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Pattern Formation in the Longevity-Related Expression of Heat Shock Protein-16.2 in *Caenorhabditis elegans*

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Abstract

Aging in *Caenorhabditis elegans* is controlled, in part, by the insulin-like signaling and heat shock response pathways. Following thermal stress, expression levels of small heat shock protein-16.2 show a spatial patterning across the 20 intestinal cells that reside along the length of the worm. Here, we present a hypothesized mechanism that could lead to this patterned response and develop a mathematical model of this system to test our hypothesis. We propose that the patterned expression of heat shock protein is caused by a diffusion-driven instability within the pseudocoelom, or fluid-filled cavity, that borders the intestinal cells in *C. elegans*. This instability is due to the interactions between two classes of insulin-like peptides that serve antagonistic roles. We examine output from the developed model and compare it to experimental data on heat shock protein expression. Given biologically bounded parameters, the model presented is capable of producing patterns similar to what is observed experimentally and provides a first step in mathematically modeling aging-related mechanisms in *C. elegans*.

Keywords

Aging; Diffusion-Driven Instability; Insulin-like Signaling; Reaction Diffusion Model

1 Introduction

Aging in *Caenorhabditis elegans* is linked to many physiological processes including the heat shock response and insulin-like signaling. The expression levels of heat shock proteins (HSPs) in *C. elegans* decrease with age, suggesting HSP expression is a biomarker of aging (Lund et al, 2002). In isogenic populations of *C. elegans* a fourfold variation in lifespan following thermal stress is predicted by the intestinal transcription of a green fluorescence protein (GFP) reporter, connected to the promoter of a small heat shock protein gene, *hsp-16.2* (Rea et al, 2005). The expression of this GFP reporter is not uniform across all

intestinal cells but rather shows distinct spatial patterning, with magnitudes that vary in conjunction with the worm's lifespan (Seewald et al, 2010). Patterns consistently show elevated expression of GFP in the anterior and posterior intestinal cells and decreased expression in the middle intestinal cells (Mendenhall et al, 2015). The mechanism behind the patterned expression is currently unknown. With the use of mathematical modeling, we aim to explore possible mechanisms that lead to the emergence of *hsp-16.2* patterns in intestinal cells. We predict that the patterned gene expression is caused by an activatory/inhibitory interaction between two classes of insulin-like peptides (ILPs). Modeling offers a means to understand how these two ILP classes diffuse through the pseudocoelomic fluid and end up reproducibly activating gene expression in different cells to different degrees. This level of detailed modeling offers feasible explanations for why patterns of gene expression may change in different biological scenarios, including during aging.

The heat shock response is characterized by the increased production of HSPs. Many HSPs act as chaperones and help stabilize and refold proteins denatured by elevated temperature or other proteotoxic stressors (Haslbeck et al, 2005). The heat shock response is activated both autonomously within a cell and via intercellular signaling pathways. In *C. elegans* subjected to heat shock, neuronal signals amplify the cell autonomous heat shock response (Prahlad and Morimoto, 2011; Prahlad et al, 2008). Genetic evidence has shown that the insulin-like signaling system interacts with thermosensory neurons to control both the average heat shock response and the interindividual variation observed in the heat shock response (Prahlad et al, 2008; Mendenhall et al, 2017). We propose that the patterned expression of *hsp-16.2* results from signals downstream of neuronal signaling rather than from the cell autonomous response. In this paper we explore the mechanism behind the patterned expression of heat shock proteins in intestinal cells

We hypothesize that the neuronal regulation of the heat shock response acts through the insulin-like signaling pathway (Prahlad and Morimoto, 2009). The insulin-like receptor encoded by *daf-2* binds any of the 40 different agonizing or antagonizing insulin-like peptides to inhibit or permit the nuclear translocation of the forkhead transcription factor encoded by *daf-16*. Furthermore, DAF-16 likely plays a role in the transcription of small HSP genes, including *hsp-16.2* (Hsu et al, 2003; Hartwig et al, 2009; Walker and Lithgow, 2003), and decreasing nuclear DAF-16 has a direct impact on the level of ILP transcription (Tepper et al, 2013). These interactions suggest that ILPs regulate their own production through positive and/or negative feedback loops.

We propose that the spatial patterning of *hsp-16.2* reporter expression along intestinal cells arises due to a diffusion-driven instability involving ILPs and the DAF-2 receptor. Diffusion-driven or Turing instabilities provide an explanation for how patterns can emerge in biological systems (Murray, 2001). Researchers have used these instabilities to explain a wide range of biological phenomenon, from embryonic development to animal coat pattern formation. One class of reaction diffusion systems that is known to produce patterns involves both an activator and an inhibitor species. The activator stimulates its own production as well as the production of the inhibitor, while the inhibitor blocks both its own production and the production of the activator. Furthermore, the inhibitor diffuses at a faster rate than the activator. We hypothesize that the insulin-like signaling pathway falls into this

class of reaction diffusion systems. The set of ILPs that act as DAF-2 antagonists represents the activator and the set of ILPs that act as DAF-2 agonists represents the inhibitor. The interaction between the two classes of ILPs in the presence of their diffusive properties leads to an instability that results in patterned ILP concentrations. As a result DAF-2 is activated in a spatially dependent manner. The transcription of *hsp-16.2*, in turn, demonstrates a similar spatial patterning.

Here, we develop and analyze a mathematical model to examine this system, and we show that the model is capable of producing patterns reminiscent of what is observed experimentally. The model simulates the ILP-driven response to heat shock (i.e., the diffusion of insulin-like peptides through the pseudocoelom along the surface of intestinal cells) and not the actual heat shock incident. In the methods, we describe the mathematical model development and detail a new analysis of previously collected data on *hsp-16.2* reporter expression in *C. elegans*. We then present the results of the mathematical model and examine the required parameter constraints to obtain diffusion-driven instability. We compare the model predictions to the analyzed data and conclude that the model successfully reproduces the *hsp-16.2* reporter expression patterns. Our results suggest that two groups of worms exist with different *hsp-16.2* expression profiles. This is the first mathematical model of the neuronally controlled heat shock response in *C. elegans*. The model can be used to predict how biological parameters affect HSP expression and, ultimately, can help assess the effect of specific biological perturbations on aging.

2 Methods

2.1 Model Development

The standard framework for the study of pattern formation in reaction diffusion equations involves an activator A and an inhibitor B. For a system with two spatial dimensions, this can be qualitatively described by

$$\begin{aligned} A_t(t, x, y) &= \text{activator diffusion} + \text{activator reactions} \\ B_t(t, x, y) &= \text{inhibitor diffusion} + \text{inhibitor reactions} \end{aligned} \quad (1)$$

where the subscripted t represents the derivatives of these concentrations with respect to time. We can more quantitatively describe the model using a general conservation law model:

$$\begin{aligned} A_t(t, x, y) &= D_A \nabla^2 A + F(A, B) \\ B_t(t, x, y) &= D_B \nabla^2 B + G(A, B), \end{aligned} \quad (2)$$

where D_A and D_B are diffusion coefficients and F and G are functions modeling the activator and inhibitor reactions, respectively.

In this framework, the activator A represents the ILPs that act as DAF-2 antagonists, and the inhibitor B represents ILPs that act as DAF-2 agonists (Figure 1). The DAF-2 antagonists activate the production of both classes of ILPs by allowing DAF-16 nuclear translocation. The DAF-2 agonists block DAF-16 nuclear translocation, and, thus, inhibit the production of both classes of ILPs. The *C. elegans* genome contains 40 genes encoding possible ILPs. Of these genes, eight have been shown to be transcriptionally regulated by DAF-16 (Tepper et al, 2013; Murphy et al, 2003; Kaletsky et al, 2016). For example, both *ins-6* and *ins-18* are positively regulated by DAF-16. *INS-6* acts as a DAF-2 agonist and is therefore a member of the inhibitor class B (Hua et al, 2003), and *INS-18* acts as a DAF-2 antagonist and belongs to the activator class A (Pierce et al, 2001). The only ILP gene that is known to decrease in response to DAF-16 nuclear translocation is *ins-7*, which codes a DAF-2 agonist. Thus, the characteristics of *INS-7* do not clearly match the characteristics of the inhibitor class. However, we hypothesize that, although *ins-7* is downregulated by nuclear DAF-16, the net effect of DAF-16 on ILPs that are DAF-2 agonists is stimulatory. An alternative model was explored in which DAF-16 led to a net decrease in DAF-2 agonist production, but we found that this model was not capable of pattern formation (See Appendix A).

