020). Therefore, the vulva defect prevents a simple conclusion regarding the role of *dcp-66* in the collapse.

Transcriptomic analysis reveals other divergently regulated pathways

To determine if the divergent effects of *dcp-66* and *let-418* extend to other cellular pathways beyond stress responses, we performed transcriptomic analysis in the absence of stress. RNA-seq was conducted using 150bp paired-end reads from polyA selected RNA isolated from wild-type, *dcp-66* RNAi, *let-418(n3536)*, and double *let-*

418(n3536); *dcp-66* RNAi treated worms. Each condition was analyzed with three biologically independent replicates harvested at young adulthood, before the onset of egg laying. Each sample generated between 37-47 million clean reads. The samples each had a Q20 score > 95% indicating good sequencing quality.

Differential gene expression analysis was performed using DESeq2 with the false discovery rate (FDR) constrained to < 0.05 by Benjamini-Hochberg adjustment. Analysis of the data revealed that 742 genes were significantly affected by *dcp-66* RNAi relative to control (N2) worms, the *let-418(n3536)* mutation affected 2,016 genes, and the combination of *let-418(n3536); dcp-66* RNAi affected 3,817 genes (**Figure S1 and File S1**). Gene ontology (GO) analysis was used to determine which cellular pathways were enriched (**Figures S2-S4 and File S2**).

Comparison of these datasets revealed that the majority of genes are uniquely affected by only one NuRD subunit: 514/742 (69%) of *dcp-66* sensitive genes were not affected by *let-418* and 1788/2,016 (89%) of *let-418* sensitive genes were not affected by *dcp-66* (**Figure 6A**). The overlap between the two subunits contained 228 differentially expressed genes that were hierarchically clustered using the Euclidean method. Visualization of these genes with a heatmap indicated four distinct classes (labeled I-IV, **Figure 6B**). 99 genes were upregulated in both conditions (class I), 39 genes were upregulated in *dcp-66* RNAi but downregulated in *let-418(n3536)* animals (class II), 33 genes were downregulated in *dcp-66* RNAi

but upregulated in *let-418(n3536)* animals (class III), and 57 genes were downregulated in both (class IV). Selected GO terms and associated gene IDs are shown for each class (see **File S3** for a complete list). The increased expression of nucleosome assembly genes (class I) is consistent with the role of NuRD in chromatin remodeling. The decreased expression of mitochondrial electron transport chain genes in both *dcp-66* and *let-418* inhibited animals (class IV) is consistent with recent literature showing a connection between NuRD and the mitochondria (Shao *et al*. 2020b; Zhu *et al*. 2020). Most intriguingly, approximately 1/3 (32%) of all genes coordinately regulated by *dcp-66* and *let-418* were divergently expressed. Importantly, the divergent effects of *dcp-66* and *let-418* extend to embryogenesis (class II) and the innate immune response (class III). Interestingly, genes affected in the double *let-418(n3536); dcp-66* RNAi animals were largely similar to the effects observed in *let-418(n3536)* worms alone (**Figure 6B**). Together, this analysis shows that the divergent effects on gene expression by *dcp-66* and *let-418* extend beyond stress responses.

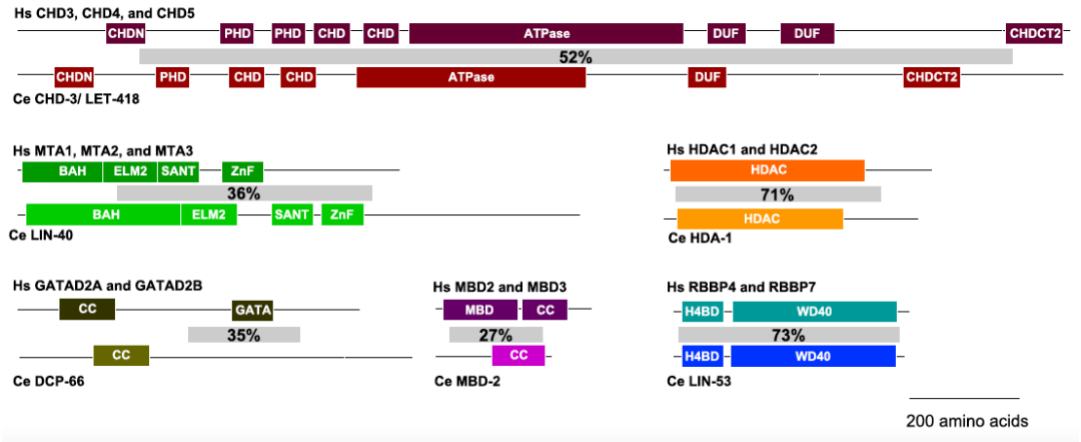

Figure 1. The core subunits and domains of NuRD are conserved in *C. elegans*.

The domain architecture and conservation (% identity) between human (Hs) and *C. elegans* (Ce).

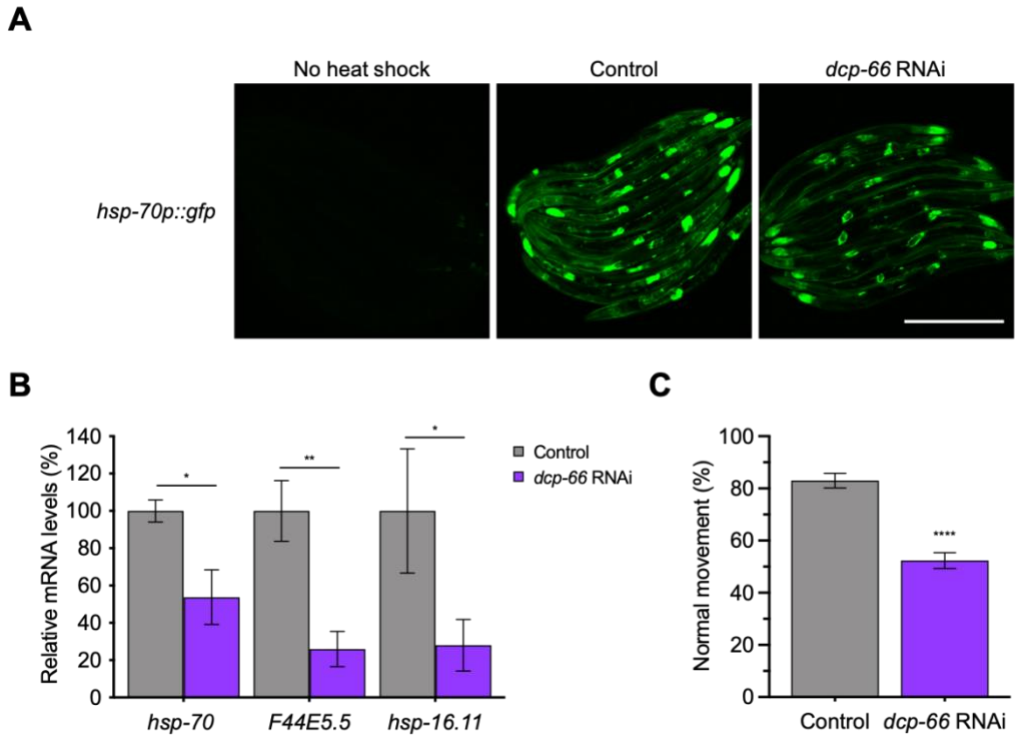

Figure 2. *dcp-66* is a positive regulator of the HSR. (A) Fluorescence from an *hsp-70p::gfp* reporter strain raised on vector only (control) or *dcp-66* RNAi and exposed to 1 hour of 33°C heat shock compared to animals maintained at 20°C (no heat shock). Worms were recovered for 8 hours at 20°C before imaging. Scale bar = 500 μm. (B) Quantitation of relative mRNA levels using RT-qPCR for three heat shock inducible genes (*hsp-70*, *F44E5.5*, and *hsp-16.11*) in wild-type (N2) worms raised on vector only (control) or *dcp-66* RNAi and harvested immediately following 1 hour of 33°C heat shock. (C) Thermorecovery of wild-type (N2) worms raised on vector only (control) or *dcp-66* RNAi and exposed to 6 hours of 33°C heat shock and recovered for 48 hours at 20°C. Values plotted in (B) and (C) are the mean of at least four experimental replicates and error bars are ± SEM. Student's t test was used to determine statistical significance. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$.

