BE 159: Signal Transduction and Mechanics in Morphogenesis

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Winter, 2017

5 Segmentation clocks

The precursor to vertebrae are called *somites*, illustrated in Fig. 7. In this lecture, we explore the mechanisms for **somitogenesis**, the process by which these somites are formed.

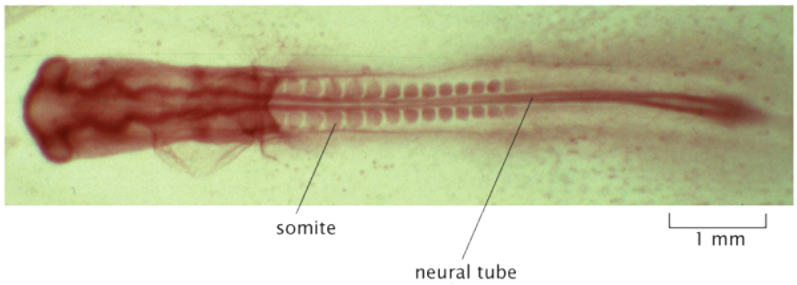

Figure 7: Somites in a developing chick embryo. Image take from Phillips, et al., *Physical Biology of the Cell*, 2nd Ed., Fig. 20.21, 2012.

5.1 Basics of somitogenesis

It is believed that somitogenesis happens as a result of oscillatory gene expression and arrest of this oscillation at a specified position along the anterior-posterior axis of the developing embryo. At the tail end of the embryo, cells in the presomitic mesoderm (PSM) (Fig. 8) exhibit oscillatory expression of certain genes. In zebrafish, these genes are *her1* and *her7*, related to the pair-rule gene *hairy* in *Drosophila* (hence the name; *her* is short for *hairy-related*). As the *her* genes oscillate, the organism is growing, so the PSM keeps moving posteriorly. If the oscillating cells are far enough from the posterior, they arrest. The position where the arrest occurs is called the **arrest front**. If a cell is in a peak of the *her* oscillation when it arrests, it will have one fate, but if it is in a valley, it will have another fate. Thus, the alternating structure of the somites is formed. Note that the distance between the arrest front and the posterior end of the organism may fluctuate, but in many models is taken to be constant.

5.2 The clock-and-wavefront model

The **clock-and-wavefront model** was proposed by Cooke and Zeeman in 1975 and was one of the first models put forward to describe somitogenesis. This model is based on the following assumptions.

a) All cells in the PSM are oscillating.

b) Coupling between the cells induces synchrony in oscillation, setting the oscillation frequency to be T.

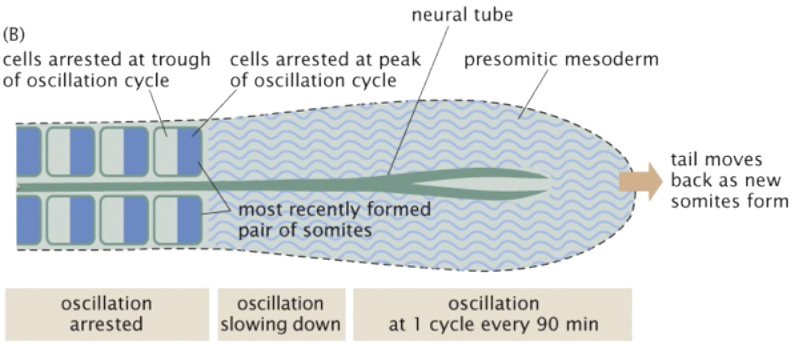

Figure 8: A schematic of the clock and wavefont model for somitogenesis.

c) Waves arrest at a front at the anterior.

d) The arrest front moves posteriorly with a speed v.

In this model, since all cells in the PSM are oscillating *in unison*, meaning that they have both the same frequency and the same phase, the dynamics of the extension of the PSM during growth is irrelevant. Only the motion of the front into the oscillating cells is important.

An important conclusion of this model is that the size of the somites is $s = v/T$. This is the distance traveled by the arrest front between peaks in the oscillators. The model can be tested indirectly by measuring somitogenesis over a time interval of set length, as shown in Fig. 9. We measure the length of the region of somites, D_s, and the position of the arrest front, W_a and W_b and the beginning and end of the time interval, respectively.

