

# Cellular Dialogs: Cell-Cell Communication Through Diffusible Molecules Yields Dynamic Spatial Patterns

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## Summary

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Scientific editor: Quincey Justman, Ph.D.

**Editorial Decision after first round of review:** Revision invited Sept. 18, 2019  
*Minor changes anticipated*  
Revision received Oct. 16, 2019

**Editorial Decision after second round of review:** Accepted Dec. 4, 2019

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## Editor's View

**Scientific context and initial assessment.** Cell Systems's relationship with models: #itscomplicated. We very rarely see models as ends, in-and-of themselves. We reject almost all papers that aim to build a model. This is because to us, models are not the end, they're they the beginning. They're *useful*. Models are ways of sharpening thinking and moving rapidly down a decision tree when you're trying to formulate an approach to a problem. It's much easier to ask, "Am I on the right track, yes or no?" with a model than with an experiment.

But the best models are worth publishing on their own. The best models are creative and capture something essential about the system studied or the question asked. They facilitate a very long string of "Am I on the right track, yes or no?" questions. They provide useful starting points for experiments when there isn't one that's supported by rigorous empirical evidence. They allow access to big numbers and long timescales that are not practically feasible outside the computer. In short, they allow you to explore ideas when biology makes that difficult.

Upon first reading, I thought this paper belonged among The Best. It reminded me, happily, of Conway's Game of Life, which is an elegant and important demonstration that very simple "rules," in a life-like context, can produce exceedingly complicated and rich "games." Like the Game of Life, this paper lets you into a whole world, complete with sensible and internally consistent rules of the authors' devising. It is an elegant and appealing world, and it made me curious immediately. I wanted to turn all its knobs, see what happened, and then compare-and-contrast that to what I know about natural systems. I also liked that this world of the authors' devising is aimed at understanding spatial problems, which are so important but tend to get short shrift.

However, Cell Systems almost always rejects papers that are about the authors' conception of the world, rather than the real one. We are firm that Cell Systems's mandate as a journal is to study real biology, not idealizations or ideations of it. But we also augment that statement to include, "now or someday." This is because today's idealized thought experiments can open the door to real-world empirical tests later, and we want to support that. So our augmented mandate is: study real biology, not idealizations or ideations of it, now or someday.

Internally, we editors have two ways of discussing this issue and understanding whether a manuscript runs counter to our augmented mandate. The first is that we ask whether the work "builds a castle in the clouds." A castle in the clouds might seem very appealing, but it has no foundation; you could never go out and test the conclusions of a castles-in-the-clouds paper because the assumptions at its base are untestable. We don't like castles built in clouds. The second is trickier. We say, "Assume the earth is a cube." This is our shorthand for trying to understand whether the foundational, simplifying assumptions of the modeled world are appropriate, and also whether the authors are making choices that are personally or mathematically useful for their story but actually degrade the problem. A cubed earth is almost never appropriate; a sphere is more accurate and likely simpler.

So do these authors build a castle in the clouds? No. Their approach proceeds from a limited number of simple and commonsense assumptions that are testable in the lab, which is excellent. Is the authors' earth a cube? That is a trickier question, one that usually needs a reviewer's expert eye. It's often not possible to ask the question until you really dig into the equations. But this means that to review the manuscript properly, we need someone who can dig and recognize cubed earths.

**Review strategy.** I chose reviewers who study spatial patterning of cells and who appreciate the challenge of connecting the small time- and length-scale activities of molecules inside cells to the much longer time- and length-scale actions of coordinated groups of cells. I also wanted the perspectives of both physicists and engineers to be represented, because these fields bring different perspectives and tend to ask questions differently. I wanted this paper to benefit from both perspectives.

**Editorial decisions.** The reviewers loved this paper. Their comments were aimed to help it along. I particularly appreciated the major question raised by Reviewer 2 (second paragraph), because it struck me as one of those questions where every answer is interesting and will promote deeper understanding.

I'll also note that the authors asked me if they could update the paper during the revision, and fold in new work that strengthened the paper, but that the reviewers didn't ask for. Of course the answer was yes,

but that's the reason that the revised manuscript is substantially larger than the initial one; it was the authors' choice.

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### Editorial decision letter with reviewers' comments, first round of review

Dear Hyun,

I hope this email finds you well. As I mentioned earlier in my personal email, the reviews are back on your manuscript and I've appended them below. You'll see that the reviewers were delighted by your manuscript. They found it to be compelling and their comments are intended to strengthen an already strong piece of work. We're happy to invite a revision.

I usually try to guide revisions, but this one needs very little in the way of guidance. I'll just encourage you to discuss the highlighted comments from Reviewer 2 in a fulsome way. It is my impression that we know comparatively little about chaotic dynamics in living systems, so any way to develop intuition around this topic is welcome and all answers are interesting. I also suspect that the additional work you've done since submitting the paper may be useful here.