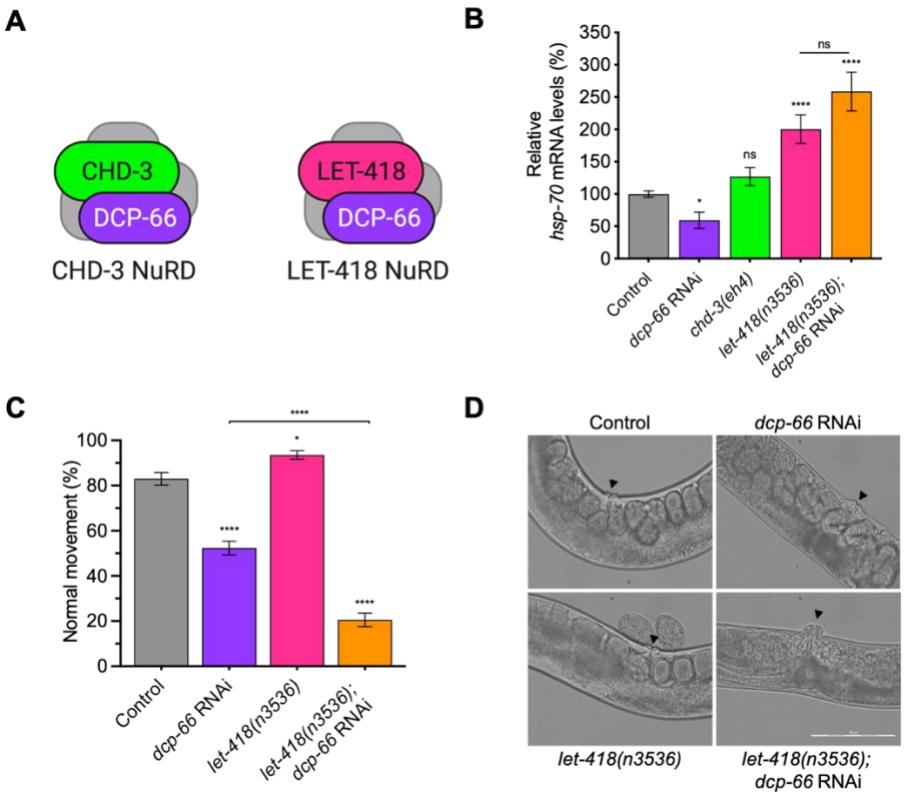

Figure 3. *let-418* is a negative regulator of the HSR. (A) Schematic of the two NuRD complexes in *C. elegans*. (B) Quantitation of relative mRNA levels using RT-qPCR for *hsp-70* following heat shock (1 hour at 33°C). (C) Thermorecovery of worms exposed to 6 hours of 33°C heat shock and recovered for 48 hours at 20°C. (D) Brightfield images of control (N2), or *let-418(n3536)* animals raised on vector only or *dcp-66* RNAi. Arrowheads indicate the vulva. Scale bar = 100 μm. Values plotted in (B) and (C) are the mean of at least three experimental replicates and error bars are ± SEM. One-way ANOVA was used to determine statistical significance. *p < 0.05, ****p < 0.0001, ns indicates p > 0.05.

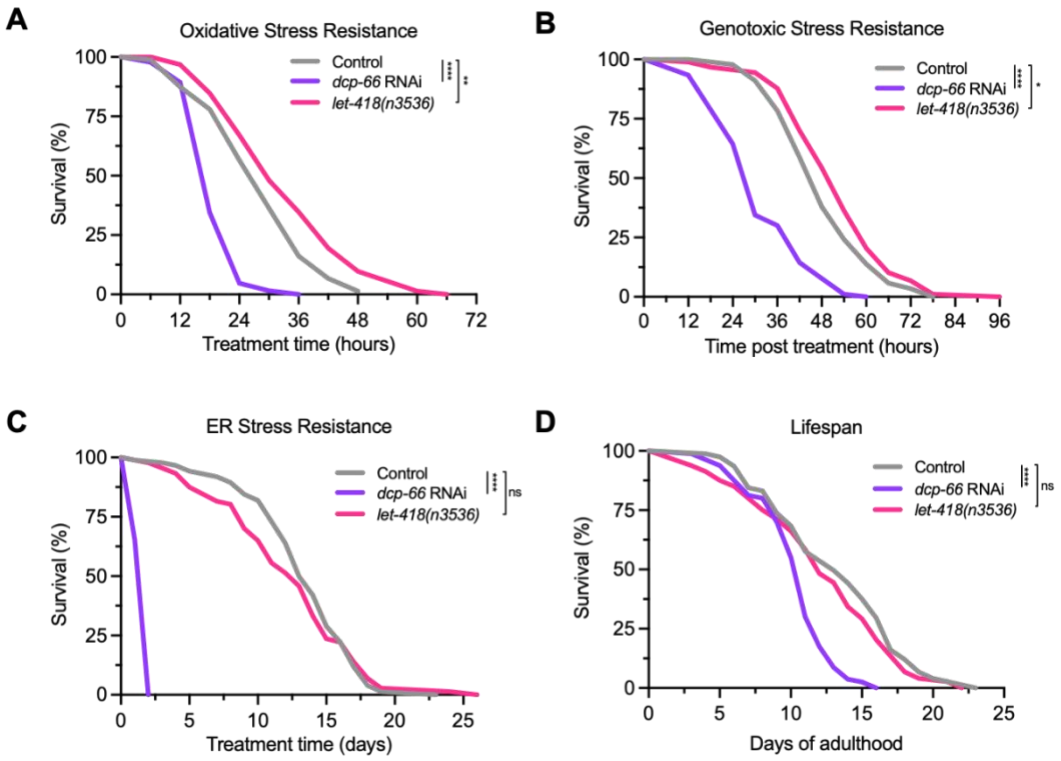

Figure 4. *dcp-66* and *let-418* divergently regulate multiple stress responses. Survival of control (N2) or *let-418(n3536)* animals on vector only or *dcp-66* RNAi after exposure to (A) oxidative stress using 30 mM paraquat, (B) genotoxic stress using 1500 J/m^2 UVC, or (C) ER stress using 10 mM DTT. (D) Lifespan of control or *let-418(n3536)* animals on vector only or *dcp-66* RNAi. For lifespan analyses, animals were shifted to RNAi and maintained at 25°C from the L4 larval stage. All stress resistance and lifespan analyses contained n ≥ 80 animals. Statistical significance was calculated using the log-rank (Mantel-Cox) test. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns indicates p > 0.05.

