INTRODUCTION

Rates of tissue aging within an animal are coordinated through a complex network of intra- and intercellular signaling pathways. Due to the complexity of this multifactorial aging mechanism, a variety of molecular targets have been identified as age determinates across a number of model organisms ranging from yeast to primates (Kenyon, 2010). In multicellular organisms, the ability of these molecules to act across tissues and connect organ systems plays a critical role in animal physiology and age determination. The nervous system is particularly well-suited to coordinate age-related processes due to its long-term plasticity and capacity for regenerative growth.
system has long been appreciated as the hub of information transfer and communication between the body’s other organ systems (Miller et al., 2020). Modulating critical regulators of cellular adaption to stress in the nervous system initiates systemic alterations with cytoprotective and age-defying potential (Douglas et al., 2015; Durieux et al., 2011; Taylor & Dillin, 2013). Receipt of these neural-born signals in the peripheral tissues is essential for the transmission and initiation of distinct cellular responses. Communication between the brain and intestine has become increasingly appreciated in the context of human health and can occur through direct synaptic innervation of the tissues, neuroendocrine means, systemic fluctuations in metabolism, or the immune response (Ambrosini et al., 2019; Boehme et al., 2020; Carabotti et al., 2015; Jena et al., 2020). To date, the molecular and cellular details underlying these integrated, trans-organ signaling mechanisms and their role in age regulation remain unclear.

The heat shock transcription factor, HSF-1, is an ancient regulator of cellular adaption to thermal stress with evolved roles as an aging regulator in the nervous system of the nematode, Caenorhabditis elegans (Morimoto, 1998; Morley & Morimoto, 2004). While HSF-1 activates the expression of protein folding enzymes to ensure proteome integrity amid thermal destabilization (Akerfelt et al., 2010; Morley & Morimoto, 2004), it has also developed as an important aging regulator in part through its ability to cooperate with the FOXO transcription factor, DAF-16 (Hsu et al., 2003). Elevating the expression of HSF-1 exclusively within the nervous system extends animal lifespan and requires the activity of enteric DAF-16 (Douglas et al., 2015; Morley & Morimoto, 2004). Neural hsf-1 was recently shown to reduce signaling through the bone morphogenetic protein (BMP) pathway for adaptation to elevated, nonpermissive temperatures (Chauve et al., 2021). As a member of the greater transforming growth factor β (TGF-β) signaling pathway in C. elegans, DBL-1 is a neural-produced BMP ligand (Morita et al., 1999; Suzuki et al., 1999) that signals to peripheral tissues through its binding to the heterodimeric receptor complex composed of the type 1 receptor, SMA-6, and type 2 receptor, DAF-4 (Gumienny & Savage-Dunn, 2013; Roberts et al., 2010; Wang et al., 2002). In addition to regulating body size and male tail development (Morita et al., 1999; Suzuki et al., 1999), DBL-1 has been linked with olfactory learning, reproductive aging, lipid metabolism, and the innate immune response (Clark et al., 2018; Luo et al., 2010; Zhang & Zhang, 2012; Zugasti & Ewbank, 2009). DBL-1 signal transduction in peripheral tissues acts through the nucleocytoplasmic SMAD proteins (SMA-2, SMA-3, and SMA-4) (Gumienny & Savage-Dunn, 2013). SMA-3, in particular, co-occupies some of the same DNA regulator elements as DAF-16 and jointly regulates the expression of select genes (Qi et al., 2017). Since DAF-16 is required for many DBL-1 functions including larval development (Kaplan et al., 2015), response to environmental nanopoly styrene (Liu et al., 2020), and lipid accumulation (Clark et al., 2018, 2021; Liu et al., 2020), we sought to determine whether DBL-1 signaling might play an important role in HSF-mediated age regulation.

Herein we report that the nonautonomous mode of age regulation by neural HSF-1 acts through this BMP/DBL-1 signaling pathway (Figure 1a). Both the neuron-specific ligand, DBL-1, and its dedicated receptor in the intestine, SMA-6, were required for lifespan extension by neural HSF-1. We find that neural HSF-1 represses dbl-1 transcription thereby reducing BMP signal transduction in the intestine via a
SMA-3-mediated negative feedback loop entailing the transcriptional repression of membrane trafficking regulators linked to intracellular lipid surveillance (Watterson et al., 2022). In turn, this reduces steady-state protein levels of the SMA-6 receptor and further dampens signal transduction. Our studies are consistent with another report that impaired DBL-1 signaling extends C. elegans lifespan (Luo et al., 2009). Moreover, the ability of HSF-1 to coordinate BMP/DBL-1 signaling across tissues resides upstream of enteric DAF-16. While previous reports have demonstrated that neural hsf-1 utilizes a distinct transcellular signaling mechanism involving chaperone activation to protect against acute heat stress (Douglas et al., 2015), thermal adaption by neural hsf-1 under mild yet chronic heat stress conditions at 25°C appears to utilize a similar DBL-1 dependent mechanism (Chauve et al., 2021). Consistent with these previous studies, we identify the neuron-specific BMP ligand, DBL-1, as a critical signaling molecule required for neural HSF-1 to extend lifespan and report how its transmission to the intestine modulates surface residency of the SMA-6 receptor through a SMAD negative feedback loop.

2 | RESULTS

2.1 | Neural hsf-1 requires both the DBL-1 ligand and its intestinal SMA-6 receptor for lifespan extension

