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CRITICAL COMMENTARY

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An interdisciplinary approach to investigate collective cell migration in neural crest

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Abstract

The neural crest serves as a powerful and tractable model paradigm for understanding collective cell migration. The neural crest cell populations are well-known for their long-distance collective migration and contribution to diverse cell lineages during vertebrate development. If neural crest cells fail to reach a target or populate an incorrect location, then improper cell differentiation or uncontrolled cell proliferation can result. A wide range of interdisciplinary studies has been carried out to understand the response of neural crest cells to different stimuli and their ability to migrate to distant targets. In this critical commentary, we illustrate how an interdisciplinary collaboration involving experimental and mathematical modeling has led to a deeper understanding of cranial neural crest cell migration. We identify open questions and propose possible ways to start answering some of the challenges arising.

KEYWORDS

collective cell movement, interdisciplinary studies, mathematical modeling, neural crest

1 | INTRODUCTION

Collective migration is a common phenomenon that is observed to arise at many different length scales ranging from the micron level to kilometers. For example, in embryonic development many processes rely on cells moving collectively either in sheets or as individuals[1,2]; in humans, a major area of research is the understanding of crowd dynamics[3-5]; in insects, the phenomenon of swarm dynamics is well-studied.[6-8] These are only a small sample of the vast number of examples of this phenomenon. In this Critical Commentary, we narrow our focus to one example, namely, cranial neural crest (NC) cell migration in chick and illustrate how an interdisciplinary approach combining experiments and computational modeling has enabled us to investigate a number of key biological questions concerning this collective behaviour.

NC cells make a vital contribution to structures that include the head, heart, and peripheral nervous system. NC cells delaminate from the dorsal neural tube and traverse along well-defined migratory routes to precise targets. NC cells exhibit a wide range of collective behaviors depending on the organism and axial level of origin (Figure 1). This great variety of emerging behaviors makes the NC system an important model paradigm for collective cell migration. Therefore, the insights gathered from studying NC cell behavior are not only crucial to understanding embryogenesis and the reasons for certain developmental defects, but could potentially provide important insights for other collective cell migration systems, including regulation of wound healing, and metastatic cancer progression.[9-11]

The wide range of potential signaling mechanisms involved in the collective migration of NC cells makes it challenging to identify the key factors that ensure successful invasion (defined here as collective occupation of the whole domain). In addition, there are many different types of cell-cell and cell-tissue interactions, such as cell-adhesion and cell-induced tissue alterations, possibly involved and these

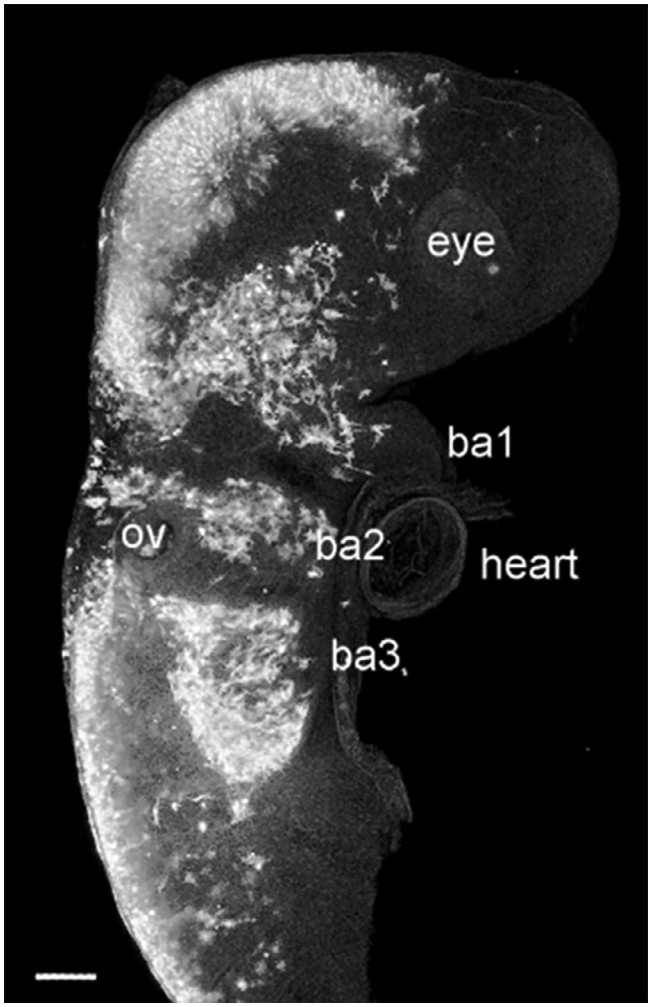

FIGURE 1 Migration of NC cells in the chick embryo, ba1-3—branchial arch 1-3, respectively, ov—otic vesicle. The image is taken after NC cells reach ba2. The scalebar is 150 microns (white)

have to be carefully investigated. Crucially, the NC cell system is experimentally tractable, and so it is possible to generate the appropriate data to investigate certain signaling mechanisms. However, the consistency of experimental hypotheses in the light of observations can sometimes be challenging to assess due to the sheer number and complexity of the signaling pathways involved. Mathematical models can incorporate multiple simultaneous and interacting inputs and compute outputs which are beyond our intuitive thinking. Therefore, models can be used to validate experimental hypotheses and help unravel the mechanisms that drive collective cell migration.

