Rectified directional sensing in long-range cell migration

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How spatial and temporal information are integrated to determine the direction of cell migration remains poorly understood. Here, by precise microfluidics emulation of dynamic chemoattractant waves, we demonstrate that, in Dictyostelium, directional movement as well as activation of small guanosine triphosphatase Ras at the leading edge is suppressed when the chemoattractant concentration is decreasing over time. This ‘rectification’ of directional sensing occurs only at an intermediate range of wave speed and does not require phosphoinositide-3-kinase or F-actin. From modelling analysis, we show that rectification arises naturally in a single-layered incoherent feedforward circuit with zero-order ultrasensitivity. The required stimulus time-window predicts ~5 s transient for directional sensing response close to Ras activation and inhibitor diffusion typical for protein in the cytosol. We suggest that the ability of Dictyostelium cells to move only in the wavefront is closely associated with rectification of adaptive response combined with local activation and global inhibition.
Long-range directional cell migration during embryonic development and wound-healing is directed by gradients of attractant cues that are often dynamic and self-enhancing. In Dictyostelium and neutrophils, localized activation of small guanosine triphosphatases (GTPases) and their downstream effectors such as phosphoinositide-3-kinase (PI3K) recruit signalling molecules at the cell cortex to form the leading edge. It is widely accepted that cells sense direction by comparing the attractant concentrations across the cell body. Such spatial sensing, however, constitutes a challenge in long-range migration, because the attractant gradient must be sustained over long distances, and the cells must be able to sense a wide range of gradient steepness in the background of various mean concentrations. Aggregation of Dictyostelium discoideum appears to have partially solved this problem by self-enhancing cell-to-cell relay of chemotactic cyclic AMP (cAMP) in the form of non-dissipating waves. However, because gradient reverses during the wave passage, it remains unclear how cells avoid futile back-and-forth movement. This is the so-called 'back-of-the-wave' problem in Dictyostelium cell aggregation.

Chemotaxis of D. discoideum amoebae is mediated by G-protein coupled receptor signalling with multiple redundant pathways; target of rapamycin complex 2 (TORC2), PI3K, PIP2/PLA and guanylyl cyclases. Localized activation of the small GTPase Ras at the leading edge of migrating cells constitute one of the earliest events of the symmetry breaking. The null-mutants of Gbeta display no chemotaxis, and Ras activation is completely abolished. Multiple guanine-nucleotide-exchange factors (GEFs) and GTPase-activating proteins (GAPs) that regulate conversion between the GTP and guanosine diphosphate-bound form of Ras have been identified. While heterotrimERIC G protein is activated non-adaptively as evidenced by the persistent dissociation between Gbeta and Ga subunit, activation of Ras, as well as their downstream targets such as PI3K, are adaptive, meaning their activities return to the pre-stimulus level under spatially uniform persistent stimulation. The two protein kinase B isoforms in Dictyostelium are regulated by TORC2 and PI3K, and their persistent stimulation. The two protein kinase B isoforms in Dictyostelium are heavily impaired in their chemotactic ability. Protein kinases B suggest possible links to cell motility as their activities return to the pre-stimulus level under spatially uniform persistent stimulation.

Recent advances in microfluidics have seen techniques that allow more accurate and rapid control of concentration gradients in time and space. Continuously applied flow of attractant in combination with Percoll density gradients supports mechanically stable concentration gradients that can be monotonically increased or decreased in time, however, gradient reversal is difficult by design. The dual-layer pyramidal mixer does allow orientation reversal, however, not without introducing unwanted transients. These approaches are not easily compatible with the travelling wave stimulus, where precise displacement of a set of continuous up and down gradients is required. More recently, laminar flows from three independent inlets were combined and focused to generate gradients that could be varied continuously in time. Unlike other techniques described above, gradient generation based on flow-focusing supports continuously changing gradients with finely controlled time constants and concentration range. In this study, we extend the flow-focusing approach to create bell-shaped gradients that can be displaced continuously in space to emulate travelling wave stimulus. By combining quantitative live-cell imaging analysis and dynamically controlled gradients, we elucidate how spatio-temporal information of the extracellular chemoattractant concentrations is encoded at the level of Ras activation. Furthermore, from mathematical analysis, we explore how an adaptive feedforward network can implement a rectifying circuit that filters out input signals based on temporal information.

Results

Cell movement in cAMP waves in vivo and in vitro. The relation between the cAMP waves and cell movement has been conventionally estimated from the periodic changes in the light scattering caused by the cell-shape change. Because such analyses failed to separate changes in cell motility and the chemoattractant concentrations, we first revisited this aspect by employing a more direct measurement of cAMP. The oscillations of intra- and extracellular cAMP concentrations occur synchronously, and the changes in the level of intracellular cAMP serve as a good indicator of the cAMP-induced cAMP relay in the chemoattractant field. Figure 1a shows a snapshot.
of the cAMP waves and cell migration in the aggregation field (Supplementary Movie 1). Cells moved directionally in the wavefront, however, no reverse movement was observed in the waveback (Fig. 1b). Thus the ‘wave paradox’ remains in defiance to the gradient-sensing paradigm, which nonetheless bases its claim on many other experimental observations in Dictyostelium.

