The Role of Noise-Induced Excitable Dynamics of Rho Family GTPases in the Regulation of Actin Cytoskeleton

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The Role of Noise-Induced Excitable Dynamics of Rho Family GTPases in the Regulation of Actin Cytoskeleton

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Introduction:

The mechanism of pattern formation in biological systems has long been a topic of intensive research and debates in scientific community. The first works on patterning of embryonic cells can be dated back to 1900 when Hans Driesch, known for his experimental work in cellular embryology, declared that the physics and chemistry of his time were unable to explain self-regulation during embryogenesis [1]. Since then, multiple theoretical models were proposed for the understanding of biological morphogenesis. These models were based on the seminal paper of Alan Turing and have been worked upon further by the works other scientists such as of Gierer and Meinhardt [2, 3]. These models have recently regained attention of the research community due to improved experimental techniques that can test theoretical predictions [4].

Turing's reaction-diffusion (RD) model provided an argument that dynamic biological systems with pattern-forming capabilities can emerge from a two-component reaction-diffusion system in the presence of an unstable homogeneous state [4, 5]. Since then, such reaction-diffusion systems have been thoroughly investigated, and multiple chemical component systems are known to display the patterns accurately reproducible by the model equations [4]. However, while the reaction-diffusion theory provides a valuable framework for understanding the phenomenon of self-organized pattern formation, it is difficult to relate simple two-component models to real biological systems with multiple interacting substances or species [6]. In his papers, Alan Turing mentioned that an interaction of three substances could generate not only static patterns but also traveling waves and out-of-phase oscillations. However, he was unaware of any biological example for such out-of-phase oscillation and did not provide a biological interpretation [2]. Hence, the RD models of biological systems were rarely used. It was not until recent studies have shown several compelling examples that gradually alleviated much of the skepticism surrounding Turing theory [4]. In the works of Holmes and Edelstein-Keshet, the RD model was applied to provide insights into the mechanisms driving various cellular morphological events by Rho-GTPases [7, 8]. Furthermore, the RD model was extended to include noise, which naturally occurs in real biological systems. In the works of Goldenfeld [9], the RD model has been shown to generate a wide variety of spatial patterns driven by intrinsic noise that are comparable to deterministic system but also have important distinctions.

With recent studies relieving the original shortcomings of Turing model and extending the reaction-diffusion theory to more realistic multi-component biological systems, RD-based modeling became a reliable groundwork for further investigations into cellular morphogenesis. The goal of this study is to use extended versions of a previously studied RD model [7] to investigate the pattern formations of Rho-GTPases and their regulation of the F-actin cytoskeleton. In particular, I study how intrinsic noise affects patterning and wave propagation in a cell of an arbitrarily complex shape. This study will provide further insight into the underlying processes that occur during embryonic development. The advancements of this computational methodology may lead to a better understanding of biological processes such as metastasis where the F-actin/Rho-GTPases play a pivotal role.

Literature Review:

Cell morphogenesis is a general phenomenon in eukaryotes which involves the migration of cells driven by chemical stimuli and moving through interactions with other cells and the extracellular environment [10]. In particular, Rho GTPases, a family of signaling proteins, play a pivotal role by acting as molecular switches to regulate F-actin cytoskeletal and cell adhesion dynamics. However, in many diseases such as cancer, these switches are either 'on' for too long or become active in the wrong place [11]. In recent publications, multiple researchers recognize the difficulties of modeling such complex protein interactions because chemical morphogenesis can arise from linear instabilities, generating periodic wave patterns [4]. However, most recently, studies have shown that in the Turing model, intrinsic noise can play a fundamental role in the regulation of pattern formation [9]. Interestingly, the theoretical predictions were later verified in the experimental biological system [12]. Since noise is a ubiquitous phenomenon in biological systems, these findings provide motivation to further study specific dynamic regimes induced by noise for a better understanding of the observed cellular morphologies and dynamics.

Turing's reaction-diffusion (RD) model is well known for its ability to explain self-organized pattern formation in the development of animal embryos. Although its prevalence has been widely debated [4], a number of compelling examples have gradually alleviated much of the skepticism surrounding the model [7, 9, 12]. The theory has since been expanded to include mathematical models that feature a mutual interaction of elements and a set of parameters that result in spontaneous pattern formations [1]. A study conducted by Holmes *et. al*. [7] shows the application of one such mathematical model that describes F-actin as a local inhibitor and Rho GTPases, which switch between active and inactive states in the presence of autocatalytic feedback. The goal of that 1D study was to determine how the spatiotemporal wave patterns are initially formed and the various types of dynamics that could be generated with different parameters. Holmes *et. al.* was able to reproduce a broad range of dynamic behaviors including static polarization and boundary localized patterns, single waves, reflecting waves, and other exotic patterns [7, 8]. However, while their model provided a broad conceptual framework for interpreting transitions between different dynamical behaviors, the study is a minimal representation of F-actin/Rho GTPase signaling network. As a result, a detailed description that involves cellular events should be further examined to draw connections between the dynamics predicted by the model and phenomena that are observed experimentally.