In order for the mathematical model to give rise to patterns, the diffusion rates between the two classes of ILPs must be significantly different. Specifically, the inhibitor B must diffuse at a faster rate than the activator A . We examined the biological feasibility of this by quantifying the surface hydrophobicity of *INS-18* and *INS-6*, which serve as representatives for the activator and inhibitor class, respectively. A more hydrophobic surface leads to a stickier protein which in turn slows the rate of diffusion. We used I-TASSER to find the top five 3D structural models for each peptide (Roy et al, 2010; Yang et al, 2015; Zhang, 2008) and PBDViewer to find the area of hydrophobic patches on the surface of the protein (Guex and Peitsch, 1997). Since none of the five models for either *INS-18* or *INS-6* obtained a C-score greater than -1.5 (the threshold C-score to validate the physical model), we decided to look at the average surface hydrophobicity across the models. We performed two t-tests to compare (1) the total hydrophobic surface area and (2) the relative hydrophobic surface area between the two peptides. The results, presented Section 3.2, show that the models for *INS-18* have a larger relative hydrophobic surface area than the models for *INS-6*. This is in agreement with the requirement that the ILPs in the inhibitor class diffuse faster than ILPs in the activator class.

To mathematically model the reactions in the system, we first derive equations for the extracellular DAF-2 and ILP interactions and then incorporate the intracellular effects when DAF-2 is activated. We use Michaelis-Menten kinetics with competitive inhibition to model the extracellular binding of the two classes of ILPs to the DAF-2 receptor. We assume the binding of ILPs to DAF-2 happens on a much faster time scale than other reactions in the system. In this analysis we use the amount of agonist (B) that is bound to DAF-2, denoted as BR , as a proxy for the activation of an intracellular pathway. The concentration of ILP agonist bound to the DAF-2 receptor is approximated by the following Michaelis-Menten relationship,

$$BR = \frac{R_0 B}{K_{D,B} \left(1 + \frac{A}{K_{D,A}} \right) + B}, \quad (3)$$

where $K_{D,B}$ is the dissociation constant of the DAF-2 agonist class of ILPs, $K_{D,A}$ is the dissociation constant of the DAF-2 antagonist ILPs, and R_0 is the total concentration of DAF-2 receptors. To relate Equation 3 to an intracellular pathway, we introduce a new variable P that represents an intracellular product formed by the activation of DAF-2. The change in P with time is approximated as

$$\frac{dP}{dt} = \frac{V_{\max} B}{K_{D,B} \left(1 + \frac{A}{K_{D,A}} \right) + B} - k_7 P, \quad (4)$$

where $V_{\max}$ is the maximum rate of product formation, and k_7 is the specific rate at which the product P leaves the system. We assume the system rapidly reaches equilibrium and, thus, calculate P explicitly by setting Equation 4 equal to zero,

$$P = \frac{V_{\max} B}{k_7 \left(K_{D,B} \left(1 + \frac{A}{K_{D,A}} \right) + B \right)}. \quad (5)$$

When DAF-2 is activated, a complex set of intracellular processes takes place that leads to the phosphorylation of DAF-16, blocking nuclear translocation (Ewbank, 2006). We note that the product P can be viewed as an intermediate molecule in this process such as, for example, AGE-1. For the mathematical model, we simplify this system and incorporate a direct effect of P on the production of A and B . As the amount of P in the system increases there is less nuclear DAF-16 (assuming total DAF-16 within a cell is constant) and therefore the production of A and B decreases. We use a hill function to describe this effect and write the final reactions as

$$F(A, B) = k_1 - k_2 \frac{P^r}{H^r + P^r} - k_3 A \quad (6)$$

$$G(A, B) = k_4 - k_5 \frac{P^r}{H^r + P^r} - k_6 B. \quad (7)$$

where H is the concentration of P at which the effect of P on the nuclear translocation of DAF-16 is half maximized, and r is the hill-coefficient that determines the steepness of the hill function.

The system given by Equations 2, 5, 6, and 7 was made dimensionless using the following substitutions,

$$\begin{aligned} t^* &= D_A t / L^2 & x^* &= x / L & y^* &= y / L \\ d &= D_B / D_A & \gamma &= k_3 L^2 / D_A & u &= A k_3 / k_1 \\ v &= B k_3 / k_4 & p &= P k_7 / V_{\max} & a &= k_2 / k_1 \\ b &= k_5 / k_4 & c &= k_6 / k_3 & h &= H (k_7 / V_{\max}) \\ k_{D,A} &= K_{D,A} k_3 / k_1 & k_{D,B} &= K_{D,B} k_3 / k_4 \end{aligned}$$

resulting in the following set of equations

$$\frac{\partial u}{\partial t^*} = \gamma f(u, v) + \nabla^2 u \quad (8)$$

$$\frac{\partial v}{\partial t^*} = \gamma g(u, v) + d \nabla^2 v \quad (9)$$

where

$$f(u, v) = 1 - a \frac{p(u, v)^r}{h^r + p(u, v)^r} - u \quad (10)$$

$$g(u, v) = 1 - b \frac{p(u, v)^r}{h^r + p(u, v)^r} - cv \quad (11)$$

$$p(u, v) = \frac{v}{K_{D,B} \left(1 + \frac{u}{K_{D,A}} \right) + v} \quad (12)$$

Here L represents the length of the worm. For notational simplicity, from here forward, we will use x and y to represent x^* and y^* , respectively. We determined approximate solutions to the PDE by linearizing the system about the spatially homogeneous steady state (u_0, v_0) , calculated by setting Equations 10 and 11 equal to zero. The steady state of this system was

calculated numerically using a Newton method as implemented in the nlqslv package of R (Hasselman, 2016). We solved the linearized system on a cylindrical domain (Figure 2). The shape of the domain was approximated based on the dimensions of *C. elegans*, where the length of the domain l_1 represents the length of the worm and the height of the domain l_2 represents the circumference of the worm. Since we are working in nondimensional coordinates where the characteristic length L is equal to the length of the worm, $l_1 = 1$. In *C. elegans* the length to maximum diameter ratio is approximately 20 (Hirose et al, 2003). Thus we set $l_2 = 0.2l_1$. The following boundary conditions are imposed on the domain ($W = [u - u_0, v - v_0]$):

$$\begin{aligned}\frac{dW}{dx} \Big|_{x=0, l_1} &= 0 \\ \frac{dW}{dy} \Big|_{y=0} &= \frac{dW}{dy} \Big|_{y=l_2}.\end{aligned}$$

Periodic boundary conditions in the y direction are used to approximate diffusion on a 2D cylindrical surface. Using these boundary conditions, the solutions to the linear problem take the form

$$\mathbf{w}(x, y, t) = \sum_k c_k e^{\lambda t} \mathbf{W}_k(x, y) \quad (13)$$

where the time independent eigenfunctions are

$$\mathbf{W}_k(x, y) = \mathbf{B} \cos\left(\frac{n\pi}{l_1}x\right) \left(\mathbf{C} \sin\left(\frac{2m\pi}{l_2}y\right) + \mathbf{D} \cos\left(\frac{2m\pi}{l_2}y\right) \right) \quad (14)$$

and the wavenumber k is given by

$$k^2 = \left(\frac{n\pi}{l_1}\right)^2 + \left(\frac{2m\pi}{l_2}\right)^2. \quad (15)$$

Here λ is the eigenvalue that determines the temporal growth of the modes and it is given by the roots of

$$\lambda^2 + \lambda(k^2(1+d) - \gamma(f_u + g_v)) + dk^4 - \gamma(df_u + g_v)k^2 + \gamma^2(f_u g_v - f_v g_u) = 0 \quad (16)$$

(see Murray, 2001 for details). The partial derivatives of f and g are calculated at the steady state values of the system (u_0, v_0). The expression $\lambda(k^2)$ will be referred to as the *dispersion relation*. Modes with $\lambda(k^2) > 0$ grow as time increases, and represent the possible patterns that can be produced.

