Model Parameters

The model parameters are tabulated in Table 2. They were arrived at as follows:

- ω_0, , ω_n: We fit the histogram of reversal frequencies computed from the model to that measured by Welch and Kaiser (1) to determine $\omega_n \approx 3\omega_0$. This ratio is consistent with ref. 2. To get absolute values for the frequencies, we fit the positions of the computed peaks to their measured positions (1), yielding $\omega_1 \approx 0.2\pi$.
- $\delta\omega$: The variance in phase velocity can be estimated from the width of the first peak in the experimental histogram (see figure 7 of ref.1), yielding $\delta\omega \approx 0.4\omega_0 \approx 0.08\pi$. This value can be used to estimate the effective diffusion in phase space: $D_\phi \approx \delta\phi^2/\tau \approx \pi\langle\delta\omega\rangle^2/\omega$.
- v, δv: The gliding speed varies from experiment to experiment ($v \sim$ 3-10 µm/min), because different experimental conditions and cell densities can influence the drag coefficient and gliding motor efficiency. However, the *relative* fluctuations in rippling phase is about the same $\delta v/v \sim 0.3$. Therefore, we scaled the dimensionless parameters to fit each experiment.

$\Delta\phi_R$: The refractory period can be estimated from the ratio of the refractory time to the reversal time at low C-signaling levels. An upper bound to the refractory period can be estimated from the position of the first significant peak in (figure 7 of ref. 1), which is ~1-1.3 min. The reversal time is ~4 min. This gives $\Delta\phi_R \leq 0.2\pi$.

Numerical Solution of the Model Equations

The definition of the model variables is summarized in Table 1. Eq. **3** can be represented as (3, 4)

$$\frac{\partial}{\partial t} n(x,y,\phi,t) = \left(L_x + L_y + L_\phi(n)\right) \cdot n(x,y,\phi,t) \ , \qquad \textbf{[7]}$$

where the L_i are convection-diffusion operators for each dimension, e.g.,

$$L_x = ?v_x \frac{\partial}{\partial x} + D_x \frac{\partial^2}{\partial x^2} \ , \qquad \textbf{[8]}$$

The iterative solution can be obtained by factoring space matrix operators (3, 4), i.e.,

$$n(t + 2\delta t) = \mathbf{T}_x \mathbf{T}_y \mathbf{T}_\phi \mathbf{T}_\phi \mathbf{T}_y \mathbf{T}_x \cdot n(t), \qquad \textbf{[9]}$$

where $\mathbf{T}_i[\delta t]$ formally denote time-stepping operators:

$$n(x,y,\phi,t + dt) = \mathbf{T}_i[\delta t] \cdot n(x,y,\phi,t) \qquad \textbf{[10]}$$

for one-dimensional convection-diffusion: $\frac{\partial n}{\partial t} = L_i n\left(x,y,\phi,t\right)$.

To construct each operator, we use separate algorithms for the convection and diffusion parts. Diffusion is treated by the Crank-Nicholson algorithm (4). The convection part is handled with Van Leer's second order upstream-centered difference scheme (5). Because v_x changes sign at $\phi = \pi$, different schemes were used for $\phi \in (0, \pi)$ and $\phi \in (\pi, 2\pi)$. A predictor-corrector method was used to account for density dependence of phase velocity (4).

For example, for $v_x > 0$, the following expression for $\mathbf{T}_x[\delta t]$ is

$$\mathbf{T}_x[\delta t] = \left(\mathbf{I} - \frac{D\delta t}{2(\delta x)^2}\mathbf{L}_D\right)^{-1}\left(\mathbf{I} + \frac{D\delta t}{2(\delta x)^2}\mathbf{L}_D - \frac{v\delta t}{\delta x}\mathbf{L}_{\mathrm{upc}}\left(\mathbf{I} + \frac{D\delta t}{2(\delta x)^2}\mathbf{L}_D + \frac{1}{2}(1 - \frac{v\delta t}{\delta x})\mathbf{L}_{\mathrm{cc}}\right)\right), \qquad \textbf{[11]}$$

where $\mathbf{I}$ is the identity matrix, $\mathbf{L}_D$, $\mathbf{L}_{\mathrm{upc}}$, and $\mathbf{L}_{\mathrm{cc}}$ are tridiagonal matrices of the form