One such cellular phenomenon is the presence of intrinsic noise. Originally, the Turing theory postulated that cellular morphogenesis could arise from an unstable homogenous state, giving rise to periodic wave patterns in reaction-diffusion systems but only those with rapidly diffusing inhibitors and a slowly diffusing activator [10]. Recent studies have satisfied these requirements and have extended the theory by including noise in the system to test the previous empirical theoretical predictions in the experiment. Karig *et*. *al*. used the Turing model with induced noise to explore a synthetic bacterial population where the signaling molecules form a stochastic activator-inhibitor system [12]. They were able to reproduce disordered patterns on a spatial scale from an initially homogeneous system and properties of the obtained patterns closely followed the theoretical prediction. Their results suggest that Turing-type pattern-formation mechanisms provide a groundwork for a unified picture of biological morphogenesis, arising from a combination of stochastic gene expression and dynamical instabilities [12]. However, it is still

unknown if applying such stochastic approach to a F-actin/Rho GTPase system makes it possible to design and control molecular underpinnings of biological pattern formations.

The above studies conducted by computational biologists and mathematicians provide compelling evidence of the ability of generalized Turing models to characterize the cell morphodynamics driven by Rho GTPases and their regulation of the F-actin cytoskeleton. The current study aims to investigate a particular dynamic behavior directly relevant to the observed protrusive activity of motile cells: a single decaying travelling wave of actin polymerization. For that I use the inhibitor decay rate as a control parameter. This approach will serve as a framework for a comprehensive representation of actin reorganization during cellular morphodynamics. The current study assesses the role of intrinsic noise in the system. Explicitly including the noise provides a more realistic representation of the underlying cell processes driven by Rho GTPase signal transduction by random initiation of wave propagation.

Methods:

Reaction-diffusion model

The central motif that I will be using to model actin dynamics is an autocatalytic model, coupled with an inhibitor. As previously mentioned, this type of signaling motif has been studied previously, but the particular GTPase/F-actin model was proposed by Holmes and Edelstein-Keshet [7]. The definitions and values of all parameters used are provided in **Table 1**. Here F-actin works as the local inhibitor and the autocatalytic feedback between active (A) and inactive (I) GTPases forms a bistable system. The biological interpretation of regulatory mechanism of the inhibitor, here F-actin (F), is less clear, but has been hypothesized that it could function through PI3K or adhesion complexes [7]. As shown in **Figure 1**, the model is described mathematically by three equations. Equations 1a and 1b describe the dynamics of active and inactive forms of Rho-GTPase. Equation 2 describes the regulation of F-actin. Equation 3 shows in detail the kinetic regulation of Rho-GTPase activity, which consists of activation rate (involving positive feedback from active form of Rho-GTPase). I used forward Euler's method [13] to obtain the discretized approximation for numerical solution.

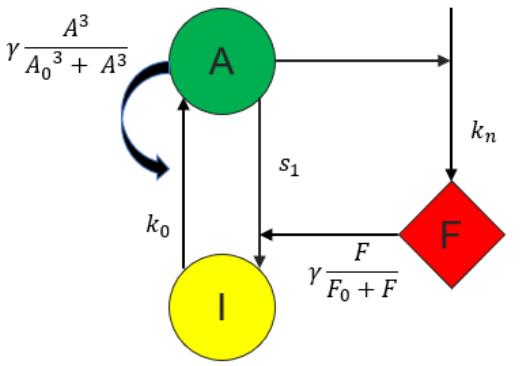


Figure 1
Schematic representation of the model used in this study.

$$1a)\ \frac{\partial A}{\partial t} = D_A \Delta A + f + \alpha\xi$$

$$1b)\ \frac{\partial I}{\partial t} = D_I \Delta I - f + \alpha\xi$$

$$2)\ \frac{\partial F}{\partial t} = \varepsilon(k_n A - k_s F) + \alpha\xi$$

$$3)\ f = \left(k_0 + \gamma \frac{A^3}{A_0^3 + A^3}\right) I - \delta \left(s_1 + s_2 \frac{F}{F_0 + F}\right) A$$

In this model, time and spatial coordinates are reported in arbitrary units with a dt step of 0.001 and dx step of 0.02. The parameter k_0 refers to a basal activation rate of Rho-GTPase and γ represents the positive feedback. The parameter k_S refers to basal inhibitor decay rate of F-actin. Parameters $s_1$and s_2 set the relative weighting of the basal inactivation of GTPases and F-actin respectively. δ refers to the overall timescale of the GTPase degradation. *D* refers to the diffusion coefficients of *A*, *I*, and *F* respectively. Neumann's no flux boundary condition is used to represent the fact that GTPases and F-actin do not leak out of the cell [8]. This system has been previously investigated in 1D setup [7], and depending on the parameters, can lead to various regimes form of polarization to single and periodic traveling waves and wave trains, which are often observed in living eukaryotic cells.

