Contact inhibition of locomotion probabilities drive solitary versus collective cell migration

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Contact inhibition of locomotion (CIL) is the process whereby cells collide, cease migrating in the direction of the collision, and repolarize their migration machinery away from the collision. Quantitative analysis of CIL has remained elusive because cell-to-cell collisions are infrequent in traditional cell culture. Moreover, whereas CIL predicts mutual cell repulsion and ‘scattering’ of cells, the same cells in vivo are observed to undergo CIL at some developmental times and collective cell migration at others. It remains unclear whether CIL is simply absent during collective cell migration, or if the two processes coexist and are perhaps even related. Here, we used micropatterned stripes of extracellular matrix to restrict cell migration to linear paths such that cells polarized in one of two directions and collisions between cells occurred frequently and consistently, permitting quantitative and unbiased analysis of CIL. Observing repolarization events in different contexts, including head-to-head collision, head-to-tail collision, collision with an inert barrier, or no collision, and describing polarization as a two-state transition indicated that CIL occurs probabilistically, and most strongly upon head-to-head collisions. In addition to strong CIL, we also observed ‘trains’ of cells moving collectively with high persistence that appeared to emerge from single cells. To reconcile these seemingly conflicting observations of CIL and collective cell migration, we constructed an agent-based model to simulate our experiments. Our model quantitatively predicted the emergence of collective migration, and demonstrated the sensitivity of such emergence to the probability of CIL. Thus CIL and collective migration can coexist, and in fact a shift in CIL probabilities may underlie transitions between solitary cell migration and collective cell migration. Taken together, our data demonstrate the emergence of persistently polarized, collective cell movement arising from CIL between colliding cells.

1. Introduction

Contact inhibition of locomotion (CIL) refers to cessation of cell migration in the direction of cell–cell collision [1–3]. Cessation of movement is rapidly followed by repolarization of the migration machinery away from the collision [4–10]. CIL operates in many cell types [2,6,11–15], is critical for coordination of cell movements in multicellular settings and has recently been implicated during migration in developing Xenopus laevis, Danio rerio and Drosophila melanogaster [5,7,16–18] as well as during invasive prostate cancer progression [6]. Despite recognition of CIL over half a century ago, methods for its in vitro study have remained largely unchanged, and involve either placing two tissue explants in close proximity in culture, or directly observing rare, serendipitous collisions between dissociated cells [1,3,4,19,20]. As a result, these methods have limited quantitative insight into CIL. The strength and lifetime of the CIL signal as well as the frequency and extent of the CIL response are not known, making it difficult to build quantitative models that explain collective cell behaviour. For example, CIL predicts that cells repolarize away from other cells upon collision, leading to a well-dispersed population of single
2. Material and methods

2.1. Cell culture and reagents
NRK-52E cells (ATCC) were chosen as model cells for migration, because they have broad leading edges and clearly demarcated polarity [10], and were cultured in 10% fetal bovine serum (Gibco, Carlsbad, CA) in Dulbecco’s modified Eagle medium (Gibco). Cells were G_{0}-synchronized prior to entry into S-phase (equation (3.1)), and the repolarization rate was measured from the proportion of total cells observed that repolarized within a specific time interval (equation (3.3)), and the repolarization rate was calculated as per equation (3.3) with the appropriate value of Δt. The entire assembly was incubated in 0.2% (v/v) Pluronics F127 to prevent protein adsorption to exposed PDMS. For all PDMS barrier experiments, cells transiently expressed EGFP, and fibronectin conjugated to AlexaFluor-568 (Invitrogen) was used to visualize the micropattern.