The speed of the arrest front is $v = (W_b - W_a)/n$, where n is the number of somites that are formed in the interval. (Developmental time is usually measured in units of number of somites.) The rate of somite formation is D_s/n. If the clock-and-wavefront model is true the rate of somite formation and the speed of the arrest front should be equal, so we should have $D_s = (W_b - W_a)$. This was tested in Gomez et al., *Nature*, **454**, 335–339, 2008, and the result is shown in Fig. 10. For each of four species, the ratio of the rate of arrest front movement to that of the formation of somites, $(W_b - W_a)/D_s$, is approximately unity, suggesting that the oscillation frequency is tuned with the front velocity, as given by the clock-and-wavefront model. However, note that this ratio is decidedly below unity for zebrafish.

The clock and wavefront model also makes valuable predictions about the size and number of somites in mutants. Consider first the ratio of the sizes of somites in wild type and mutant embryos. Here, we assume the mutations affect the genetic oscillations and not the speed of the arrest front. Specifically, for the purposes of

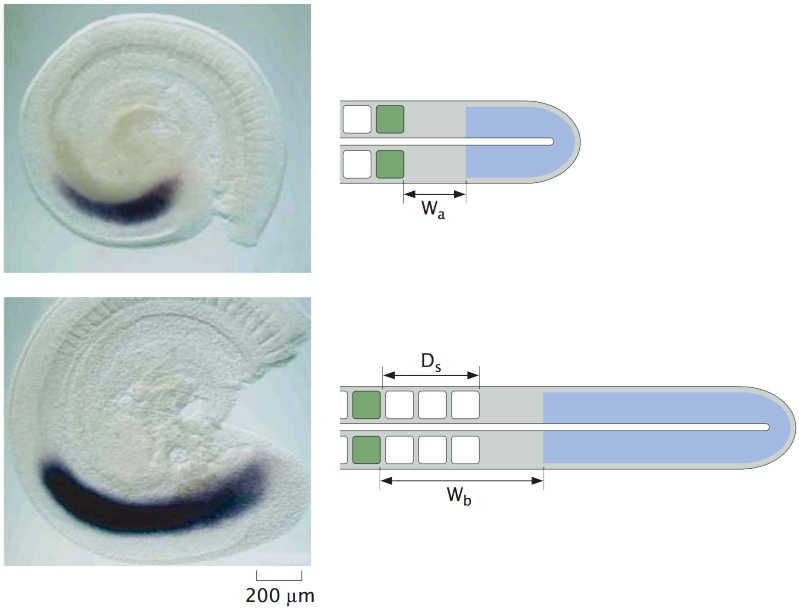

Figure 9: A schematic of a measurement of the arrest front speed and rate of somitogenesis in a corn snake embryo. The gene MSGN1 is stained to signify the location of the PSM. The arrest front is at the anterior edge of this stained region. The somites are also clearly visible. W_a and W_b are the positions of the arrest front at the beginning and end of a developmental time interval, and D_s is the length of somite region formed during the same time interval. From Phillips, et al., *Physical Biology of the Cell*, 2nd Ed., 2012, adapted from Gomez et al., *Nature*, **454**, 335–339, 2008.

discussion, we will take $T_{\text{mut}} > T_{\text{wt}}$.

$$\frac{s_{\text{mut}}}{s_{\text{wt}}} = \frac{vT_{\text{mut}}}{vT_{\text{wt}}} = \frac{T_{\text{mut}}}{T_{\text{wt}}}. \tag{5.1}$$

So, we get larger somites with slower oscillations. Now, consider the ratio of the number of somites over the developmental time T_{dev}.

$$\frac{n_{\text{mut}}}{n_{\text{wt}}} = \frac{T_{\text{dev}}/T_{\text{mut}}}{T_{\text{dev}}/T_{\text{wt}}} = \frac{T_{\text{wt}}}{T_{\text{mut}}}, \tag{5.2}$$

which says that we get fewer somites.

5.3 PSM cells do not oscillate in unison

Despite some indirect experimental evidence supporting the original clock-and-wavefront model, such as in the Gomez et al. paper, direct observations of *her1* dynamics in the PSM show that the gene expression in the cells does not oscillate in unison. This was