Simulation setup and choice of control parameter
Gaussian noise with the amplitude α, was applied in this system (see [13] for details of implementation) to investigate its ability to induce excitable dynamics and change the overall dynamics of the system.

The overall motivation of this project is to investigate the system dynamics in a 2D setup in an arbitrarily shaped cell. This is in contrast to different studies done in a 1D representation [7]. I investigate the formation of single travelling waves, which can be induced by a finite stimulus and represents an example of excitable dynamics. As a control parameter I used k_s, which represents the rate of inhibitor decay (disassembly of F-actin mesh).

Briefly, the simulation setup without noise can be described in three steps. First, the value of the inhibitor decay rate parameter, k_s, is arbitrarily chosen. Then, a simulation of the model is run in order to find the concentration values in the stable homogeneous state. Afterwards, the homogenous concentrations are implemented back into the system with stimulus induced excitation to observe what kind of wave patterns are formed. This regime has a few important things to note. Firstly, differentiating k_s parameters greatly change the overall concentrations of active and inactive forms of GTPase. Thus, in order to observe the changes that each parameter causes, I select the initial homogeneous concentrations where Rho-GTPase is in inactive from (*A = 0, I = 1*). As a result, I numerically calculated the values of Rho-GTPase concentration for which there is a stable homogeneous state, and these values were used later in the simulation as an initial stimulus to ensure that the system is excited from a steady state.

In the simulation with noise, I introduced the same noise factor for active/inactive GTPase and F-actin in the form of Gaussian random variable, ξ, with an amplitude, α, as an adjustable parameter [13]. The amplitude of noise was implemented in a range from 0 to 2. All simulations with noise used the same parameters and solution methods as one without noise as stated above.

Table 1
Parameters used in this study. Units of concentration and time are all in arbitrary units (AU).

Parameter Name	Value	Units
$\boldsymbol{k_0}$	0.05	[AU of time]$^{-1}$
$\boldsymbol{s_1}$	0.1	[AU of time]$^{-1}$
$\boldsymbol{s_2}$	1.2	[AU of time]$^{-1}$
$\boldsymbol{A_0}$	0.4	[AU of conc.]
$\boldsymbol{F_0}$	0.5	[AU of conc.]
$\boldsymbol{\gamma}$	1	[AU of time]$^{-1}$
$\boldsymbol{\delta}$	1	Unitless
$\boldsymbol{\varepsilon}$	0.1	Unitless
$\boldsymbol{k_n}$	1	[AU of time]$^{-1}$
$\boldsymbol{k_s}$	$0-2$	[AU of time]$^{-1}$
$\boldsymbol{D_A, D_I}$	$\frac{10^{-3}}{3}, \frac{10^{-1}}{3}$	[AU of length]^2 / [AU of time]
$\boldsymbol{L}$	0.05	[AU of length]

$\boldsymbol{\alpha}$	$0-2$	[AU of conc.] / [AU of time]
$\boldsymbol{\xi}$	Gaussian random value with zeros mean and STD = 1	Unitless

Results:

System dynamics without noise

Before implementing noise in the system, I investigated the dynamics of the system without random perturbations. **Supplemental Videos 1, 2, and 3** provides three simulations with different values of k_s and with different cell shape (**Figure 2**). **Supplemental Video 1** demonstrates propagation of single traveling wave in a cell of arbitrary shape ($k_s = 0.0625$). This k_s value was also implemented in a setup with circular geometry of the simulation domain, shown in **Supplemental Video 2**. Here we observe that the cell geometry does not affect the formation of a single spiral wave for a k_s value of 0.0625. For k_s values above 0.1, reflecting waves were observed (**Supplemental Video 3**), which eventually broke the front of the wave due to boundary effect. Also, waves mostly reflected in the regions of the simulation domain with high curvature.

In order to better visualize the propagation of waves occurring in these different scenarios, **Figure 2** displays scaled images of the concentrations of active GTPase (green) and F-actin (red) at different time points of the three previous videos. The concentration values were scaled from 0 to 1 by dividing the difference of the current concentration value and the minimum value of concentration by the difference of the maximum and minimum values. In all simulations the front of F-actin (inhibitor) follows the front of active GTPase (activator) with some time delay. In the cases of decreased k_s (rate of inhibitor decay) both fronts reach the boundary, and the system is no longer active. However, in the case of increased k_s the strength of the inhibitor is decreased. As a result, at the boundary with high curvature the activator is not turned off completely and the wave reflects to continue propagation in the opposite direction. Also, in the case of increased k_s, due to the interaction with the boundary, the wave front can break, which leads to the formation of spiral waves. We can conclude that regulation of the rate of inhibitor decay in the system controls transition from reflection waves to a single decaying wave and helps to avoid the formation of spiral waves.