2.2. Micropatterned substrates
Surface and microwell patterns were generated via microcontact printing [28]. Physical barriers were formed by casting polydimethylsiloxane (PDMS; Dow Corning, Midland, MI) against a silicon wafer patterned with approximately 350 μm tall photore sist and cleaning with EtOH. For experiments in the electronic supplementary material,电影 S2, the barrier was coated with Pluronics F127 to prevent protein adsorption to exposed PDMS. For all PDMS barrier experiments, cells transiently expressed EGFP, and fibronectin conjugated to AlexaFluor-568 (Invitrogen) was used to visualize the micropattern. Patterned culture reveals cell repolarization. (a) Time-lapse microscopy of cells 6–20 h after plating, and trajectories of 10 representative cells (lower right) 30 min before (blue) and after (red) the collision. In phase contrast micrographs, blue arrowheads indicate cell migration direction 1 h before the collision, yellow asterisks indicate the site of the collision at 0 h, and red arrowheads indicate the direction of cell migration 1 h after the collision. (b) X- and y-velocities of cells 30 min before (blue) and after (red) the collision. Means ± s.e. from three independent experiments. (c) Cells were fixed and immunostained 20 h after plating on micropatterns. (d,e) Time-lapse sequence, trajectories and velocities were measured as in (a,b) from cells on micropatterns. In (d), micropatterned stripes are indicated by purple shading. All scale bars, 25 μm.

2.3. Immunofluorescence and microscopy
Cells were fixed in pre-warmed 2% paraformaldehyde in microtubule stabilizing buffer (1 mM ethylene glycol tetraacetic acid, 1 mM MgSO4, 4% (w/v) poly(ethylene glycol) 8000 and 1% (v/v) Triton X 100 in 0.1 M piperazine-1,4-bis(2-ethanesulfonic acid), pH 6.75) for 10 min at 37 C, and labelled with antibodies in 10% goat serum. Fixed samples were imaged via widefield epifluorescence with a 60×, NA 1.4 Plan Apochromat objective on a Zeiss Axiosvert 200M, and images were acquired with an AxioCam HRM using AxiVision software (Carl Zeiss, Thornwood, NY). Live samples were imaged via phase contrast microscopy with a 10×, NA 0.3 Plan Fluor objective, and a custom-built environmental chamber mounted on a Nikon Eclipse Ti with the Nikon perfect focus system (Nikon Instruments, Melville, NY), using a Hamamatsu C4742 camera (Hamamatsu Corporation, Bridgewater, NJ) and MetaMorph software (Molecular Devices, Downingtown, PA).

2.4. Experimental measurement of repolarization
Repolarization was determined from phase contrast, time-lapse recordings using a 10×, NA 0.3 Plan Fluor objective and a time interval of 4 min. Morphological repolarization was defined as a cell entering the collision with its lamellipodium facing the opposite cell before collision but after collision redirecting the lamellipodium away from the opposite cell, concurrent with migration away from the site of collision and opposite cell (figure 1d and electronic supplementary material, movie S2). The proportion of total cells observed that repolarized within a specific time interval (Δt) was recorded and plotted as a cumulative probability in figure 2c. Polarization was described as a two-state transition (equation (3.1)), and the repolarization rate was calculated as per equation (3.3) with the appropriate value of Δt.

2.5. Agent-based model
We created an agent-based model to predict the result of head-to-head collisions between two single cells or between a single cell and a train of cells. The model was formulated according to the following rules.

— An agent was defined as either a single cell or a train of cells, and had a speed and polarization as prescribed below.
Figure 2. CIL is triggered upon head-to-head collision between cells. (a) Time-lapse microscopy of cells 6–20 h after plating, showing (i) persistent migration of a single cell, (ii) head-to-head collision between cells, (iii) head-to-tail collision between cells in which the rear cell repolarizes and the (iv) lead cell remains persistent, and (v) collision of a cell with an inert barrier. In (v), transmitted light optics were poor, so the cell is expressing GFP (green), is migrating along a fluorescent, micropatterned stripe (red), and collides with an inert topographical barrier (dashed line). In (ii–v), the collision occurs at 0 min. All scale bars, 25 μm. (b) The probability of repolarization within 2 h of (i) beginning a persistent migration, or (ii–v) colliding with another cell or barrier as indicated in (a). Arrowheads in (a) indicate the cell (rear or lead) whose probability was measured for (b). In (ii), either cell of the head-to-head collision was measured. (c) For head-to-head collisions between single cells (situation ii), 58% of total cells had repolarized within 40 min of the collision.