Mutations of dbl-1 and its dedicated type I transmembrane serine/threonine kinase receptor, SMA-6, are involved in age modulation (Luo et al., 2009). Moreover, neural hsf-1 was recently shown to reduce DBL-1 signaling at the nonpermissive temperature of 25°C (Chauve et al., 2021). We hypothesized that this particular BMP signaling mechanism is required for lifespan extension by neural hsf-1. Indeed, the dbl-1(nk3) null mutation abolished lifespan extension by neural hsf-1 (Figure 1a,b, Figure S1a, Table S1). While dbl-1 expression was necessary for lifespan extension by neural hsf-1, we next examined its sufficiency. Under our experimental conditions, elevating the expression or activity of dbl-1 through transgenic dbl-1::GFP overexpression (Schultz et al., 2014) or RNAi of its negative regulator, lon-2 (Gumienny et al., 2007; Taneja-Bageshwar & Gumienny, 2013) did not impact lifespan and does not appear to be sufficient to promote lifespan extension (Figure S1b–d and Table S1). Due to the requirement of this BMP signaling ligand to extend lifespan, we next examined the necessity of its dedicated type I receptor, SMA-6, in the peripheral tissues (Figure 1a). Consistent with DBL-1 being required for lifespan extension, preventing receipt of this neural-born DBL-1 ligand via sma-6(wk7) mutation also abolished lifespan extension by neural hsf-1 (Figure 1c, Figure S1e, and Table S1). Previous studies have highlighted the importance of the intestine for neural hsf-1 to extend animal lifespan (Douglas et al., 2015). Rescuing sma-6::GFP expression exclusively in the intestine was sufficient for the neural hsf-1 to extend the lifespan in the sma-6(wk7) mutant background (Figure S1f and Table S1). Thus, neural hsf-1 requires both the neural-born DBL-1 ligand and its dedicated BMP receptor, SMA-6, in the intestine to modulate the aging process. Yet how neural hsf-1 modulates this transcellular signaling paradigm was unclear.

2.2 | Neural HSF-1 represses expression of the BMP ligand, DBL-1

As a well-established regulator of worm body length, DBL-1 expression levels directly correlate with animal body length (Morita et al., 1999; Suzuki et al., 1999; Wang et al., 2002). Transgenic animals expressing hsf-1 in the nervous system demonstrated smaller body size throughout development and a 10% reduction in body length as egg-laying Day 1 adults (Figure S2a,b). Since neural hsf-1 was reported to modulate DBL-1 activity (Chauve et al., 2021), we examined whether these morphological differences in body size correspond with dbl-1 expression levels. Consistent with this prior study (Chauve et al., 2021), analysis of transcriptomic datasets confirmed a 2-fold reduction in dbl-1 expression in neural hsf-1 transgenic animals (Figure 2a). By contrast, transcript levels for a different TGF-β signaling ligand, DAF-7, were not changed in neural hsf-1 transgenic animals, further highlighting the selectivity of HSF-1 in the BMP branch of the greater TGF-β signaling pathway (Figure S2c). Three predicted consensus heat shock elements were identified within the 5’ untranslated region of DBL-1, suggesting a direct mode of transcriptional regulation (Figure 2b). Analysis of previously reported ChIP-seq datasets (Labbadia & Morimoto, 2015) revealed enrichment of HSF-1 binding at two of the three predicted heat shock elements within the DBL-1 promoter (Figure 2c). In a complementary fashion, activation of HSF-1 via transient heat shock reduced dbl-1 transcript abundance but had no significant effect on daf-7 transcription (Figure 2d and Figure S2d). Conversely compromising hsf-1 activity via the hsf-1(sy441) hypomorphic mutation activated dbl-1 transcription by over 4-fold (Figure 2e). Comparison of transcriptional profiles between dbl-1(nk3) null mutants and neural hsf-1 transgenic animals showed a significant overlap of differentially regulated genes (Figure S2e–g and Table S2). Moreover, analysis of transcripts differentially regulated in neural hsf-1 animals identified TGF-β signaling (cel04350) as the 4th most significantly enriched KEGG pathway (Figure 2f). Consistent with HSF-1 and its dependence on the DAF-16/FOXO transcription factor for age regulation (Douglas et al., 2015; Hsu et al., 2003), both FOXO signaling (cel04068) and insulin resistance (cel04931) were significantly enriched in KEGG pathway analysis (Figure 2f). Collectively, these data suggest that neural hsf-1 binds the DBL-1 promoter and mediates its transcriptional repression resulting in physiological defects regarding body length.

2.3 | Neural hsf-1 reduces peripheral DBL-1 signaling

Since HSF-1 represses expression of the neural-produced DBL-1 ligand, we examined how these transcriptional changes in the nervous
system elicited a complementary response in the animal’s peripheral tissues (Wang et al., 2002). Worm morphology suggested that neural hsf-1 initiated a periphery response as indicated by shortened body length of adults (Figure S2a,b). Monitoring whole-body fluorescence from two distinct DBL-1 pathway transcriptional reporters, spp-9:p::GFP (Roberts et al., 2010) and RAD/SMADp::GFP::NLS (Tian et al., 2010), enabled the spatial characterization and quantification of DBL-1 signaling to peripheral tissues. In keeping with spp-9 expression being inversely proportional to DBL-1 levels (Madhu et al., 2020; Roberts et al., 2010), we observed enhanced spp-9:p::GFP fluorescence in transgenic animals expressing neural hsf-1 (Figure 3a,b and Figure S3a), indicating a reduction in DBL-1 signaling. We also observed that expression of full-length hsf-1 in the nervous system was sufficient to activate spp-9 transcription in the hsf-1(sy441) hypomorphic background (Figure S3b), further supporting the neuronal origins of the DBL-1 signaling mechanism. Consistent with previous reports (Chauve et al., 2021), neural hsf-1 transgenic animals showed reduced fluorescence from the RAD/SMAD transcriptional reporter (Tian et al., 2010) throughout larval development and into day 1 of adulthood (Figure S3c-e), further confirming that neural hsf-1 reduces DBL-1/BMP signaling in peripheral tissues.

We then asked whether DAF-16/FOXO, which is required for lifespan extension by neural hsf-1 (Douglas et al., 2015), is also needed for DBL-1 signaling. Reduced expression of daf-16 via RNAi treatments did not impact spp-9:p::GFP fluorescence in the animal’s peripheral tissues (Figure 3b), suggesting it may act downstream of, or in parallel with, DBL-1 signaling to impact age regulation. In further support, activation of spp-9 transcription by neural hsf-1 still occurred in daf-16(mu86) null mutant animals (Figure 3c). To examine the combined impact of FOXO and BMP signaling on lifespan, co-administering daf-16 and dbll-1 RNAi significantly reduced lifespan when compared to dbll-1 alone (Figure 3d and Table S1), highlighting that both BMP and FOXO signaling might serve distinct yet complementary roles with respect to lifespan determination by neural hsf-1. Overall, repression of dbll-1 transcription by neural hsf-1 reduces systemic BMP signaling seemingly independent of DAF-16.