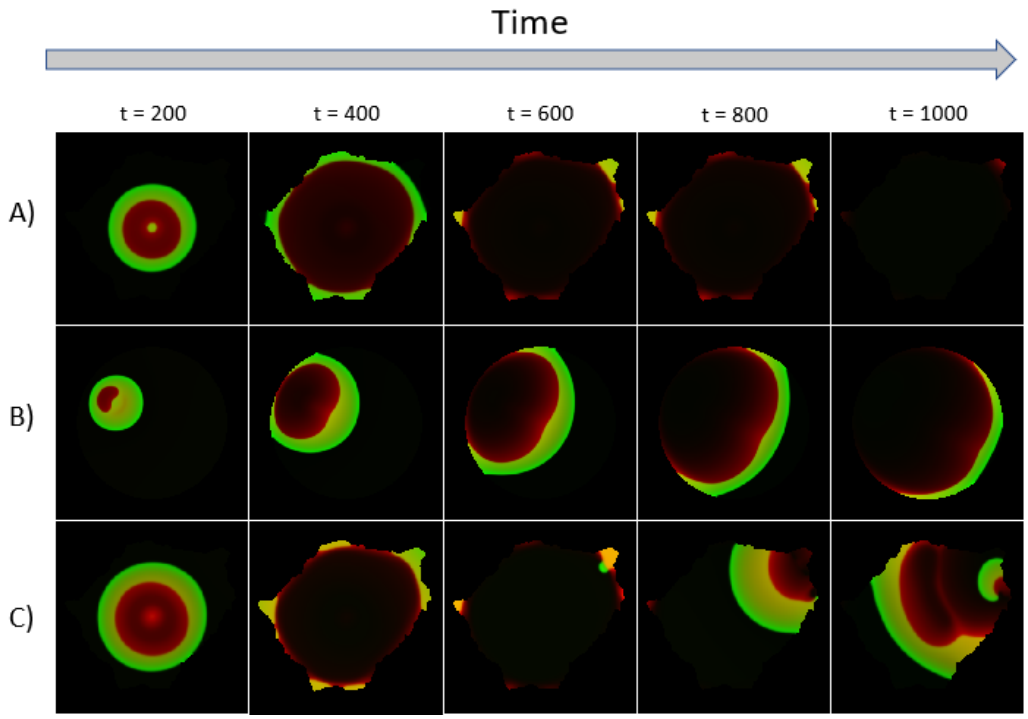


Figure 2
Merged images with two channels representing active GTPase (green) and F-actin (red) at different time of simulation A, B, and C correspond to the results shown in **Supplemental Videos 1, 2, and 3**.

Noise induced excitation of the system

While we were able to reproduce single traveling waves (shown in **Figure 2**), which are often observed in biological system we did not account for the effect of noise, which is an intrinsic property of biological system. In this section, noise was implemented as a Gaussian variable and the value of noise was controlled by changing its amplitude.

In our previous simulations without noise, for k_s value between 0.0625 and 0.1 we obtained a single traveling wave with initial stimulus that induced the propagation of the wave. In the system with noise, we used homogenous initial conditions. For $k_s = 0.0625$ and noise amplitude equal to 1.75, we obtained single travelling waves initiated by random fluctuations in the concentration level of Rho-GTPases as shown in **Supplemental Movie 4** and **Figure 3** (simulation A). Unlike the simulations in **Figure 2**, the waves were much noisier and generated at the periphery of the cell. Additionally, after propagation of a single wave new waves were generated much like in real biological systems.

To further evaluate the role of noise, different values of k_s and noise were used to observe its effects on the system. When lower values of noise were implemented, the system continued to have perturbations and decay, but did not induce excitable dynamics. However, for higher values of noise, different observations can be made as shown in **Supplemental Movie 5** and **Figure 3** ($k_s = 0.0625$, noise amplitude 2.0, simulation B). Waves were quickly generated in two locations at the periphery and traveled outwards until two fronts of excitation met and reached the boundary of the simulation domain. We can conclude that the amplitude of noise controls the fluctuations of active GTPase concentration and can lead to formation of multiple wave fronts.

We also investigated the regulation of inhibitor decay rate in a noisy system ($k_s = 0.1$, noise amplitude 1.75, simulation C). As shown in **Supplemental Movie 6 and Figure 3**, not only were spiral patterns observed, but multiple wave fronts were observed. In conclusion, the amplitude of noise controls the propagation of wave fronts, where higher amplitudes induced a greater number of waves.