The speed of each agent was 30 μm h−1, unless the agent was within the collision distance (10 μm) of, and polarized toward, another agent. In the latter case, the speed was defined to be 0.

The polarization of each agent was either left or right.

Initial polarization was defined such that a head-to-head collision occurred early in model simulations.

Polarization could change only when agents collided head-to-head (came within the collision distance). Such a head-to-head collision triggered a repolarization signal, characterized by a magnitude and lifetime. The magnitude was probabilistic (events min−1), as based on experimental data (curve fit in figure 4c). The lifetime was varied systematically in the model (figure 6).

The timestep of model simulations was 1 min. In each timestep, the following steps were performed: (i) check for a collision, (ii) simulate repolarization if appropriate, (iii) simulate movement if appropriate, and (iv) update positions. These steps were iterated as long as specified (figure 6). At the end of the simulation, entrainment was measured by assessing whether the agents were polarized in the same direction and were within 15 μm of one another. Simulations were implemented in MATLAB (The MathWorks, Natick, MA), and at least 1000 simulations were performed per condition.

3. Results

We followed migration of dissociated NRK-52E cells on a two-dimensional surface via time-lapse microscopy and observed collisions between cells. It appeared that cells ceased migrating in direction of the collision, and repolarized such that they migrated in a different direction before the collision (figure 1a and electronic supplementary material, figure S1). However, fewer than 16% of cells collided with another single cell within a 12 h observation period, even after pre-selecting for cells that appeared on the verge of collision (data not shown). Moreover, average cell velocity both before and after the collisions was zero, because migration in this two-dimensional, unpatterned setting was unconstrained (figure 1b). Taken together, our observations suggested the existence of CIL in these cells, but also illustrated the difficulties of describing the process.

To direct cells to experience a high frequency of visually clear, head-to-head collisions, we micropatterned parallel stripes of fibronectin with a period of 25 μm. This width was chosen to be similar to cell size, and permitted us to constrain cell migration to quasi-one-dimensional tracks [29,30]. Cells localized polarity markers in a typical fashion on the micropatterns: cortactin localized to the leading edge, the centrosome preceded the nucleus in the direction of migration, α-tubulin accumulated near the front of migrating cells and myosin IIB accumulated in the rear (figure 1c). Cells migrated with a similar speed as on unpatterned surfaces (29 ± 14 μm h−1), and spontaneously switched direction infrequently (figure 2a(i),b(i)). Time-lapse microscopy showed that 53% of cells underwent collisions with another single cell during a 12 h observation period (data not shown).

Upon collision, cells remained in contact with the other cell for approximately 60 ± 5 min, indicative of transient ‘co-attraction’ between cells [31] (see electronic supplementary material, figure S1). Collision also frequently triggered repolarization. That is, cells entered the collision with their lamellipodium facing the opposite cell but repolarized their lamellipodium upon collision, and migrated away from the collision in opposite directions (figure 1d and electronic supplementary material, movie S2). In fact, the magnitude of velocity parallel to the micropatterns before and after the collision was similar, albeit oppositely polarized (figure 1e). We conclude that polarization and migration speed are similar on patterned versus unpatterned surfaces, but collisions on patterned surfaces occur more consistently and frequently compared with unpatterned surfaces.