2.4 | Intestinal SMA-6 protein levels are reduced through repressed membrane trafficking

Canonical DBL-1/BMP signal transduction is composed of multiple transmembrane serine/threonine kinase receptors. Upon ligand binding, the type I BMP receptor, SMA-6, and its complementary type II transmembrane receptor, DAF-4, are recruited into a heterotetrameric complex at the plasma membrane which in turn activates...
the intracellular receptor-regulated r-SMAD proteins, SMA-2 and SMA-3. SMA-2, SMA-3, and co-Smad SMA-4 then form a complex that enters the nucleus and regulates transcription of target genes (Gumienny & Savage-Dunn, 2013) (Figure 1a). While daf-4 transcript levels remained unchanged, we observed DBL-1-dependent transcriptional activation of sma-6 in neural hsf-1 transgenic worms (Figure S4a). Conversely, the DAF-7 type I receptor, daf-1, which also heterodimerizes with DAF-4, is repressed in a DBL-1-dependent manner (Figure S4a), suggesting a potential inverse relationship between signaling by DBL-1 versus DAF-7. However, further studies are required to elucidate the interplay between these different TGF-β signaling pathways.

Despite elevated sma-6 transcript abundance upon neural hsf-1 expression, we observed that steady-state protein levels of ectopically expressed SMA-6::GFP in the intestine were reduced by 79% at late L4 larval/Day 1 adult stages as indicated by western blot analysis (Figure 4a and Figure S4b). Consistent with reducing dbl-1 signaling by neural hsf-1, dbl-1(nk3) mutants showed a similar 72% decrease in enteric SMA-6::GFP protein levels, while pathway activation via lon-2 RNAi did not significantly affect SMA-6::GFP levels (Figure 4a and Figure S4b). We confirmed that reducing sma-6 expression via RNAi was sufficient to decrease BMP signal transduction in peripheral tissues as evidenced by increased fluorescence of the spp-9p::GFP reporter strain (Figure S4c). Thus, reducing dbl-1 expression by neural hsf-1 or gene deletion compromises steady-state protein levels of its dedicated SMA-6 receptor, which corresponds with reduced BMP signaling.

Intrigued by the loss in intestinal SMA-6::GFP protein levels by neural hsf-1, we further investigated how reducing BMP signaling by neural hsf-1 might impact steady-state protein levels of the SMA-6 receptor. Several regulators of BMP/TGF-β signaling have the capacity to bind and modulate BMP/DBL-1 receptor dynamics, including trafficking to endosomes for recycling or degradation (Gumienny & Savage-Dunn, 2013). Thus, we examined whether reduced DBL-1 signaling by neural hsf-1 and signal ablation in dbl-1(nk3) mutants elicited a dose-dependent change in the extracellular DBL-1 pathway regulators LON-1, LON-2, SMA-10, CRM-1, DRAG-1, and ADT-2 (Gumienny & Savage-Dunn, 2013). Transcript abundance for lon-1, sma-10, and drag-1 was significantly changed in neural hsf-1 transgenic animals, but sma-10 was the only extracellular regulator whose transcriptional repression corresponded with dbl-1 levels in the cell (Figure 4b). SMA-10, a LRIG (leucine rich and immunoglobulin domains) homolog physically binds SMA-6 and regulates its activity (Gumienny et al., 2010). Moreover, loss of sma-10 aberrantly affects endocytic trafficking and reduces signal strength of the SMA-6 receptor (Gleason et al., 2017). We hypothesized that reduced dbl-1 signaling by neural hsf-1 represses sma-10 expression in peripheral tissues thereby disrupting surface residency and steady-state expression of SMA-6 in endothelial cells, which ultimately reduces BMP signal transduction.

Since the loss of SMA-10 disrupts the endocytic dynamics of SMA-6 (Gleason et al., 2017), we examined other critical regulators of membrane transport and endocytosis with the potential to

![Figure 3](https://example.com/fig3.png)

**Figure 3** Neural hsf-1 represses DBL-1 signaling in the peripheral tissues independent of DAF-16. (a, b) Transgenic Day 1 adult worms harboring the spp-9p::GFP transcriptional reporter in wild-type, WT, (PMD145) and neural hsf-1 (PMD93) backgrounds. (a) Fluorescence micrographs, scale = 100 μm and (b) Relative spp-9p::GFP fluorescence by large-particle flow cytometry. Worms cultured on control empty vector or daf-16 RNAi. Mean with 95% confidence interval, ****p < 0.0001 and ns, not significant by one-way ANOVA with the Tukey test, n = 924 (PMD144, EV), n = 1843 (PMD144, daf-16 RNAi), n = 1282 (PMD64, EV) and n = 993 (PMD64, daf-16 RNAi) over multiple independent trials. (c) Relative transcript abundance for spp-9 determined by qPCR. Wild type or control (light gray, N2), neural hsf-1 (blue), daf-16(mu86) (dark gray), and daf-16(mu86); neural hsf-1 (orange). Left to right, **p = 0.0074, 0.0039, and 0.0043 by two-way ANOVA with the Sidak multiple comparison test, n = 3. (d) Lifespan analysis of control (N2) and neural hsf-1 transgenic worms at 20°C with the respective RNAi combinations. See Table S1.
impact SMA-6 surface residency. In addition to FOXO and TGF-β signaling, KEGG pathway analysis identified a significant enrichment of transcripts differentially regulated in neural hsf-1 animals which were associated with endocytosis (cel04144) (Figure S2f). Of the 29 endocytosis-annotated transcripts that were differentially regulated by neural hsf-1, 27 of them were significantly repressed (Figure 4c). Therefore, reducing dbl-1 signaling by neural hsf-1 corresponded to the global repression of endocytosis-related genes. In further support, ablation of dbl-1 signaling displayed a similar yet more robust repression of the same endocytic genes (Figure 4c). As critical regulators of secretory and endocytic transport (Pfeffer, 2017; Stenmark, 2009), Rab GTPases are the largest family of small G-proteins within the cell (Stenmark, 2009). We observed that reducing and ablating DBL-1 signaling in neural hsf-1 transgenics and dbl-1(nk3) mutants resulted in approximately a 2-fold reduction in the transcription of several Rab GTPases with roles in regulating the early endosome (RAB-5), late endosome (RAB-7), basolateral exocytosis (RAB-8), and endocytic recycling (RAB-11.1) (Figure 4c)
and Figure S4d). Consistent with our previous study (Watterson et al., 2022), we detected transcriptional activation of the RAB-11.1 paralog, rab-11.2, which likely occurs through loss of RAB-11.1 expression and subsequent activation of the intracellular lipid surveillance response (Figure 4c and Figure S4d). Thus, reducing BMP/DBL-1 signaling in the nervous system has the potential to modulate lipid surveillance and nuclear hormone receptor, NHR-49, activity in the intestine. We confirmed NHR-49 activation in both neural hsf-1 and dbl-1(nk3) animals as indicated by the transcriptional induction of an established NHR-49 target (Van Gilst, Hadjivassiliou, Jolly, et al., 2005; Van Gilst, Hadjivassiliou, & Yamamoto, 2005) in the acyl-CoA synthase, acs-2 (Figure S4e). In further support, the same elevated nonpermissive temperatures, which have been reported to impact lipid metabolism genes in the intestine by neural hsf-1 (Chauve et al., 2021), were sufficient to activate lipid surveillance as indicated by increased fluorescence of the rab-11.2p::YFP transcriptional reporter at 25°C (Figure S4f).

