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A chemical flow system mimics waves of gene expression during segmentation

M. Kærn[a,*], M. Menzinger[a], Axel Hunding[b]

[a] *Department of Chemistry, University of Toronto, Toronto, Ontario M5S 3H6, Canada*
[b] *Department of Chemistry, University of Copenhagen, DK-2100 Copenhagen, Denmark*

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Abstract

The early vertebrate developmental process of somitogenesis involves bands of gene expression that form periodically at the posterior end of the presomitic mesoderm (PSM) and traverse it with decreasing width and velocity. We have constructed a chemical flow system that, based on the novel flow-distributed oscillator (FDO) mechanism of wave pattern formation, reproduces key physical features of the PSM and observe concentration waves having similar spatio-temporal behavior. This suggests that the gene expression waves can be understood qualitatively in terms of phase dynamics in an open flow of a self-oscillating medium and that chemical flow systems can be used to mimic and model biological pattern formation during axial growth. In fact, expressions for wavelength and wave velocity derived from phase dynamics are found to be in quantitative agreement with measurements from both the biological and the chemical systems. This indicates that they, despite their significant differences, have common dynamics.

Keywords: Pattern formation; Somitogenesis; Segmentation; Growth; Kinematic phase waves; Flow-distributed oscillators (FDO)

1. Introduction

The developmental process of somitogenesis (reviewed by Gossler and Hrabê de Angelis [1]) creates a number of blocks of mesodermal tissue called somites. Recent studies [2–6] have shown that somitogenesis involves kinematic [2] waves of gene expression that propagate through the presomitic mesoderm (PSM). These waves appear to be important in the developmental process but their origin remains somewhat obscured. Nevertheless, the fact that the waves are kinematic means that they arise from phase dynamics in an oscillatory medium [7]. Phase dynamics have been investigated intensively in closed chemical sys-

* Corresponding author.

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tems (see e.g. [7–11]) and several models involving phase dynamics have been suggested to account for biological segmentation [2,12–15]. It has, for instance, been suggested that the cells in the PSM contain 'segmental clocks' [16] and that the spatio-temporal wave behavior arises from the slowing down and ultimate arrest of this oscillation [2,16]. However, these models do not explicitly consider axial growth, which is an integral part of the developmental process.

Segmentation in many biological systems, including vertebrate, occurs during axial growth of either an internal structure or the body as a whole. Axial growth, or equivalently an open flow, thus plays a significant role in biological pattern formation and morphogenesis. To see this equivalence consider a cell that is laid down at the terminal growth boundary of an extending structure. While the cell typically remains stationary with respect to the body as a whole, its distance to the growth boundary increases in time as cells are being added to the growing form. Hence, cells are effectively 'carried away' from the growth boundary and axial growth creates, relative to the growth boundary, a steady flow of cells through the growing form (the PSM). Changing the reference frame to one where the terminal growth boundary is stationary shows that axial growth and open flows are indeed equivalent processes.

The presence of axial growth and the equivalence between axial growth and an open flow suggests that the waves observed during somitogenesis are formed as the result of reaction, e.g. the above mentioned oscillation, and convection. Such systems have been studied only recently [17–22] and to a much lesser extent than reaction–diffusion systems. To investigate the possibility that reaction-convection is involved in biological segmentation, a chemical flow system based on flow-distributed oscillators (FDO) [21–23] was constructed. This system reproduces key features of somitogenesis and we observed, given appropriate conditions, concentration waves having spatio-temporal behavior similar to that of the gene expression waves in the PSM. Thus, the chemical flow system mimics the gene expression waves in the PSM and may be used as a model system for the investigation of pattern formation in biology. Furthermore, it is shown, by deriving the wavelength and the wave velocity of the phase waves, that the spatio-temporal wave behavior in the chemical flow and the biological growth systems can be accounted for quantitatively by the same set of universal expressions.

2. Waves in somitogenesis

During somitogenesis, somite pairs form periodically (every 90 min in the chick) at the anterior end of the PSM. The somites appear on each side of the neural tube in a bilaterally symmetric and synchronous fashion. While somites form at the anterior end, hence removing cells from the PSM, cells are continuously added to its posterior end, such that the length of the PSM is kept relatively constant. The horizontal box in Fig. 1a (redrawn from [24]) shows how a prospective somite (squares) entering the PSM to the left is carried over the entire length of the PSM during the formation of 12 somite (circles) pairs (roughly 18 h). Note that only one of two identical PSM halves is shown for clarity of the figure. The transport of the cells within the prospective somite is evidently equivalent to an open flow of cells in the reference frame where the posterior PSM boundary is stationary.