The consistency of cell repolarizations on micropatterns permitted us to quantitatively analyse such events in different contexts. Individual cells that migrated on the stripes spontaneously repolarized with a probability of 0.14 ± 0.09 within 2 h of beginning a persistent migration (figure 2a(i),b(i) and electronic supplementary material, movie S3). By contrast, a cell that collided with another cell head-to-head repolarized with a probability of 0.87 ± 0.09 within 2 h of the collision (figure 2a(ii),b(ii) and electronic supplementary material, movie S2). We also observed occasional, ‘rear-end’ collisions between cells. In these situations, the lead cell repolarized with a probability of 0.18 ± 0.13 within 2 h of the collision, and the rear cell repolarized with a probability of 0.59 ± 0.12 within 2 h of the collision (figure 2a(iii),iv),b(iii),iv) and electronic supplementary material, movies S4 and S5).
Significantly lower repolarization of the lead cell (0.18 ± 0.13) compared with the rear cell (0.59 ± 0.12) suggests the rear cell encounters CIL signals from the lead cell, but not vice versa. Modest repolarization of the rear cell (0.59 ± 0.12) compared with head-to-head collision (0.87 ± 0.09) suggests that the CIL signal operates most efficiently when two leading edges of cell abut. The cumulative time distribution of repolarization probability following head-to-head collision between single cells indicated that 58% of cells (126 total cells over three experiments) had repolarized within 40 min of the collision (figure 2c). We conclude that CIL occurs probabilistically, operates most efficiently when two cells collide head-to-head, and largely occurs within 40 min of a head-to-head collision between single cells.

Physical presence of the opposing cell might trigger CIL by preventing forward motion and new cell–extracellular matrix adhesion. Indeed, topographical features can direct cell migration along ridges via contact guidance [32–34]. To address such topographically induced repolarization, previous studies examined collisions between live and fixed cells [2,14]. Because these studies found conflicting results as to whether topography was sufficient to induce repolarization, we tested whether topographically induced repolarization operates here. We placed a non-adhesive PDMS block perpendicular to the micropatterned stripes prior to seeding cells. Cells colliding with the barrier migrated in the opposite direction with a probability of 0.27 ± 0.07 (figure 2a,v),b(v) and electronic supplementary material, movie S6). This was higher than expected for spontaneous repolarization in the absence of such a barrier (0.14 ± 0.09), but lower than the probability observed following cell–cell collisions (0.87 ± 0.09). Coating the PDMS barrier with E-cadherin, the primary receptor involved in initial cell–cell adhesion, also did not induce repolarization: cells collided with the barrier, spread against it, and subsequently migrated on the barrier itself (see electronic supplementary material, figure S2 and movie S7). Thus, these results indicate that a physical barrier to forward migration is not sufficient to induce CIL. These data support our assertion that collision between single, living cells induces strong CIL.

The observed repolarization probabilities illustrate the relative strength of the CIL signal, but did not describe the repolarization probabilities in absolute terms. Because our experimental setting essentially restricted cell migration to a single plane, we described CIL with a two-state transition:

\[ L^k \rightarrow R. \]  

(3.1)

In this description, the probability \( P \) of repolarizing in time interval \( \Delta t \) from left (L) to right (R) or vice versa was calculated as

\[ P = 1 - \exp(-k \times \Delta t), \]  

(3.2)

where \( k \) represents the repolarization rate in \( \text{min}^{-1} \). Repolarization rates were obtained from the experimentally observed probabilities, by solving equation (3.2) for \( k \):

\[ k = \frac{-\ln(1 - P)}{\Delta t}. \]  

(3.3)

The repolarization rate can be interpreted as repolarization events per minute. Because the probabilities in figure 2b were computed over a 120 min interval, \( \Delta t = 120 \text{ min} \) was used to compute \( k \) from these probabilities (see electronic supplementary material, figure S3). These data more clearly describe the extent to which head-to-head collisions trigger the strongest CIL signal. We conclude that cells on micropatterned lines repolarize with probabilities that can be described via a two-state transition.

Prior studies have not assessed whether CIL is a coupled response between two cells. To address this, we considered head-on collisions between single cells and asked whether CIL occurs statistically independently (figure 3a). Specifically, if CIL did not involve coupling between cells, we should expect to observe both, one or no cells repolarizing with probabilities of \((0.87) \times (0.87) = 0.76, (0.87) \times (1 - 0.87) \times 2 = 0.23\) and \((1 - 0.87) \times (1 - 0.87) = 0.02\), respectively (figure 3b, white bars). Our experimental results followed these expectations very closely (figure 3b, black bars), leading us to conclude that CIL occurs statistically independently, consistent with no coupling between cells.