We used the CountourPlot3D and RegionPlot3D functions in Mathematica to numerically identify regions of parameter space that can lead to pattern formation (Wolfram Research Inc., 2016). These functions initially evaluate the criteria for pattern formation on a 3D grid and then uses an adaptive algorithm to evaluate the function on select subdivided regions to obtain the regions where the specified criteria are met. We generated two sets of region plots. First we examined which reaction parameters satisfied the Routh-Hurwitz criterion. Then we incorporated spatial properties, including diffusion and domain size, to determine how these properties affect the number of possible modes that can result.

2.2 Data Analysis

We compared our model output to data on *hsp-16.2* reporter expression in *C. elegans* (Mendenhall et al, 2015). Our previous study (done by the Mendenhall lab) obtained relative *hsp-16.2* reporter expression levels in each of the 20 intestinal cells for 28 worms. Expression was measured 24 hours after heat shock using a *C. elegans* strain that contained a GFP reporter coupled to the promoter of *hsp-16.2* ($P_{hsp-16.2}$ -GFP). Thus, GFP fluorescence quantified in individual cells serves as a reporter of *hsp-16.2* transcription. We used the concentration of the relatively pure GFP signal in the nucleus as a proxy for the whole cell. Since nuclear fluorescence was over 97% correlated with the cytoplasmic signal (see Mendenhall et al, 2015, Figure 3), we would expect cells with larger nuclear $P_{hsp-16.2}$ -GFP concentrations to have larger cytoplasmic concentration as well. We then assume (1) a constant concentration of ILPs in the pseudocoelom leads to the same concentration of $P_{hsp-16.2}$ -GFP in the cell regardless of cell size and (2) changes to the local ILP concentrations would manifest in changes to the concentration of $P_{hsp-16.2}$ -GFP in the cell.

To analyze the available data, we first averaged the GFP signal in cells within the same intestinal cell ring. This was done to account for technical measurement error attributable to differences in animal orientation and sampling depth. There are a total of 9 intestinal cell rings in the *C. elegans* intestine; 4 cells in the anterior most cell ring and 2 cells in all the other cell rings. Cells within the same cell ring are at approximately the same distance down the length of the worm. Since our analysis predicts that variations in HSP production are only observed along the length of the worm and not along the circumference of the worm (see Section 3.1), we assume the rate of *hsp-16.2* transcription is the same for cells within a single ring. Next, we used a rolling window to average across cell rings and smooth the data. For interior cell rings, the window length was three. For example, the value at the 4th cell ring was averaged with the 3rd and 5th to obtain an updated value at the 4th cell ring. For the edge cell rings we averaged the value of that cell ring with its one neighbor. Finally, we normalized the data for each worm by dividing by the mean *hsp-16.2* transcription value across all intestinal cells. This analysis resulted in nine spatial data points for each worm.

To compare this data to predictions from the mathematical model, we decomposed the data into the relevant trigonometric modes. Due to the zero flux boundary conditions of the system, we used only cosine modes for the analysis (see Equation 14). Here, again we assume that $m = 0$ and thus the system reduces to a one dimensional problem. The nine normalized data points obtained for each worm lead to the inclusion of nine modes in the projection analysis. The contribution of each mode, $n = 0, 1, 2, \dots, 8$, is given as

$$p_n(x) = \alpha_n \cos\left(\frac{n\pi(x-1)}{8}\right) \quad (17)$$

where the expansion coefficients, α_n , are

$$\alpha_n = \frac{1}{16}(y_1 + \cos(n\pi)y_9) + \frac{1}{8} \sum_{i=2}^8 \cos\left(\frac{n\pi(i-1)}{8}\right)y_i, \quad n = 0, 8, \quad (18)$$

$$\alpha_n = \frac{1}{8}(y_1 + \cos(n\pi)y_9) + \frac{1}{4} \sum_{i=2}^8 \cos\left(\frac{n\pi(i-1)}{8}\right)y_i, \quad n = 1, 2, \dots, 7. \quad (19)$$

Here y_i represents the normalized data from the i th intestinal cell ring. Using the expansion coefficients obtained for each worm, we grouped the worms using a k-means clustering analysis. We assessed the validity of this clustering approach using a bootstrap analysis as implemented in Flexible Procedures for Clustering package in R (Hennig, 2015).

2.3 Model simulations

To examine how patterns form and what modes dominate with time, we simulated the reaction diffusion model using R v3.2.3 (R Core Team, 2015). The PDE was simulated over a 2D grid using a finite difference approach as implemented in ReacTran (v1.3.2) (Soetaert and Meysman, 2009). The grid over which the system was solved was 100×20 cells. This resolution corresponds to an approximate resolution of 10 μ m which is much smaller than the length of an intestinal cell (approximately 100 μ m) but much larger than the size of a protein. To perform simulations we used one of two initial conditions. The two sets of initial conditions correspond to hypothesized mechanisms that explain how the non-cell autonomous heat shock response is initialized in *C.elegans*. In Case I the domain was initialized using the homogeneous steady state values for each ILP class and Gaussian noise was added. This scenario is meant to represent the case where the heat shock response is initialized by neurons throughout the body of the worm. Each grid cell was initialized with the following dimensionless quantity of the two classes of ILPs

$$\begin{aligned} u_{ij} &= u_0 + \epsilon_{u,ij} \\ v_{ij} &= v_0 + \epsilon_{v,ij} \end{aligned}$$

where $\epsilon_{u,ij}$ and $\epsilon_{v,ij}$ were drawn from normal probability distributions with means at u_0 and v_0 and standard deviations of $0.10u_0$ and $0.10v_0$, respectively. This level of variance was chosen to allow us to explore the effect of noise on the evolution of the system when it is perturbed from the homogeneous steady state. The initialization method avoids assumptions about where the pattern is initialized.

In Case II for model initialization, we explore our hypothesis that the non-cell autonomous heat shock response is initialized by an ILP stimulus at the head of the worm. Here, ILPs are added to one edge of the domain, corresponding to a release at the head of the worm. In this case the first column of the grid was initialized with the reaction steady-state values and the rest of the grid had no ILPs. Specifically, if u_{ij} , v_{ij} represent the initial concentration of u , v , in grid cell (i, j) , for $i=1,2,\dots,20$, the initialization concentrations are given as

$$\begin{aligned} u_{i1} &= u_0 \\ v_{i1} &= v_0 \\ u_{ij} &= 0 \quad j = 2, 3, \dots, 100 \\ v_{ij} &= 0 \quad j = 2, 3, \dots, 100. \end{aligned}$$

Each simulation was run for a dimensionless time t^* of 2.0. We chose this value of t^* because it represents the same time scale as the experimental data from Mendenhall et al, 2015. Specifically, we have that $t^* = tD_A/L^2$, and we estimate that the length of the worm $L = 1000\mu\text{m}$ and the diffusion rate D_A is between 10 and $100 \mu\text{m}^2/\text{s}$, i.e., the diffusion rate is between that of a protein in water and that of a protein in a cell (Milo and Phillips, 2015). This corresponds to a time between 12 hours and 6 days, which is on the same time scale as the data from the experiment, obtained 24 hours post heat shock. When determining the steady-state of the system, in some cases it was necessary to run the simulation for a dimensionless time t^* of 3.0.