$$\mathbf{L}_D = \begin{bmatrix} -2 & 1 & \cdots & 1 \\ 1 & -2 & 1 & \cdots \\ \vdots & \vdots & \vdots & \vdots \\ 1 & \cdots & 1 & -2 \end{bmatrix}, \quad \mathbf{L}_{cc} = \frac{1}{2}\begin{bmatrix} 0 & 1 & \cdots & -1 \\ -1 & 0 & 1 & \cdots \\ \vdots & \vdots & \vdots & \vdots \\ 1 & \cdots & -1 & 0 \end{bmatrix}, \quad \mathbf{L}_{upc} = \begin{bmatrix} -1 & 1 & \cdots & 0 \\ 0 & -1 & 1 & \cdots \\ \vdots & \vdots & \vdots & \vdots \\ 1 & \cdots & 0 & -1 \end{bmatrix} \qquad \textbf{[12]}.$$

Stability of Unidirectional Waves

A rigorous linear stability analysis of the homogeneous steady state is forbidding, because the eigenfunctions of the linearized model equations cannot be expressed in terms of known elementary or special functions. Therefore, we present a heuristic stability analysis that sheds light on conditions that are necessary and sufficient for existence of the rippling pattern.

It is easy to check that n = constant is a steady state of the model equations. Consider the following perturbation of the uniform distribution of the right-moving cells. Let $n_+(x, t) = n_+ + f(x-v\cdot t)$, where n_+ is the constant average density of right-moving cells, and f is a small perturbation in the form of a traveling periodic train of pulses:

$$f(z) = \begin{cases} \dfrac{\delta n}{2}, & 0 \le z < \dfrac{\lambda}{2} \\ -\dfrac{\delta n}{2}, & \dfrac{\lambda}{2} \le z < \lambda \end{cases} \quad . \qquad \textbf{[13]}$$

Here $\delta n << n_+$ is a small perturbation amplitude, $z = x - vt$, and λ the wavelength of the wave train. Natural time and space scales in the model are the reversal period, τ, and the distance covered between the reversals: $v\cdot\tau$. Indeed, we show below that the wavelength of the ripples is $\lambda \sim 2\, v\tau$. Therefore, we choose $\lambda = 2v\tau$, so that $f(z + \lambda) = f(z)$.

Consider two "average" cells that reverse at the same time: cell **F** originates from the front of the ridge at $z = \lambda/2$, and cell **R** originates from the rear of the ridge at $z = 0$; see Fig. 6a. We place our coordinate system on the moving perturbation so that the right-moving cells are stationary, while the left-moving cells glide with velocity $2v$ to the left. In the unperturbed homogeneous system, both cells would glide to the left for time τ covering a distance $\lambda = 2v\tau$ (in the moving coordinate system), and reverse again at the front and back, respectively, of the next ridge to the left. However, in the ripple phase, cell **F** from the front of the ridge traverses the trough towards the end of the sensitive part of its cycle. In the low cell density in the trough, the speed of its clock is close to its "solitary" speed, ω_0, so that its path is long before it reverses. Consequently, cell **F** would reverse closer to the center of the next ridge. Conversely, cell **R** that originated from the back of the ridge travels through the crest during the sensitive part of its clock cycle where the elevated density increases the phase velocity of its clock (making it more likely to reverse); this decreases the distance it glides. Consequently, cell **R** will turn to the right before the back of the ridge, i.e., closer to the wave center. Thus the two cells converge, ending up closer together at the end of one cycle than they started out, thus increasing the density in the center of the second ridge.

This argument demonstrates that periodic perturbations of the uniform density whose period is close to $\lambda = 2v\cdot\tau$ lead to pattern growth. This process is opposed by the effects of fluctuations in spatial and phase velocity, which disperse inhomogeneities. We can make this argument more quantitative as follows.