To test whether the rectified motion originates from hidden cues such as asymmetry in the profile of extracellular cAMP concentrations, cell–cell contacts or other cofactors known to
affect chemotaxis, we studied isolated cells under artificially generated cAMP waves. Waves of a size similar to natural waves ($L_p = 850 \mu m$ in width) that are spatially symmetric were generated by flow-focusing in a microfluidic channel (Fig. 1c; Supplementary Fig. 1). When cAMP waves were applied at a period of 7 min per $L_p$, cells migrated directionally towards the incoming waves (Fig. 1d, see also Supplementary Movie 2) just as they would in the aggregation field. The speed of cell migration increased in the wavefront and reached 7 $\mu m$ min$^{-1}$ comparable to that during the early stage of cell aggregation (Supplementary Fig. 2). For fast waves ($<2$ min per $L_p$), no migration was observed (Fig. 1e). For slow waves ($>10$ min per $L_p$), cells first moved towards the incoming wave but then reversed their migratory direction in the waveback (Fig. 1f). As summarized in Fig. 1g, cells migrated directionally towards the incoming waves only when the transit time was between 3 and 10 min per $L_p$, which overlaps well with the time period of the native cAMP waves. These observations indicate that the rectified movement is not absolute and that the temporal-scale rather than the spatial asymmetry of the wave is critical. Interestingly, for slow waves, the mean displacement became negative most likely due to the 'Doppler' effect (see Supplementary Note; see also Supplementary Fig. 6).

Ras activation in dynamically changing gradients. To gain insights on the selective movement towards the cAMP wave, directional sensing under a single pulsatile wave was quantified by monitoring translocation of fluorescent protein fused to a Ras-binding domain (RBD), which binds to the activated form of Ras. (Fig. 2a–c). For transit time of $<2$ min per $L_p$, translocation of RBD to the plasma membrane occurred uniformly (Fig. 2d,e). For passage time of 7 min per $L_p$, RBD translocated only towards the side facing higher cAMP concentrations during the first 2 min (Fig. 2f; Supplementary Movie 3). During the following 5 min while the level of membrane-bound RBD returned to the pre-stimulus level, there was no reversal in the RBD distribution. For slow waves, RBD localized in the direction facing higher cAMP concentrations both in the wavefront and the waveback (Fig. 2h), consistent with the reversed cell movement in the waveback (Fig. 1f).

To delineate possible mechanisms of rectified movement, we carried out a systematic survey on Ras activation (RBD translocation) and cell movement under all possible combinations of sign in the space and the time derivatives (Fig. 3). First, temporally increasing (Fig. 3a,b) or decreasing (Fig. 3c,f) spatial gradients were imposed by moving a gradient at a constant speed. As expected, in temporally increasing spatial gradients, Ras was activated at the cell edge facing the higher concentrations of cAMP (Fig. 3c), and cells migrated accordingly (Fig. 3d). In contrast, no Ras activation (Fig. 3g) nor directional cell migration (Fig. 3h) was detected in temporally decreasing gradients. Unlike the back of the travelling wave that cannot be experienced without first being exposed to the wavefront, here the cells were first allowed to fully adapt to spatially uniform cAMP concentrations (> 5 min) before experiencing the gradient. Thus suppression of directional sensing appears not to require history of the past gradient and cell polarity.

The importance of temporal information was further vindicated by the ‘inverse wave’ (Fig. 3i,j), where the signs in space and time derivatives were inverted with respect to the normal wave. Again, there was no Ras activation (Fig. 3k) and no net cell movement (Fig. 3l) in the temporally decreasing gradient. Not until the cAMP concentrations started to increase in the second slope (Fig. 3k,l; $t > 2.6$ min), did Ras activation (Fig. 3k) and cell migration (Fig. 3l) become detectable. Moreover, when the temporal gradient was reversed by retreating the inverse wave while retaining the orientation of the spatial gradient (Fig. 3m,n), directional sensing at the level of Ras and cell movement were again suppressed (Fig. 3o,p; non-shaded time-windows).

Although there was slight retention of directional movement (Fig. 3p; see also Fig. 1d) and Ras activity (Fig. 3c) after the rising phase, Ras activity was never sustained under temporally decreasing gradients (Fig. 3o; see also Fig. 2g). Furthermore, although treatment with the PI3K inhibitor LY294002 (LY) diminished the peak amplitude of the response, it had minimal effect on the selectivity of the response to temporally increasing gradients (Fig. 4a,b; Supplementary Fig. 3a–f). Similarly, in cells immobilized by Latrunculin A treatment, although suppression of Ras activation at the rear of the cells became less prominent, the response itself was still selectively observed for temporally increasing stimuli (Fig. 4c,d; Supplementary Fig. 3g–i), suggesting that the downstream excitatory feedback circuit is not necessary for the rectification. These observations further indicate that the transient response is suppressed for temporally decreasing mean concentrations of the chemotactant and that this occurs at the level of or upstream of Ras.

We should note that there appears to be an additional effect from cell memory as evidenced by extended cell migration in the wake of wave stimulation (Fig. 1d right panel $t > 3$ min). Interestingly, there was almost no detectable RBD translocation during the later phase of this movement (Fig. 1d $t > 5$ min), indicating that, once established, polarity can be maintained in the absence of marked Ras activation. To test whether such memory effect plays a role in the suppression of directional sensing, we studied travelling wave stimuli with elevated background levels of cAMP (Fig. 5a–h). Under such conditions, there was marked cell polarization and movement in random direction prior to gradient exposure. As soon as the cells were exposed to the rising wavefront, RBD localized to the side facing the higher concentrations of cAMP, and cells reoriented and moved in the correct ascending direction. Because travelling wave stimulus on top of 10-nM background cAMP still elicited the rectified response (Fig. 5e–h), lack of gradient sensing in the decreasing gradient of the inverse wave down from 10 nM cAMP (Fig. 5i–l; $t < 2.4$ min) is difficult to explain by the memory effect of absolute cAMP concentrations. These results further demonstrate that temporal increase in the chemotactant concentrations is essential for Ras activation and reorientation.