To quantify the duration of quasi-steady states, we calculated the rate of convergence with respect to an approximate L1 norm. To do this we considered the value of species u in the top row of the domain. Only the top row was considered because, after a short time interval, the concentration of u changes spatially only in the x -direction. We subtracted the mean value of u in the top row to obtain a function, $\tilde{u}(t)$, that could be directly compared to the predicted $e_n(x, t) = A(t) \cos(n\pi x)$. The difference between $\tilde{u}$ and e_n , which we denote $d(t)$, was calculated by summing the absolute difference between e_n and eu at the midpoint of each of the 100 cells and multiplying by the size of the grid cell in the x -direction. The rate of convergence was found to be roughly linear by considering $d(m+1)/d(m)$, where $m+1 - m = 0.01$.

The source code for all the analyses performed is available on GitHub¹.

3. Results

3.1 Reaction diffusion model predicts patterns along the length of the worm

The developed reaction diffusion model is capable of generating patterns reminiscent of intestinal *hsp-16.2* reporter expression patterns in *C. elegans*. The model has a total of 9 dimensionless parameters (Table 1). In order to explore the model's properties, where possible we set parameter values equal to biologically reasonable values. This was done to verify that patterns could occur under biologically feasible conditions. The parameters a and

¹ ReationDiffusionCElegans repository from MathBioCU group on GitHub (<https://github.com/MathBioCU>).

b were set equal to 1.0, implying that the expression of ILPs is completely under the control of DAF-16. That is, as p approaches infinity, all DAF-16 is blocked from entering the nucleus and the net ILP production rate approaches zero. The parameter c was set equal to one under the assumption that the two classes of ILPs decay at the same rate (i.e., $k_3 = k_6$). For the remaining four parameters that describe the reactions in the system (h , r , $k_{D,A}$, and $k_{D,B}$) we sought to find biologically reasonable parameter values that together could result in diffusion driven instability.

We determined ranges for biologically feasible values for $k_{D,A}$ and $k_{D,B}$ by first determining ranges for the values of the dissociation constants ($K_{D,A}, K_{D,B}$), ILP production rates (k_1 , k_4), and ILP degradation rates (k_3) as follows. In humans, insulin likely binds to its receptor in two stages, leading to two dissociation constants of approximately 40 and 170 nM (Subramanian et al, 2013). In our model we assume a single binding stage for all ILPs in *C. elegans* where the dissociation constant lies at a similar value (i.e. between 40 and 170 nM). Although it is possible that the true dissociation constant for a specific ILP lies outside this range, it is a reasonable estimate due to the biological similarity between DAF-2 and the human insulin receptor (Kimura et al, 1997) as well as between ILPs and insulin (Pierce et al, 2001). The degradation half-life of proteins can vary drastically from 10 minutes to several days (Belle et al, 2006; Cambridge et al, 2011). This leads to degradation rates that vary between 10^{-6} s^{-1} and 10^{-3} s^{-1} . As for production rates, the maximum protein production rate in *E. coli* ranges from 1 to 10^6 molecules per generation, where the generation time is 21.5 minutes (Li et al, 2014). To translate the production rate units from molecules to molarity, we used a pseudocoelom volume range of 40 – 80 pL (Banse and Hunter, 2012). Putting this all together, we estimated that $k_{D,A}$ and $k_{D,B}$ values are likely between 10^{-3} and 10^7 . This large range is primarily due to the uncertainty in ILP production and degradation rates. However, in subsequent sections, we find that only a portion of this range meets the requirements for pattern formation.

Biological ranges for the values of h and r are more elusive. The value of h depends on H , V_{max} , and the degradation rate k_7 . Both H and r are challenging to estimate since they describe the effect of DAF-2 activation on DAF-16 nuclear translocation, and the kinetics of this pathway have not been experimentally examined. Thus, to explore the pattern formation space we set a lower bound on h of 0.05 but note that h could potentially take on smaller values. Furthermore, the largest value of r we explore is 10, but again this is not a biologically strict upper bound.

To have a diffusion driven instability the parameter values must satisfy the following Routh-Hurwitz stability criterion

$$\begin{aligned} f_u + g_v &< 0 \\ f_u g_v - g_u f_v &> 0. \end{aligned} \quad (20)$$

For diffusion to destabilize this system, $df_u + g_v > 0$. These inequalities imply that that either $f_u < 0 < g_v$ or $g_v < 0 < f_u$. We found these criteria were satisfied when $k_{D,A} < 2$, $k_{D,B} > 10$, and $r > 1$ (Figure 3). When performing the grid search with adaptive refinements, we set the

minimum value of $k_{D,A}$ and $k_{D,B}$ equal to 0.001, the biological constraint obtained previously. We used integer values for r of 1, 2, 4, 6, 8, and 10. At $r = 1$, there were no regions that led to diffusion driven instability.

Although the constraints given by Equation 20 are necessary for pattern formation, they are not sufficient. Whether patterns can form and the number of possible modes also depends on the size of the domain and the difference in diffusion rates between the two classes of ILPs. For our purposes, the parameter γ determines the size of the domain while d gives the ratio of diffusion rates. An estimate for γ depends on the characteristic length L of the domain, the diffusion coefficient D_A , and the rate of ILP degradation, k_3 . As discussed previously the protein degradation rate and diffusion rates are likely between 10^{-3} and 10^{-6} s^{-1} and $10 - 100 \mu\text{m}^2/\text{s}$, respectively (Belle et al, 2006; Cambridge et al, 2011; Milo and Phillips, 2015). The characteristic length is equivalent to the length of the worm, or 1.0 mm. This leads to possible values of γ ranging from 0.01 to 100. Furthermore, we set a lower bound on the parameter d based on the requirement that, for diffusion driven instability to occur, d must be greater than the critical diffusion value d_c given by the roots of following equation (see Murray, 2001 for derivation):

$$d_c^2 f_u^2 + 2(2f_v g_u - f_u g_v) d_c + g_v^2 = 0. \quad (21)$$

For the example parameter set given (Table 1), the critical diffusion value d_c is 2.97, implying the inhibitor class B must diffuse at least 3 times faster than the activator class A.

We calculated the dispersion relation (Equation 16) as a function of γ at different values of d to determine how γ and d affect the number of possible modes (Figure 4). For this calculation all other parameter values were obtained from Table 1. Larger values of γ and d lead to a greater number of possible modes. In total when $d = 10$ and $\gamma = 100$, one of five modes can arise (Figure 4). These modes correspond to setting $n = 1, 2, 3, 4, 5$ and $m = 0$ in Equation 14. For larger values of γ the first mode ($n = 1$) eventually is no longer possible. In this analysis we found that, when setting $d = 10$ and $\gamma = 100$, there were no modes possible in which $m > 0$. This implies that fluctuations in the HSP expression only occur along the length of the worm and not along the circumference of the worm.

To determine the dimensionless dissociation constants with more precision, we examined which parameter values resulted in patterned modes and how many modes were possible. We did this by using the parameters given in Table 1 but varied r , $k_{D,A}$, $k_{D,B}$, and h to determine the number of possible stable modes as a function of the parameter values (Figure 5). Given the constraints on the parameter values specified previously, to obtain at least one patterned mode, $k_{D,A}$ must be less than 0.2, $k_{D,B}$ must be less than 2.5, and h must be less than 1. The parameter values become further constricted when we require that the system have at least two modes that grow with time.

3.2 Hydrophobicity analysis suggests INS-18 has a larger relative hydrophobic surface area than INS-6

For patterns to arise from diffusion-driven instability, the two species in the model must diffuse at different rates. To determine if this is likely, we examined the surface hydrophobicity of two representatives from each class; INS-18 which belongs to the activator class and INS-6 which belongs to the inhibitor class. We generated five 3D models of each peptide and, for each of the 10 models, quantified the absolute hydrophobic surface area and the relative hydrophobic surface area (i.e., the ratio of the hydrophobic surface area to the total surface area). We performed two t-tests; in the first we compared the absolute hydrophobic surface area between the two sets of models (INS-18: $1147 \pm 50 \text{ \AA}^2$; INS-6: $1040 \pm 66 \text{ \AA}^2$), and in the second, we compared the relative hydrophobic surface between the two sets of models (INS-18: 0.251 ± 0.011 ; INS-6: 0.183 ± 0.011)². The relative hydrophobic surface area of the INS-18 models is significantly larger than the INS-6 models ($p=0.001$). Whereas, the INS-18 models have a trend towards a larger absolute hydrophobic surface area ($p=0.019$). This evidence suggests that INS-18 is a “stickier” protein, implying it is more likely to be attracted to objects in the pseudocoelom which would result in a slower rate of diffusion. This is in agreement with the model requirement that the activator diffuse at a slower rate than the inhibitor.