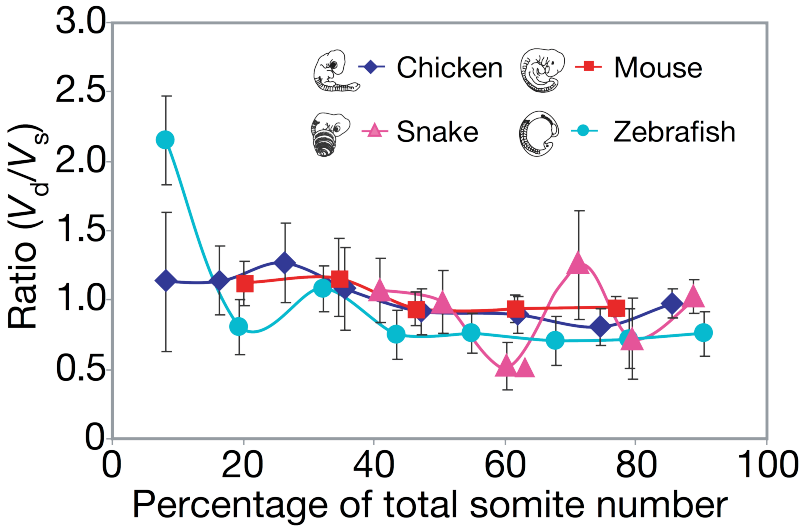

Figure 10: The ratio of the rate of the arrest front movement (V_d) to that of formation of somites (V_s). For all four species, this ratio is approximately unity. Adapted from Gomez et al., *Nature*, **454**, 335–339, 2008.

seen before by fixing and staining embryos at certain time point, but was more firmly demonstrated when Soroldoni, et al. (*Science*, **345**, 222–225, 2014) developed a reporter for *her1 in vivo*. As seen in Fig. 11, the expression of *her1* is not uniform. Further, we see that **kinematic waves** of *her1* expression travel toward the anterior where they are arrested.[3]

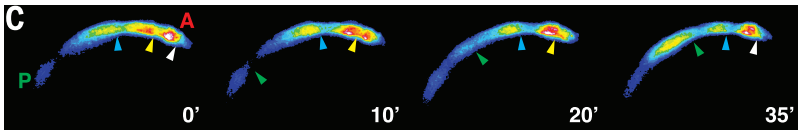

Figure 11: Image of *her1* expression over time in a developing zebrafish embryo. Waves of expression (each wave is identified and color coded with arrowheads) travel toward the posterior where they are arrested. Figure taked from Soroldoni, et al., *Science*, **345**, 222–225, 2014.

To understand how kinematic waves work, I borrow the analogy from the Soroldoni paper. Think about a stock ticker. Each light flicks on and off and there is some coupling to neighboring lights. The result is a movement of an image of lights across the ticker, even though each light bulb is stationary.

Since the observation of kinematic waves automatically eliminates the clock-and-wavefront hypothesis with a uniform oscillation frequency, we need to take a more careful look at the oscillators.

[3]This is better seen through a movie of this process, http://science.sciencemag.org/highwire/filestream/595541/field_highwire_adjunct_files/0/1253089s1.avi, though the link may not work for you because *Science* is a closed journal.

5.4 Generic description of oscillatory gene expression in somitogenesis

To more generically describe somitogenesis, Soroldoni et al. describe the oscillations in the PSM generically as a function of space at time. To do this, we define a generic description of an oscillatory function. For any function $Q(x,t)$ that is oscillatory in time, we can write the dynamics at position x as

$$Q(x,t) = q_0 + q(x,t)\cos\phi(x,t), \tag{5.3}$$

where $\phi(x,t)$ is the **phase** of the oscillation, q_0 is the baseline, and $q(x,t)$ is the amplitude. The parameters q_0 and $q(x,t)$ capture the strength of the oscillatory signal, while the frequency information is captured in the phase. As an example, we get a pure cosine wave that is uniform in space if $\phi(x,t) = \omega t$, and a pure sine wave if $\phi(x,t) = \omega t - \pi/2$. The period of both of these waves is $T = 2\pi/\omega$.

Our analysis will focus on the phase of the oscillations, which is a result of the temporal dynamics of gene expression and coupling to neighboring cells. Let the x-position of the posterior end of the PSM be $x = 0$ and let $a(t)$ be the x-position of the anterior end of the PSM (the arrest front). We define $\phi_A = \phi(a(t),t)$ as the phase of the oscillators at the anterior and $\phi_P = \phi(0,t)$ as the phase of the oscillators at the posterior. Then, the number of kinematic waves, K, in the PSM is given by the total difference in phase, modulo 2π.

$$K = \frac{\phi_P - \phi_A}{2\pi} \tag{5.4}$$

Note that we do not wrap the phase shift here, i.e., $\phi = 2\pi$ is not the same as $\phi = 4\pi$.

To investigate how the number of kinematic waves changes in time, we compute the time derivative.

$$\frac{\mathrm{d}K}{\mathrm{d}t} = \frac{1}{2\pi}\left(\frac{\mathrm{d}\phi_P}{\mathrm{d}t} - \frac{\mathrm{d}\phi_A}{\mathrm{d}t}\right) = \frac{\omega_P - \omega_A}{2\pi} = \frac{1}{T_p} - \frac{1}{T_A}, \tag{5.5}$$

where we have defined

$$\omega = \frac{\partial\phi}{\partial t}. \tag{5.6}$$

This tells us that if the number of kinematic waves changes in time, then the period at the anterior is different than that at the posterior. This can be observed experimentally, and is one way of seeing a non-flat phase profile across the PSM.