The cell density in the ridges is dispersed by the diffusion term, $D\partial^2 n/\partial x^2$, in Eq. **3**. (Note that D is the effective diffusion coefficient combining effects of D_x and D_ϕ). Using the natural length and time scales defined above, D can be estimated as $D \sim (\delta x)^2/\tau$, where $\delta x \approx \delta(v\tau) = v\delta\tau + \tau\,\delta v$ is the displacement variance over a half-period of the clock. Then the magnitude of the diffusion term is $D\partial^2 n/\partial x^2 \sim (\delta x)^2(\delta n)/(\tau\lambda^2)$. The ridge density increases in proportion to the advection term, $-\,\partial/\partial x(V\cdot n)$, where $V = (\ell_1 - \ell_2)/\tau$ is the effective convergence rate of the cells. The convergence velocity, V, can be estimated as $(v\delta t/\tau)$, where $\delta t \approx \delta(\Delta\phi_S/\omega) \approx -\Delta\phi_S\delta\omega/\omega^2$ is the difference in the traveling time between the two cells. Differentiating Eq. **5** for the density dependence of the angular velocity in the sensitive state, we find that $\delta t \approx q\Delta\phi_S\omega_0\delta n/(4n_+(\omega_0+\omega_n/2)^2)$, when $n_+ = n_w$. Note that if the average cell density deviates too much from the optimal wave density, n_w, then the difference in the traveling time is negligible. Similarly, if the refractory period is too small, the difference in the traveling time is negligible, as well: cells from the front and rear of the ridge would be affected equally by the inhomogeneity of the density. The magnitude of the advection term can be estimated as $-\partial/\partial x(Vn) \sim Vn_+/\lambda \approx Vq\Delta\phi_S\,\omega_n\delta n/(4\tau\lambda(\omega_0+\omega_n/2)^2)$. Comparing the magnitudes of the diffusion and advection terms, we derive the following inequality for the criterion of instability of the uniform steady state:

$$\underbrace{\frac{q}{4}\frac{v\,\Delta\phi_S\,\omega_n}{(\omega_0+\omega_n)^2}}_{\text{Focusing}} > \underbrace{\frac{(\delta x)^2}{2v\tau}}_{\text{Dispersion}}, \qquad \textbf{[14]}$$

where $\delta x \approx v\delta\tau + \tau\delta v$. Substituting the model parameters from Table2, we see that both sides of the inequality are of order ~0.01/min. In the simulations that produce waves, the left-hand side is indeed larger, so that the system is in the regime where the uniform cell distribution is unstable. Note that inequality **14** is valid for estimating a lower bound for $\Delta\phi_R$; it does not hold when $\Delta\phi_S$ is close to π.

The argument above does not demonstrate that there are no instabilities at other wavelengths. However, for wavelengths different from $2v\tau$, synchronization of the cycle will be less precise, whereas stochastic effects remain the same magnitude. Thus, the wavelength is $\sim 2v\tau \approx 80$ µm (about 10 body lengths). This prediction agrees with both our numerical simulations and the experimental observations (50-100 µm).

As the left-hand side of inequality **14** increases, and the right-hand side decreases, the amplitude of the ripples grows. Far from the bifurcation, the width of the ridges has to be of order δx. Then, almost all right-moving cells are aggregated in the ridges, whereas left-moving cells are distributed almost uniformly in the troughs. This means that the ratio of the densities of the cells in the crests and troughs is $\sim(\lambda/\delta x)$. The wavelength of the rippling pattern $\lambda \approx$ 50-100 µm; the displacement variance $\delta x \approx$ 10-20 μm. Thus, the theory predicts that the width of the crests is a few body lengths, and that the cell density there is 3-10 times higher than that in the troughs. Thus the stability analysis predicts that the clock and gliding have to be relatively precise. We observed numerically that the uniform pattern was stable when δx is less than 20 μm. These predictions agree with experimental observations.

When the average cell density, n_{av}, deviates significantly from the optimal wave density n_w, then the effect of the clock's synchronization becomes smaller. Numerically, we observed that when $n_?^{av} < n_w/2$, or $n_?^{av} > 2n_w$, regular patterns did not appear. The same is true when the density parameter q is small. Numerical runs produced ripples at $q = 4$. Less regular patterns evolved at $q = 3$, and there was no pattern formation at $q = 1,2$ (note that q need not be integer).

Another prediction of the stability analysis is that if the angular velocity of the clock in the sensitive state does not accelerate enough, then the uniform state is stable (this is clearly true in the extreme case, $\omega_n = 0$, when there is no density dependence). A less obvious prediction is that if ω_n is too large, there can be no pattern formation. Numerical analysis confirms that when ω_n rises significantly above $4\omega_0$, the patterns became irregular. Note that the refractory period of the clock should be short enough so that cells sense the rising density of the trailing wave. Indeed, numerical simulations show that the ripples disappear when $\Delta\phi_R$ increases from 0.2π to 0.5-0.6π. On the other, hand rippling requires that cells are not sensitive to the signaling from the cells in the crest they just reversed in. That gives a lower bound on $\Delta\phi_R$, which appears to be about 0.05π. The acceptable ranges of dimensionless parameters are summarized in Table 2.