Due to their role in endocytic recycling and lipid surveillance, the RAB11 family of GTPases acts as critical regulators of protein residency at the apical cell surface (Welz et al., 2014). Similar to smo-10 mutants (Gleason et al., 2017), reducing rab-11.1 transcription via RNAi was sufficient to reduce cell surface residency of enteric SMA-6::GFP with a larger portion of the fusion protein decorating FM4-64 fluorescently-labeled endocytic vesicles (Figure 4d). Moreover, the overall signal intensity of SMA-6::GFP was reduced with rab-11.1 RNAi as evidenced by large-particle flow cytometry (Figure 4e). In combination, reduced expression of critical regulators of membrane trafficking such as RAB-11.1 and extracellular regulators such as SMA-10 is sufficient to dampen BMP signaling in the peripheral tissues by reducing surface residency of the type I BMP receptor, SMA-6.

### 2.5 | SMA-3 mediates repression of membrane trafficking

It remained unclear how reducing DBL-1 signaling initiated transcriptional repression of these critical regulators of membrane trafficking and receptor dynamics. As intracellular signal transducers of DBL-1 signaling in the periphery, SMA-2 and SMA-3 represent the receptor-regulated Smads (R-Smads) while SMA-4 is the Co-Smad (Savage et al., 1996) (Figure 1a). These Smad proteins engage in dynamic nucleocytoplasmic translocation with nuclear retention being stimulated by pathway activation (Inman et al., 2002). We confirmed that smo-3 RNAi, and to a lesser extent smo-4 RNAi, reduced BMP signaling as indicated by increased fluorescence of the spp-9p::GFP reporter (Figure 5a). No transcriptional fluctuation was observed in neural hsf-1 transgenic animals for these intracellular transduction components, SMA-2, SMA-3, and SMA-4 (Figure S5a). Knowing that SMA-10 was capable of regulating SMA-6 activity in the periphery (Gleason et al., 2017), we examined whether SMA-3 might impact smo-10 transcript levels. While smo-6 transcription was unaffected, smo-3(e491) mutant animals significantly altered the transcription of smo-10 (Figure S5b). Analysis of previously reported chromatin immunoprecipitations (Madaan et al., 2018) identified a SMA-3-binding peak within the 5′ untranslated regions immediately upstream of the smo-10 gene (Figure 5b). Furthermore, we observe SMA-3-binding peaks within the first kilobase upstream of the translation start sites for rab-5, rab-7, and rab-11.1 (Figure 5b), all of which were repressed in neural hsf-1 and dbl-1(nk3) mutant backgrounds (Figure 4c and Figure S4d). Predicted binding sites for HSF-1, heat shock elements, were not identified in the promoter regions of these membrane trafficking genes (Table S3). Thus, their transcriptional fluctuations are likely due to DBL-1/BMP signal transduction in the peripheral tissues. Unlike the RAB GTPases that were repressed in neural hsf-1 and dbl-1(nk3) mutant backgrounds, the lipid surveillance responsive transcript, rab-11.2, was activated in the same worm strains without a detectable SMA-3-binding peak (Figure 5b). Overall, reducing receipt of the DBL-1 signal promotes a BMP negative feedback loop within the intestine. Our data suggest that this occurs in part through SMA-3 and its ability to repress critical factors involved in membrane trafficking and receptor dynamics, which ensure steady-state levels and cell surface residency of the SMA-6 receptor. However, activation of lipid surveillance under these same conditions does not appear to be directly regulated by BMP signaling and SMA-3 but rather appears to be an indirect consequence of reduced RAB GTPase function and membrane trafficking.

### 2.6 | Neural HSF-1 does not require DAF-16/FOXO to regulate DBL-1 signaling

The FOXO transcription factor, DAF-16, is required in the worm intestine for neural hsf-1 to extend C. elegans lifespan (Douglas et al., 2015). We sought to understand whether BMP signaling acts to bridge HSF-1 activity in the nervous system with DAF-16 in the intestine. As previously mentioned, our data indicate that reducing BMP signaling by neural hsf-1 resides upstream of DAF-16 activity in the intestine (Figure 3b,c). Since DAF-16 complexes with SMA-3 to regulate the transcriptional output of target genes (Qi et al., 2017), we hypothesized that DAF-16 activity converges with SMA-3 to mediate transcriptional repression of membrane trafficking regulators. As a regulator of GTPase activity, the Ras homolog enriched in the brain, RHEB-1, was one gene whose promoter region was reported to be co-occupied and its expression mutually regulated by DAF-16 and SMA-3 (Qi et al., 2017). We confirmed SMA-3 binding within a kilobase of the untranslated promoter region upstream of the RHEB-1 translational start site (Figure S5c) and this corresponded to reduced rheb-1 transcription in both neural hsf-1 and dbl-1(nk3) mutant strains (Figure S5d). Thus, a previously characterized promoter mutually occupied by both DAF-16 and SMA-3 showed repression of its respective transcript upon reduction and ablation of dbl-1 signaling.