I just used the word **phase profile** loosely to mean how the phase of the oscillators varies across the PSM. Let's codify that more concretely. We define the phase profile $\psi(x,t)$ as

$$\psi(x,t) = \phi(x,t) - \phi_P(t), \tag{5.7}$$

which is simply how the phase varies as we move away from the posterior. Then, we can write

$$\begin{aligned}\omega_A = \frac{\mathrm{d}\phi}{\mathrm{d}t} &= \frac{\mathrm{d}\phi_P}{\mathrm{d}t} + \frac{\mathrm{d}\psi(a(t),t)}{\mathrm{d}t} \\ &= \omega_P + \frac{\partial\psi(a(t),t)}{\partial t} + \frac{\mathrm{d}a}{\mathrm{d}t}\frac{\partial\psi(a(t),t)}{\partial x} \\ &= \omega_P + \omega_W + \omega_D, \end{aligned} \tag{5.8}$$

So, the difference in oscillation frequency between the anterior and posterior is $\omega_A - \omega_P = \omega_W + \omega_D$. We have defined $\omega_W \equiv \partial\psi(a(t),t)/\partial t$. This is the change in frequency that is inherent to the oscillators. Soroldoni, et al. call $2\pi/\omega_W$ the "dynamic wavelength," which gives the change of the wavelength of the kinematic waves in time. We have also defined

$$\omega_D = \frac{\mathrm{d}a}{\mathrm{d}t}\frac{\partial\psi(a(t),t)}{\partial x}. \tag{5.9}$$

This describes how the anterior phase differs from the posterior due to the Doppler effect. Since the PSM is shortening during development, the anterior is rushing into the kinematic waves, so the observed frequency is higher. Specifically, the speed of the observer is $\mathrm{d}a/\mathrm{d}t$ and the traveling wave has a wavelength of $2\pi(\partial\psi/\partial x)^{-1}$.

5.5 Assessment of models in terms of ω_A and ω_P

Soroldoni, et al. can measure the phase profile and can therefore deduce ω_D and ω_W. What do different models predict?

Clock-and-wavefront model. In this model, $\phi(x,t) = \omega t$, since all oscillators oscillate in unison with a constant frequency ω. Thus, $\phi(x,t) = \phi_P(t)\ \forall x$, so $\phi(x,t) = 0$. Thus, $\omega_D = \omega_W = 0$ and $\omega_A = \omega_P$.

Steady-state PSM. One might consider the scenario where the PSM is in steady state. That is to say that it does not grow or shrink, $a(t) = a_0$, and there is no modulation of the phase portrait, $\phi(x,t) = \omega t + \psi(x)$. In this case, ψ is not a function of time, so $\omega_W = 0$. The length of the PSM, a, is also not a function of time so, $\mathrm{d}a/\mathrm{d}t = 0$, meaning that $\omega_D = 0$. So, again, we have $\omega_A = \omega_P$ under this model.

Scaling wave pattern. In this model, the phase profile is a time-independent function of the *normalized* PSM length.

$$\phi(x,t) = \omega t + \psi\left(x/a(t)\right), \tag{5.10}$$

and we may have $\mathrm{d}a/\mathrm{d}t \neq 0$. Then, we have

$$\omega_W = \frac{\partial \psi(x/a(t))}{\partial t} = -\frac{1}{a}\frac{\mathrm{d}a}{\mathrm{d}t}\left.\frac{\partial \psi}{\partial (x/a(t))}\right|_{x/a(t)=1}, \tag{5.11}$$

and

$$\omega_D = \frac{\mathrm{d}a}{\mathrm{d}t}\frac{\partial \psi(x/a(t))}{\partial x} = \frac{1}{a}\frac{\mathrm{d}a}{\mathrm{d}t}\left.\frac{\partial \psi}{\partial (x/a(t))}\right|_{x/a(t)=1}, \tag{5.12}$$

So, in this case, ω_D and ω_W have equal magnitude and opposite sign. Thus, we again have $\omega_A = \omega_P$. Therefore, if we find experimentally that $\mathrm{d}K/\mathrm{d}t \neq 0$, we must have $T_P \neq T_A$, so therefore $\omega_P \neq \omega_A$, and none of these three models can be true.