Stability of Bidirectional Waves

A similar argument allows us to derive a crude estimate for the stability condition for the interpenetrating (standing) waves. Consider the cell density concentrated in two rectangular pulses (Fig. 6b). Initially, all cells in the right pulse have just turned from right to left, whereas all cells in the left pulse have just turned from left to right. Let the width of the pulses be $W_p <$ $\lambda/2$, and the distance between the pulses λ at the moment they turn. Let Δx be the difference between the distances two cells glide before turning; this is the "focusing length": the pulse gets narrower by Δx. We place our coordinate system on the right pulse. Consider two cells from the left pulse: one in its front (**F**), another in its rear (**R**). **R** will reverse when it encounters the front of the right pulse, i.e., when the pulses collide. This cell spends all its signal sensitive period gliding through a low density of counter-moving cells. Therefore, the following approximate relation holds:

$$(\lambda/2v)\omega_0 = \pi, \text{ or } \lambda = 2v\tau. \qquad \textbf{[15]}$$

This is the same formula for the wavelength of the rippling pattern derived above. Cell **F** will spend the time $((\lambda - W_p)/2v)$ gliding with phase velocity ω_0, and $((W_p - \Delta x)/2v)$ gliding with phase velocity $\omega_0 + \omega_n$ in the high density of oppositely moving cells (see Fig. 1). Thus for cell **F**, the following relation holds:

$$((\lambda - W_p)/2v)\omega_0 + ((W_p - \Delta x)/2v)(\omega_0 + \omega_n) = \pi. \qquad \textbf{[16]}$$

Solving Eqs. **15** and **16** for Δx:

$$\Delta x = W_p\omega_n/(\omega_0 + \omega_n)\ . \qquad \textbf{[17]}$$

For the wave trains to be stable, the focusing length, Δx, must be greater than or equal to the dispersion because of variations in speed and phase velocity, δx:

$$W_p\omega_n/(\omega_0 + \omega_n) \geq \delta x\ . \qquad \textbf{[18]}$$

When the two sides of Eq. **18** are equal, the waves are stable:

$$W_p = \delta x \cdot (\omega_0 + \omega_n) / \omega_n \leq \lambda/2 \qquad \textbf{[19]}$$

Eq. **19** shows that a stable pattern of interpenetrating (standing) waves can exist if

i. $\delta x < \lambda/2 = v\tau$ (i.e., $\delta(v\tau) < v\tau$). Otherwise, the crests merge, and the pattern disappears.

ii. $\omega_n >> \omega_0$.

iii. The signaling sensitive period, $\Delta\phi_S$, is long enough that a cell (e.g., **F**) that encounters an oppositely moving crest is sensitive.

iv. The refractory period is long enough for a cell to pass through a crest after reversing ($\Delta\phi_R \sim W_p \cdot \omega_0/2v$).

Wave Length Depends on C-Signal Intensity

Sager and Kaiser diluted wild-type (csgA$^+$) cells with a mutant strain (csgA$^-$) that could respond to C-signal but could not produce C-factor, thus decreasing the net C-signaling experienced by wild-type cells. They found that the mean distance between ripple peaks in a unidirectional wave train increased (2), and the amplitude of the wave decreased with decreasing fraction of wild-type cells. The conclusion drawn from these observations is that the average number of C-signal reversals per unit time will decrease in proportion to the percent of C-signal enabled cells in a population. Wild-type cells will receive C-signal only from other wild-type cells, so the probability of a successful C-signaling event is diminished in proportion to the percent of csgA$^-$ cells. Fig. 8a shows that the model can quantitatively reproduce this behavior if we suppose that the total phase velocity in Eq. **5** is a linear function of the fraction of wild-type cells: $\omega = \omega_0 + \omega \cdot n + \omega_n(n_\pm)$. Fig. 8b shows that including this dependence does not significantly alter the results of the model. Because the wavelength is approximately $2v\tau$, and $\tau = \pi/\omega$, the wavelength depends hyperbolically on the fraction of wild-type cells: $\lambda \sim 1/\text{fraction of wild-type cells}$.