Fig. 1a also shows the experimentally observed spatio-temporal expression of mRNA from the gene *c-hairy1* within the PSM (see Stern and Vasiliauskas [24] for details) and how the period of the cellular gene expression cycle changes as prospective somites mature. The cells in the posterior half of the PSM express mRNA in a periodic, synchronous fashion, leading to a wide band of gene expression (gray in Fig. 1a) appearing every 90 min. This posterior oscillation initiates a wave-like band of gene expression that propagates in the anterior direction with decreasing width and velocity. When the expression wave after 180 min reaches the anterior end of the PSM, the gene is expressed only in the posterior half of the newly formed somite. At this stage, the expression wave has a wavelength equal to the length of one somite and it propagates away from the posterior end of the PSM at a velocity equal

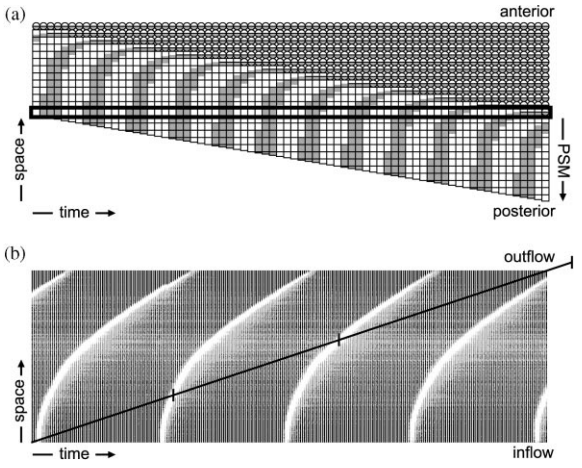

Fig. 1. (a) Space–time plot showing the spatio-temporal behavior of *c-hairy1* mRNA expression waves in the chick PSM over an 18-h period corresponding to the formation of 12 somites (redrawn from [24]). Each column represents a snapshot of the PSM taken every 30 min. Somites (circles) are formed from prospective somites (squares) periodically at the anterior end of the PSM. New cells are continuously added posteriorly and the prospective somite entering the PSM at the left moves through the PSM and matures into somite S_1 after 18 h (horizontal box). The horizontal box emphasizes the flow of cells through the PSM and the pronounced slowing down of the expression cycle (gray = on, white = off) near the anterior end. Note how the wide posterior expression band arising from a homogeneous oscillation travels through the PSM with decreasing width and velocity. (b), Space–time plot of experimentally observed concentration waves (white = high ferriin) in the BZ medium, mimicking the waves of gene expression in the PSM. Frames (256), taken at 1-s intervals, and showing 61 cm of the flow reactor, are stacked from left to right. The diagonal line shows how a volume elements entering the flow reactor at the left is eventually flushed out to the right ($v = 0.238$ cm/s). The intrinsic period T is seen to increase along this line while the period T' at the inflow (bottom) remains constant. As a result, white bands are formed periodically at the reactor inlet and propagate downstream with decreasing width and velocity.

to the rate of posterior PSM growth. It is noted that the posterior oscillation period T' matches the period of somite segmentation and that the PSM grows by one somite length for each somite formed (see [24] for details). Hence, the posterior growth rate and the velocity at which a prospective somite, and hence its cells, is carried through the PSM is given by $v = l/T'$, where l is the length of one somite.

Based on the illustration in Fig. 1a, key physical features of somitogenesis appear to be: (a) axial growth of a structure comprised of (b) cells containing a periodic process that are added at a growth boundary with (c) a periodically recurrent phase of the oscillatory process which (d) is slowed down as the distance between the cell and the growth boundary increases. Restated in physical terms these features corresponds to (a) an open flow of (b) oscillatory media subject to (c) periodic forcing at the inflow boundary with a (d) period that increases in the downstream direction.

To investigate if the above features (a)–(d) are sufficient to generate waves with the observed spatio-temporal behavior, they were reproduced in a chemical flow system. In these experiments, the oscillating ferroin-catalyzed Belousov–Zhabotinsky reaction medium was pumped through a vertically mounted flow reactor (a 10-mm inner diameter glass tube) filled with 1-mm glass beads to obtain a uniform (plug-flow) profile (features a and b). The flow reactor was fed from below by the outflow of a continuously stirred tank reactor (CSTR) using a peristaltic pump. Conditions in the CSTR were such that volume elements entering the flow reactor oscillated with a constant period (feature c). The volume of the CSTR was chosen large such that its oscillation period coincides with that at the flow reactor inlet (see below). Finally, a downstream increasing period (feature d) was achieved by heating the CSTR and the reactor inlet to 45°C and allowing free cooling of the upper part of the flow reactor.