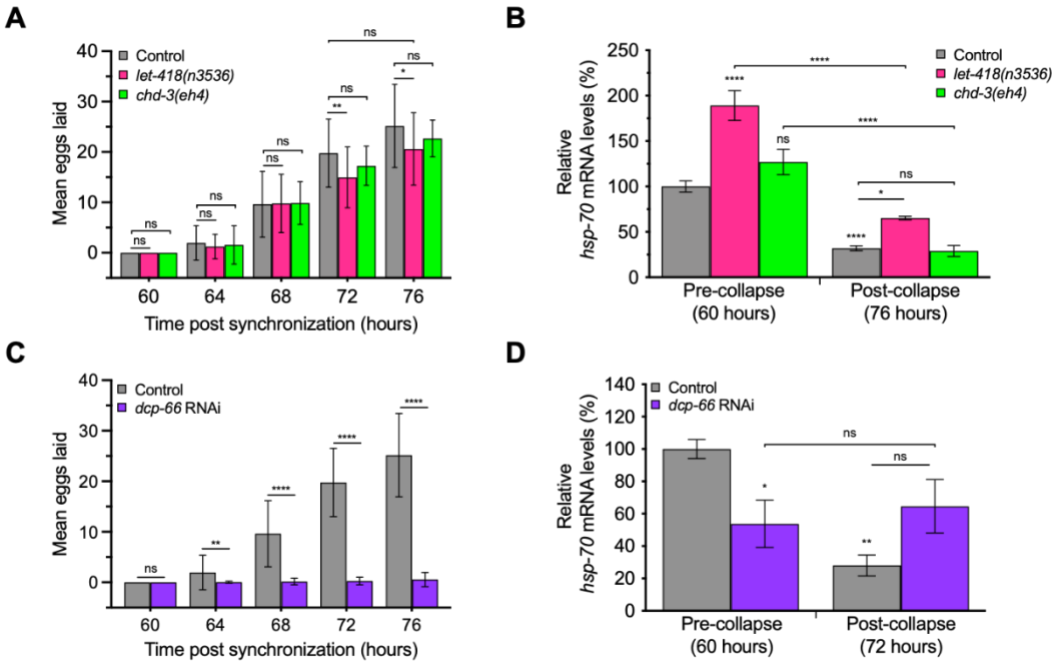

Figure 5. *dcp-66* and *let-418* divergently regulate the HSR prior to the onset of egg-laying. (A, C) Time course for egg laying in control (N2) worms raised on vector only compared to (A) *let-418(n3536)* and *chd-3(eh4)* or (C) *dcp-66* RNAi animals. n ≥ 30 animals. Error bars are ± SD. (B, D) Quantitation of relative mRNA levels using RT-qPCR for *hsp-70* following heat shock (1 hour at 33°C) in (B) *let-418(n3536)* and *chd-3(eh4)* or (D) *dcp-66* RNAi animals compared to control (N2) worms. Values plotted are the mean of at least three experimental replicates and error bars are ± SEM. One-way (A, C) or two-way (B, D) ANOVA was used to determine statistical significance. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$, ns indicates $p > 0.05$.

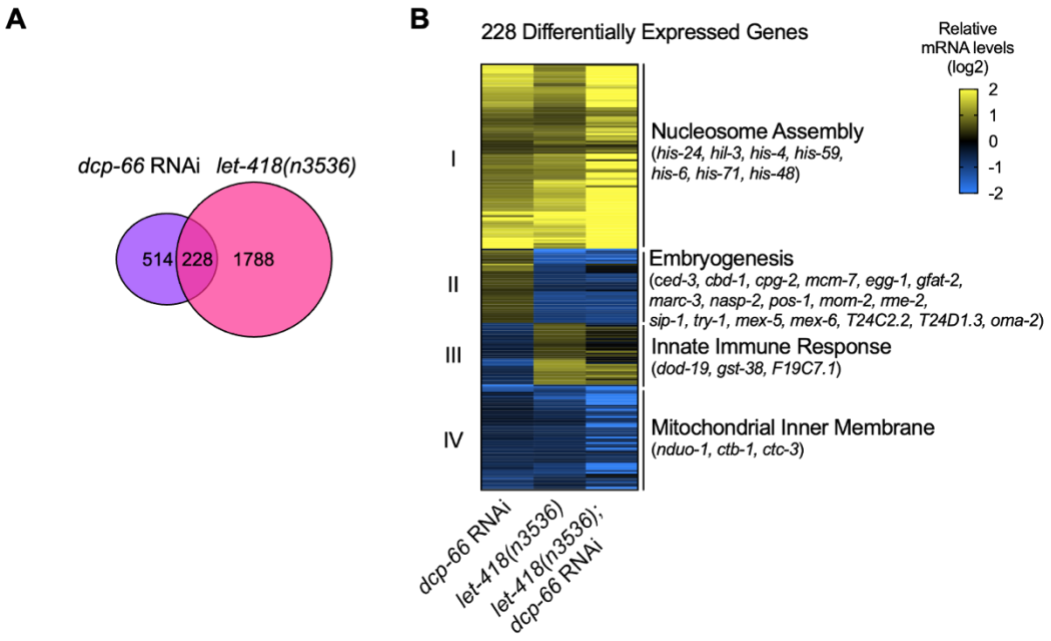

Figure 6. Transcriptome analysis of *dcp-66* and *let-418* reveals distinct categories of genes that are similarly or divergently regulated. (A) Venn diagram of the total number of significant (p-adj < 0.05) differentially expressed genes (DEGs) in *dcp-66* RNAi and *let-418(n3536)* animals compared to control (N2). (B) Heatmap of the (log2) fold change of 228 shared DEGs in *dcp-66* RNAi and *let-418(n3536)* animals vs control. Genes (rows) were hierarchically clustered using the Euclidean method. The relative mRNA levels for the same 228 genes in double *let-418(n3536); dcp-66* RNAi animals vs control were added after clustering. Colors show the magnitude of change in gene expression (*yellow*: increase, *black*: no change, *blue*: decrease). Four classes of differentially expressed genes (I-IV) and selected gene ontology (GO) terms are indicated. For GO terms, p values were calculated using Fisher's exact test and a complete list can be found in **File S3**.

