

To whom it may concern
Chaos, Editorial Office:

Through this letter, I am resubmitting the manuscript entitled "Hybrid reaction-diffusion and clock-and-wavefront model for the arrest of oscillations in the somitogenesis segmentation clock", with reference number CHA21-AR-00106, to be considered for publication in Chaos. We have modified the manuscript taking into consideration all the Reviewers' comments and criticism. Please find below our response to the Reviewers.

Sincerely

Moisés Santillán

Response to Reviewers

We thank both reviewers for their comments and criticisms, which greatly helped us to improve our work. We have modified our manuscript, taking into consideration all their comments. Particularly, we changed the manuscript title to better reflect our conclusions, and practically rewrote the Results section, to improve the storytelling and clarify the contributions of our work. Below, we explain in detail the changes we made in our paper, to account for the Reviewers comments. All the performed modifications are marked with red fonts in the manuscript PDF file.

Reviewer 1

1. In the analysis of the equations (A1) I could not see in figure 6 (a) the point where the limit cycle became unstable and the new stable fixed points in the diagram. This is vital for the manuscript as it is the main mechanism for patterning.
Fig. 6 contains bifurcation diagrams for the system in the absence of diffusion of both the activator and the repressor. Under these circumstances, the limit cycle is always stable. It destabilizes when repressor diffusion is included. We have modified the manuscript to clarify this point.
2. To further understand the phase space portrait equations (A1) when "beta" is low enough, it must be shown the stability and type of fixed points coexisting, if the oscillation is perturbed at a given phase, to which fixed point the system will land on?
As can be seen in Fig. 9, the purely kinetic system (A1) has no overlapping steady-states branches for the parameter set values taken into account. This implies that, in absence of diffusion, only periodic orbits branches and steady-states co-exist. However, our dynamic proposal for somitogenesis lies on the Turing-Hopf bifurcation, which consists of having a transport process that produces unstable oscillations. These oscillations do not persist in the long term as spatially periodic stationary state arise by means of having resonant wave modes for the real part of eigenvalues only. As is shown in figure 11(c), the imaginary part of the eigenvalues are non-zero for small wave modes only, and so oscillating features are transitory. To avoid confusion, we have extended our discussion in Appendix A to thorough describe the way diffusivity and background regulatory input of the activator play a relevant role on the spatio-temporal dynamics, where self-sustained spatial stable oscillations become unstable to yield Turing patterns in the end.
3. The introduction of the diffusion constant " D_a " is unnecessary as it only introduces confusion on how this system works. Two diffusion constants are only necessary if the patterning emerged from an instability due to the spatial coupling (Turing type), and indeed the most interesting part of this model is that the patterning emerges from a different mechanism. The spatial analysis should be done by

having only the diffusion constant " D_r ".

The Reviewer is right in that only repressor diffusion is necessary to explain a spatial instability of the Turing type. However, by including activator diffusion, the limit cycle turns stable at high β values. This allows us to couple the system with a β wavefront and get a clock-and-wavefront behavior. In summary, incorporating activator diffusion is necessary to get a hybrid PORD and clock-and-wavefront model. We have largely modified the manuscript to make this clear.

4. A plot showing the relationship between segment size and velocity is necessary, as it is one of the central parameters in models of the segmentation clock. The following paper should be cited (doi.org/10.2976/1.3027088). Also it should be cited when mentioning the slowing of oscillations that generate the traveling waves.
The suggested plot has been included, and the mentioned paper has been properly cited. Thanks for the suggestion.
5. In equations 6 a and b, the white noise term is " dW/dt " and not " dW ", as W is the Wiener process which is the time integral of white noise.
We have corrected this error. Thanks for pointing it out.
6. Wnt and Fgf oscillate in chicken and mouse, but it does not oscillate in zebrafish.
We have clarified this point in the manuscript. Thanks.
7. The word "substance" is used when the authors mean "morphogen".
We have substituted the word substance by more adequate terms, like morphogen and proteins, along the manuscript.
8. In equations 6 a and b, what is the exact meaning of " \bar{a} " and " \bar{r} " are the mean values of the deterministic oscillation? or is the value of the unstable fixed point?
They represent the value of the stationary fixed point. We have changed the notation to \bar{a}^* and \bar{r}^* for clarity.
9. In page 10, $k^2 = 0$ is mentioned, but it is not mentioned explicitly that k is the Fourier mode.
We have corrected this.
10. In equation (8) the letter " K " should be omitted as it can be confused with a dissociation constant.
We have substituted K for $K_{1/2}$,s to make clear that it is a half saturation constant.
11. When the coefficient of variation $C_v = 0.1$ the segments are irregular, can you explain why?
We have offered the following explanation in the manuscript modified version: "This happens because one of the effects of adding noise is altering the phase of the oscillating genes, and this modifies the timing of their interaction with the morphogen wavefront"