While we expect similar transcriptional signatures between reduced dbl-1 signaling by neural hsf-1 and signal ablation via dbl-1(nk3) mutants, we anticipate select differences, which likely account for their longevity effects. To investigate this, we compared a core set
of 37 daf-16 transcriptional targets, which were compiled across several studies (Kumar et al., 2015; McElwee et al., 2003; Murphy et al., 2003; Riedel et al., 2013), and observed significant changes in 62% of these core transcripts in neural hsf-1 animals while dbl-1(nk3) null mutations modulated 54% of these core genes (Figure 5c,d). Select daf-16 targets including fat-5, cdr-2, and hacd-1 with subtle transcriptional changes were confirmed by qPCR (Figure S5e). While neural hsf-1 transgenic animals tended to exhibit stronger
activation of these core daf-16 targets, the dbl-1(nk3) mutants had a more repressive effect. Of the six DAF-16 transcriptional targets, which were significantly activated in neural hsf-1 transgenics and repressed in dbl-1(nk3), the carboxylesterase,GES-1, the stearoyl-CoA desaturase, FAT-5, and the superoxide dismutase, SOD-3, have been implicated in previous aging studies (Honda & Honda, 1999; Libina et al., 2003; Murphy et al., 2003). Furthermore, the hydroxyacyl-CoA dehydrogenase, HACD-1, in addition to GES-1 and FAT-5 play important roles in lipid metabolism while SOD-3 and the cadmium-responsive, GST-domain containing, CDR-2, protect against cellular redox imbalances, which are closely linked with mitochondrial energetics and metabolic output. It is possible that this subset of genes with strong metabolic links are acting through lipid surveillance mechanisms to impact age determination via neural hsf-1. 

Overall, our studies support a model in which neural hsf-1 regulates DBL-1 ligand production in the nervous system while reducing signal transmission has the potential to extend animal lifespan by driving a negative feedback loop in the BMP/DBL-1 signaling pathway through SMA-3 and activating intracellular lipid surveillance. Dampening BMP/DBL-1 signaling is further enhanced by the reduced expression of small G proteins involved in membrane transport and the extracellular regulator, SMA-10, which maintains the SMA-6 receptor at the basolateral surfaces of intestinal epithelia.

3 | DISCUSSION

Herein, we provide molecular details underlying a transcellular signaling paradigm involving the heat shock transcription factor, HSF-1, in the nervous system and its ability to modulate animal physiology and age progression across tissues through the TGF-β/BMP signaling pathway. Previous studies have identified that HSF1 activity in cancer-associated fibroblasts promotes malignancy through two stromal-derived signaling molecules; TGF-β and the stromal-derived factor, SDF1 (Scherz-Shouval et al., 2014). More recently in C. elegans, it was shown that hsf-1 overexpression in the nervous system can reduce BMP/DBL-1 signaling in peripheral tissues (Chauve et al., 2021). Our studies highlight BMP/DBL-1 signaling as a means by which neural hsf-1 extends animal lifespan and places this signal transduction mechanism upstream of the FOXO transcription factor, DAF-16. Specifically, we postulate that neural hsf-1 represses dbl-1 expression through binding to consensus heat shock elements within its 5’ promoter region. Decreasing neural expression of dbl-1 reduces BMP signal transduction in intestinal epithelia, in part, through loss of SMA-6 receptor availability at the cell surface, which occurs through SMA-3-mediated repression of smo-10 and several Rab GTPases. Previous studies have demonstrated that downstream nucleocytoplasmic SMAD proteins, SMA-3 and SMA-2, directly interact with DAF-16 (Qi et al., 2017) and provide a likely point of convergence between BMP signaling and DAF-16-mediated age regulation. While previous studies report a modest lifespan extension in dbl-1(nk3) mutants (Luo et al., 2009), removal of dbl-1 alone was not sufficient to extend lifespan in our experimental paradigm but was required for neural hsf-1 to extend lifespan. We hypothesize that in addition to reducing dbl-1 signaling, transcriptional fluctuations observed in neural hsf-1 and not in dbl-1(nk3) null mutants alter critical molecular pathways, which are needed in addition to reduced BMP/DBL-1 signaling to prolong aging. Knowing that DAF-16 is also required for lifespan extension by neural hsf-1 (Douglas et al., 2015) yet its overexpression has been reported to yield modest lifespan extensions (Henderson & Johnson, 2001), perhaps both reduced DBL-1 signaling and DAF-16 activation work synergistically to provide robust lifespan effects. However, additional studies are required to further understand how BMP/DBL-1 signaling impacts the molecular dynamics of FOXO within the nucleus.

BMP signaling plays an important role in growth and development and thus has implications in several human disease states (Wang et al., 2014). Yet reducing DBL-1/BMP activity in C. elegans and subsequently restricting growth through development possesses age-defying properties (Luo et al., 2009). This is reminiscent of another well-established age-extension paradigm in reduced insulin signaling (Kenyon et al., 1993), which also requires DAF-16 expression in the intestine (Libina et al., 2003; Uno et al., 2021). In both cases, whether it be insulin or BMP signaling, restricting but not ablatting its signaling capacity can extend the lifespan in a FOXO-dependent manner. Consistent with our observation regarding age regulation by neural hsf-1, BMP signaling acts upstream of insulin signaling and DAF-16 in the context of lipid metabolism (Clark et al., 2018, 2021). Recent studies have shown that neural hsf-1 regulates metabolic genes, which control fatty acid saturation and thus have the capacity to impact membrane fluidity and overall lipid availability in the intestine (Chauve et al., 2021), the major site of lipid storage in the worm (Ashrafii et al., 2003). Perhaps similar to the disposable soma theory of aging (Kirkwood & Holliday, 1979).
restricting organismal growth and development earlier in life as observed in neural hsf-1 transgenics might enable these animals to reinvest their molecular resources and metabolic reserves later in life. Yet the same metabolic genes regulated by neural hsf-1 over-expression (Chauve et al., 2021) were also reported to be similarly modulated upon hsf-1 RNAi treatments (Brunquell et al., 2016). Thus, further studies are required to understand this inexplicable link between HSF-1, BMP signaling, and lipid metabolism. While our studies focused on the gut–neuron axis, BMP signaling to other tissues yields alternate physiological consequences as demonstrated by BMP/DBL-1 signaling to the hypodermis, which is an important determinant in animal body length (Wang et al., 2002). Our studies show that rescuing SMA-6 expression exclusively in the intestine of the short-lived sma-6(wk7) enables neural hsf-1 to extend lifespan. This is consistent with the requirement of DAF-16 in the intestine for neural hsf-1 to extend animal lifespan (Douglas et al., 2015). Yet in both cases, a lifespan extension typical of neural hsf-1 was not fully achieved by the enteric rescue of either SMA-6 or DAF-16, suggesting that signaling between other tissues like the hypodermis or the germline, likely plays a complementary role in age regulation by the heat shock factor.