Rectified response in a directional sensing model. Many of the essential properties of directional sensing and the adaptive Ras activation have been understood from the framework of the so-called local excitation global inhibition (LEGI) model and its variants. (Fig. 6a). A detailed modelling has been proposed that maps this scheme primary to the regulation of Ras between its GTP- and guanosine diphosphate-bound forms. The basic LEGI framework assumes two mediators; activator ‘A’ and inhibitor ‘I’ of the output R, ‘A’ and ‘I’ are both positively regulated by the input signal ‘S’. For spatially uniform input, the model is described by the following equations:

\[
\frac{dA(t)}{dt} = k_\alpha S - \gamma_A A(t) + \theta_A
\]

\[
\frac{dI(t)}{dt} = k_\gamma I(t) + \theta_I
\]

\[
\frac{dR(t)}{dt} = A(t)F(R) - I(t)G(R)
\]

where $F(R)$ and $G(R)$ are functions shown in Fig. 6a (yellow box; basic LEGI). In the first and second equations, $k_\alpha$, $k_\gamma$, and $\gamma_A$, $\gamma_I$ determine the rate of increase and decrease of the activator ‘A’ and inhibitor ‘I’.
and the inhibitor 'I' molecules, respectively. \( \theta_a \) and \( \theta_i \) are basal activation rates that determine imperfectness of adaptation. Because we observed that adaptation of RBD translocation was slightly imperfect (Supplementary Fig. 4), here \( \theta_a \) and \( \theta_i \) are assumed to be small but non-zero. The first and second terms in the third equation describe activation and deactivation of \( R \) by \( A \) and \( I \), respectively. Upon spatial uniform increase in 'S', due to a higher rate of activation, there would be a transient rise in the output 'R' followed by its return to the pre-stimulus level by the action of the inhibitor 'I' (Supplementary Fig. 5a). Thus the model describes well the adaptive 'temporal sensing' property of the chemotactic response to spatially uniform stimuli. The other essential feature of the LEGI scheme is that the inhibitor 'I' diffuses fast and acts globally. Thus, for a stationary gradient stimulus, while the activator level mirrors the local receptor occupancy, the inhibitor level traces its average. Consequently, the ratio \( Q = A/I \), which dictates the output \( R \), would always transmit the relative difference in the input signal S from the background (Supplementary Note; see also Supplementary Fig. 5b). From this 'spatial sensing' property, intracellular gradient of the ratio \( Q \) within the cell faithfully mirrors the applied attractant gradient irrespective of the temporal change. Although a possible resolution to the 'wave paradox' may be provided for rapidly changing signals where the response transients could significantly deviate from the stationary state, the response transmits the relative difference in the input signal \( S \) from the background. The output 'R' describes well the adaptive 'temporal sensing' property of the chemotactic response to spatially uniform stimuli. The other essential feature of the LEGI scheme is that the inhibitor 'I' diffuses fast and acts globally. Thus, for a stationary gradient stimulus, while the activator level mirrors the local receptor occupancy, the inhibitor level traces its average. Consequently, the ratio \( Q = A/I \), which dictates the output 'R', would always transmit the relative difference in the input signal 'S' from the background (Supplementary Note; see also Supplementary Fig. 5b). From this 'spatial sensing' property, intracellular gradient of the ratio 'Q' within the cell faithfully mirrors the applied attractant gradient irrespective of the temporal change. Although a possible resolution to the 'wave paradox' may be provided for rapidly changing signals where the response transients could significantly deviate from the stationary state, the basic LEGI scheme nevertheless predicts symmetric responses in...
both wavefront and waveback (Fig. 6b; see Methods for equations). How would the discrepancy between the model prediction and the observed rectification be resolved?

As a natural extension of the basic LEGI model, let us examine a case where the kinetics $F(R)$ and $G(R)$ follow the Michaelis–Menten form (Fig. 6a; cyan box) thereby equipping the LEGI circuit with an ultrasensitive transfer function (Fig. 6c; see also Supplementary Fig. 5k; Supplementary Note for analysis). The output response predicted from the model are now largely consistent with the observed Ras response for the travelling wave stimulus (Fig. 6d,e) as well as monotonically changing gradients (Fig. 6f–i), inverse waves (Fig. 6j,k) and alternating gradients (Fig. 6l,m). Owing to simplicity of the ultrasensitive model, the basis of rectification can be well described by the response to uniform stimulation. The basic LEGI model predicts a strong undershooting response for a spatially uniform and temporally decreasing stimulus (Supplementary Fig. 5a–c; refs 50,52). In contrast, the ultrasensitive LEGI circuit does not respond to the temporally decreasing stimulus (Fig. 7a cyan; Supplementary Fig. 5d–f) due to strong suppression at work in the zero-order regime. This is in accordance with the observed changes in the level of membrane-bound RBD to increase and decrease in spatially uniform cAMP concentrations (Supplementary Fig. 4a; see also Fig. 7b cyan). Immediately after the release from the prolonged exposure to spatially uniform cAMP concentrations, Ras activity quickly recovered the pre-stimulus level without an undershoot (Supplementary Fig. 4a). Together with the apparent absence of membrane-bound RBD prior to stimulation.

Figure 3 | Chemotaxis and directional sensing in dynamically changing gradients are rectified by temporal information. (a–d) Temporally increasing gradient. (e–h) Temporally decreasing gradient. (i–l) Inverse wave. (m–p) Alternating gradient. The sign of the space (blue)/time (grey) derivatives of the stimulus are indicated by ‘+’ and ‘−’ (o.p). Schematics (a,e,i,m), and the space–time plot (b,f,j,n) of the stimulus. RBD localization in the positive and negative direction (c.g.k,o; left panel; blue and orange) and representative confocal images (c.g.k.o; right panels). Cell displacement (d.h,l,p; magenta). Time series of the extracellular cAMP level ($c$,g,k,l,p; green). Time $t = 0$ indicates the point at which the stimulus concentration was above (c,d) or below (g,k,l) a threshold (0.01% of the maximum for c and d; 99.5% for g, h, k and l). Data were averages over $n = 13$ (c,d), 22 (g,h), 21 (k,l) and 6 (o,p) cells. Error bars indicate s.e.m. Scale bar, 10 μm.
The results are in line with the perfect shutdown of the resting-state response in the ultrasensitive regime.