Reviewer 2

1. In my opinion, the change is a relatively incremental discussion compared to the PORD model. But before I get to this, there is another issue, primarily due in fact to Cotterell et al : the PORD model is not fundamentally different from another classical model, the Meinhardt model for segmentation, which patterns in the absence of a wavefront exactly as described by the author of the current paper. This connection of the PORD to the Meinhardt model is [briefly] discussed in Francois et al, Curr Op Syst

Biology, 2018, with some mathematical derivations in Supplement of the paper, showing e.g. that the system in absence of diffusion is a relaxation oscillator, etc... . As an illustration, Fig 2B of that paper is virtually identical to the present Fig 1, and while the precise terms/details of the equations are different, both the structures of the equations (nuclines, etc...) and the properties (autoactivation + repression, initial conditions, mechanism of stabilization) appear very similar to me. I would invite the authors to make more explicit this connection, or to explain in which ways those models are fundamentally different. This is not a minor issue since, again, many properties of the PORD model discussed here are virtually identical to Meinhardt's model, and of course Meinhardt himself discussed limitations and fixes of his segmentation model (e.g. in his 1982 book, see also his 1992 review in Reports on Progress of Physics). Now going back to the new model, this kind of models classically stabilize because of diffusion, which explains why there can be irregular patterns if initial conditions are randomized. The authors then add an extra « Wavefront » parameter to induce a transition from a cycle to the pattern, arguing that this would stabilize the system. This adds "some" robustness. Overall, I find the idea potentially interesting, but the novelty of the idea is not entirely clear to me. But on top of the important theoretical discussion, better connections to biology and explanations about what happens would be really welcome in my opinion.

We have made a great effort to contrast our model with previous ones, and clarify what its main contribution is. Namely, we start that developing an equivalent Meinhardt-PORD model, and then expand it (by considering activator diffusion) to efficiently couple it with a morphogen wavefront. The model thus amended is in fact a hybrid Meinhardt-PORD and clock-and-wavefront model, which retains the advantages of both paradigms. Please see also the responses to the rest of the Reviewer comments.

2. My major recommendation is to discuss better the connections to the current model (and to the PORD) to other existing models. Some of my comments below relate to this issue because the authors do not give all the details needed to understand how their model practically work (in my opinion).

We included in the manuscript a brief revision of the Meinhardt model, and thoroughly discussed its connections with the PORD model and ours.

3. Meinhardt discussed how morphogens can bias reaction diffusion based patterning in his own model, I am wondering if the authors could compare their work to his.

We thank the Reviewer for pointing this out. Meinhardt indeed coupled its model with a morphogen gradient, but it was a static gradient. In the present work, we managed to efficiently couple it with a morphogen wavefront, like the ones observed in vertebrates.

4. A general question is what defines a somite. For instance, when p11 it is said that when "the limit cycle turns unstable, a somite is formed", so I gather that implicitly the authors assume that a somite is defined by a steady state. But part of the discussion (in the biological literature as well as in Meinhardt's papers already) is that the definition of a proper somite includes not one but two states. Meinhardt argues that the alternation of two steady states A/B is necessary to define a segment. In the biology papers, rostral caudal markers have been argued to be necessary to define somites, so that somites without markers are not "proper" somites. So, in the authors' model, what defines a somite, for instance in Fig 3B? Are boundaries stabilized by diffusion? If we switch off diffusion, what happens and how realistic is it?