3.3 Worms fall into two distinct groups based on HSP expression

To determine if the modes resulting from the hypothesized diffusion driven instability can explain experimental data on HSP expression patterns in *C. elegans*, we performed a clustering analysis. Worms were grouped using the expansion coefficients obtained from projecting trigonometric modes onto the data (Figure 6a). This analysis reveals that *C. elegans* fall into two distinct groups based on HSP expression patterns. Using bootstrap methods, we found that grouping the worms into two clusters had strong support with mean Jaccard similarity scores of 0.92 and 0.89. However, when the number of groups was increased to three, this support decreased to 0.51, 0.79, and 0.60 for the three clusters.

Using the two cluster approach, we determined the dominate modes for each cluster (Figure 6b). For both clusters, the $n = 0$ mode has significant contributions to the overall signal. This mode includes the contributions from the cell autonomous heat shock response which we would expect to be constant across all intestinal cells. For Cluster A the first and second mode contribute significantly to the observed pattern, and for Cluster B the second mode contributes significantly to the observed pattern. To determine if these specified modes are sufficient to explain the data, we overlaid the predicted spatial profiles using only these modes onto the the individual worm data (Figure 6c) and the mean worm data (Figure 6d). The predicted profiles using only the the specified dominate modes demonstrate a good match with the data.

3.4 Pattern formation in the Simulations

Our previous analysis reveals that a linearized version of the mathematical model produces stable oscillatory solutions. This result, however, does not guarantee that patterns will

²Values are given as Average $\pm$ Standard Error.

emerge from the full nonlinear model. Accordingly, we generated computational simulations of the nonlinear system. This allowed us to both check the validity of our analysis and verify that the model can reproduce the data on *hsp-16.2* transcription in *C. elegans*. These simulations are meant to reproduce the portion of the heat shock response that is due to differences in the reception of diffusible ILPs by individual intestine cells.

We performed a series of computational simulations of the full PDE system where we varied the values of γ and d and kept other parameter values constant (see Table 1 for values used). These simulations provide evidence supporting the existence of stable patterns, where the steady state is represented by a single dominate mode. We next compared these results to the clustering analysis presented previously. The model provides an explanation for how the pattern of a single dominate mode observed in Cluster B emerges (see Figure 6). However, the steady-state results do not explain the pattern of two equally dominate modes observed in Cluster A. We hypothesize that Cluster A is in a quasi-steady state where two modes are competing for dominance.

To determine if a quasi-steady state is possible, we examined whether we can predict the transition from a first mode dominate to a second mode dominate state based on the parameter values. In turn, we predict a quasi-steady state representing a combination of the first two modes may exist near this transition region. While our analysis of the linearized model predicts that several modes could exist, it does not predict which mode will dominate. There are analytical tools to perform such predictions and, for low-dimensional modes, they typically yield that the dominant mode will be the fastest growing mode or the mode for which $\text{Re}(\lambda)$ is maximized (Murray, 2001). To see if this method could be used to predict the transition point from a first mode to a second mode dominate state, we compared the fastest growing mode to simulation results for the steady state (Figure 7). When the model is initialized using random noise around the steady-state for the reactions, we are able to roughly predict the transition region as a function of γ and d . However, the exact transition point is sensitive to the initial conditions of the simulation.

To further explore this result, we performed two sets of simulations with different initialization conditions at points in parameter space near the transition region. Specifically, we set $\gamma = 30.2$ and $d = 5$. In the first set of simulations, we initialized the domain with Gaussian noise around the steady-state. In this scenario the signal that induces the non-cell autonomous heat shock response is released at many points throughout the worm's body. These simulations showed that the second mode ($n = 2$) is dominate. We found with this parameter combination that the system sometimes enters a quasi-steady state (Figure 8 and 9). However, the progression into the final steady state is sensitive to the specific Gaussian noise present in the initialization. In the example shown in Figure 8, the system quickly (by $t^* = 0.2$) enters a state that represents the superpositioning of two modes. The system continues to evolve, and at $t^* = 3.24$ the difference between the function and the final stationary mode is < 0.007 (see Section 2.3 for details on this calculation)³. The rate of convergence to the stationary mode was linear. In the second example the convergence rate

³Due to errors in numerical approximation, the difference between each simulation and the final stationary mode plateaus between 0.006 and 0.007.

was again linear, but the steady state was reached more rapidly (Figure 9). The difference between this simulation and the final stationary mode is < 0.007 at $t^* = 1.45$. Thus, if we obtain experimental measurements at $t^* = 2.00$ (a time of approximately 1 day), these results suggest we could observe either a single stationary mode or the superpositioning of multiple modes.

We next performed simulations where the pattern was initialized at the left end of the domain. Biologically this corresponds to a release of ILPs at the anterior region of the worm's intestine and corresponds to the hypothesized mechanism that neurons at the head of the worm release signals into the pseudocoelom in response to heat shock. We found that at multiple values of γ and d the observed pattern orientation was accurately reproduced (an example is shown in Figure 10). The simulation predicts increased *hsp-16.2* transcription at the head and the tail of the worm and this matches experimental observations. This is in contrast to the simulations initialized using Gaussian noise across the domain (Figure 8 and 9). These simulations predict reflected versions of the pattern may arise (i.e., decreased transcription of *hsp-16.2* at either end of the domain compared with the middle). Thus, the initial conditions may explain why only one orientation of mode 1 and 2 are observed experimentally.

4 Discussion

We developed a reaction diffusion model that predicts experimentally observed patterns in the expression of a reporter for *hsp-16.2* (Mendenhall et al, 2015). This work provides insight into the mechanism of the non-cell autonomous heat shock response in the nematode *C. elegans*. We hypothesize that the transcription of *hsp-16.2* is dependent on a complex interplay of insulin-like peptides signaling at a multicellular level. This complex system warrants the use of mathematical tools to understand how changes in the system affect the total levels of *hsp-16.2* transcription. Furthermore, since *hsp-16.2* expression is correlated with lifespan, the development of a mathematical model that connects heat shock to intestinal *hsp-16.2* transcription allows us to study how alterations to kinetic or diffusive parameters in this system might be related to aging.

We explored the possible values of four parameters in the system by combining biological intuition with the requirement for diffusion driven instability. Information on these parameter values allows us to accurately simulate the system under different conditions and provides us with additional biological insight. To obtain patterns, the reaction parameters must satisfy the Routh-Hurwitz conditions. Furthermore, parameter values are constrained by the spatial and diffusive properties of the system, including the difference in the diffusion rate between the two classes of ILPs and the domain size. Ultimately, when setting the minimum value of h to 0.05 and the maximum value of r to 10, to obtain patterned modes $k_{D,A}$ can range from 0.001 to 0.2 and $k_{D,B}$ can range from 0.001 to 2.5 (see Table 1 for the values of other parameters). Given the assumptions in the parameter estimation, it is possible that the actual values of the parameters lie outside this range. However, this result illustrates that patterned modes can arise over a large range of biologically feasible parameter values.