The ultrasensitive model reduces to the basic LEGI model in the limit of high Michaelis–Menten constants ($K_i$ in Fig. 6a), therefore predicts an undershooting response to a temporally decreasing uniform stimulus (Fig. 7a brown; high $K_i$ ($K_i=0.1$); and Supplementary Fig. 5g–j). Consequently, directional sensing occurs both in the wavefront and the waveback (Fig. 7c,d), in marked contrast to the rectified response at low $K_i$ (Fig. 7f). To test the model predictions, we took advantage of a high occurrence of spontaneous Ras activation in weakly starved cells (≈40% total cells; Fig. 7b brown; $t<0$ min). In these cells, there was a transient undershoot of Ras activity upon release from a uniform cAMP stimulus (Fig. 7b, 100–200 s). Moreover, these cells sensed the gradient not only in the wavefront but also in the waveback (Fig. 7g), hence exhibited back-and-forth movement (Fig. 7h). These behaviours are in striking contrast to the more asymmetric response observed for weakly starved cells without spontaneous RBD localization (Fig. 7b cyan; Fig. 7ij).

**Model prediction of the essential parameters.** The origin of the timescale dependence (Figs 1g and 2) can be identified by analysing the behaviour of the ultrasensitive LEGI circuit in moving gradients (Fig. 8; see also Supplementary Fig. 6 for travelling wave stimulus). For low $K_i$ ($K_i=0.01$), we see that the response can be classified qualitatively into three regimes depending on the propagation velocity $V_S$ (Fig. 8). When $V_S$ is large ($V_S=1,000 \mu m \text{ min}^{-1}$), there is a spatially uniform increase in the output $R$ in temporally increasing gradients (Fig. 8a). In contrast, $R$ remains at the basal level in temporally decreasing gradients (Fig. 8d). For small $V_S$ ($V_S=10 \mu m \text{ min}^{-1}$), the output $R$ is always greater on the side facing higher concentrations of $S$ irrespective to its time derivative (Fig. 8c,f). At an intermediate signal velocity ($V_S=120 \mu m \text{ min}^{-1}$), the output $R$ rises only in the temporally increasing gradients, and this is spatially restricted towards the side facing higher concentration of $S$ (Fig. 8b,e).

The upper bound of signal velocity provides us with an estimate of diffusion constant of the inhibitor $I$ independent of the model details. In order for a cell to sense the moving gradient of the chemoattractant, time required for the inhibitor to spread out within a cell $(2l)^2/2D$ must be shorter than the time lag of signal detection between the two ends of a cell, $2l/V_S I$ is the cell radius (Fig. 6a) and $D$ is the diffusion coefficient of the inhibitor. In other words, the inhibitor that initially increased at the cell front must immediately diffuse intracellularly and reach the other end of a cell before the stimulus does, otherwise the response transients would be equal in magnitude between the two ends of the cell (see Fig. 8a). Hence, we obtain the upper bound for the propagation speed

$$V_I < V_{fast}^* \equiv D/I$$

In principle, chemotactic gradients travelling faster than this limit could only be perceived as spatially uniform stimulation. From the limit of directional sensing, we obtained $V_{fast}^\sim \approx 240 \mu m \text{ min}^{-1}$ (Figs 1g and 2) thus for cell radius $l=7.5 \mu m$, we estimate the diffusion constant of the inhibitor to be approximately $D \approx 30 \mu m^2 \text{ s}^{-1}$, which matches well with those reported for green fluorescent protein (GFP) and GFP-tagged protein in the cytosol. The result is suggestive of an inhibitor protein that shuttles between the plasma membrane and the cytosol as the higher mobility in the cytosol would dominate its diffusion. Although other mechanisms such as tension-based global inhibition cannot be ruled out, our analysis indicates that the diffusion process is sufficiently fast to meet the required global effect. While the analysis assumed activator diffusion to be negligibly small for the sake of mathematical analysis, the estimate and the overall model behaviours hold as long as the diffusion constant of the activator is more than one order of magnitude smaller ($\leq 3 \mu m^2 \text{ s}^{-1}$; Supplementary Fig. 7a–c). Because membrane-bound protein diffusion falls well within this range, it may be that the activator molecule is more strongly sequestered to the plasma membrane.

On the other hand, the lower bound of signal velocity for the rectified migration readily reveals that the pulsatile response is
essential for directional sensing in dynamically changing gradients. In a gradient \( S(x, t) \), a cell at the position \( x = 0 \) at time \( t \) experiences \( S(+l, t) \) at the positive end \((x = +l)\) and \( S(-l, t) \) at the negative end \((x = -l)\). For slowly moving gradients \((V_S < V_{fast})\), the concentrations of the activator and the inhibitor at both ends of a cell are approximately \( A_+(t) \approx \gamma_1^{-1}k_S(S(+l, t) - \gamma_1^{-1}) \) and \( I_+(t) \approx \gamma_1^{-1}k_I(S(+l, t) - \gamma_1^{-1}) \). Here, \( S_0(t) = [S(+l, t) - S(-l, t) + S(-l, t) - S(+l, t)]/2 \) is the spatial average of the input signal. Hence, 

\[
Q_+(t) = \frac{A_+(t)}{I_+(t)} \\
\approx Q_0 \left[ 1 + \frac{\delta S(\pm l, t)/\partial t}{S(\pm l, t)} (\gamma_1^{-1} - \gamma_a^{-1}) + \frac{1}{2} \frac{\delta S(\pm l, t)/\partial x}{S(\pm l, t)} (2l) \right]
\]