And regarding that new work, from your description, it seems to complement the existing paper beautifully and strengthen it in important ways. We'd be delighted if you included it within the revision. The current version of the paper is as clear as a bell, so I'm not worried about diluting or confusing your key points, but if you find that the paper is becoming bloated or unwieldy let me know we'll work it out. This can happen at any stage, so don't be too concerned. Remember, too, that it's absolutely fine to include more supplementary figures, etc. if need be.

I hope you find this feedback helpful. If you have any questions or concerns about the revision, I'd be happy to talk about them, either over email or by phone. More technical information and advice about resubmission can be found below my signature. Please read it carefully, as it can save substantial time and effort later.

I look forward to seeing your revised manuscript.

All the best,

Quincey

Quincey Justman, Ph.D.  
Editor-in-Chief, *Cell Systems*

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### Reviewers' comments:

Reviewer #1: In this study, the authors develop open-source simulation software to explore how multicellular patterns can self-organize through diffusible biochemical cell-cell communication. This software package allows for facile simulations of hundreds to thousands of cells modeled as cellular automata with tunable properties communicating with either one or two diffusible signals that either activate or repress patterns and modeled by reaction diffusion equations. By varying the parameters informing these "cellular dialogues", both static and dynamic patterns are formed and the authors focused on analyzing the dynamic patterns to uncover how these patterns could potentially form in natural systems. They find that dynamic spatial patterns form through a three-step process where first gene expression patterns become more correlated between the cells, then these patterns fluctuate, and finally the longer-term dynamic spatial pattern such as spiral waves emerges.

#### General comments

This is a very thoughtful and comprehensive study that asks and answers the question not of how these patterns do form in a handful of systems, but how they could form and thus what we should be keeping an eye out for or may be missing in our search for mechanisms underlying natural dynamic pattern formation. The results are for the most part clearly presented with helpful visuals to guide the reader through key concepts. The software package is easy to deploy and use to both re-create the results presented in the paper, as well as to potentially probe questions about the parameters found in specific biological systems. Overall, this work would be an excellent fit for publication in *Cell Systems* if the following minor concerns can be addressed.

#### Specifically:

\* With regards to Figure 3:

o Figure 3E would be easier to interpret if the shapes were outlined as it is sometimes challenging to tell on a computer monitor without zooming in what the shape is for some colors. The companion Figure S2 has a similar issue, though the darker colors the data is there make the shapes easier to distinguish

\* With regards to Figure 4:

o In Figure 4E, it could be helpful to make the nodes of the directed graphs the corresponding color in the

simulation.

o In Figure 4F, the colors of the lines on the spider plots can be challenging to see on the colored backgrounds. Perhaps the lines could be made bolder so the color is clearer or another change?

\* With regards to Figure 5:

o The importance of the results in Figures 5C and D could be emphasized more in the main text or they could be moved to the supplement.

o An alternate visual for Figure 5F that captures the variation and distributions presented in Figure S8A would be exciting.

There are also some of what appear to be minor typographical errors that should be rectified or clarified before publication. For example:

\* With regards to Figure S7:

o In the main text, it is said to correspond to Figure 6C bottom left and feature cells randomly arranged in space. However, the caption states it corresponds to Figure 6C top right, where cells are arranged on a lattice, and this appears to be the case when viewing the video. The authors should provide the additional video and update the references in the text.

\* With regards to Figure S8:

o It is stated in the figure caption that this is related to Figure 4, but from the main manuscript, the supplemental table of contents, and the Figure 5 legend, it appears to be related to Figure 5.

Reviewer #2: Dang et al. have produced a well explained and comprehensive study of the patterns that can be generated through all possible two-molecular species interactions on large cellular grids. They categorise the networks according to their possible behaviours and show the tree-like structures of their relationships to each other - showing that closely related networks generate similar behaviours and revealing general principles behind whether the networks will generate no dynamic pattern, dynamic temporal patterns, vs dynamic spatial patterns. Categorising all the possible two molecule networks in this way could be helpful for biologists to make predictions about the types of network that could underlie their system of interest, or to predict the behaviour of particular networks on a tissue level scale. The theoretical work presented by the authors provides an intuitive understanding of the requirements for travelling spatial waves and they show that their findings are relatively robust to noise, parameter values and cellular properties.