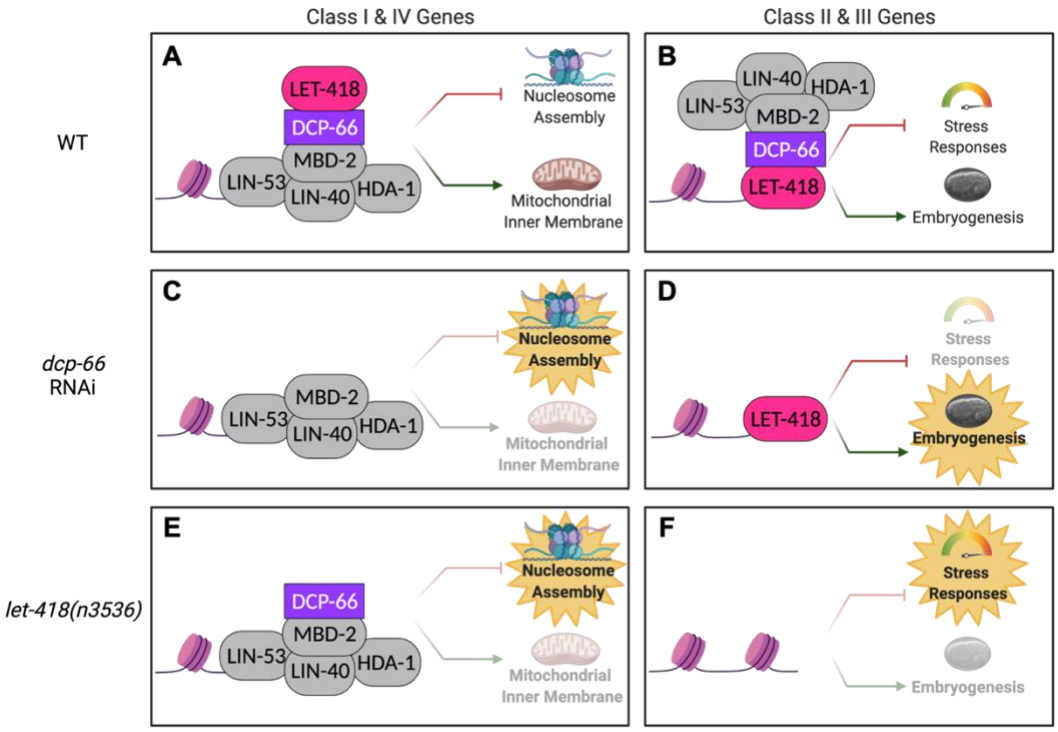

Figure 7. A model for transcriptional regulation by *let-418* NuRD. In wild-type (WT) worms, the NuRD complex is recruited to promoters through interactions with other NuRD subunits at class I and IV genes (A) or via direct interactions with *let-418* at class II and III genes (B). Inhibition of the *dcp-66* subunit results in no recruitment of the *let-418* core at class I and IV genes (C), and also does not inhibit *let-418* recruitment at class II and III genes (D). In the absence of *let-418*, only the non-remodeling subunits of NuRD (E) or no NuRD subunits (F) are recruited. Therefore, with respect to the *let-418* core, inhibition of *dcp-66* and *let-418* have similar effects on some genes (class I and IV genes; where no *let-418* core is recruited), but divergent effects on other genes (class II and III genes; where *dcp-66* does not prevent recruitment of the *let-418* core).

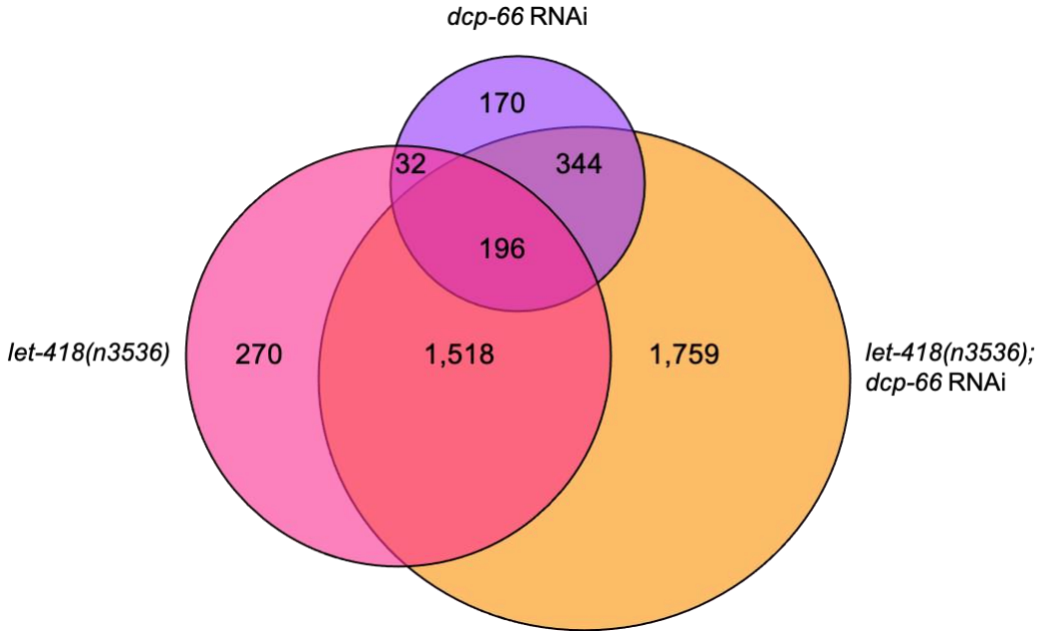

Figure S1. *let-418* and *dcp-66* differentially regulate thousands of genes. Venn diagram of differentially expressed genes (p-adj < 0.05) revealed by RNA-seq analysis (see **File S1** for a complete list).

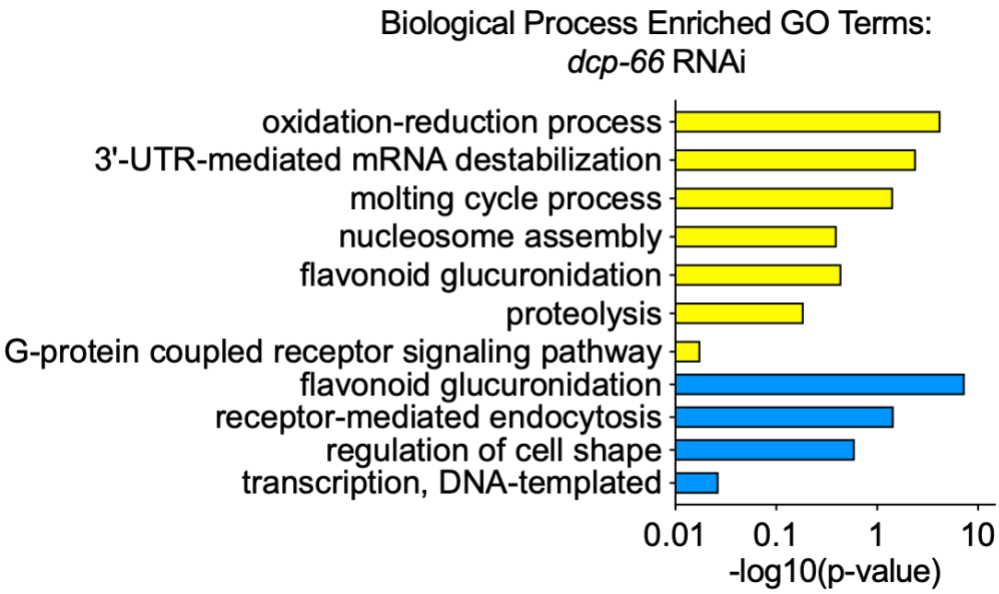

Figure S2. *dcp-66* RNAi enriched GO terms. The 10 most significant upregulated (*yellow*) and downregulated (*blue*) GO terms in the biological process category from each functional annotation cluster associated with *dcp-66* inhibition compared to (N2) control (see **File S2** for a complete list).