where \( Q_0 = \gamma_a^{-1}k_I/\gamma_1^{-1}k_S \). The second and the third term represents the response in \( Q_+ \) to temporal and spatial changes in \( S \), respectively. In the slow limit of propagation speed, that is, stationary gradient, the second term vanishes, therefore \( Q_+ < Q_0 < Q_+ \) always holds for a positive stationary gradient \( \delta S(\pm l, t)/\partial x > 0 \) (Supplementary Fig. 5i). In temporally decreasing gradients with non-zero propagation velocity, \( \delta S(\pm l, t)/\partial t < 0 \) thus again \( Q_+ \) always satisfies \( Q_+ < Q_0 \). Since rectification requires that changes in \( Q_+ \) not be conveyed to downstream \( R \), \( Q_+ > Q_0 \) must be satisfied in order for a cell to sense the gradient direction. By combining these conditions and the relationship \( \delta S/\partial t = -V_S(\delta S/\partial x) \), we arrive at the lower bound for the rectified directional sensing 

\[
V_S > V_{slow}^* \equiv \frac{l}{\gamma_1^{-1} - \gamma_a^{-1}}
\]

From our migration assays, \( V_{slow}^* \approx 90 \mu \text{m min}^{-1} \) (Fig. 1g) thus for a cell radius \( l = 7.5 \mu \text{m} \), we obtain \( \gamma_1^{-1} - \gamma_a^{-1} \approx 5 \mu \text{s} \), which is close to the observed transient of the RBD translocations.

Although chemotactic response in migratory cells is often characterized by the pulsatle response that peaks in the timescale of seconds, such as that observed here for Ras activation, its role in chemotaxis has not been well defined. The above inequality states that the gradient must travel a distance longer than the cell size within the time-window of the transient response in \( R \) (but no faster than the upper bound \( V_{fast} \)). Otherwise, the time-window would be long enough to support slow relaxation dynamics of \( R \) to its stationary state (that is, stationary spatial
sensing scheme), which does not discriminate temporally negative and positive changes in the chemoattractant concentrations. The current analysis corrects the misconception in the field that cells must be in a refractory period of chemotactic response for a few minutes while they experience the waveback gradient. Although refractoriness associated with excitatory dynamics could explain rectified movement towards the propagating waves, the reported refractory periods are 16.5 s for Ras29 and 30 s for PI3K59, which are both too short to explain the lack of response in the waveback. The rectified adaptive sensing predicts a spatially localized response transient of a seconds timescale, not minutes.

To summarize, the above analysis clarifies the upper and lower bounds of the stimulus velocity that supports rectified directional sensing (Fig. 8g,h). In the ultrasensitive regime (low $K_I$), for stimulus within the time-window of rectification ($V_{\text{slow}} < V < V_{\text{fast}}$), a large intracellular gradient of $R$ is expected for the temporally increasing gradients (Fig. 8g, red curve) while it nearly vanishes for temporally decreasing gradients (Fig. 8g, cyan curve). In other words, a rectified sensing circuit implements low-pass filters with different cutoff times for rising and falling gradients (see also Supplementary Fig. 6; Supplementary Notes).

At high $K_I$ where rectified directional sensing becomes compromised (Fig. 7c,d; $K_I = 0.1$, Supplementary Fig. 5g–i), the intracellular gradient of $R$ (the ratio $R_+ / R_-$) always takes similar values in the rising and falling gradients (Fig. 8h). The time dependence is consistent with an earlier observation of cell movement in slowly diminishing gradients34 and may explain discrepancies between earlier works32,33.

### Discussion

The present study utilized precise and continuous displacement of a pulsatile gradient, thereby faithfully emulating the travelling wave stimulus of cAMP experienced in the aggregating field of cells. We demonstrated that cells are able to exhibit directional migration towards the incoming waves as observed in vivo. Cell migration in travelling waves of cAMP was indeed rectified, meaning that the cells migrated towards the incoming waves and did not reorient in the waveback. To the best of our knowledge, this is the first clear demonstration of *Dictyostelium* chemotaxis in artificially generated travelling waves of chemotactic cAMP. Our observations indicate that no asymmetry in the gradient steepness between the wavefront and the waveback nor cell–cell contact is required for directional migration.

By generating various forms of dynamic gradients—inverted waves, transiently increasing or decreasing gradients, the current analysis demonstrated that Ras does not transduce gradient information when the mean concentration of cAMP is decreasing in the appropriate timescale. One of the key aspects of directional

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**Figure 6 | An ultrasensitive LEGI circuit implements rectification.** (a) A schematic of the incoherent feedforward circuit in the LEGI model. One-dimensional space was considered where a cell of length $2L$ was positioned at $x = 0$ (see Methods for details). Space was discretized and coarsely so that variables $A$, $I$ and $R$ were considered only at the ends of a cell; denoted by $A_+$, $I_+$, $A_-$, $I_-$, $R_+$ and $R_-$. The ‘+’ and ‘−’ signs indicate the direction in the chamber. (b) Numerical simulations of the basic LEGI equation for the travelling wave stimulus. The output in the positive side ($R_+$, blue) and the negative side ($R_-$, orange) of a cell. In travelling wave stimulus, the positive side faces the rising side of the incoming wave. (c) The resting state of $R$ plotted against the change in the activator/inhibitor ratio $Q$. (d–m) Numerical simulations of the ultrasensitive model; travelling wave stimuli (d,e), temporally increasing (fg) and decreasing (hi) gradients, inverse travelling wave (jk) and alternating gradient (lm). The sign of the space (blue)/time (grey) derivatives of $S$ are indicated by ‘+’ and ‘−’ (m). See Supplementary Table 1 for model parameters.
Figure 7 | Asymmetry in the response to temporally increasing and decreasing stimulus. (a) Adaptive response to spatially uniform increase and decrease of the signal input (a, green line and shaded area) in the ultrasensitive LEGI model for high K_i (K_i = 0.1) (a, brown) and low K_i (K_i = 0.01) (a, cyan). (b) Ras response to spatially uniform increase and decrease of cAMP (between 0 and 1 μM cAMP; green shaded area) in weakly starved cells with (b, brown; n = 24) or without (b, cyan; n = 20) spontaneous RBD localization. (c–f) Directional sensing response in the ultrasensitive LEGI model for high K_i (K_i = 0.1) (c) and low K_i (K_i = 0.01) (e). Schematics of chemotactic response to traveling wave stimulus without (d) or with (f) rectification. (g–j) Ras response (g,i) and cell movement (h,j) in travelling wave stimulus in cells with (g,h, n = 22) or without (i,j, n = 19) spontaneous Ras localization. Time t = 0 indicates the point at which the stimulus concentration exceeded a threshold (0.01% of the maximum). Error bars indicate s.e.m. Scale bar, 10 μm.