Fig. 1b shows successive snapshots of the flow reactor, stacked from left to right. A volume element that enters at the inflow (bottom) is carried along the diagonal line to the outflow (top). The intrinsic period increases along this line due to the decreasing temperature of the medium. Close to the inflow, the CSTR and the lower end of the flow reactor oscillate in synchrony. This gives rise to periodic, wide bands of high ferroin concentration (oxidized ferriin, white in Fig. 1b). The synchronous oscillations at the reactor inlet develop into concentration waves that propagate downstream with decreasing width and velocity in qualitative agreement with the

observations in the PSM (Fig. 1a). Thus, the behavior of the gene expression waves appears, by analogy, to be the result of a biological mechanism that, in terms of physics, is equivalent to the FDO mechanism. In this mechanism, waves are formed as the result of phase dynamics in an open flow (axial growth) of oscillatory media (oscillating cells) subject to forcing at the inflow (growth) boundary.

3. Phase dynamics

To derive expressions for the wavelength and the wave velocity of waves induced by the FDO mechanism, consider a volume-element that is injected into the flow at the inflow boundary and is carried downstream at velocity v. First, we neglect that the oscillation is slowed down as a volume-element is carried downstream and initially investigate features (a) through (c) only. Thus, each volume-element is assumed to oscillate with a period T that is independent of the distance x to the inflow boundary. This assumption allows for analytic treatment without knowledge of the analytic form of $T(x)$ (feature d) and the derived expressions provide insights into the spatio-temporal dynamics of the simplest manifestations of the FDO mechanism. Furthermore, Fig. 1a suggests that T is constant at the posterior half of the PSM corresponding to constant period close to the reactor inlet (Fig. 1b).

The residence time a of the volume element, measured as the time spent in the flow reactor, is at constant flow given by $a = x/v$. Thus, when the volume element has moved the distance x, its oscillation phase has advanced by $\Delta\phi = a/T$. If it entered the flow with phase $\phi_0(\tau) = \tau/T'$, where T' is the periodicity at the inflow boundary, the phase at x at time $t = a + \tau$ is given by

$$\phi(x,t) = \phi_0(t-a) + \frac{a}{T} = \frac{x}{v}\left(\frac{1}{T} - \frac{1}{T'}\right) + \frac{t}{T'} \tag{1}$$

The phase equation in Eq. (1) can be used to calculate the velocity and wavelength of the concentration waves. The velocity is derived [8] from the fact that the phase is an exact differential of space and time, i.e. from $d\phi(x,t) = \partial_t\phi dt + \partial_x\phi dx = 0$, while the wavelength is the distance between cells with a phase difference of one. The expression of the wavelength λ and the wave velocity $c = -\partial_t\phi/\partial_x\phi$ are then readily obtained from Eq. (1) as

$$c = \frac{-v}{R-1}, \tag{2a}$$

$$\lambda = \frac{vT'}{|R-1|}, \tag{2b}$$

where $R = T'/T$ is the parameter that determines the behavior of the phase waves.

Eq. (2a) predicts four different types of spatio-temporal wave behavior. They are: stationary waves ($c = 0$) for $R = \infty$, upstream traveling waves ($c < 0$) for $R > 1$, homogeneous oscillations ($c = \infty$) for $R = 1$ and downstream traveling waves ($c > 0$) for $R < 1$. The decreasing wave velocity observed in Fig. 1b can thus be understood qualitatively, since the experimental value of R decreases (T increases) in the downstream direction from an initial value of one. Furthermore, since Eq. (2b) predicts a decreasing wavelength under these conditions, the downstream decreasing width of the oxidized band can also be accounted for.

In addition to the experiment in Fig. 1b, we did a number of experiments, summarized in Fig. 2, where the value of R was constant, i.e. without a temperature gradient [features (a) through (c) only]. We observed all four types of wave behavior predicted by Eq. (2a) and, as shown in Fig. 2, there is quantitative agreement between phase dynamics [Eqs. (2a) and (2b)] and our experimental observations.