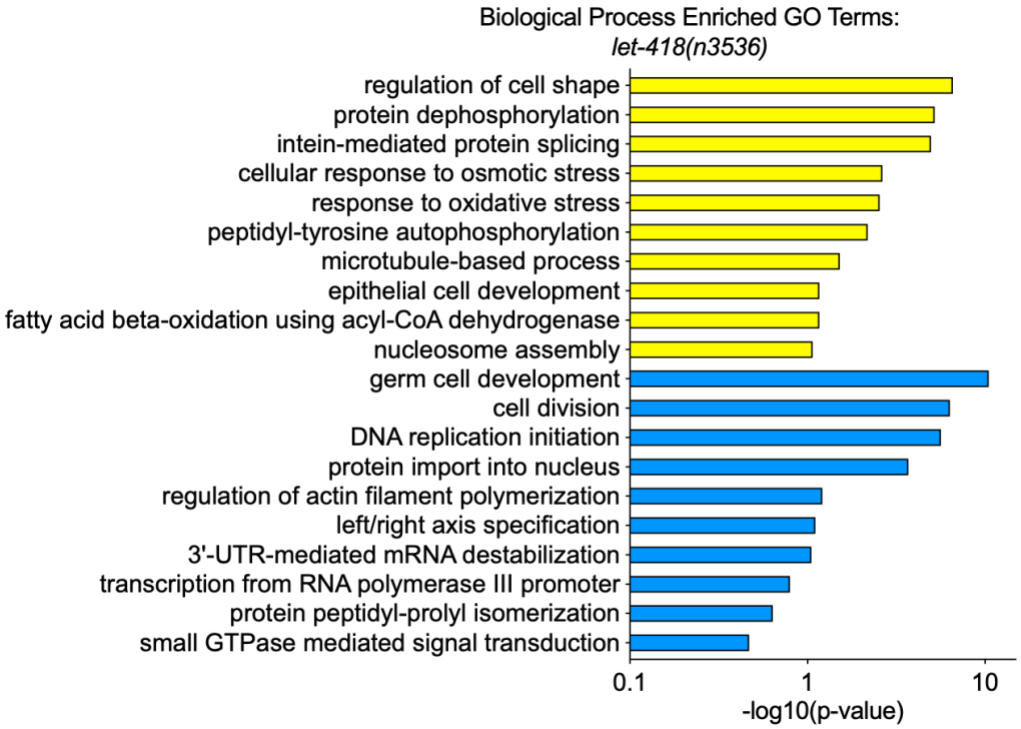

Figure S3. *let-418(n3536)* enriched GO terms. The 10 most significant upregulated (*yellow*) and downregulated (*blue*) GO terms in the biological process category from each functional annotation cluster associated with *let-418* inhibition compared to (N2) control (see **File S2** for a complete list).

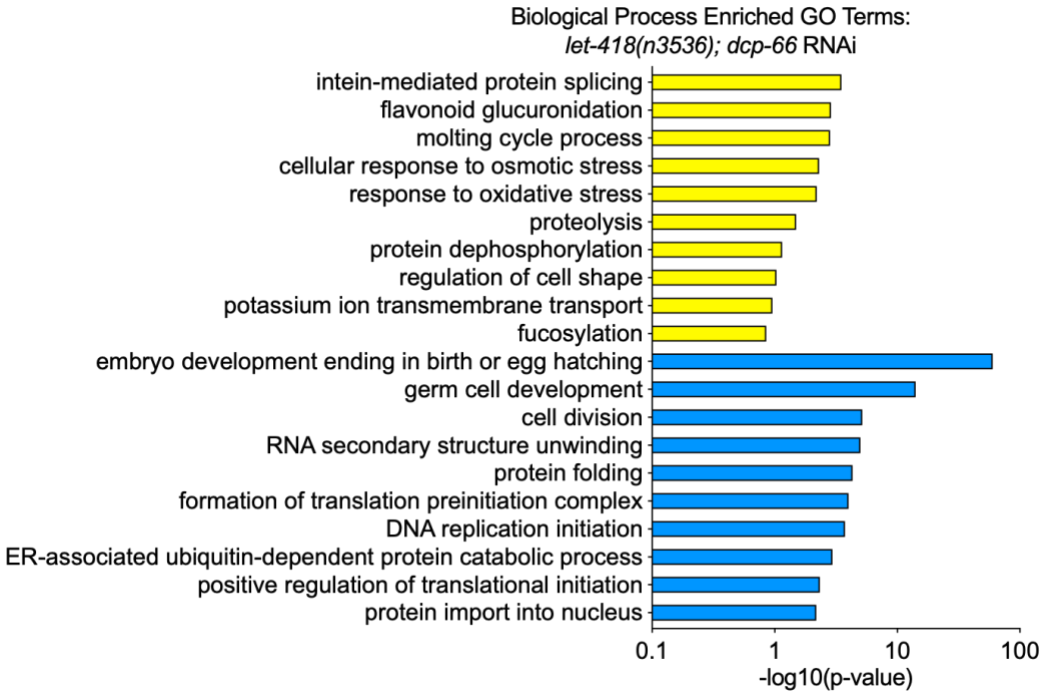

Figure S4. *let-418(n3536); dcp-66* RNAi enriched GO terms. The 10 most significant upregulated (*yellow*) and downregulated (*blue*) GO terms in the biological process category from each functional annotation cluster associated with *let-418; dcp-66* inhibition compared to (N2) control (see **File S2** for a complete list).

DISCUSSION

In this manuscript, we have identified two subunits of the NuRD chromatin remodeling complex as regulators of multiple stress responses in *C. elegans*. Surprisingly, these subunits work in a divergent manner, whereby *dcp-66* promotes and *let-418* prevents stress resistance. Genomic analysis extends this discovery to other pathways, including genes involved in embryogenesis and innate immunity. Taken together, our work provides new insights into the behavior of NuRD subunits within the complex, reveals new cellular pathways influenced by NuRD, and uncovers a potential mechanism for stress response coordination.

The finding that NuRD regulates the HSR in worms complements previous research. During larval development, ModENCODE data shows that LET-418 (CHD4) localizes to HS gene promoters in the absence of stress, suggesting that the mechanism of HSR regulation could involve chromatin remodeling at these loci (Celniker *et al.* 2009). However, in *Drosophila*, CHD4 was shown to promote both splicing and 3' end formation of HS genes during stress, suggesting that NuRD involvement could extend beyond recruitment to promoters (Murawska *et al*. 2011). Furthermore, in the context of breast cancer cells, HSF1 and the MTA subunit of NuRD were shown to physically interact and influence the regulation of estrogen receptor sensitive genes (Khaleque *et al*. 2008). Though each of these examples highlight a connect between NuRD and the HSR, they suggest distinct regulatory mechanisms in different organisms. Together, these data clearly indicate that

regulation of the HSR by NuRD is conserved through evolution and justify the need for additional mechanistic experiments.

The NuRD complex has been extensively studied in the context of stem cell differentiation, cell division, and development. Our discovery of NuRD-mediated HSR regulation was facilitated by the unique features of our experimental system. In particular, somatic cells in *C. elegans* are post-mitotic, which enables analysis of the NuRD complex in the absence of cell division. In addition, our use of *dcp-66* RNAi and a hypomorphic allele of *let-418* allows the organisms to reach adulthood. Similar approaches have been used to identify novel roles for the NuRD complex in muscle maintenance and the activation of the mitochondrial unfolded protein response (mt-UPR) in *C. elegans* (Müthel *et al*. 2019; Shao *et al*. 2020b; Zhu *et al*. 2020).