Because repolarization rates were very high for cells engaging in head-to-head collisions, we expected that cells would remain well distributed as might occur with particles exhibiting mutually repulsive forces. Instead, we observed multicellular trains of closely contacting cells that migrated in a highly coherent and persistent manner (figure 4a and electronic supplementary material, movie S8). Moreover, immunofluorescent staining suggested that cells within such trains were stably coupled based on accumulation of E-cadherin at cell–cell contacts, and was supported by localization of actin and microtubule cytoskeletons between adjacent cells (figure 4b). These data are consistent with many studies showing that cells accumulate cadherins at contacts and juxtapose cytoskeletons upon cell–cell adhesion [35–37].

Interestingly, initial observations suggested that the persistence of trains appeared to be higher with longer trains. That is, upon collision with a single cell, longer trains were less likely to repolarize than shorter trains. To quantify this, we experimentally measured the probability of an \( n \)-cell train repolarizing within 40 min of a head-to-head collision with another cell, and observed a rapid decrease in repolarization with \( n \) (figure 4c). A curve fit of the relationship between the probability of repolarization, \( P \), and number of cells, \( n \), is given by \( P(n) = \exp(-[n - 1 - \beta]) \). The fit parameter \( \beta \) is found by the constraint that \( P(n = 1) = 0.58 \) (figure 2c); hence, \( \beta = 0.546 \). Although the curve fit is empirical, it captures the experimentally observed features that (i) \( P(n = 1) = 0.58 \), (ii) \( P \) decreases rapidly with increasing \( n \), and (iii) \( P(n \rightarrow \infty) = 0 \). Irrespective of mechanisms that cells engage when they are part of a train, we observe trains to move collectively and repolarize length-dependently.
Such collectively moving trains could have arisen from pre-existing cell clusters that already were present during initial seeding onto the substrate, or could have emerged from collisions between single cells. Surprisingly, experimental observations suggested the latter: single cells became ‘entrained’ over the course of the experiment (figure 5a and electronic supplementary material, movie S9). Although the percentage of cells in trains at the beginning of the experiment was less than 40% of total cells, within 9 h this percentage had more than doubled to 88%, and by 14 h this percentage had eclipsed 93% (figure 5b). Together, our prior data indicated that CIL is operational in these cells, but we also observed the emergence of collectively migrating trains.

To reconcile these seemingly paradoxical observations, we constructed an agent-based model in which a cell collided head-to-head, on a stripe, with either another single cell or a train composed of two to six cells (figure 6a). In the model, collision triggered probabilistic repolarization as per the experimental probabilities we previously found (figure 4c). We converted probabilities to rates using equation (3.3) and Δt = 40 min. We systematically varied the length of time, τ, between the collision and duration over which the repolarization rate was non-zero. By iterating the model 1000 times, we measured the rate of entrainment (figure 6b). Our simulations predicted that, for τ = 80 min, entrainment rapidly and monotonically increased with increasing train length (figure 6c, grey curve). That is, for increasing n, an n-cell train was increasingly likely to transition to an n + 1 cell train upon head-to-head collision with a single cell.

To experimentally test this prediction, we measured the rate of entrainment following a head-to-head collision between a single cell and either another single cell or trains composed of two to six cells. We found good agreement between the model prediction and experimental data (figure 6c). To quantify the agreement, we computed $R^2$, which represents the extent to which the model captures experimental data. We computed $R^2$ while systematically varying τ to assess the optimal value of this free parameter in our model. We found that $R^2$ varied biphasically with τ, with an optimum $R^2$ at τ = 80 min (figure 6d). We posit that for low τ, neither agent repolarizes, whereas for high τ, both agents repolarize. Only for intermediate values of τ does exactly one agent repolarize such that entrainment occurs. Our data indicate that the time between the signal trigger (collision) and cell response (entrainment) was 80 min.