4. Discussion

The derivation of the wave velocity [Eq. (2a)] and the wavelength [Eq. (2b)] relies only on the phase dynamics in an oscillatory media and is independent of how the flow or the oscillation is generated. An oscillatory medium [7] represents a continuous limit of a large population of weakly

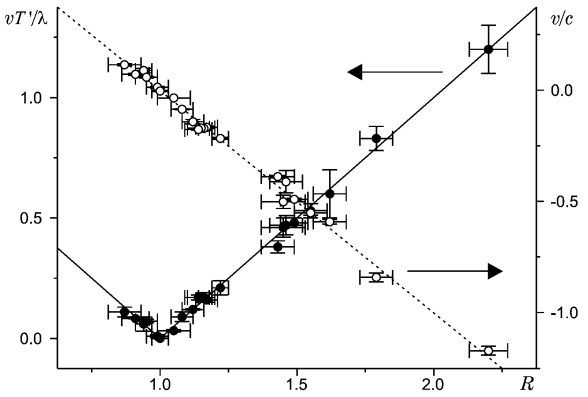

Fig. 2. Quantitative agreement between the FDO mechanism and experiments for constant R. Data is plotted as vT/λ (left axis, open circles) and v/c (right axis, closed circles) vs. R. Full and broken lines corresponds to Eqs. (2a) and (2b), respectively. v was measured as the velocity of the liquid/gas meniscus when the flow reactor was filled, T' was measured potentiometrically in the CSTR and T, c, λ were obtained by analysis of the recorded intensity maps. Typical CSTR premixed feed stream concentrations were [ferroin] = 7.5×10^{-4} M; [malonic acid] = 0.4 M; [sodium bromate] = 0.3 M; [sulfuric acid] = 0.15 M. The period in the flow reactor depends on the concentrations of the above species; the temperature of the medium; the velocity at which volume elements are carried through the flow reactor and on the diameter of the packing material. The period in the CSTR was altered, without affecting that in the flow reactor, by changing the volume of the CSTR; its volumetric flow rate; by adding small amounts of bromide to the feed-stream or by placing a pre-reactor in the feed-stream.

coupled self-oscillating elements whose interactions do not significantly change the amplitude or the form of individual oscillations. The expressions in Eqs. (2a) and (2b) are thus universal in the sense that they are independent of the nature of the elements that constitute the oscillatory medium and of the particularities of their weak mutual interaction. In the chemical flow system, the oscillating elements are diffusively coupled sub-volumes containing an oscillating chemical reaction while they, in vertebrate development, are cells that periodically express certain genes that may, for instance, be phase linked. Phase dynamics does not require for instance reaction–diffusion or cell–cell interactions to be absent and as shown (Fig. 2), the FDO predictions are indeed quantitatively valid in a chemical flow system when such interactions are present. In fact, they are expected to be valid in any system where an open flow (or growth) of an oscillating medium (physical, chemical or biological) is subject to constant or periodic boundary forcing at the inflow (or growth) boundary.

As it is evident from Fig. 1a, there are two regions of the PSM where the intrinsic cellular oscillation period T can be considered constant and the wave behavior should be one of the types predicted by phase dynamics [Eqs. (2a) and (2b)]. Fig. 1a shows that two cells in the posterior end of the PSM oscillate with the same period and that the intrinsic period T equals the forcing period $T'(R = 1)$ in this region. The experimental observation of a homogeneous oscillation in the posterior PSM half is thus consistent with the predictions by phase dynamics since a wave with infinite velocity and wavelength is predicted for $R = 1$.

The gene expression in Fig. 1a is seen to be constant at the anterior end of the PSM and within the array of mature somites. Hence, the gene expression cycle has been arrested such that $T = \infty$ and $R = 0$ in this region. For this value of R, phase dynamics predict a downstream traveling wave with velocity $c = v$ and wavelength $\lambda = vT'$. These predictions are also in perfect agreement with experimental observations. In Fig. 1a, the anterior gene expression band moves away from the posterior PSM boundary at a velocity c equal to the rate v of posterior growth. In this case, the segmental pattern is stationary with respect to the mature somites and the individual cells. Furthermore, since the growth (or flow) rate is $v = l/T'$ (see above), the space-periodicity of the anterior gene expression is predicted to be one somite length $\lambda = vT' = l$. This is in quantitative agreement with the wavelength of the gene expression wave observed experimentally.

The qualitative similarities of the wave behavior (Fig. 1) suggest that the waves in the two different systems are the result of their common features. In both cases, the spatio-temporal wave behavior can be accounted for by wave pattern formation in a flow of oscillatory media with boundary forcing, if the control parameter R decreases monotonically from a value of one at the

inflow/growth boundary. Furthermore, we have shown that the expressions for the wave velocity and wavelength derived from the universal FDO mechanism are quantitatively valid in both the PSM (Fig. 1a) and in the chemical flow experiments (Fig. 2). While it may be coincidental that phase dynamics naturally predict the correct distance between regions of gene expression, it appears that the spatio-temporal behavior of the gene expression waves is adequately captured by phase dynamics in flows of oscillatory media. Thus, chemical flow systems may be used to model biological pattern formation. Future theoretical, numerical and experimental investigations of open flow systems may thus provide further insight into the physical and chemical basis of morphogenesis in organisms, such as vertebrate, annelids and short- and intermediate germ band insects, where segmentation takes place during axial growth.