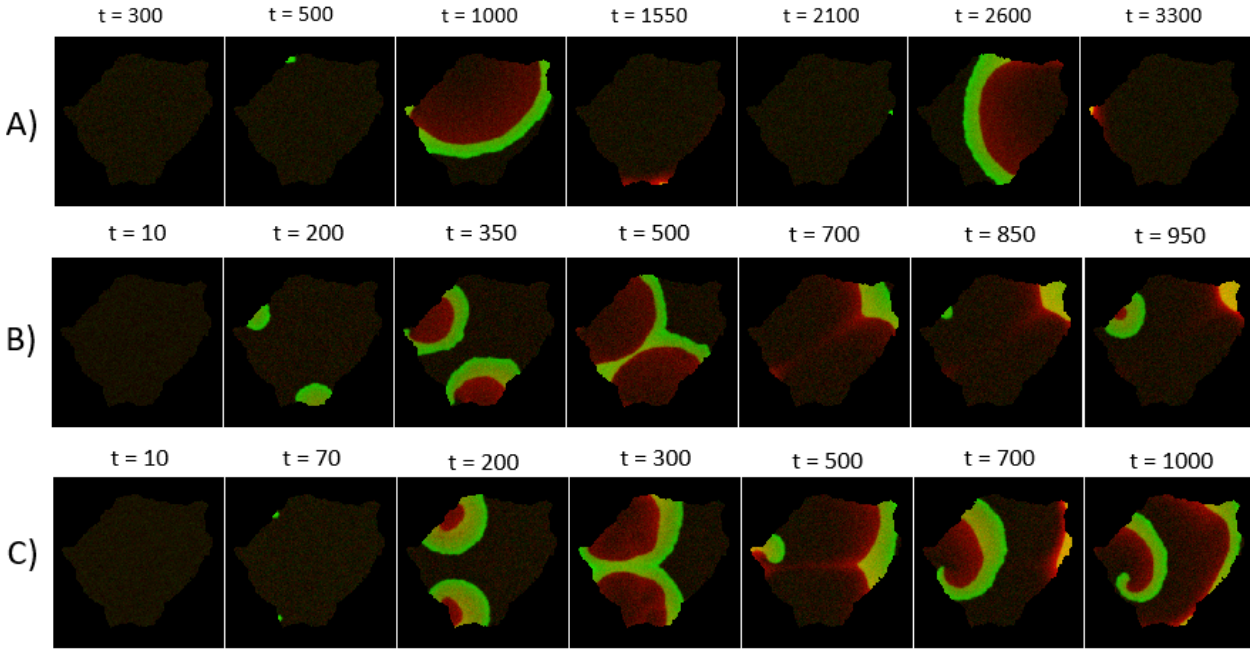


Figure 3
Merged images with two channels representing active GTPase (green) and F-actin (red) at different time of simulation D, E, and F correspond to the results shown in **Supplemental Videos 4, 5, and 6** (scaling of the signal was performed in the same way as in **Fig. 2**). **A)** Screenshots of simulation with noise induced single traveling waves ($k_s = 0.0625$ and amplitude of noise equal to 1.75) was used for components representing Rho-GTPases and F-actin). **B)** Screenshots of simulation of multiple travelling waves induced by noise ($k_s = 0.0625$ and amplitude of noise equal to 2 was used for components representing Rho-GTPases and F-actin). **C)** Screenshots of simulation of multiple travelling waves induced by noise ($k_s = 0.1$ and amplitude of noise equal to 1.75 was used for components representing Rho-GTPases and F-actin).

Note that in simulations with noise, the locations in which waves were induced varied. In a system without noise, the wave was induced by a stimulus at a specified location in the center of a simulation domain and traveled outwards toward the boundary until it decayed or reflected. See **Figure 2** for such dynamics. However, in systems with noise, active GTPases are constantly perturbed. The speed at which these perturbations decay is different at different locations of the simulation domains and depends on the boundary conditions. Furthermore, active GTPases at the boundary were observed to induce excitable dynamics. To evaluate why waves occurred at the edge, I took the absolute differences in the concentration of active GTPase for consecutive frames, before the wave was induced, and averaged over time as shown in **Figure 4**. Due to no flux boundary conditions, in the center of the domain, active GTPases can diffuse in any direction, but at the border it can only diffuse inwards. Thus, on average the perturbation of concentration of active GTPase is higher at the edges (lighter color) of the domain. Since active GTPase decays slower at the periphery, excitable dynamics are most likely to be induced there.