In a head-to-head collision between single cells, a two-cell train will result only when exactly one of the cells repolarizes. We therefore reasoned that the probability of single cell repolarization would affect the probability of a two-cell train emerging. Indeed, two-cell train emergence was quite sensitive...
Figure 6. An agent-based model predicts the emergence of trains. (a) A head-to-head collision between a single cell and a train of cells can result in four outcomes. Entrainment, in which the single cell repolarizes but the train remains persistent, is indicated by the black arrow. The rate of this transition from a head-to-head collision to entrainment is indicated by $k$. (b) Schematic of the model, in which a collision triggers a non-zero rate of repolarization. The magnitude of the rate of repolarization is given by length-dependent repolarization as measured in figure 4c. The duration of the rate of polarization ($\tau$) is systematically varied in model simulations. At the end of the simulation, the rate of entrainment $k$ is measured. (c) Entrainment rate $k$ versus train length, as predicted by the model for $\tau = 80$ min (grey line) and measured experimentally (black line). Simulated rates are based on 1000 simulations. Experimental rates are based on at least three independent experiments. ($d$) $R^2$, a measure of experimental data predicted by the model, versus $\tau$ was computed to assess the ideal value of $\tau$. Note that $R^2$ is maximum at $\tau = 80$ min. (e) The time for one cell to, through a series of head-to-head collisions and entrainments, give rise to a seven-cell train was computed based on model predictions versus single cell repolarization rate. Arrowhead, experimentally observed rate $(2.2 \times 10^{-2} \text{ min}^{-1})$.

4. Discussion

Traditional culture suggested the presence of CIL, but was fraught with challenges including rare collisions, uncertainty surrounding how to categorize collisions, and lack of precision in defining repolarization. Micropatterns and modelling addressed these challenges permitting a large number of frequent, reproducible and quantifiable cell–cell collisions, and generating precise, quantitative and experimentally falsifiable predictions, respectively. Experiments on micropatterns revealed that CIL is a probabilistic event, head-to-head collision between cells induced the most robust CIL, cells repolarized statistically independently and the probability of a train repolarizing depended on the number of cells within the train. Based on these data, we formulated a model that predicted monotonically increasing entrainment with length of the train; this novel prediction was quantitatively verified experimentally. Together, these findings highlight the utility of our dual approach combining micropatterning and modelling.

The CIL signal upon head-to-head collision between individual cells was strong relative to other repolarization signals. It has been shown in a variety of contexts that cell–cell contact triggers cell polarity [5,7,10,38,39]. In many cases, homophilic binding between cadherin receptors on adjacent cells underlies the polarity signal, although the specific cadherin isoform and downstream signalling pathways involved are perhaps context-specific. Moreover, binding of ephrin ligands to cognate Eph receptors has been shown to underlie CIL in several cell types, including neural crest cells and prostate cancer cells [6,15,40]. Despite these inroads in understanding that highlight which molecules might be involved in the process, no study to date has quantitatively described the probabilistic nature of CIL, its relative strength, and its dependence on context. Our data revealed the stochasticity of CIL triggered by cell–cell collision and suggest that the context of cell–cell collisions (e.g. head-to-head versus head-to-tail) could have important effects on this CIL signal.

Why spontaneous repolarization in freely migrating cells is infrequent remains unclear. Cell–matrix adhesions are well known to direct cytoskeletal structure [41–45] and can trigger polarity signals via activation of Cdc42 and actin polymerization [46–48]. In contrast to the repulsive effects of cell–cell adhesions, cell–matrix adhesions appear to attract cell polarity via a process that could be called ‘adhesion promotion of locomotion’ (APL). It may be that CIL and APL alone can explain some of the differences in repolarization rates observed between pairs of cells. For example, upon
head-to-head collisions cells lose an APL signal and gain a CIL signal; we observed that both cells are likely to repolarize. Similarly, upon head-to-tail collisions the lead cell receives APL and CIL signals that act synergistically in the migratory direction, whereas the tail cell receives an APL signal in the migratory direction but a CIL signal in the direction opposite migration. Consistently, we observed that the lead cell most likely persists and the rear cell most likely repolarizes. We propose that freely migrating cells receive APL signals from cell–matrix adhesions to confer persistence but no CIL signals to induce repolarization, and therefore are predominantly persistent.