The requirement for pattern formation led to constraints on the parameters that control the feedback in the system. The parameter h represents how strongly ILPs regulate their own production; as h increases more stimulus is required for the same response size. Thus, an upper bound on the value of h of 1.0 implies that a minimal feedback strength is required for pattern formation to occur. We found no lower bound on the value of h , suggesting that there is not a maximal feedback strength. The Hill coefficient r , which determines the sharpness of the feedback response must be greater than one, suggesting some cooperativity in the system may exist. There are several signaling motifs that could lead to a Hill coefficient greater than one (Zhang et al, 2013). One such process involves non-processive multisite protein phosphorylation by a single kinase. The nuclear translocation of DAF-16 is potentially blocked when DAF-16 is phosphorylated by AKT at one or multiple sites (Lin et al, 2001). Thus, it may be the requirement for multi-site phosphorylation of DAF-16 that leads to the large Hill coefficient predicted by the model.

To derive these parameter constraints, the values of three other parameters were set to constant values. By setting a and b equal to one, we assumed ILP transcription was completely dependent on DAF-16 nuclear localization. However, this is likely not the case since there could be other transcription factors that affect ILP transcription. If a and b were less than one, this would imply that there is a baseline level of ILP production without DAF-16 nuclear localization. Values of a and b greater than one are not biologically possible, since this would lead to negative production rates. The third parameter value c was set equal to one, implying that the two classes of ILPs decay at the same rate. This is an assumption that warrants future investigation. Very little is currently known about ILP degradation in *C. elegans*. In higher level organisms, such as rats and humans, insulin is typically endocytosed by cells with insulin-receptors and is degraded within the cells (Bai and Chang, 1995; Duckworth et al, 1998). These processes likely depend on properties of the ILPs such as specific and non-specific binding affinities that determine the likelihood of endocytosis and subsequent degradation.

The model presented here provides a mechanistic explanation for the data obtained on *hsp-16.2* reporter expression patterns in *C. elegans*. A previous study found that *hsp-16.2* reporter expression in intestinal cells was greatest in the anterior region, followed by the posterior region, and finally least in the middle of the worm (Mendenhall et al, 2015). We sought to use the developed model to provide an explanation for this spatial profile. Since the mean spatial profile of the 28 worms studied did not clearly match one of the modes predicted by the model, we hypothesized that the worms fell into multiple groups with different expression patterns. Using a clustering analysis, we found that the worms could be divided into two groups. One of these groups had a clear single dominate mode; however, the second group had two modes that contributed equally to the spatial profile. Due to a lack of bootstrap support, we did not divide the worms up into more than two groups.

The cluster with two dominate modes may represent worms that have not yet reached a steady state in their *hsp-16.2* transcription spatial profiles. To explore this possibility we performed simulations to determine if the system could get temporarily stuck in a state between the first two modes. A method for predicting whether the first or second mode dominates is to determine what mode grows the fastest according to the linearized model.

We predicted that if two modes grow at a roughly equal pace, then the system may reside in a quasi-steady state that represents a superpositioning of the two modes. In our simulations we found that this was indeed the case (Figure 8 and 9); however, the dynamics of the system ultimately depended on the initial conditions. This dependency could explain why some worms appear to have reached a steady state 24 hours after heat shock and other worms have not.

The mathematical model presented demonstrates how patterns can emerge but represents a simplification of the actual signaling pathways within *C. elegans*. There are over 40 ILPs that have been identified in *C. elegans*, and the action of each ILP does not necessarily fit precisely into one of the two classes of ILPs included in the model. For instance, in the model DAF-16 only has a stimulatory effect on the production of ILPs. In reality, DAF-16 increases the production of some ILPs and decreases the production of others. This leads to ILP agonists that are both self-activating (e.g., INS-7) and self-inhibiting (e.g., INS-6) (Hua et al, 2003; Murphy et al, 2003). This is in contrast to our model where, we treat the ILP class that agonizes DAF-2 as purely self-inhibiting. The model is meant to capture the net effect of DAF-16 on all ILP agonists, and, thus, we do not include ILP agonists that are both activated and inhibited by DAF-16. We did however explore a model where the dimensionless parameter b is negative, implying DAF-16 has a net inhibitory effect on ILP agonists. We were able to show that when b is less than zero it is not possible to satisfy the requirements for diffusion driven instability (see Appendix A). Given this result we propose that although INS-7 acts through a self-activating loop, there are other self-inhibiting DAF-2 agonists that counteract this effect.

Here, we present a specific mechanism to explain the patterns observed in *hsp-16.2* reporter expression, however other mechanisms may contribute to the observed patterns. We hypothesize that ILPs act downstream of thermosensory neurons. In an alternate mechanism, thermosensory neurons may stimulate other neurons and lead to a signal propagation through the peripheral nervous system. However, the intestine in *C. elegans* is not directly innervated (Altun and Hall, 2009), so a diffusive process is still essential. It is also possible that serotonin itself diffuses through the pseudocoelom and directly activates the heat shock response in intestinal cells. However, experiments have not yet revealed the presence of serotonin receptors on intestinal cells and this mechanism does not explain why DAF-2 is an essential component of the process. Finally, patterned HSP expression may be, in part, caused by coelomocytes in the pseudocoelom. Coelomocytes are large, endocytotic cells situated in relatively constant positions in the pseudocoelomic cavity (Fares and Grant, 2002). Their endocytotic properties make it likely that they function similar to immune cells. There are 6 coelomocytes located within *C. elegans*, and one study has suggested that they may endocytose ILPs (Kao et al, 2007). The six coelomocytes are located in pairs at the anterior, the mid body, and the posterior of the worm. If coelomocytes endocytose a large amount of ILPs this would affect the ILP concentration in a spatially dependent manner and result in cell to cell variation in the level of DAF-2 activation. Due to these potential alternate mechanisms, future work is needed to validate the mathematical model. For example, the model predicts that DAF-16 nuclear localization would occur in a patterned fashion, similar to the *hsp-16.2* transcription patterns. Thus, an experiment measuring the

localization of DAF-16 within individual intestinal cells would help validate the proposed mechanism.

Although the model provides an explanation for how patterns in protein expression arise, the initialization of these patterns is not explicitly included. We hypothesize that the patterning is initiated by a stimulus at the head of the worm. The data shows *hsp-16.2* reporter expression is consistently higher in anterior and posterior intestinal cells compared with middle intestinal cells. However, given random initialization conditions, the model predicts that the reflected version of this pattern, in which expression levels are minimized at the edges, would also be observed. When performing simulations where ILPs were initially only present at the left end of the domain, we found the resulting pattern had an orientation that matched the data (Figure 10). This asymmetric initialization provides an explanation for why reflected versions of the pattern are never observed in the data. Finally, we note that as the model currently stands, ILP transcription will occur regardless of the initialization conditions. However, under healthy conditions this transcription should not occur. Thus, the model assumes the stressor has affected the system in some way as to allow for ILP transcription. Future work could involve exploring the nature of this alteration to the system. A deeper understanding of the initialization process could provide insight into how different magnitudes of HSP-16.2 arise.

To study how aging affects the heat shock response, we must first fully characterize this system in healthy worms. With this goal in mind, we have proposed a mechanism for the formation of a patterned heat shock response in the optimally functioning young adult intestinal cells. The development of this model has led to three significant biological insights/predictions. The first is that diffusion plays an intimate role in forming the cell-specific pattern of the heat shock response. To the authors' knowledge this is the first work that has suggested the magnitude of the heat shock response depends on the diffusion rate of ILPs in the pseudocoelom. It is plausible that changes in the viscosity of the pseudocoelom could impact the diffusion of ILPs and alter the intestinal heat shock response, and the model allows us to predict this relationship. The second finding is that the interaction of 40 ILPs can potentially be reduced to the interactions of two classes of ILPs. Thus, rather than studying all 40 ILPs, experiments can focus on quantifying the average characteristics of the two classes. In turn, the model can be utilized to determine how these characteristics impact the heat shock response. Ultimately, the model may help identify which ILPs play the most significant role in the pattern formation. The third finding is a biological prediction that we would expect to observe similar spatial dependencies in all proteins that are regulated by DAF-16. The hypothesized spatial patterning of genes under the control of DAF-16 is significant due to the extensive role of DAF-16 in protein regulation. Specifically, DAF-16 controls a large number of genes that are associated with aging (Murphy et al, 2003). However, future work will be required to determine the exact relationship between spatially-dependent gene expression and aging.