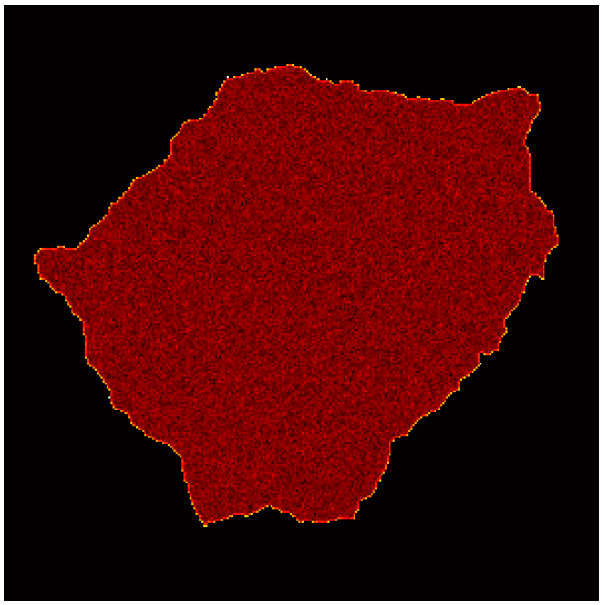


Figure 4
Effect random fluctuation of active form of Rho-GTPase at different locations of the simulation domain. Length scale of cell is 2.2531 in A.U. of length.

Light area along the perimeter of the simulation domain represents high values of the random fluctuations at the periphery, which induce excitation of the wave (see text for detailed description). Due to no-flux boundary conditions the effect of noise in the center of the cell is less significant.

Another observation was that the time it takes for the system to become active was smaller for the larger values of noise. The wave was first generated around the 200th iteration in simulation A (noise amplitude 2.0), whereas in simulation B (noise amplitude 1.75), the wave did not generate until the 500th iteration (shown in **Figure 3**). In order to analyze and detect the time of wave onset, I used the pipeline as shown in **Figure 5**. First, I compute the amplitudes of active GTPase for each frame in a simulation. After computing the logarithmic values, I applied a moving average of these values. Then, I computed the gradient and graphed the moving average of the gradient. Finally, a threshold was chosen to evaluate the first frame in which we observe a spike (this is the point where the computed gradient of the log-transformed amplitude is increasing sharply). The length of the characteristic cell was computed by finding the square root of the area of each pixel divided by pi ($\sqrt{dx^2/\pi}$). In **Figure 6**, the pipeline was conducted in multiple simulations with different amplitudes of noise and graphed in order to evaluate the effect the amplitude of noise had on the time of wave onset. It shows that as the value of noise increases, the wave generates faster. Additionally, I observed that as the values of noise increase, the time that is needed to induce waves is decreasing exponentially.

A) Compute Ampl. of active GTPase for each frame in simulation
(Ampl. = max A – min A)

↓

B) Compute Logarithmic values of Ampl.
log(Ampl.)

↓

C) Compute moving average of logarithmic values of Ampl.
movmean(log(Ampl.))

↓

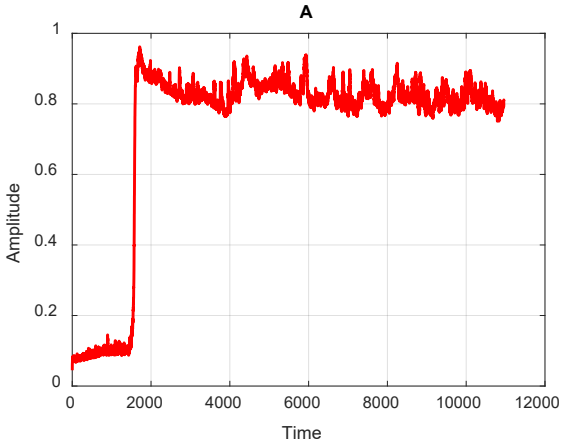
D) Compute and graph gradient of moving average of logarithmic values of Ampl.
gradient = gradient(log(Ampl.))

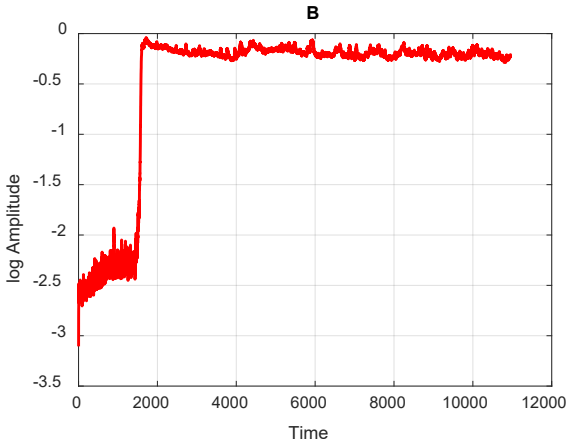
↓

E) Compute moving average of the gradient
movmean(gradient)

↓

Find first frame where gradient is above threshold
green threshold is arbitrarily chosen