We have used the advantages of our approach to show that NuRD can regulate multiple stress responses. Our genomic analysis also implicates the NuRD complex in the regulation of innate immunity and embryogenesis. Since each of these responses are transcriptional, a chromatin remodeling complex is well-positioned to be involved in regulation. In each of these cases, the NuRD complex appears to function as a modulator, rather than a central regulator, meaning that it is not a required component of each pathway but rather plays a role in fine-tuning these responses. Consequently, NuRD could function to integrate multifaceted environmental signals, thereby affecting a diverse set of physiological processes in a

coordinated manner to optimize organismal survival, growth, reproduction, and development.

Unexpectedly, we found that *dcp-66* and *let-418* subunits of NuRD regulate stress responses in a divergent manner. These divergent effects extend to other cellular pathways. However, many genes and pathways are affected similarly by both subunits. Here, we propose a model that can explain how *dcp-66* and *let-418* can have divergent effects on some genes but similar effects on others (**Figure 7**). Our model has three key parts. First, the LET-418 subunit contains the core chromatin remodeling activity. Second, DCP-66 links LET-418 to the rest of the NuRD complex (Spruijt *et al*. 2020; Low *et al*. 2020). Finally, the NuRD complex is recruited to promoters via different mechanisms.

In our model, recruitment of NuRD via different subunits accounts for the divergent sensitivity to *dcp-66* and *let-418* inhibition. Genes that recruit the NuRD complex through interactions with the LET-418 core are divergently affected (class II and III genes, **Figure 7B**). For these genes, inhibition of *let-418* prevents recruitment of the entire NuRD complex (**Figure 7F**), but inhibition of *dcp-66* does not (**Figure 7D**). Therefore, the effects of *dcp-66* inhibition are distinct from *let-418* inhibition. In contrast, genes that recruit the NuRD complex through interactions with any other NuRD subunit are similarly affected by *dcp-66* and *let-418* inhibition (class I and IV

genes, **Figure 7A**). For these genes, inhibition of either *dcp-66* or *let-418* prevents the LET-418 core from localizing to the promoter (**Figure 7C and 7E**).

In the literature, the recruitment of NuRD by different subunits is well-established. Many transcription factors recruit NuRD through interactions with the MTA paralogs. For example, NuRD is recruited to the CDH1 promoter in breast cancer via association of the TWIST transcription factor with the MTA2 subunit (Fu *et al*. 2011). Other transcription factors that recruit NuRD via interactions with MTA paralogs include: BCL11B in T lymphocytes (Cismasiu *et al.* 2005), BCL-6 in B lymphocytes (Fujita *et al*. 2004), estrogen receptor in breast cancer (Mazumdar *et al*. 2001; Toh and Nicolson 2009), FOG1 and FOG2 (Hong *et al*. 2005; Roche *et al*. 2008), the MYC oncogene (Zhang *et al*. 2005), and the P53 tumor suppressor (Luo *et al*. 2000; Moon *et al*. 2007). In contrast, NuRD can be recruited via interactions with the MBD subunit to promoters containing methylated DNA or by transcription factors including c-Jun, which targets NuRD to activator protein 1 (AP-1) associated genes (Zhang *et al*. 1999; Aguilera *et al.* 2011). Alternatively, NuRD can be recruited via interactions with the CHD3 or CHD4 core subunits. For example, NAB2 and ZIP recruit NuRD through direct binding to CHD3 or CHD4 (Srinivasan *et al*. 2006) (Li *et al.* 2009). CHD4 also contains poly(ADP-ribose) (PAR) binding motifs that could drive association with promoters that have PAR polymerase (PARP) activity (Silva *et al*. 2016).

Differential recruitment of the NuRD complex through distinct subunits enables the complex to act on a wide variety of genes yet maintain the capacity for pathway-specific gene regulation. In support of this possibility, we have found that sensitivity to the two different subunits correlates with distinct functional classes. Moreover, NuRD subunits are subject to extensive post-translational modification, indicating a potential regulatory mechanism. Furthermore, this model could extend to the CHD-3 containing NuRD complex. The partial redundancy between CHD-3 and LET-418 enables further combinatorial complexity.

This simple model not only explains how inhibition of NuRD subunits can have differential effects on stress responses, but it can also help to interpret previous findings in other contexts. For example, the NuRD subunit MBD is required to maintain global cellular repression in induced pluripotent stem cells, which promotes transcriptional reprogramming and cellular differentiation (Rais *et al*. 2013). However, in *C. elegans* germline stem cells, LET-418 prevents differentiation (Käser-Pébernard *et al*. 2014). Furthermore, NuRD is distinct from other chromatin remodelers in that it both activates and represses tumorigenesis (Lai and Wade 2011). In regards to proliferation of cancer cells, the NuRD subunit CHD4 acts as a repressor in colon cells, while GATAD2 acts as an activator in thyroid cells (Xia *et al*. 2017; Wang *et al*. 2017). These differential effects of NuRD are highly context-specific and were thought to arise from the interaction of NuRD with other proteins (Lai and Wade 2011). However, our model provides an alternative explanation for the

opposing effects of NuRD complex subunits that are often observed in different types of cancer. As NuRD mutations and HSR misregulation are both associated with cancer, determining the mechanism behind NuRD-mediated HSR regulation is an important step in understanding the elaborate relationship between NuRD, the HSR, and disease.

Table S1

All primer pairs used in this study.

Target	**Forward**	**Reverse**
C12C8.1	GTACTACGTACTCATGTGTCGGTATTT	ACGGGCTTTCCTTGTTTTCC
F44E5.5	CAACTGCTGGTGATACCCATCTC	CTTGAAAGTGTTCTCTTGGCACG
hsp-16.11	GGCTCAGATGGAACGTCAA	GCTTGAACTGCGAGACATTG
18S	TTGCGTCAACTGTGGTCGTG	CCAACAAAAAGAACCGAAGTCCTG

Data Availability

Strains and plasmids are available upon request. The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, and tables. Supplementary materials including supplemental figures (**Figures S1-S4**), all DEGs (p-adj < 0.05) (**File S1**), enriched GO terms for all DEGs (**File S2**), enriched GO terms associated with 228 DEGs referenced in Figure 5B (**File S3**), and DNA sequences of oligonucleotide primer pairs used in this study (**Table S1**) are available online. Sequencing data have been deposited in NCBI SRA under accession code PRJNA692471.

The following dataset was generated:

Author(s)	Year	Dataset Title	Dataset URL	Database and Identifier
Golden NL, Guisbert EA	2021	Transcriptomic profiling of young adult *C. elegans* deficient in *dcp-66* and *let-418* NuRD subunit expression	https://dataview.ncbi.nlm.nih.gov/object/PRJNA692471?reviewer=cgvkhbf9ui7bupgm1o694kkk4s	NCBI Sequence Read Archive, PRJNA692471

ACKNOWLEDGEMENTS

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PERSPECTIVES AND FUTURE DIRECTIONS

In this dissertation work, we sought to gain insight into how the HSR is regulated to better understand how it becomes misregulated during disease. In chapter one, we addressed a major issue in the current methodologies used for HSR studies in *C. elegans* and in chapter two, we identified a new way in which the NuRD chromatin remodeling complex works. The importance of these discoveries to the *C. elegans*, HSR, and chromatin remodeling fields is discussed below.