sensing in migrating cells is that it operates independent of cell motility50,61. Under a stationary gradient stimulation, Ras activation and PIP3 synthesis at the leading edge are observed even when cell motility is suppressed by Latrunculin13,60. Our present results clarified that this also applies to the ability of cells to filter out temporally decreasing gradients. Rectification is observed at the level of Ras activity, and this requires neither cell motility nor feedback from downstream PIP3 signalling.

The present results indicate that spatial sensing and temporal sensing can be understood under a unified framework. We have introduced the ultrasensitive LEGI model as a plausible and minimal extension of the basic LEGI framework and made use of its simplicity to analyse the basis of rectification. Our analysis suggests that rectification is separable from downstream amplification and/or an excitable circuit thus arises at or very close to the level of a LEGI-like circuitry. Biochemically, the rectifying property is expected to originate from regulation of Ras or its upstream signalling. Other variants of the LEGI model can also support rectification, however, these models require additional downstream signalling modules to realize the characteristic nonlinear transfer function (Supplementary Fig. 8; see Supplementary Note). For example, addition of the downstream PI3K-mediated amplification step (extended LEGI model Fig. 3b in ref. 52, amplified-LEGI53) to the basic LEGI model can provide
Figure 8 | Rectified directional sensing can be attributed to the low-pass filter characteristics in the ultrasensitive circuit. (a–f) Time series from the numerical simulations in the ultrasensitive LEGI model ($K_0 = 0.01$). Response in the positive ($+$) side ($R_+$, blue) and the negative ($-$) side ($R_-$, orange) of a cell to monotonic gradients that are increasing (a–c) or decreasing (d–f) in time. The spatial-temporal profiles of $S$ were the same as in Fig. 6f–i. Monotonic gradients move from right to left (in the ($-$) direction) for temporally increasing gradients, and left to right (in the ($+$) direction) for temporally decreasing gradients. Hence, the positive ($+$) direction always faces higher concentrations of the monotonic gradients. The propagation velocity $V_S$ for fast- ($V_S = 1,000 \mu m \min^{-1}$; a,d), intermediate- ($120 \mu m \min^{-1}$; b,e) and slow- ($10 \mu m \min^{-1}$; c,f) moving gradients. Dashed lines (c,f) indicate the analytically obtained stationary states of $R_+$ and $R_-$ for a given signal gradient evaluated at each time point. (g,h) Maximum values of the ratio $R_+/R_-$ in response to temporally increasing (red) and decreasing (cyan) spatial gradients in the ultrasensitive LEGI model at $K_0 = 0.01$ (g) and $K_0 = 0.1$ (h). Corresponding wave transit time for $L_p = 800 \mu m$ is indicated (g,h; top x axis label). Asymmetry in directional sensing to temporally increasing and decreasing stimulus is highlighted by the shaded regions. The characteristic velocity, $V_{fast}$ and $V_{slow}$ are indicated in black dashed lines. See Supplementary Table 1 for model parameters.

necessary nonlinearity to support rectification (Supplementary Fig. 8e–l). However, the present analysis of LY-treated cells indicates that PI3K is required for overall amplification of Ras signal, but not for rectification (Fig. 4a,b; Supplementary Fig. 3a–f). While it is possible that Ras itself constitutes the amplifying/rectifying module downstream of an unidentified LEGI module close to the G-protein coupled receptor, the responses at the level of the heterotrimeric G-protein observed so far have been non-adaptive thus not LEGI-like in their property.

The other plausible source of nonlinearity is the feedback from F-actin that supports excitability. In latrunculin-treated cells, RBD translocation was again rectified meaning that it occurred in temporally increasing gradients, not in decreasing gradients. Note, however, that RBD localization appeared more graded in space (Fig. 4c,d; Supplementary Fig. 3g–j) suggesting that nonlinearity that enhances the difference between the leading and trailing end of the cells has a separate origin than the rectification. In the light of the LEGI scheme, these observations suggest that either sequestering of the activator or diffusion of the inhibitor is F-actin dependent (Supplementary Fig. 7d–f). This is plausible considering that a membrane scaffold that associates with RasGEFs is known to translocate to the plasma membrane in a F-actin-dependent manner. Finally, we should note that the system must operate near the point of inflection in the Q–R curve. This requires fine-tuning of the stationary state $Q_0$ (Supplementary Fig. 5e), which also determines the pulse form of $R$ and the degree of imperfectness of adaptation. Future works should address how such robustness is achieved in relation to complexities of the signalling network that were omitted in the current model.