Our data suggest that HSF-1 in the nervous system communicates to DAF-16 in the intestine through BMP/DBL-1 signaling. Previous studies have demonstrated that the homeobox domain protein, CEH-28, activates DBL-1 expression specifically in M4 neurons (Ramakrishnan et al., 2014). Our data provide additional regulatory information regarding the controlled expression of this DBL-1 ligand as HSF-1 appears to bind consensus heat shock elements in the 5′ promoter region of DBL-1. It remains unclear whether HSF-1 acts in these M4 neurons in conjunction with CEH-28 to modulate DBL-1 expression or whether it is acting in other parts of the nervous system to repress dbl-1 expression and promote longevity. Upon receipt of the DBL-1 ligand in peripheral tissues, signal transduction ultimately leads to the nuclear accumulation of SMA-2/SMA-3 (Gumienny & Savage-Dunn, 2013), which can co-occupy some of the same DNA regulator elements as DAF-16 (Qi et al., 2017). We observe the SMA-10 and several RAB GTPases are significantly repressed by neural hsf-1 and in dbl-1 mutants. Consistent with the removal of dbl-1 being sufficient to repress transcription of these genes, we observed SMA-3 binding to the promoter regions of these membrane trafficking genes. Thus, under times of growth and development, DBL-1 would likely promote the expression of these genes to help establish polarity and maximize nutrient uptake through ensured cell surface residency of nutrient transport machinery. While neural hsf-1 reduces dbl-1 expression and extends animal lifespan, dbl-1 ablation does not extend animal lifespan in our laboratory, yet it still acts to repress transcription of sma-10 and several Rab GTPases, and reduce steady-state levels of intestinal SMA-6::GFP. Thus, further characterization of nuclear interactions between SMA-3 and DAF-16 might help identify additional factors that contribute to lifespan extension through reduced DBL-1 signaling.

4 | METHODS

4.1 | Caenorhabditis elegans strains and maintenance

Worm strains were maintained at 15°C on an OP50 E. coli lawn grown on nematode growth medium (NGM) plates and all experiments were performed at 20°C unless otherwise noted in the text. Age synchronization was performed by treatment with hypochlorite of gravid animals to obtain eggs. The following strains were obtained from the Caenorhabditis Genetics Center (CGC): N2 (wild-type (WT)), CF512 (nrf-3[b26] II; fem-1[hc17] IV), NU3 (dbl-1[nk3] III), LT186 (sma-6[wk7] II), CB491 (sma-3[e491] IIII), LW2436 (RAD-SMAD: jjls2277 [pCXT51[5′RLR::pes-10p(deleted)::GFP] + LiuFD61[mech-7p::RFPI]); I or IV).

The following strains were obtained from external laboratories: AGD1289 (neural hsf-1 FL: uthls368[rab-3p::HSF-1 FL, myo-2p::tomato]) worm strain was obtained from the laboratory of Andrew Dillin. LT620 (wkEx52 [spp-9p::GFP]) was created by the Richard Padget group. RT2495 (unc-119(ed3); pws1921[vha-6p::SMA-6::GFP]) was created by the Barth Grant group.

The following strains were generated for this study: PMD22 (dbl-1[nk3] V; uthls368[rab-3p::HSF-1 FL, myo-2p::tomato] strain) by crossing strain NU3 with AGD1289, PMD23 (sma-6[wk7] IIII; uthls368[rab-3p::HSF-1 FL, myo-2p::tomato] strain) by crossing strain LT186 with AGD1289, PMD145 (wkls52(spp-9p::GFP); integrated on X chromosome) by integrating strain LT620, PMD93 (spp-9p::gfp [integrated]; uthls366[rab-3p::HSF-1 FL, myo-2p::tomato] strain) by crossing strain PMD145 with AGD1289, PMD79 (uthls368[rab-3p::HSF-1 FL, myo-2p::tomato] strain; jjls2277 [pCXT51[5′RLR::pes-10p(deleted)::GFP] + LiuFD61[mech-7p::RFPI]; integrated on LG I or V; RAD-SMAD), PMD35 (vha-6p::SMA-6::GFP; uthls368[rab-3p::HSF-1 FL, myo-2p::tomato] strain) by crossing strain RT2495 with AGD1289, PMD87 (vha-6p::SMA-6::GFP; dbl-1[nk3]) by crossing strain RT2495 with NU3, PMD74 (vha-6p::SMA-6::GFP; sma-6[wk7]) by crossing strain RT2495 with LT186, PMD69 (uthls368[rab-3p::HSF-1 FL, myo-2p::tomato] strain; sma-6[wk7]; vha-6p::SMA-6::GFP [intestine]), PMD144 (wkEx52 [spp-9p::gfp [extrachromosomal]]; PMD74 [wkEx52 [spp-9p::gfp [extrachromosomal]]; uthls368[rab-3p::HSF-1 FL, myo-2p::tomato] strain]) by crossing strain PMD144 with AGD1289. PMD64 (wkEx52 [spp-9p::gfp]; uthls368[rab-3p::HSF-1 FL, myo-2p::tomato] strain).

4.2 | RNAi administration

RNA interference experiments were performed using RNAi strains acquired from C. elegans RNAi feeding libraries created by Ahringer and Vidal. Each library consists of HT115 E. coli containing the L4440 empty vector (EV) plasmid that houses various RNAi constructs for the knockdown of specific genes. Within this study, HT115 E. coli containing the L4440 empty vector (EV) was used as control versus other various RNAi constructs. RNAi was initially cultured in liquid
Terrific Broth (TB) for 14–16 h at 37°C then induced with 1 mM IPTG for 4h at 37°C. Bacterial cultures were concentrated 1/10 via centrifugation at 4400 g for 10 min, seeded onto RNAi plates (60 mm or 100 mm, NGM containing 100 µg/ml carbenicillin and 1 mM IPTG added 24 h prior to seeding), and allowed to dry for 1–2 days at room temperature, protected from light.