We have included the following text in the manuscript, where we explain what defines a somite in our model: "Further notice in Fig 2A that, once the system has reached a stationary pattern, the high-activator-concentration stripes are much shorter than those corresponding to low activator concentration. This behavior is different from that of the Meinhardt-PORD model, which renders alternated equally sized stripes. Meinhardt interpreted them as corresponding to the anterior and posterior phenotypes (AP) of a somite. Meinhardt also pointed out that this pattern was not sufficient

to explain somitogenesis, as another periodic stripe (S) was needed, at least, to determine somite boundaries via the pattern: SAPSAPSAPS, and suggested that a more complex gene network was required to reproduce this pattern. In our model, we propose that a high activator level is the signal that triggers the mesenchymal-epithelial transition, and so the high-activator-concentration stripes can be interpreted as corresponding to the boundaries between adjacent somites (S stripes). Hence, somites occupy the low-activator-level regions in the final stationary pattern. As in the Meinhardt model, ours does not explain all segmentation features. In particular, it does not account for the differentiation between the anterior and posterior somite phenotypes, and a more complex network would be required to do so." Regarding the role of diffusion, without activator diffusion, our model is equivalent to the Meinhardt one, and repressor diffusion induces a spatial instability. Thus, patterning appears via a Turing mechanism. Furthermore, activator diffusion induces back spatial stability at high beta values, preventing patterning. This allows us to couple the system with a morphogen wavefront to yield a clock-and-wavefront behavior. We have modified the manuscript to clarify these points.

5. I suspect that in the authors' mind, the pattern is clearly defined by two « states » (the yellow and blue region). Is it a bistable system (possibly "blurred" by diffusion)? Then, the idea to have a transition from oscillation to bistability fits several other models of somitogenesis, that should be mentioned. In fact those models precisely have a wavefront inducing a bifurcation, just like what the authors build here, so it is a bit unclear to me what the fundamental difference is with such models. For instance, the "irregular" pattern could also just be a bistable system stabilizing with random initial conditions. We have included the following text in the manuscript revised version to address this point: "The behavior depicted in Figs. 4 and 5 is similar to several other models for somitogenesis, in which the wavefront causes a transition from oscillation to bistability \cite{Francois2007}. What makes the present model different is the dynamic mechanism driving segmentation. In here, when parameter β (which accounts for the interaction of the gene network with the wavefront) locally decreases below a given threshold, the system dynamics turns spatially unstable. Hence, any local inhomogeneity is magnified and causes a biphasic pattern of gene expression. This mechanism has a couple of characteristics that make it appealing: a) since the system behavior changes when the value of β is modified, a β gradient gives rise to local inhomogeneities, and so no special initial condition is needed to seed patterning; and b) the fact that the system is spatially stable for large β values, should make the patterning process robust to variability of the initial conditions and to added random noise (spatial stability attenuates the inhomogeneities induced by these sources of variability)."
6. Another related point is the size of the pattern. If the speed is multiplied by 2 (on Fig 5), it seems the pattern size is also multiplied by 2, which fits the expectation of the clock and wavefront model (size of the pattern = period times speed). Is it correct?
The Reviewer is correct. We have verified this point. See Fig. 5 in the manuscript revised version.
7. The authors argue that the modified model includes some "robustness". But there is no real metric of this robustness, just simulations in presence of noise compared for instance to Fig 2 where irregular patterns are shown due to very specific initial conditions. What happens if one uses the initial conditions of Fig 2 for Fig 5? More generally, the notion of robustness is a bit fuzzy and I am wondering if the authors could find a way to quantify their model vs the PORD model in terms of robustness (to noise? to initial conditions?)
We have quantified the system robustness to two different types of fluctuations: initial-condition random fluctuations, and added white noise. See Figs. 6 and 8 in the manuscript revised version.
8. Lastly, again coming back to other model and classical experiment on somitogenesis, it is generally argued that the system creates essentially two kinds of stripes, of equal size (possibly corresponding to

rostra-caudal markers in somites). But then, on Fig 4 and Fig 6 the blue region is much smaller than the yellow region. How realistic is it? In general, more connections to biology and possible predictions of the current model not present in the PORD model would be welcome...

[Se response to point 4.](#)