Modeling of somitic segmentation in vertebrates

Lecture 28
Somites are embryonic tissue structures that form shortly after the end of gastrulation and simultaneously with the beginning of organogenesis. Somites are visible as small roundish pillow-looking elements formed by mesenchimal cells on both sides of the neural tube. Normally vertebrate organisms form about 50 – 70 of such somites although snakes are known to have some 3 – 4 hundred. The somites form sequentially from the presomitic mesoderm in the head to tail order, one by one. Later in the course of embryonic developments, somites give rise to formation of periodic body structures which are visible in the adult organism as vertebra, ribs, muscles, blood vessels and nerves.

This process has attracted attention of developmental biologists and mathematical modelers at least since 1970s. What captivated the interest of scientists was the time and space periodicity of the process which occurs in nature with remarkable robustness and reproducibility.
The Clock and Wavefront model

Already in 1975 Cooke and Zeeman proposed a “clock and wavefront” theory which phenomenologically correctly describes the formation of somites from the presomitic mesoderm (PSM):

This model proposes combination of coupled intracellular oscillators and the propagation of “competence wave”. The molecular mechanisms of both phenomena were of course unknown at the time.

The first model which was destined to have a long life and become a prototype for the latter developments was model known under somewhat poetic name “The Clock and Wavefront”. This model was formulated on the conceptual level as no biological details were known at the time. It assumes that all cells of the PSM have internal oscillator of fixed period and the neighboring cells are phase connected so that the phase of the oscillation forms nicely periodic (sinusoid on the picture) distribution in space. At the same time, there exists a steadily propagating front of terminal differentiation which moves from the head to tail with constant speed. As the front passes, the oscillation is stopped and the phase of cell oscillation is fixed. Particular phase of the oscillation is transformed into specific developmental program, e.g., boundary creation. Provided constant speed of the wave and fixed period of the intracellular oscillations, the process results in steady creation of new somites.
What to model? Where to start and where to stop?

The past three decades of research on somitic segmentation have seen several waves of modeling approaches:

- Early conceptual models: were based on the assumption of “pre-patterning” of PSM by surrounding tissue, did not address question how periodicity emerges
- Mathematical phenomenological models: considered various flavors of the “clock and the wavefront” model. Meinhardt (1986) proposed a model based on the activator-inhibitor mechanism. Separation of somites due to periodically growing mechanical tension was proposed by Belintsev (1987)
- With arrival of molecular biology and genetic information, the modeling studies begin to address issues of the nature of intracellular oscillations and the cell-cell interaction.

 Needless to say that the regular nature of somitic segmentation attracted a great deal of attention among theoretical biologists, mathematicians and biophysicists. The first wave of studies while pointing to the robustness of the somitogenesis, ascribed the periodicity to the pre-existing pattern laid out by some other process. That did not really help and we will not consider those studies. The first breakthrough in the understanding came with the clock and the wavefront model proposed by Cooke and Zeeman. Naturally, a number of theorists proposed their own mechanisms that can account for the “wave and the clock”. We will consider a few examples of this type. Finally due to the recent progress in biological understanding the new modeling stream has considered molecular nature of the potential mechanisms that stand behind the “clock and wavefront”.

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Phenomenological approach to modeling

Here we consider two examples of models which formally describe the kinetics of the process without detailing the underlying biological mechanism.


\[
\frac{\partial a}{\partial t} = \frac{M}{T} \cdot \theta(vt - r) \cdot \theta(h_0 - h(t - t_0))
\]

\[
\frac{\partial h}{\partial t} = ka - dh + D \frac{\partial^2 h}{\partial r^2}
\]

\[a(0, r) = h(0, r) = 0, \quad r \in [0, L]\]

\[\frac{\partial h}{\partial r}(t, 0) = \frac{\partial h}{\partial r}(t, L) = 0\]

\[\theta(x) = 1 \text{ for } x \geq 0 \text{ and } 0 \text{ otherwise}\]

Here \(a\) is the density of cells “initiated” to become a somite boundary by the propagating wave. Activated cells produce diffusible inhibitor \(h\) which prevents cells adjacent to the activated ones to become activated.

In the other model (Meinhardt, 1986) the activator and inhibitor oscillate homogeneously until the oscillations are “fixed” at the boundary of the domain. Then as shown below, the pattern extends from the boundary towards posterior.

The models presented on this slide are typical representatives of the mathematical ways to formalize the “clock and wavefront” concept. The first model however assumes that local oscillation is not necessary as far as lateral inhibition of cells is in place. Once the concentration of inhibitor exceeds the critical value \(h_0\) for time longer than \(t_0\), the transition to activated state stops. Thus, the presence of inhibition accounts for the spatial periodicity of somite boundary initiation. The model takes the presence of the propagating wave front as given reality and does not consider the origin of this phenomenon. Other models used different mechanisms and focused on varying aspects of the somitogenesis. For example, Meinhardt’s model is more concerned with the anterior-posterior polarization of the somites which requires different granularity of the model.