4.3 Caenorhabditis elegans lifespan analysis

Worms were age-synchronized using hypochlorite treatment of gravid animals for egg retrieval. Eggs were transferred onto RNAi plates seeded with EV control or different RNAi constructs. 100 µg/ml 5-fluorouracil-2'-deoxyribose (FUDR) was supplemented to media plates on Day 1 of adulthood to avoid progeny that would disrupt the synchronization of the worm population. Each lifespan was set up to consist of 10 plates per condition, with at least 10 worms per plate, and at least 100 worms per condition. Plates were scored every day or every other day for “censored” or “dead” worms. Worms that were censored were those that were missing from the plate, bagged with progeny, exploded/ruptured, or desiccated. Whole plates were only censored in the event of fungal/bacterial contamination. Worms that showed no response to touch with a platinum wire at the head or tail were scored as dead. To alleviate transgenic silencing observed in neural hsf-1 transgenic animals (AGD1289), worms were thawed from frozen stocks and allowed to recover for 3–4 generations prior to lifespan analysis.

4.4 Quantitative PCR

Quantitative reverse-transcriptase PCR (qPCR) was performed as previously described Egge et al. 2019. Worms were age-synchronized by hypochlorite treatment and grown on EV control or experimental RNAi at 20°C until the L4 stage of development. Total RNA was extracted by three rounds of freeze/thaw using TRIzol (ThermoFisher Scientific), followed by a chloroform and isopropanol precipitation extraction process. RNA pellets were rinsed twice with 75% ethanol, air-dried, and re-suspended in 20–50 µl molecular biology grade water. A DS-11 FX+ spectrometer (DeNovix) was used to measure RNA concentration including 260/280 and 260/230 ratios. Reverse transcription was conducted using the QuantiTect Reverse Transcription kit (Qiagen). 1 µg of RNA was used to synthesize cDNA according to the manufacturer’s guidelines. For qPCR, 20 µl reactions were performed using the iTaq™ Universal SYBR® Green Supermix kit (Bio-Rad) with 5 ng cDNA per well in a CFX384 Real Time System (Bio-Rad). Each sample was loaded in technical triplicate, and three biological replicates were analyzed for each condition. The relative transcript levels for each target gene and two housekeeping genes, tba-1 and Y45F10D.4, were calculated with the ∆Ct method. The geometric mean of the two housekeeping transcripts was used to normalize target gene expression. The following primers were used for qPCR analysis:

hsf-1 FWD: 5′-TCAGACAGTTGAATATGTACGG-3′
hsf-1 REV: 5′-CTTGATCTGATCTGTTCCAG-3′
dbl-1 FWD: 5′-AATCTGTTGTCCTGTGCTC-3′
dbl-1 REV: 5′-CGGATGTCGCCAGACTCTC-3′
daf-7 FOR: 5′-CTTCTTTCCCTCCACATGTG-3′
daf-7 REV: 5′-AGGTACTCTGTGGTGCTGT-3′
sp-9 FOR: 5′-GTGGAGAAAATATCCCTTCCG-3′
sp-9 REV: 5′-GCGTAGTTTTAGACAAGTCT-3′
sma-6 FWD: 5′-AGGGTCGCGGTGATCGCAAAGG-3′
sma-6 REV: 5′-TACGACCCAGTAACTCCTCCG-3′
sma-10 FWD: 5′-AGTTTTGGCAAGAAGACACGTG-3′
sma-10 REV: 5′-ATCCAACTTCCCAGTAAAGC-3′

All amplifications were performed at 95°C for 1 min, followed by 40 cycles at 95°C for 15 s, 60°C for 1 min, and a melt curve analysis consisting of 60 cycles at 65–95°C, 0.5°C/cycle (increment), 0.5°C/s (ramp).

4.5 Protein extraction and western blotting

All western blots were performed on late L4 larvae/day 1 adult worms. Animals have washed off the plates with liquid M9 buffer, centrifuged at 1000 g for 30 s, and washed twice with M9 before being transferred to 1.5 ml Eppendorf tubes and rapidly flash frozen in liquid nitrogen. Frozen worm pellets were thawed on ice and worm extracts were generated by glass/zirconia bead disruption in non-denaturing lysis buffer (50 mM Hepes pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton, EDTA-free mini-protease inhibitor cocktail [Roche]). Crude lysates were subject to centrifugation at 8000 g at 4°C for 5 min prior to protein determination with a BCA protein quantification kit (Thermo Scientific). Lysates were supplemented with 2x Laemmli sample buffer, boiled at 90°C for 10 min, resolved by SDS-PAGE, transferred to 0.22 µm nitrocellulose membranes, and subject to western blot analysis (Egge et al., 2019). Electrophoresis was performed at a constant 100 V and protein size was compared with the prestained protein ladder (EZ-RUN, ThermoFisher).

All antibodies were prepared in 5% BSA/PBST. The membrane was incubated with primary antibody overnight at 4°C followed by the secondary antibody for 1 h at room temperature. Rabbit anti-α-Tubulin (Cat. No. ab4074, Abcam) primary antibody was used at 1:15,000, and secondary anti-IgG goat polyclonal antibody horseradish peroxidase (Cat. No. 20403-1ML, Biotium) was used at 1:5000. Both the rabbit anti-GFP antibody (ThermoFisher, A6455) and the anti-IgG goat polyclonal antibody horseradish peroxidase (Cat. No. 20403-1ML, Biotium) were used at 1:5000. Bands were visualized after incubation with Clarity Western ECL Substrate (Bio-Rad, 1705061) and detection on film was performed using a Konica Minolta SRX-101A film processor. Samples were run in triplicate and ratioed band intensities were averaged for each biological replicate.
4.6 | Microscopy

For representative fluorescence micrographs of transcriptional reporter stains, five to six worms per condition were aligned on NGM/carb plates in a drop of M9 supplemented with 100 mM levamisole. Imaging was performed on a Zeiss Axio Zoom.V16 set to 100× magnification. Images were acquired using transmitted light and standard filter settings for excitation and emission of fluorescence probes and recorded on a CCD camera (Zeiss AxioCam 503 mono). Zeiss ZEN software was used to control acquisition. Exposure settings and additional processing parameters remained consistent among samples in each experiment.