We observed trains of cells form over time, and posit that once formed these trains are an integrated, coherent unit rather than a collection of independently acting cells. First, the actin cytoskeleton of a train of cells resembled that of a single cell (compare figure 1c with figure 4b): f-actin accumulated at the sides and rear of both single cells and entire trains of cells. Second, trains repolarize collectively and length-dependently (figure 4c), indicating that trains migrate as a collective. Third, entire trains spread and migrate on an E-cadherin-coated barrier with similar probability as single cells (see electronic supplementary material, figure S2b). Because trains accumulate E-cadherin at cell–cell adhesions as expected (figure 4b), we hypothesize that cell–cell adhesions within trains signal differently from newly formed cell–cell adhesions that induce CIL. Indeed, cell–cell adhesions reinforce in response to exogenoe force [49–51], and cell–cell adhesion triggers rearrangement of the cytoskeleton [10,35,52]. It is possible that an interplay between cell–matrix and cell–cell adhesions underlies the difference in how cell–cell adhesions signal in trains versus initial E-cadherin-mediated binding; vinculin, well-studied for its role in regulating cell–matrix adhesion, has been implicated in regulating mecanosensing at cadherin-based cell–cell junctions [53,54]. Moreover, in contexts ranging from convergent extension during embryonic morphogenesis to cancer metastasis, cell–matrix and cell–cell adhesion systems are intimately connected [55]. In light of this evidence, it would be surprising if cell–matrix and cell–cell adhesion systems did not interact to mediate CIL.

Our relatively simple experimental setting does have the limitation that migration is restricted to essentially one dimension, but it may nonetheless be relevant to a number of physiologic migration patterns. Specifically, migration patterns normally ascribed to two or three dimensions such as fibrillar migration, capillary sprouting or lateral-line migration in the zebrafish embryo may be mimicked by the standardized ‘one-dimensional’ setting studied here [30,56–58]. We predict a biphasic relationship between the probability of CIL and the emergence of collective cell migration (figure 6c): when CIL is either unlikely or very likely collective cell migration emerges very slowly, only intermediate CIL probabilities rapidly give rise to collective cell migration. Thus, by tuning the CIL probability cells might transition from solitary to collective migration and vice versa with more or less rapidity. Rapid transitions between solitary and collective migration have been observed for example during embryonic development when macrophages migrate collectively along the ventral axis then solitarily in a classic CIL manner [16,17], or upon starvation in Dictyostelium discoideum when cells transition from solitary to collective migration [59]. Our study reveals that CIL is a probabilistic process, and this stochasticity could drive transitions between solitary and collective cell migration. This experimental setting can be more closely linked to physiologic migration patterns by, for example, changing the width of the stripes, the cell type(s), the chemistry and mechanics of the substrate, and the growth media composition. It would be interesting, for example, to place cells from differing stages of embryogenesis or cancer progression in our assay to assess their ability to undergo CIL and collective migration.

The migration dynamics of cell populations are critical to multicellular organisms. Efforts to describe such dynamics in cells are largely focused on single phenomenon, and as such behaviours such as CIL, collective migration and single cell motility are largely thought of as unrelated processes. By contrast, our studies show that CIL, entrainment and collective migration are in fact linked. Interestingly, our model suggests that depending on the likelihood of CIL, population dynamics could shift between modes of migration, and this could perhaps underlie differences observed between cell types and contexts [21,22]. The strategy by which local interactions give rise to global migration patterns is not unlike that used by collectively behaving groups of animals [60,61], and perhaps represents a biological strategy whereby individuals act autonomously to influence collective behaviour [62–64]. Our data therefore highlight the abilities of cells to not only probe their local environment via local interactions, but also use such local interactions as a mechanism to give rise to collective behaviour.

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