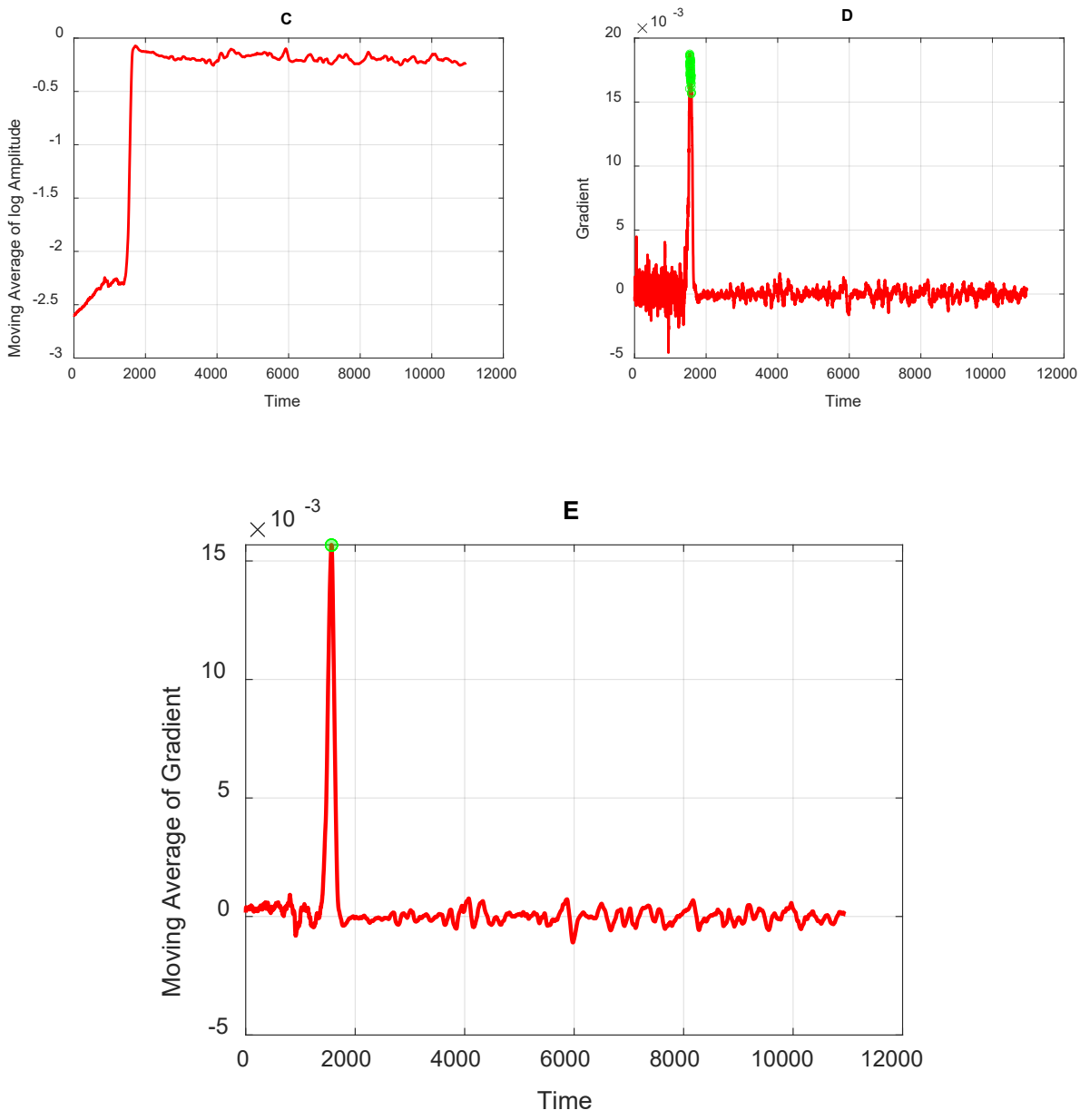


Figure 5

The pipeline used to analyze and detect the time of wave onset. Graphs evaluated the values of amplitude active GTPase concentrations are taken from a simulation using default values shown in **Table 1** and an amplitude of noise of 0.70. Ampl. stands for amplitude. The threshold (green dots) is chosen by selecting a slightly lower value than the maximum value observed on graph E. In this simulation, the threshold had a value of 0.01565.