In *Dictyostelium*, the rectified directional sensing together with the self-generated gradients enable long-distance cell migration by circumventing dissipation of the guidance cue and recycled use of spatial gradients. In sperm, the gradient perceived by the cell
becomes a periodic stream of chemoattractant due to looping cell
migration, thus in essence utilizes dynamic sensing62. Directional
migration in neutrophils also appears to involve spatio-temporal
mechanisms40,63,64. A similar rectified sensing may allow the
migration in neutrophils also appears to involve spatio-temporal
infliction and inflammation. Although whether periodic travelling
waves exist in migrating systems besides Dictyostelium remains
an open question, neutrophil aggregation to the wound site is
mediated by self-amplified signals3. The signals perceived by the
cells in such developing fields of attraction are likely to be
complex in their temporal patterns. The present insights on
spatio-temporal sensing and rectification should be useful for
the analysis of these and other cellular sensing.

Methods DNA construct and cell strains. An expression vector for RFP tagged
with the RBD of human Raf1 protein was based on GFP-RBD34,24. RFPmars65 was PCR
amplified using primers 5'-AGATCTACTGATCGATCAAGATGATGTAATT-3'
(BglII-RFP) and 5'-GAATTCGACCTGCACTGTTGAATGCTTAATT-3'
(RFPAATAA-EcoRI) using pHygRFPmars66 as a template. The GFP-RBD
expression vector was replaced with RFPmars, purified and sequenced. The vector
confers resistance to G418 and expression of RFP-RBD under a strong promoter.
AX4 cells expressing the cAMP sensor Epac1-camps were employed. Cells were grown axenically in modified HL-5
phosphate buffer (PB) (20 mM KH2PO4, 20 mM Na2HPO4, pH 6.5) at 6.5
x 106/ml. The expression vector was replaced with RFPmars, purified and sequenced. The vector
was transformed with pCy5-RFPmars and selected for G418 and hygromycin resistance. All transformants were cloned for further analysis.

Cell preparation. For time-lapse imaging analysis of cell aggregation, the laboratory wild-type strain Ax4 of D. discoideum cells expressing the cAMP sensor Epac1-camps67 were employed. For cell association experiments, cAMP-expressing cells
were mixed with RFP-expressing cells in a 98:2
ratio. This is more than one order of magnitude smaller than the shear force required to
induce migration (<0.5 Pa)68 or to detach the cells (<1 Pa)69.

Live-cell imaging. Image data were obtained using an inverted microscope (IX-81;
Olympus) equipped with a confocal multibeam scanning unit (CSU-X1; Yokogawa)
and an EM-CCD camera (Evola 12.0; Photometrics). To record GFP,
fluorescence, a bandpass filter (510–550 nm; BA510-550, Olympus) and a broad-
spectrum filter (>757 nm; BA575IF, Olympus) were used, respectively. For
FRET-based cAMP measurements, an excitation filter (BP425–445HQ, Olympus) and a
dichroic mirror (DM450, Olympus) were used. Bandpass filters (BA460–510HQ,
Olympus; BA515–560HQ, Olympus) were used for cyan fluorescent protein and
eyellow fluorescent protein fluorescence, respectively. Objective lenses were used ×4
(PlanApo, numerical aperture (NA) 0.16) for the quantification of the stimulus
profiles, ×20 oil immersion (PlanApo, NA 0.85) and ×60 oil immersion
(PlanApo N, NA 1.45). Fluorescence images were acquired at 1–30–s
intervals and stored as Tagged Image File Format (TIFF) files. Obtained data
were later analysed using ImageJ and MATLAB (MathWorks). All live-cell imaging
was performed at 22°C.

Image processing. Cell tracking and fluorescence signal quantification were performed with custom programs written in ImageJ and MATLAB (MathWorks).
To acquire changes in the FRET efficiency from Epac1-camps-expressing cells, the
ratio of the fluorescence intensities in the cyan fluorescent protein (Icyt) and yellow fluorescent protein (Imem) channels were averaged over a 40-μm square region
around the centre of a cell at each time point. The mean ratio (Icyt/Imem) was further
averaged at each phase of an oscillation period to normalize by subtracting
linear trends of the signal (normalized Icyt/Imem).

For quantification of RBD translocation to the cell membrane, a 1-μm-wide region
inside the cell outline and the cytosolic region were defined by binarization.
For dynamically changing gradients, translocation of RFP-RBD to the cell
membrane was quantified by defined the thresholding algorithm in the
the membrane region in the direction θ (0°<θ<2π) from the center: Imem(θ), t=
. The angle θ=π/2 faces the positive direction (right-hand side of the chamber). As an
indicator of RBD localization, the averaged intensity of the membrane-cytosolic
ratio was weighted by the alignment with the wave direction by computing,
Icyt(θ,t)/Imem(θ,t) = (1/2)∑(i,j)∈θ exp[−(i−cθc)2/(2σ2)]Δθ/Δx for the positive half of a cell
periphery, and Icyt(θ,t)/Imem(θ,t) = (1/2)∑(i,j)∈θ exp[−(i−cθc)2/(2σ2)]Δθ/Δx for the remaining half.
Here, the discretized angle θi = θi for 1≤θi≤2πN where N=90. For
normalization, the mean intensity of the cytosolic fluorescence, Icyt(θ=π/2,t) was used.
We occasionally encountered extremely polarized cells with marked movement in the
z axis direction. These cells were excluded from the present analysis due to
difficulty in accurate tracking of the RBD translocation.

For quantification of the spatial and temporal changes in the cAMP levels, bias
due to non-uniform illumination was removed following the flat-field correction
method. In brief, the spatial and temporal profiles of the RBD translocation
Icyt(x,y,t) were obtained by calculating
Imem(x,y,t) = (Imem(x,y,t) − Cmax − Cmin)/Cmax − Cmin x
Imem(x,y,t) + Imem(x,y,t) + Cmem. Here, I(x,y,t) was fluorescence profile of the fluorescein
indicator observed during the stimulus experiments. The intensity profiles of the maximum
Imax(x,y) and the background Ibackground(x,y) were obtained by capturing
fluorescence of uniform concentration fields of the cAMP solution with or without
fluorescein, respectively. Cmax and Cmin were the maximum and minimum
concentration of CAMP at the source, respectively. The calibration data
were obtained prior to stimulus experiments per chamber on a daily basis.