Overall I really enjoyed reading the study, which seems very well suited to Cell Systems. I do have one major question concerning the biological relevance of some of the simulations. A key finding is that the systems that generate travelling waves do so in a 3 stage process which, prior to stable travelling waves being established, involves a long period (~1 week assuming a time step (or molecular change of state) takes one minute) of seemingly chaotic dynamics, where the pattern fluctuates unpredictably across the tissue and across time. I wonder how this relates to known biological processes. If the systems have to pass through this long chaotic phase before more travelling waves emerge, is what is happening in the simulations biologically relevant? **It would be useful if the authors added more discussion of this. Could it**

be that properties of the biological systems (such as tissue structure or growth or external signals influencing the system) mean that travelling waves can be established more quickly without having to go through these long period of fluctuations? Have chaotic fluctuations ever been observed experimentally (perhaps during de novo pattern formation in tissue culture scenarios)?

Some minor comments:

Generally it's not very clear how the findings here relate to previous modelling work. In the introduction, on page 3, lines 86-89, it's not very clear what is missing from previous models that the authors will address here. Also, have previous modelling studies shown similar behaviours for some of the circuits studied here?

p8, 226-228 "if collective oscillations were to arise from synchronisation of individually oscillating cells, then we would expect a period of four timesteps or less" - give more explanation of why this is the case.

Since the relative rates of diffusion of activator and inhibitor molecules is important in Turing patterning, I was wondering what the role of relative diffusion rates was on patterning. Would it be worth discussing this in the main text?

Fig 7c. Spelling mistake - circadian clock rather than circadian clock.

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### Authors' response to the reviewers' first round comments

Attached.

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### Revised manuscript

Attached.

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### Editorial decision letter with reviewers' comments, second round of review

Dear Hyun,

The reviews are back on your manuscript and I'm very pleased to let you know that your manuscript is

now "accepted in principle," that is, pending our receipt of your properly formatted, final files. Congratulations!

This email contains a lot of detailed information. All of it is important, so please read it very carefully. The bulleted list below highlights final steps in the editorial process, our formatting checks, and, after acceptance, your manuscript's transfer to our production department for typesetting and publication. As always, please let me know if you have any questions.

I'm looking forward to going through these last steps with you. More technical information can be found below my signature, and please let me know if you have any questions.

All the best,  
Quincey

Quincey Justman, Ph.D.  
Editor-in-Chief, Cell Systems

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### Editorial Notes

*Title:* Your title is very elegant, but I worry that it's not as effective as it could be because "cellular dialogs" is not a standard term. As you re-consider your title, note that an effective title is easily found on Pubmed and Google. A trick for thinking about titles is this: ask yourself, "How would I structure a Pubmed search to find this paper?" Put that search together and see whether it comes up is good "sister literature" for this work. If it does, feature the search terms in your title.

*Manuscript Text:* While the text is compelling and clear, I'm worried that it's rather significantly too long. You're welcome to go up to approximately 68,000 characters-with-spaces, not including the STAR Methods, the references, or the Key Changes section of the Discussion. (To be explicit, the STAR Methods, Key Changes, and references don't "count" towards your manuscript's length.) Anything longer taxes readers too much; it's simply too much for the human mind to take in all at once. When you think about what to cut, start with the Discussion. We favor slim Discussions that do not reiterate what's found in the Results beyond a brief transitional summary and are limited to around four medium sized paragraphs. If further cuts are needed, your figures can guide you: paragraphs within the Results section that only pertain to supplemental figures can be slimmed down dramatically (usually into a single sentence that calls out the supplemental figure and states its punchline) or deleted entirely. If it's appropriate, discrete details from those paragraphs can be moved into the Supplemental Figure Legends.

Also:

- **Please note this important scientific point** that we may have discussed earlier (and I'm sorry to repeat myself if we have): chaos has a formal, scientific definition, so it's important not to use it colloquially in a scientific paper. Unless you have (and present) evidence that a behavior is chaotic in the scientific sense, please replace "chaotic" with a word like "erratic" or something similar of your choosing.
- Two minor details:
  - Please err on the side of breaking paragraphs up; long paragraphs are unfriendly to the eye on the printed page.
  - Please note that house style disallows editorializing within the text (e.g. strikingly, surprisingly, importantly, etc.), especially the Results section. These terms are a distraction and they aren't needed—your excellent observations are certainly impactful enough to stand on their own. Please remove these words and others like them. “Notably” is suitably neutral to use once or twice if absolutely necessary.

*Figures and Legends:* Your figures are exceptionally clear and instructive. Kudos! Please double-check that all of the information necessary to interpret the figures is included within the Figure Legends (that is, you shouldn't need help from the main text).

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#### Reviewer comments:

Reviewer #1: The authors have done a very thorough job addressing my own and the other reviewer's concerns, and have generally improved the manuscript. I am excited to see this work in publication and congratulate the authors on their contribution to the field.

From QJ: In an informal message to me, Reviewer 2 mentioned that some of your bioRxiv references have been published as papers, and accordingly, the reference list should be updated in these cases. They are also very pleased to recommend publication of the paper!



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**Author-provided documents included with the Transparent Peer Review Record:**

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