It should be noted that as in most of other cases, any biological phenomenon allows for a number of different explanations and mathematical modeling alone cannot discriminate between them. However it can propose plausible mechanisms which can be experimentally tested when and if necessary experimental base is available.
Molecular clockwork of the “clock”

In 1997 Palmerim et al. identified the first protein – c-hairy1 – oscillating with the period of somite formation (90 min in chicken).

Since that somitic oscillations were found in mice, frogs, zebrafish and other vertebrate models. Found molecular components in all cases were linked to the delta-notch signaling pathway.

With more biological knowledge accumulated, it has become clear that there are at least two types of control network: one specific to higher vertebrates (more complex) and one typical for the lower vertebrates (less complex).

Recent studies have identified a great deal of molecular components responsible for the operation of the “clock”. These components are related to the delta-notch signaling pathway which we already visited once in the previous lecture. There it was identified as a key implementation of the lateral inhibition mechanism. In somitic segmentation it plays different but equally important role in synchronization of local oscillations. The basic part of the clock in all studied systems is the family of hairy basic helix-loop-helix transcription factors. These are transcriptional repressors that can repress a number of genes including their own genes and ligands of the delta family.
Are the “clock” and “wavefront” one thing?

The oscillations of *hairy* gene expression behave like a wave which propagates from the posterior of PSM towards the anterior. As the somite buds, the oscillations stop and the posterior part of a newly formed somite continues to express c-hairy1 in full agreement with the Meinhardt model.

With the discovery of oscillating proteins in the lab, the old modeling efforts have resumed with the doubled enthusiasm. Surprisingly, in the light of the new experimental data, the model by Meinhardt published in 1986 came the closest to the “correct behavior”. It appears that the oscillations of hairy gene expression propagate from some leading node in the growing posterior of the PSM as a wave that sweeps the entire PSM and stops shortly before the last formed somite. As the wave propagates its width shrinks and finally when the wave stops, it is equal to the size of somite. While one wave period pre-patterns the PSM, the previously patterned somite undergoes necessary structural changes as the cells inside it differentiate.
In the recent paper, J. Lewis proposed a very simple model explaining operation of the “clock” in lower vertebrates, in particular in zebrafish. His model is based on the existence of transcription and translation delays in the pair of bHLH transcription factors her1 and her7:

\[
\begin{align*}
\frac{dp}{dt} &= am(t - T_p) - bp \\
\frac{dm}{dt} &= \frac{k}{1 + p^2(t - T_m) / p_0} - cm
\end{align*}
\]

Here \(m\) and \(p\) are mRNA and protein levels respectively of either gene. Both her1 and her7 are transcription repressors which can form homo and heterodimers that repress the transcription of their own genes.

In this model Lewis proposed very simple explanation for the existence of oscillations in the lower vertebrate networks. In the particular example of zebrafish where period of somitic segmentation is only 30 min, it is feasible that the oscillations are purely due to the existence of transcription and translation delays. He estimated transcription delay to be on the order of 20 min and translation delay to be about 3 min. However, this explanation of oscillation requires that the degradation of repressor proteins and their RNAs occurs very quickly – half life ~ 3 min. This assumption is likely to be an over stretch. However the author remarks that in higher vertebrates this mechanism will not be able to explain the entire complexity of the observed network.
Local clocks communicate with the neighbors

The proposed model also incorporates the cell-cell communication which occurs through the interaction of her1-her7 transcription network with the delta-notch signaling pathway. While delta-notch signal induces production of her factors, they repress transcription of delta ligand and thus provide negative feedback.

As mentioned earlier, in this case delta-notch signaling pathway does not mediate lateral inhibition but instead synchronizes local oscillations. Remember that delta-notch represents an example of a juxtacrine signaling that operates from cell to cell through direct interaction of neighboring cells. Thus somitic segmentation appears to be an example of a developmental system which utilizes diffusible morphogens for the creation of long range gradients and employs juxtacrine relay for the establishment of the wave of synchronized oscillations.
What to take home

• Somitic segmentation is a developmental program based on the complex intracellular signaling and gene network dynamics orchestrated by cell-to-cell communication.

• Periodic formation of somites is driven by intracellular oscillatory dynamics.

• Study of somitic segmentation provides a particularly encouraging example demonstrating how mathematical modeling can provide valuable insight and practical guidance for the experiment long before all the molecular details are identified in the lab.