To estimate the duration of the stimulus (that is, wave passage time), profiles
of the wave stimulus were fitted by a Gaussian curve moving at a constant speed
S(x,t) = B0exp[−(x + Sx)2/2σ2] + B0, using the nonlinear least-squares
method. The wave passage time was defined by the time-window during which the
stimulus intensities were above 0.01% of the peak intensity at a position
x, that is, S(x,t)>10−4 B0 + B0. Estimation of the pulse width followed the same criterion (>0.01% of the peak intensity).

Quantification of Ras activation to uniform stimulus was performed as follows.
The mean fluorescence intensities from the cytosolic region of cells under mock
(0% cAMP) were fitted by a Gaussian curve moving at a constant speed
S(x,t) = B0exp[−(x + Sx)2/2σ2] + B0, using the nonlinear least-squares
method. The wave passage time was defined by the time-window during which the
stimulus intensities were above 0.01% of the peak intensity at a position
x, that is, S(x,t)>10−4 B0 + B0. Estimation of the pulse width followed the same criterion (>0.01% of the peak intensity).

Dynamically changing gradient stimulus. For precise control of concentration
profiles of extracellular cAMP in space and time, we employed a microfluidics
chamber (µ-slide 3-in-1; Ibidi) consisting of three inlets and a single outlet. Inlet
and outlet channels were connected to a pneumatic pressure regulator (MFCs;
FLEx; Fig. 1a) and precisely controlled pressure and flow rates were automated.
A pressure control was automated by the MAESFLO software (Flugient) using cust-
om-made scripts that set the flow rates from the individual inlet dynamically over
time. The rate of total flow from the three inlets was maintained at 33 µl min−1.

Figure 1a and the schematics (Figs 2a and 3a-c,m,n). As a marker for the stimulus profile, fluorescein
(Wako; molecular weight = 332) at a final concentration of 3 µM was included in the
stimulus solution. A concentration of 1 µM RBD was chosen as the stimulus
profile of all experiments, except for the inverse travelling wave experiments
(Fig. 5b–l; Supplementary Fig. 3a–c,g,h) where 10 nM RBD was also used. For
elevated basal cAMP, the buffer-only pools were replaced with either 1 or 10
nM cAMP.

For spatially uniform stimuli, the left and right inlets were connected to a pair of
sering pumps (NE-1002X; New Era Pump Systems Inc., NY), and the centre
inlet was sealed with plug. The total flow rate of the solutions was
20 µl min−1 (Fig. 7) or 120 µl min−1 (Supplementary Fig. 4). Under the present
conditions, the estimated shear stress66,68 experienced by the cell was <0.03 Pa.

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activity, we computed $I_{\text{forward}}(t) = \frac{I_{0} \text{exp}(s(t))}{\text{erf}(t)} f_{\text{st}}(t)^{-1}$.

**Mathematical modelling.** In the basic LEGI formulation $^6$, $F(R) = k_{S}(R_{m} - R)$ and $G(R) = k_{R} R$ are employed, where $R_{m} - R$ and $R$ are concentrations of $'R'$ in the inactive and the active form, respectively. As a biochemically natural extension of the LEGI scheme, we adopted the Michaelis–Menten form in the regulation of $R$, such that

$$F(R) = k_{S} \frac{R_{m} - R}{k_{S} + (R_{m} - R)} \quad \text{and} \quad G(R) = k_{R} R$$

(2)

This constitutes the so-called push-pull type reaction where two antagonistic gradients (Fig. 3e–h), we set $F(R) = k_{S} R_{m} - k_{S} R$ and $G(R) = k_{R} R$ so that equation (1) recovers the basic LEGI equation (first-order kinetics). For the simulations of directional sensing in various concentration fields, the response $A$, $I$ and $R$ at the cell ends were considered in one-dimensional space along the gradient (Fig. 6a). Under these assumptions, we obtained the following equations:

$$\frac{\text{d}A}{\text{d}t} = k_{S}(x(t) + l) - \frac{1}{4} A(x - A(t)) \quad \text{and} \quad \frac{\text{d}I}{\text{d}t} = k_{S}(x(t) - l) - \frac{1}{4} I(x - I(t))$$

(3)

To simulate inverse travelling waves (Figs 3i–l and 5i–l; Supplementary Fig. 3),

$$\frac{\text{d}A}{\text{d}t} = k_{S} A(x(t) - l) + \frac{1}{4} (A(x - A(t))) \quad \text{and} \quad \frac{\text{d}I}{\text{d}t} = k_{S} I(x(t) - l) + \frac{1}{4} (I(x - I(t)))$$

For gradient stimulation, the following signal profiles were adopted:

- **(i) Temporally changing gradients (Fig. 6f–i; Fig. 8).** To simulate inverse travelling waves (Figs 3i–l and 5i–l; Supplementary Fig. 3), we imposed $F(R) = 1 + \tanh[b(x - l + 0.5M(x))]$ where $M(x)$ is the triangular-wave function. Parameter values were $b = 0.015 \mu m^{-1}$, $l = 350 \mu m$ and $\tau = 60$ min. The important difference from the inverse wave stimulus is that the signal intensity oscillates at a period half of that of the spatial gradient (Figs 3i,m and 6j).

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Author contributions

A.N. and S.S. designed the research. A.N. and D.I. performed experiments and analysed the data. A.N. and S.S. designed and analysed the model. S.S. carried out theoretical analysis and simulations. S.S. oversaw and coordinated data collection and analysis and contributed to all aspects of data interpretation. A.N., S.I. and S.S. wrote the paper.

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