To examine SMA-6::GFP co-localization with FM4-64, approximately 100 days, 1 adult worms were rinsed off plates with M9 buffer, centrifuged (1000 g) for 30 s, and washed twice with M9 buffer. Worms were then incubated in 100 μl 0.4 mM FM4-64 (Fisher Scientific, T3166) for 2 h at 20°C in an Eppendorf Thermomixer C shaker set to 300 rpm. Following incubation, worms were washed three times with M9 and allowed to recover on NGM plates seeded with OP50 for approximately 30 min in the dark. Confocal micrographs were acquired using a Leica SP8 confocal microscope (Leica, Buffalo Grove, IL) and Leica Application Suite X (LAS X) software. Live worms were mounted in M9 supplemented with 100 mM levamisole and samples were imaged at 40× or 63× with oil immersion. All images were acquired in xyz acquisition mode at a speed of 600, and each line was imaged three times and averaged to reduce noise. Lasers 488 and/or 552 were used to excite fluorophores using hybrid detectors (HyD). Laser power, range, and gain were adjusted according to strain/experiment but remained consistent between independent repeats.

4.7 | Large-particle flow cytometry

A COPAS FP-250 flow cytometer (Union Biometrica) was used to analyze age-synchronized worms using the attached sample cup or acquired from a 96-well plate by an LP Sampler (Union Biometrica). Sample solution was comprised of M9 while the flow sheath solution contained a proprietary recipe, COPAS GP sheath reagent (PN: 300-5070-100, Union Biometrica). Flow data were collected and processed using the FlowPilot software (ver. 2.6.1, Union Biometrica). Further data processing was performed in Excel (Microsoft) and statistical analysis in Prism 9 (GraphPad). Extinction was detected using the 488 nm laser line with a 1.3 ND filter and the gain of 1.0. Propidium iodide, mCherry, and DsRed were excited using a 561 nm laser, while YFP and GFP were excited using a 488 nm laser. Gains for fluorescence detection were set to 2.0, and PMT voltage adjusted within the linear range of the instrument was consistent for each experiment (500 for YFP, mCherry, DsRed, and 450 for GFP). WormProfiler (an in-house program written for the MATLAB runtime) was used to standardize worm lengths and calculate fluorescence intensity profiles for individual worms within samples. Unless otherwise noted, the integral of the fluorescence intensity for each worm within samples was averaged, and mean values were normalized according to time-of-flight (TOF) to account for variation in worm size across RNAi conditions. Mean integral or peak fluorescence values were standardized relative to empty vector control.

4.8 | Transcriptomics and bioinformatics

Three biological repeats of age-synchronized wild-type, neural hsf-1 (AGD1289), dbl-1(nk3) (NUS), and neural hsf-1;dbl-1(nk3) (PMDF22) worms cultured on HT115 E. coli until late L4 larval stages were collected and rapidly flash frozen in liquid nitrogen. RNA was purified by chloroform/phenol extraction followed by isopropanol precipitation and two washes with 75% ethanol before resuspension in 50 μl molecular biology grade water. Quality control, mRNA purification, and paired-end 150 bp Illumina sequencing were performed by Novogene as previously described (Egge et al., 2021). Statistical analysis was performed using CLC software (version 9.5, CLC Bio). Gene expression data were compared by the Baggerly’s test and genes were considered significantly regulated when absolute fold change ≥1.5 and p-value <0.05. Deposited ChIP-seq datasets from the previous publication were analyzed using the CLC genomic workbench software v9.5. Raw data obtained from either Geo Datasets (GSE81523 for HSF-1::GFP ChIP-seq) or EnCode datasets (ENCSR992FVB for SMA-3::GFP ChIP-seq) were trimmed and mapped to the reference C. elegans genome (NCBI accession number NC_002944). With respect to HSF-1 binding within the Dbl-1 promoter in a subset of neurons, genomic reads isolated in complex with HSF-1::GFP were divided by the total input reads for the same respective genomic region. The CLC shape-based peak caller was used for all SMA-3::GFP ChIP. ChIP-enriched DNA was aligned onto input DNA; when the sequence coverage of a genomic region in the enriched DNA exceeded the input DNA, a ChIP peak score was called. ChIP peaks with their respective P values determined by CLC were generated for candidates whose transcript levels were differentially regulated as determined by RNAseq. To identify heat shock element consensus motif presence in the 5′ untranslated promoter region of the Dbl-1 gene, we examined 2.5 kilobases upstream of the Dbl-1 translation start site using the MEME suite (v4.11.1) (Bailey et al., 2015). Additional analysis for HSEs within the promoters of other genes was determined using TFBind (https://tfindb.hgc.jp/). For each gene, 1000 bp upstream of the TSS (translational start site) was searched for sites with >90% sequence similarity of the HSF-1 consensus binding site, obtained from the transcription factor matrix ID (TRANSFAC R.3.4), on both the (+) and (−) strand.

4.9 | Statistical analysis

Statistical analyses, including t-test, mixed-effects, Chi-squared, and ANOVA with post hoc multiple comparisons analysis, were conducted using Prism (version 9.0, GraphPad) and statistical analysis for large transcriptomic datasets was performed using CLC genomics
workbench (version 9.5). For large worm populations, outliers were removed using the ROUT method (Q = 1%) prior to analysis.

AUTHOR CONTRIBUTIONS
S.L.B.A., J.M., and P.M.D. involved in conceptualization; S.L.B.A., J.M., L.T., A.W., K.R.Z., B.M., T.L.G., and P.M.D. involved in methodology; S.L.B.A., J.M., L.T., A.W., K.R.Z., B.M., T.L.G., and P.M.D. involved in investigation; S.L.B.A., T.L.G., and P.M.D. involved in writing—review and edit; T.L.G. and P.M.D. involved in funding acquisition, resources, and supervision.

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CONFLICT OF INTEREST
The authors declare no competing interests.

DATA AVAILABILITY STATEMENT
All data generated and analyzed during this study are included in this article and its extended data are also available from the authors upon reasonable request. Transcriptomic data files that support the findings of this study in C. elegans will be deposited in the NCBI Gene Expression Omnibus (GEO) upon publication.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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