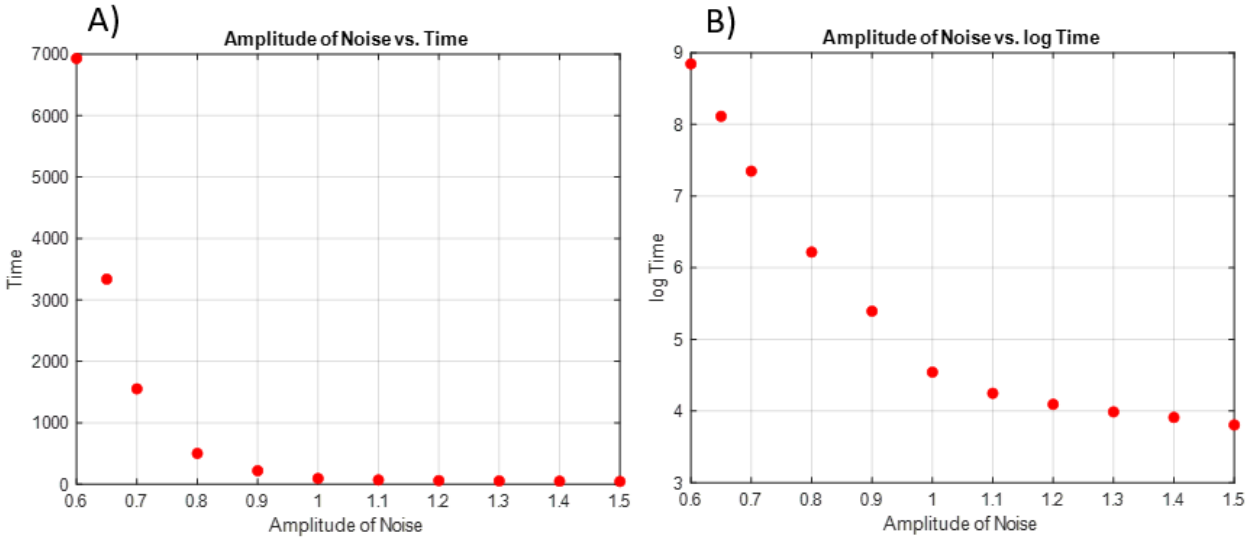


Figure 6
The dependence of the time before wave formation on the amplitude of noise. **A)** Raw data that displays the time at which wave was induced by noise depending on the level of noise. **B)** Log-transformed plot of the time-noise amplitude dependence. The linear part on the log-transformed plot corresponds to the exponential decay in the original plot.

Discussion:

Rho GTPases play a pivotal role in cell morphogenesis by acting as molecular switches to regulate F-actin cytoskeleton and cell adhesion dynamics. However, in many diseases, these switches are either 'on' for too long or become active in the wrong place. Such issues motivate my study of the mechanisms driving the dynamics of wave propagation in the cortex of an arbitrarily shaped cell. Such generalized approach provides a more realistic representation of the underlying cell processes driven by Rho GTPase.

With a careful consideration of the inhibitor decay rate, we explored how it controls the transition from reflecting waves to a single decaying wave and helps to avoid the formation of spiral waves. In experiments, one may be able to observe these wave dynamics by changing protein concentrations that affect F-actin polymerization. In this study, we observed that the amplitude of intrinsic noise controls the propagation of wave fronts, such that higher amplitudes induce a greater number of waves. In addition, waves were observed to propagate in the periphery of the domain which occurred at different time points depending on the amplitude of noise. Although more investigations are needed to understand the mechanisms driven by noise in real biological systems, this study supports an argument that noise may play an important role in the regulation of spatiotemporal dynamics of Rho family GTPases.

Additional studies of this type of models will further our understanding of the role of noise in cell-level regulation of actin dynamics by the Rho family GTPases. While this study was able to establish the role of k_s and noise, more extensive simulation scans are necessary to establish more general conclusions. By running additional simulations of varying k_s and amplitudes of noise, we may be able to observe more distinguishable patterns. In addition, the effect noise played was only established with a varying inhibitor decay rate. By changing other variables such

as the basal activation rate, other conclusions can be drawn from the system. Furthermore, an *in vivo* approach to this computational model can be conducted to draw conclusions that occur experimentally.

Supplemental Files:

Supplemental Video 1

Simulations of F-actin/Rho-GTPase dynamics without noise and parameters used in Table 1. The waves shown are Active GTPases. k_s value of 0.0625 producing a single traveling wave in an "arbitrary" shaped 302x302 cell.

Supplemental Video 2

Simulations of F-actin/Rho-GTPase dynamics without noise and parameters used in Table 1. The waves shown are Active GTPases. k_s value of 0.0625 producing a single traveling wave in a "circle" shaped 302x302 cell.

Supplemental Video 3

Simulations of F-actin/Rho-GTPase dynamics without noise and parameters used in Table 1. The waves shown are Active GTPases. k_s values above 0.1 producing spiral patterns in an "arbitrary" shaped 302x302 cell.

Supplemental Video 4

Simulation of noise induced single traveling waves ($k_s = 0.0625$ and amplitude of noise equal to 1.75 was used for components representing Rho-GTPases and F-actin). Multiple propagations of single traveling waves were observed.

Supplemental Video 5

Simulation of multiple travelling waves induced by noise ($k_s = 0.0625$ and amplitude of noise equal to 2 was used for components representing Rho-GTPases and F-actin). Higher amplitude of noise induced wave propagation in two locations. Two fronts of excitation met and reached the boundary, eventually leading to spiral pattern.

Supplemental Video 6

Simulation of multiple travelling waves induced by noise ($k_s = 0.1$ and amplitude of noise equal to 1.75 was used for components representing Rho-GTPases and F-actin). Using the same amplitude of noise in **Fig. 3** and a higher value k_s and induced wave propagation in two locations. Two fronts of excitation met and reached the boundary, eventually leading to spiral pattern.

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