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Appendix A: Alternative Mechanism

Here we explore an alternate system in which the ILP class that acts as a DAF-2 agonist, B, is repressed by DAF-16 nuclear localization. This implies that B regulates its own production through a positive feedback loop rather than through a negative feedback loop as presented in the main paper. The following system of equations describes this alternative model:

$$\frac{\partial A}{\partial t} = F(A, B) + D_A \nabla^2 A \quad (22)$$

$$\frac{\partial B}{\partial t} = G(A, B) + D_B \nabla^2 B \quad (23)$$

where

$$F(A, B) = k_1 - k_2 \frac{P^r}{H^r + P^r} - k_3 A \quad (24)$$

$$G(A, B) = k_4 + k_5 \frac{P}{H^r + P^r} - k_6 B \quad (25)$$

and

$$P = \frac{V_{\max} B}{k_7 \left(k_m \left(1 + \frac{A}{k_i} \right) + B \right)}. \quad (26)$$

The system was made dimensionless using the following substitutions

$$\begin{aligned} t^* &= D_A t / L^2 & x^* &= x / L & d &= D_B / D_A \\ \gamma &= k_3 L^2 / D_A & u &= A k_3 / k_1 & v &= B k_3 / k_4 \\ p &= P k_7 / V_{\max} & a &= k_2 / k_1 & b &= k_5 / k_4 \\ c &= k_6 / k_3 & h &= H (k_7 / V_{\max}) & k_{D,A} &= K_{D,A} k_3 / k_1 \\ k_{D,B} &= K_{D,B} k_3 / k_4 \end{aligned}$$

where all the parameters must take on positive values. This leads to the following system of equations

$$\frac{\partial u}{\partial t^*} = \gamma f(u, v) + \nabla^2 u \quad (27)$$

$$\frac{\partial v}{\partial t^*} = \gamma g(u, v) + d \nabla^2 v \quad (28)$$

where

$$f(u, v) = 1 - a \frac{p(u, v)^r}{h^r + p(u, v)^r} - u \quad (29)$$

$$g(u, v) = 1 + b \frac{p(u, v)^r}{h^r + p(u, v)^r} - cv. \quad (30)$$

$$p(u, v) = \frac{v}{K_{D,B} \left(1 + \frac{u}{K_{D,A}} \right) + v} \quad (31)$$

Ignoring diffusion and linearizing about the state (u_0, v_0) leads to the following differential equation:

$$w_t = \gamma A w, \quad w = \begin{bmatrix} u - u_0 \\ v - v_0 \end{bmatrix}, \quad A = \begin{bmatrix} f_u & f_v \\ g_u & g_v \end{bmatrix}_{(u_0, v_0)}. \quad (32)$$

For diffusion driven instability to occur, the system must be stable without diffusion, leading to the following requirements:

$$f_u + g_v < 0 \quad (33)$$

$$f_u g_v - g_u f_v > 0. \quad (34)$$

The partial derivatives of $f(u, v)$ and $g(u, v)$ are

$$f_u = \frac{ah^r rk_{D,B} v^r}{k_{D,A} Z^2} Y - 1 \quad (35)$$

$$f_v = -\frac{arv^{r-1}}{Z^2} + \frac{av^r}{Z^2} (rv^{r-1} + h^r rY) \quad (36)$$

$$g_u = -\frac{bh^r rk_{D,B} v^r}{k_{D,A} Z^2} Y \quad (37)$$

$$g_v = \frac{brv^{r-1}}{Z} - \frac{bv^r}{Z^2} (rv^{r-1} + h^r rY) - c \quad (38)$$

$$Z = v^r + h^r \left(k_{D,B} \left(1 + \frac{u}{k_{D,A}} \right) + v \right)^r \quad (39)$$

$$Y = \left(k_{D,B} \left(1 + \frac{u}{k_{D,A}} \right) + v \right)^{r-1} \quad (40)$$

Using the condition given by Equation 34 and a series of algebraic manipulations, we obtain the following inequalities that must hold for diffusion driven instability to occur

$$\begin{aligned} f_u g_v - g_u f_v > 0 \Rightarrow & \left(\frac{ah^r rk_{D,B} v^r}{k_{D,A} Z^2} Y - 1 \right) \left(\frac{brv^{r-1}}{Z} - \frac{bv^r}{Z^2} (rv^{r-1} + h^r rY) - c \right) \\ & - \left(-\frac{bh^r rk_{D,B} v^r}{k_{D,A} Z^2} Y \right) \left(-\frac{arv^{r-1}}{Z} + \frac{av^r}{Z^2} (rv^{r-1} + h^r rY) \right) > 0 \end{aligned} \quad (41)$$

$$\Rightarrow -\frac{brv^{r-1}}{Z} + \frac{bv^r}{Z^2}(rv^{r-1} + h^r rY) - \left(\frac{ah^r rk_{D,B}v^r}{k_{D,A}Z^2}Y - 1 \right)c > 0 \quad (42)$$

$$\Rightarrow -Zbrv^{r-1} + bv^r(rv^{r-1} + h^r rY) - \frac{ah^r rk_{D,B}cv^r}{k_{D,A}}Y + cZ^2 > 0 \quad (43)$$

$$\begin{aligned} \Rightarrow & -\left(v^r + h^r\left(k_{D,B}\left(1 + \frac{u}{k_{D,A}}\right) + v\right)\right)^r brv^{r-1} \\ & + bv^r(rv^{r-1} + h^r rY) - \frac{ah^r rk_{D,B}cv^r}{k_{D,A}}Y + cZ^2 > 0 \end{aligned} \quad (44)$$

$$\Rightarrow -brv^{2r-1} - br^r rv^{r-1}Y\left(k_{D,B}\left(1 + \frac{u}{k_{D,A}}\right) + v\right) \quad (45)$$

$$+ brv^{2r-1} + br^r rv^rY - \frac{ah^r rk_{D,B}cv^r}{k_{D,A}}Y + cZ^2 > 0 \quad (46)$$

$$\begin{aligned} \Rightarrow & -bh^r rv^{r-1}Y\left(k_{D,B}\left(1 + \frac{u}{k_{D,A}}\right) + v\right) + \\ & bh^r rv^rY - \frac{ah^r rk_{D,B}cv^r}{k_{D,A}}Y + cZ^2 > 0 \end{aligned} \quad (47)$$

$$\Rightarrow -bh^r rk_{D,B}v^{r-1}Y\left(1 + \frac{u}{k_{D,A}}\right) - \frac{ah^r rk_{D,B}cv^r}{k_{D,A}}Y + cZ^2 > 0 \quad (48)$$

Furthermore, for diffusion to cause instability in the system the following relation must hold

$$df_u + g_u > 0. \quad (49)$$

Taken together with Equation 33, this implies that fu and gv must have opposite signs. This leads to two possible cases. In Case 1, $fu > 0$ and $gv < 0$, and in Case 2, $fu < 0$ and $gv > 0$. For Case 1, using Equations 35 and 38, we derive the following inequalities:

$$f_u > 0 \Rightarrow \frac{ah^r rk_{D,B} v^r}{k_{D,A}} \left(k_{D,B} \left(1 + \frac{u}{k_{D,A}} \right) + v \right)^{r-1} > Z^2 \quad (50)$$

$$g_u > 0 \Rightarrow cZ^2 > +Zbrv^{r-1} - br^r \left(rv^{r-1} + h^r r \left(k_{D,B} \left(1 + \frac{u}{k_{D,A}} \right) + v \right)^{r-1} \right) \quad (51)$$

$$\begin{aligned} \Rightarrow cZ^2 > +brv^{2r-1} + bh^r rv^{r-1} \left(k_{D,B} \left(1 + \frac{u}{k_{D,A}} \right) + v \right)^r - brv^{2r-1} \\ - bh^r rv^r \left(k_{D,B} \left(1 + \frac{u}{k_{D,A}} \right) + v \right)^{r-1} \end{aligned} \quad (52)$$