To a novice HSR researcher, one can imagine the difficulty in determining which HSR inducible assays to select when there are so many different times, temperatures, and techniques proposed in the literature. Prior to our publication, a document describing how best to conduct consistent, reliable, HSF-1 dependent HSR research in *C. elegans* did not exist. This work helps guide future research by providing a set of tools that allows scientists to generate reproducible results that others can build upon. In addition, one of the major contributions of this work was the identification of an organismal assay that is HSF-1 dependent, unlike the widely-used thermotolerance assay.

Our work represents only the beginning, and there are still further optimizations that need to be made. For example, the thermorecovery assay measures motility, which signifies that it predominately relies upon a muscle-specific defect. One can envision an improvement to this assay that is instead based upon a more quantitative method;

invoking a whole-organism response, such as a live/dead assay. Determining the number of organisms alive/dead after HS at a time and temperature of which 50% of control and < 25% (or less) of *hsf-1* inhibited worms survive would be ideal. This assay would be highly effective in identifying both positive and negative regulators of the HSR, whereas the current assay identifies positive regulators well, but reaches a limit for the identification of negative regulators, as there is only a 15-20% decline in the normal movement of control worms. In addition to the thermorecovery assay, the RNA isolation method described in our protocol also has room for improvement. For example, our protocol relies on a vortexing technique that generally results in nanograms of RNA, whereas a more robust method of fractionation (*e.g.* repeated freeze-thaw, or a bead-based method) could potentially generate micrograms of RNA from a similar sample size. Another tool that the *C. elegans* HSR community would benefit from is the establishment of additional HSR reporters; *i.e. F44E5.4/5.5* and *hsp-16.11* promoter reporters, as these could reveal distinct tissue specific effects that would also be useful in identifying new regulators of the HSR.

In chapter two, we establish a new relationship between subunits within the NuRD complex. Prior to our work, a genetic interaction between *dcp-66* and *let-418* had not been tested. Although it was presumed that *dcp-66* and *let-418* both interacted as subunits of the same complex, our work reveals the nature of this genetic interaction, in our finding that *let-418* is epistatic to *dcp-66* for the majority of genes differentially expressed by both subunits of the complex.

A caveat to the genetic interactions described in our work is that we were not able to conduct a true epistasis experiment; as *dcp-66* mutants cannot reach adulthood, a viable genetic cross between *dcp-66(gk370)* and *let-418(n3536)* mutants could not be generated. Furthermore, the epistasis affect we observed at the molecular level was not conserved at the physiological level. Instead, double *let-418(n3536);dcp-66* RNAi worms had a very distinct morphology as indicated by the inability to generate viable embryos into adulthood. While our work indicates the NuRD complex is required for embryogenesis, we have not yet identified which stages of embryogenesis are interrupted. Whether the double inhibition of *let-418* and *dcp-66* represents a defect in gametogenesis, or a more general defect in the structural machinery of the somatic gonad remains to be determined. For example, some work has shown *dcp-66* is required for the correct formation of the spermatheca-uterine valve (Praslicka *et al*. 2017).

In our model, we propose that the recruitment of NuRD to promoters through either *let-418* or other NuRD subunits can explain the divergent effects on gene expression. In support of this, recent work identifying the structure of NuRD suggests that the complex is assembled in two functional parts: an MBD-GATAD2-CHD unit and an MTA-HDAC-RBBP unit, where GATAD2 connects the two enzymatic activities of the complex (Low *et al.* 2020). However there remains an additional possibility, that *dcp-66* and *let-418* are acting independently of the complex to regulate gene expression. In fact, our RNA-seq analysis shows that a large number of genes

differentially expressed by *dcp-66* and *let-418* are unique to each subunit. Although our model proposes that *let-418* acts independently of the complex to regulate the transcription of stress response and embryogenesis-related genes, it is also possible that *dcp-66* acts independently of *let-418*/NuRD. While both proteins can affect the expression of the same genes, as opposed to functioning directly at gene promoters, they could act on different regions of the same gene (*i.e. dcp-66* at the promoter and *let-418* at an enhancer or vice versa), influencing expression independent of the complex. Because *dcp-66* contains a GATA domain, it is possible that it directly associates with DNA as a transcription factor.

Similar to the developmental affects observed in *C. elegans*, mutations in SWI/SNF, NuRF, and NuRD can lead to inherited developmental disorders, intellectual disabilities, neurodevelopmental disorders, autoimmune disease, and cancer (Lu *et al*. 2008; Zhang 2011; Agaimy *et al.* 2018; Goodwin *et al*. 2018). These diseases are largely caused from disruption of the DNA damage response (DDR). When DNA damage such as double-stranded breaks (DSB) occur, each complex gets recruited to the site of DNA damage and repositions or evicts histones from the area to begin repair and maintain DNA integrity (Lans *et al.* 2012). The complexes utilize DSB repair pathways including non-homologous end joining (NHEJ) or homology directed repair (HDR) through homologous recombination (HR), however this is unique to each complex (Chiu *et al.* 2017). Of the two pathways, NHEJ is much more error-prone in that repair involves directly ligating DSBs, without using a template

as in HR. The NuRD complex gets recruited to DNA damage where it and an accessory factor (ZMYND8) that binds directly to GATAD2A work together to suppress transcription, enabling HR (Chiu *et al.* 2017). Consistent with this report, recent work utilizing the adult *C. elegans* germline shows that in the absence of either NuRD ATPase (LET-418 or CHD-3), DSBs persist, resulting in fewer offspring (Turcotte *et al.* 2018). It would be interesting to determine if in the absence of DCP-66, DSBs are also present. From the literature and our model, one might predict that DSB repair represents a LET-418 dependent process, and therefore in the absence of DCP-66, LET-418 would still be recruited to prevent DSBs.

We have established a new paradigm between *dcp-66* and *let-418* in the regulation of stress responses, however the mechanisms responsible for this regulation are unknown. As NuRD is a well-established chromatin remodeling complex, we hypothesize that DCP-66 and LET-418 regulate chromatin accessibility at HS promoters in opposite ways. This hypothesis can be tested in a series of three complementary approaches. First, the effects of *dcp-66* and *let-418* inhibition on basal chromatin accessibility at the *hsp-70 (C12C8.1)* promoter could be tested to establish if changes in NuRD subunit expression correlate with changes in chromatin. Next, DCP-66 and LET-418 localization at the *hsp-70* promoter after HS should be measured to determine if the effects on HS gene expression are direct or indirect. Finally, the genomic localizations of DCP-66 and LET-418 should be determined to correlate NuRD subunit expression at promoters to the gene expression of NuRD

subunit inhibition. This will also help distinguish between NuRD dependent vs independent effects. Together, these results will clarify how NuRD regulates stress response transcription and will identify where DCP-66 and LET-418 localize genome-wide. Here, we outline three experimental aims directed at testing this hypothesis.