References

[1] A. Gossler, M. Hrabê de Angelis, Somitogenesis, Curr. Top. Dev. Biol. 38 (1998) 225–287.
[2] I. Palmeirim, D. Henrique, D. Ish-Horowicz, O. Pourquié, Avian hairy gene expression identifies a molecular clock linked to vertebrate segmentation and somitogenesis, Cell 91 (1997) 639–648.
[3] H. Forsberg, F. Crozet, N.A. Brown, Waves of mouse lunatic fringe expression, in four-hour cycles at two-hour intervals, precede somite boundary formation, Curr. Biol. 8 (1998) 1027–1030.
[4] I. Palmeirim, J. Dubrulle, D. Henrique, D. Ish-Horowicz, O. Pourquié, Uncoupling segmentation and somitogenesis in the chick presomitic mesoderm, Dev. Genet. 12 (1998) 77–85.
[5] A. Aulehla, R.L. Johnson, Dynamic expression of lunatic fringe suggests a link between notch signaling and an autonomous cellular oscillator driving somite segmentation, Dev. Biol. 207 (1999) 49–61.
[6] I.D. Barrantes, A.J. Elia, K. Wünsch et al., Interaction between notch signalling and lunatic fringe during somite boundary formation in the mouse, Curr. Biol. 9 (1999) 470–480.
[7] A.S. Mikhailov, Foundations of synergetics I. Distributed active systems, Springer Series in Synergetics, 51, Springer-Verlag, New York, 1990.
[8] D. Thoenes, Spatial oscillations in the Zhabotinsky reaction, Nat. Phys. Sci. 243 (1973) 18–20.
[9] N. Kopell, L.N. Howard, Horizontal bands in the Belousov reaction, Science 180 (1973) 1171–1173.
[10] S. Su, M. Menzinger, R. Armstrong, A. Cross, C. Lemaire, Magnetic resonance imaging of kinematic waves and pacemaker dynamics in the Belousov–Zhabotinsky reaction, J. Phys. Chem. 98 (1994) 2494–2499.
[11] A.A. Ploezhaev, Phase waves in oscillatory media, Physica D 84 (1995) 253–259.
[12] B.C. Goodwin, M.H. Cohen, A phase-shift model for the spatial and temporal organization of developing systems, J. Theor. Biol. 25 (1969) 49–107.
[13] J. Cooke, E.C. Zeeman, A clock and wavefront model for control of the number of repeated structures during animal morphogenesis, J. Theor. Biol. 58 (1976) 455–476.
[14] A.T. Winfree, The geometry of biological time, Biomathematics, 8, Springer-Verlag, New York, 1980.
[15] H. Meinhardt, Models of biological pattern formation, Academic Press, New York, 1982.
[16] Y. Jiang, L. Smithers, J. Lewis, Vertebrate segmentation: the clock is linked to notch signalling, Curr. Biol. 8 (1998) 868–871.
[17] A.B. Rovinsky, M. Menzinger, Self-organization induced by the differential flow of activator and inhibitor, Phys. Rev. Lett. 6 (1993) 778–781.
[18] S.P. Kuznetsov, E. Mosekilde, G. Dewel, P. Borckmans, Absolute and convective instabilities in a one-dimensional Brusselator flow model, J. Chem. Phys. 106 (1997) 7609–7616.
[19] J.H. Merkin, R.A. Satnoianu, S.K. Scott, Spatiotemporal chaos in a differential flow reactor, J. Chem. Soc. Faraday T 94 (1998) 1211–1216.
[20] P. Andresén, M. Bache, E. Mosekilde, G. Dewel, P. Borckmans, Stationary space-periodic structures with equal diffusion coefficients, Phys. Rev. E 60 (1999) 297–301.
[21] M. Kærn, M. Menzinger, Flow-distributed oscillators: stationary chemical waves in a flow system, Phys. Rev. E 60 (1999) 3471–3474.
[22] M. Kærn, M. Menzinger, Pulsating wave propagation in reactive flows: flow-distributed oscillations, Phys. Rev. E 61 (2000) 3335–3338.
[23] M. Kærn, M. Menzinger, Reply to comment on flow-distributed oscillators: stationary chemical waves in a flow system, Phys. Rev. E 62 (2000) in press.
[24] C.D. Stern, D. Vasiliauskas, Clocked wave expression in somite formation, Bioessays 20 (1998) 528–531.