$$\begin{aligned} \Rightarrow Z^2 > \frac{bh^r rv^{r-1}}{c} \cdot \\ \left(\left(k_{D,B} \left(1 + \frac{u}{k_{D,A}} \right) + v \right)^r - v \left(k_{D,B} \left(1 + \frac{u}{k_{D,A}} \right) + v \right)^{r-1} \right) \end{aligned} \quad (53)$$

$$\begin{aligned} \Rightarrow Z^2 > \frac{bh^r rv^{r-1}}{c} \cdot \\ \left(k_{D,B} \left(1 + \frac{u}{k_{D,A}} \right) + v \right)^{r-1} \left(\left(k_{D,B} \left(1 + \frac{u}{k_{D,A}} \right) + v \right) - v \right) \end{aligned} \quad (54)$$

$$\Rightarrow Z^2 > \frac{bh^r rk_{D,B} v^{r-1}}{c} \left(k_{D,B} \left(1 + \frac{u}{k_{D,A}} \right) + v \right)^{r-1} \left(1 + \frac{u}{k_{D,A}} \right). \quad (55)$$

In summary, for Case 1, Z^2 must satisfy the following inequality (using Equation 40):

$$\frac{bh^r rk_{D,B} v^{r-1}}{c} \left(1 + \frac{u}{k_{D,A}} \right) Y < Z^2 < \frac{ah^r rk_{D,B} v^r}{k_{D,A}} Y. \quad (56)$$

Thus, for Case 1, using the fact that the determinate of A must be greater than zero (Equation 48) and the upper bound on Z2 given in Equation 56 we have that

$$\frac{ah^r rk_{D,B} v^r}{k_{D,A}} Y > Z^2 > \frac{bh^r rk_{D,B} v^{r-1}}{c} \left(1 + \frac{u}{k_{D,A}}\right) Y + \frac{ah^r rk_{D,B} v^r}{k_{D,A}} Y \quad (57)$$

$$\Rightarrow 0 > Z^2 - \frac{ah^r rk_{D,B} v^r}{k_{D,A}} Y > \frac{bh^r rk_{D,B} v^{r-1}}{c} \left(1 + \frac{u}{k_{D,A}}\right) Y. \quad (58)$$

However, this inequality is not possible since all the parameter values are greater than zero. A similar argument holds for Case 2. Using the same steps as shown in Equations 50 – 55, but reversing the equality sign, Z2 must satisfy the following inequality:

$$\frac{ahrk_{D,B} v^r}{k_{D,A}} Y > Z^2 > \frac{bhrk_{D,B} v^{r-1}}{c} \left(1 + \frac{u}{k_{D,A}}\right) Y. \quad (59)$$

Equation 48 and 59 imply that

$$\frac{bhrk_{D,B} v^{r-1}}{c} \left(1 + \frac{u}{k_{D,A}}\right) Y > Z^2 > \frac{bhrk_{D,B} v^{r-1}}{c} \left(1 + \frac{u}{k_{D,A}}\right) Y + \frac{bhrk_{D,B} v^r}{k_{D,A}} Y \quad (60)$$

$$\Rightarrow 0 > Z^2 - \frac{bhrk_{D,B} v^{r-1}}{c} \left(1 + \frac{u}{k_{D,A}}\right) Y > \frac{ahrk_{D,B} v^r}{k_{D,A}} Y. \quad (61)$$

Since the parameters are positive, the final term in Equation 61 can not be less than zero. Thus, for this system it is not possible for the determinate of A to be greater than zero and for fu and gv to have opposite signs. Therefore, diffusion driven instability cannot occur.

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Fig. 1. Interactions between the activator class A and inhibitor class B in the reaction diffusion model. The activator A leads to the production of HSPs while the inhibitor B does not

Fig. 2.
Domain on which the reaction diffusion model was solved with boundary conditions shown.

Fig. 3. Parameter regions that satisfy the reaction requirements for diffusion driven instability. The area inside the surfaces represent the parameter space where diffusion driven instability can occur

Fig. 4.

The dispersion relation reveals that more modes are possible given larger values of γ and d .

(a) The regions of the dispersion relation curve (Equation 16) with positive values of $\text{Re}(\lambda)$ represent the possible wavenumber k^2 under which modes can grow. In the color spectrum shown, the red end corresponds to smaller values of $\text{Re}(\lambda)$ and the pink end corresponds to larger values of $\text{Re}(\lambda)$. Due to finite domain constraints only a discrete set of modes are possible, corresponding to discrete values of k^2 (Equation 15), shown with the orange lines on the surface in each figure. Each line represents a different mode, which from left to right correspond with $n = 1, 2, 3, 4, 5$. (b) Visualization of the five different possible modes with the lowest mode ($n = 1$) on top and the highest mode ($n = 5$) on bottom. Here, the green and black colors represent larger and smaller concentrations of the species in the model, respectively.

Fig. 5.

Total number of possible modes as a function of parameter values. In the larger orange shaded region, at least one mode is possible. In the smaller blue shaded region, at least two modes are possible. The two plots show results at different values of r .

Fig. 6.

Clustering analysis reveals two distinct groups of worms. (a) Example individual worm data with interpolation using cosine modes weighted by expansion coefficients. (b) Cluster centers following a k-means analysis where clustering was done using the expansion coefficients for each cosine mode. (c) Individual worm data (thin lines) compared with predicted profiles (thick lines) using summation of the dominant modes ($n = 0, 1$ and 2 for Cluster A; $n = 0$ and 2 for Cluster B). (d) Same as (c) but now showing the mean and standard error of the individual worm data for each cluster

Fig. 7.

Comparison of the steady states obtained in simulations with predictions from the linearized model. (a) and (b) represent sets of simulations ran using two different initializations of Gaussian noise around the steady state. Each dot represents a single simulation of the full non-linear model and the color of the dot represents the dominate mode that the system eventually enters. The shaded regions represent the fastest growing mode as predicted by the linearized model. The linear model prediction roughly matches the dominate mode obtained using simulations of the full non linear model. However, the exact transition point from a first mode to second mode dominance depends on the initial conditions

Fig. 8. Simulation initialized with Gaussian noise that enters a quasi-steady state. Showing simulation results at four different values of t^* . The activator magnitude shown represents the top horizontal line shown in the domain

Fig. 9. Simulation initialized with Gaussian noise that has entered a steady-state mode by $t^* = 2$. Showing simulation results at four different values of t^* . The activator magnitude shown represents the top horizontal line shown in the domain

Fig. 10.

Results of simulation initialized using left constant conditions. (Left) Condition used to initialize simulation. (Right) Simulation results where $\gamma = 37$ and $d = 4$. All other parameters are given in Table 1. The activator magnitude shown represents the top horizontal line shown in the domain

Table 1

Parameters of the mathematical model. Values shown were used to perform model simulations. The justification for these parameter values is provided in the text

Parameter	Value	Description
a	1	Relative inhibitory effect of DAF-16 on activator based on maximum production rate. The value implies activator production is completely controlled by DAF-16.
b	1	Relative inhibitory effect of DAF-16 on inhibitor based on maximum production rate. The value implies inhibitor production is completely controlled by DAF-16.
c	1	Relative decay rate of inhibitor to activator. The value implies the decay rate of the two classes of ILPs is the same.
h	0.4	Concentration of p at which the feedback strength is half maximized. A lower value implies feedback is more sensitive to ILP concentrations.
r	10	Hill coefficient describing feedback strength. The large value implies cooperativity exists in the system.
kD,A	0.01	Dimensionless dissociation constant for the activator.
kD,B	0.01	Dimensionless dissociation constant for the inhibitor.
γ	80	Relative domain size. Reasonable biological values lie between 0.01 and 100 (see text).
d	5	Ratio of diffusion rates between the two ILP classes. Implies the inhibitor diffuses 5 times faster than the activator