1. Establish *dcp-66* and *let-418* as chromatin remodelers at the promoter of *hsp-70*. If *dcp-66* and *let-418* remodel chromatin, then they will increase or decrease chromatin accessibility at the promoters of HS genes. First, chromatin accessibility can be measured at the promoter of *hsp-70* in N2 (control), *let-418(n3536)*, and *dcp-66* RNAi worms with a DNase I digestion assay. Briefly, chromatin isolated from animals should be mock-treated or DNase I digested, and the percentage of relative digestion by DNase I at genomic regions corresponding to -100bp, -50bp, 0, +60bp, and +120bp from the transcription start site (TSS) of *hsp-70* could be measured by qPCR. As a positive control, relative DNase I digestion at the *hsp-70* promoter in N2 worms before and after reproductive maturity should be completed, as it is already established that chromatin accessibility declines at this locus at the transition to adulthood (Labbadia and Morimoto 2015). If our hypothesis is supported, then DNA will be less accessible with *dcp-66* knockdown and in an open/accessible state in *let-418(n3536)* mutants. These results will indicate that DCP-66 and LET-418 work together to increase or decrease chromatin access directly at the promoter of *hsp-70*.

2. Measure DCP-66 and LET-418 localization at the *hsp-70* promoter after HS. If DCP-66 and LET-418 remodel chromatin at the promoters of HS genes, then they will localize to these promoters. Here, the localization of DCP-66 and LET-418 at the *hsp-70* promoter could be tested with ChIP-qPCR using established protocols (Labbadia and Morimoto 2015). Animals should be exposed to heat stress followed by chromatin cross-linking, shearing, and precipitation with antibodies for LET-418, FLAG-tag (for DCP-66), and IgG. The relative percentage of signal compared to input should be measured spanning -150bp to -50bp from the TSS of *hsp-70* with qPCR. LET-418 and DCP-66 localization could also be quantified in *let-418* RNAi and *dcp-66* RNAi animals as additional controls. We expect this experiment to show that DCP-66 and LET-418 localize to the promoter of *hsp-70* before and/or after stress induction.

3. Elucidate the genome-wide locations of DCP-66 and LET-418. Our RNA-seq data indicates that *dcp-66* and *let-418* have differential effects on other genes and pathways. Here, it will be important to determine if DCP-66 and LET-418 colocalize to the promoters of these genes with ChIP-seq. Chromatin preparations could be scaled up and outsourced to Novogene for library preparation and sequencing. If our hypothesis is supported, these results would support our RNA-seq analyses and furthermore, would reveal other novel pathways regulated by NuRD. In addition, we would expect to identify promoters where DCP-66 and LET-418 bind separately, indicating their NuRD-independent roles.

The completion of this work will determine if DCP-66 and LET-418 act in opposition to modulate chromatin at the promoters of stress-sensitive genes in response to environmental cues, exposing molecular mechanisms for the fine-tuning of HS gene expression. This work would establish if both NuRD subunits are found at HS promoters and if they influence chromatin structure there. Furthermore, novel DNA-binding NuRD protein interactions will be identified and compared to RNA-seq data, clarifying direct and indirect NuRD functions.

One caveat to the proposed experiments is that a *C. elegans*-specific antibody for DCP-66 does not currently exist. Therefore, a FLAG-tagged construct or similar must be generated and microinjected into *C. elegans*. It is also possible that our hypothesis is not supported from these experiments, in which case there are several testable alternatives. For example, there are many ways in which DCP-66 and LET-418 could influence HS gene expression without localizing to HS promoters and altering chromatin accessibility. One possibility is that both proteins act within their capacity as NuRD subunits to affect the acetylation of histones, HSF-interacting proteins like p53, or even HSF1 itself (Westerheide *et al.* 2009; Lai and Wade 2011; Zelin and Freeman 2015). For example, the histone deacetylase subunit of NuRD, HDA-1, could affect HSF-1 acetylation directly. HSF1 is known to be acetylated and this modification affects DNA binding (Zelin and Freeman 2015). If chromatin accessibility is not affected, then the effects of *dcp-66* RNAi and *let-418(n3536)* on

the acetylation of histones H3 and H4 at the promoter of *hsp-70* could be tested by ChIP-qPCR.

These experiments are written with the intention of helping guide progress on this project. Importantly, outside of this work, several other HSR regulators still remain to be characterized. For example, *pyp-1*, a subunit of the NuRF chromatin remodeling complex, was first identified by our lab as one of 52 other negative regulators of the HSR (**Figure 1**) (Guisbert *et al.* 2013).

It is still difficult to monitor the ever-changing epigenome *in vivo*. Our work highlights that accessory subunits of chromatin remodeling complexes can have additional roles in responding to environmental cues and therefore, these should be further investigated. Building upon our research, future work should continue to focus on determining how the subunits within each complex interact with one another to better understand complex function. The catalytic cores of each complex should also be studied and compared in more detail; for example elucidating the mechanisms behind the rate and specific movement of each remodeler within specific cells and tissues will help establish the relationship between remodeling and cellular functions or stages of development.

Commonly used methods for nucleosome remodeling studies include DNase I footprinting and restriction enzyme cleavage with reconstituted nucleosomal DNA

in vitro (Côté *et al.* 1994; Hamiche *et al.* 1999). While these techniques distinguish between nucleosome-free and nucleosome-rich forms of chromatin, it fails to capture all of the intricacies present *in vivo*. These techniques have been replaced with more recent advances in next generation sequencing such as MNase-seq and ATAC-seq, which provide increased resolution of nucleosome remodeling *in vivo*.

Recently, the NIH has started a roadmap epigenomics project that will lead to the creation of a 'reference genome', consisting of all epigenetic modifications in every cell and tissue type. Moreover, techniques for high-resolution mapping of nucleosome positions have transformed our thinking of chromatin organization, however higher-order chromatin structure still remains a mystery. Advances in epigenome editing using CRISPR/Cas9 have recently led to the targeted introduction of epigenetic modifications such as DNA methylation that can be exploited to determine the impacts on gene expression. Ultimately, it may one day be possible to understand the dynamics of chromatin so well that we create "remodelomes"; the collection of distinct epigenetic marks and chromatin remodelers expressed in a cell. Because the entire cell lineage in *C. elegans* is known, it is likely the first complete history of the epigenome will one day be defined on a cell-by-cell basis in this organism.

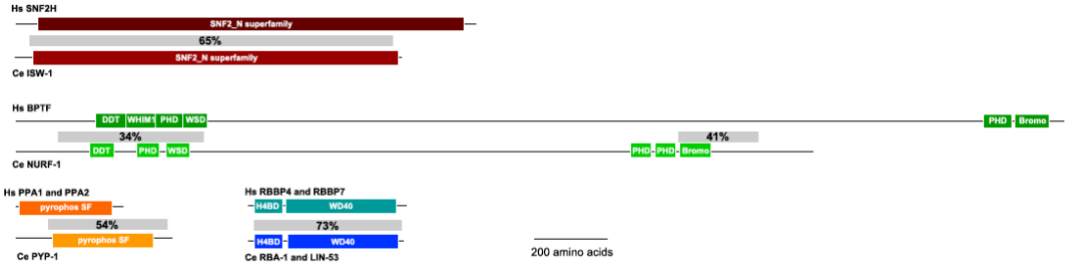

Figure 1. NuRF complex domains are conserved in *C. elegans*. The domain architecture and conservation (% identity) between human (Hs) and *C. elegans* (Ce) NuRF complex subunits. The NuRF subunit *pyp-1* has been identified by our lab as a negative regulator of the HSR (Guisbert *et al.* 2013).

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