Various experimental and computational studies have been carried out to understand the behavior of distinct NC cell populations in different species (see, for example, the reviews[12-16]). These distinct populations of NC cells emerge at different axial locations and, after migration and differentiation, contribute to diverse cell lineages, including cartilage, connective tissue, melanocytes and glia.[17,18] Most of the different populations of NC cells are guided by different signaling mechanisms. This is understandable as characteristics such as NC cell-cell adhesion, cell-microenvironment interactions, distances traversed and time to reach targets, all differ depending on the organism and axial level.

Interdisciplinary studies have demonstrated that the combination of contact inhibition of locomotion (CiL, which is a process during which migratory cells momentarily stop upon physical contact and subsequently repolarize to move in the opposite direction) and co-attraction (CoA, a mutual cell-cell attraction at distances larger than a cell diameter mediated by contacts of thin filopodia) mechanisms are sufficient to explain collective migration of NC cells in *Xenopus* and zebrafish.[19-22] *Xenopus* NC cells initially migrate as a cohesive tissue.[23] More recent studies suggest that cell polarization is also crucial for successful cell invasion but it is naturally induced by CiL and CoA, which are modulated by dynamical interactions of Rac1 and RhoA.[24]

Studies of chick NC cell migration reveal that the main migratory driving forces for these cells vary across different axial levels and involve chemotaxis. Simpson et al.[25] investigated the main mechanisms regulating successful invasion and colonization of the gut by vagal NC cells in chick embryos. Their mathematical model suggested that cell invasion is driven by a combination of motility and proliferation of the NC cells at the front of the stream, whereas the cells behind the front do not contribute to invasion. These results were verified experimentally and used to provide insights to understand Hirschsprung's disease, which is characterized by a failure of NC cells to fully migrate throughout the developing gut.[26] In contrast to Simpson et al.'s[25] model for chick vagal NC cells, McLennan et al.[27] demonstrated that chemotaxis induced by vascular endothelial growth factor (VEGF) plays a fundamental role in chick cranial NC cell invasion. Stromal cell-derived factor,[16,28] glial cell-derived neurotrophic factor,[29] brain-derived neurotrophic factor,[30] platelet-derived growth factor[31,32] and fibroblast growth factors[33] are other important chemotactic factors in NC cell migration.

The remainder of this article is organized as follows. In Section 2, we briefly introduce some basic biological knowledge of chick cranial NC cell invasion. In Section 3, we discuss a study that suggests that different cell phenotypes are necessary for successful cell invasion—these have been termed “leaders” and “followers”. In Section 4, we ask the question: how many leader cells are required to ensure robust invasion? The findings that demonstrate these phenotypes to be plastic are presented in Section 5. In Section 6, we present results that suggest cell speed is regulated by an extracellular protein signal (DAN). We conclude, in Section 7, with a short

discussion and list some open questions that still need to be addressed.

2 | WHAT IS KNOWN ABOUT CHICK CRANIAL NEURAL CREST CELLS?

Chick cranial NC cells delaminate from the dorsal neural tube, at the midbrain and hindbrain levels, including rhombomere (r) segments of the hindbrain. We focus on the cranial NC cell migratory stream that emerges adjacent to r4 and migrates through the growing embryonic tissue into branchial arch 2 (ba2) (Figure 1). Cranial NC cells differentiate and contribute to cartilage, bone, teeth, connective tissue and cranial neurons.[18] Until recently, it has been unclear what the main molecular signals that drive the collective migration of chick cranial NC cells are. An experimental study on the expression of neuropilin genes in these cells[34] helped to generate a hypothesis that VEGF, to which neuropilins bind, plays an important role in migration. McLennan et al.[27] investigated this hypothesis and discovered that indeed, VEGF acts as a chemoattractant for NC cells. The absence of VEGF at the target site, or the inability of NC cells to read out the VEGF signal by knockdown of the neuropilin-1 receptor, led to a loss of directed migration and failure of NC cells to invade ba2. These data suggested that local known inhibitory signals that typically maintain the segregation of cell migratory streams prevent the cells from further dispersion since NC cells collapsed protrusions and remained at the entrance to ba2. McLennan et al.[27] also demonstrated that NC cells are attracted by VEGF in vitro and that migrating cranial NC cells changed direction and moved towards ectopic in vivo sources of VEGF placed either adjacent to a cranial NC cell migratory stream or within a stream.[35] They also observed that before the NC cells enter the migratory domain, VEGF protein is expressed throughout the surface ectoderm directly overlying the NC cell migratory pathway and becomes expressed in the ba2 target tissue.[27] These findings suggested that a possible explanation for collective NC cell migration is chemotaxis up gradients in VEGF. To test this hypothesis for collective migration, a combined experimental and mathematical modeling study was carried out in McLennan et al.[36] and we summarize their results below.

3 | IS A CELL-INDUCED VEGF GRADIENT SUFFICIENT FOR CRANIAL NEURAL CREST CELL MIGRATION?

The NC cells migrate through a three-dimensional curved domain. It is known that the depth of the domain is about three to five cell diameters. Therefore, to investigate computationally whether a cell-induced VEGF gradient can account for collective chick cranial NC cell invasion, McLennan et al.[36] used a two-dimensional flat domain as an approximation of the full geometry. In their model, cells were represented as discrete individuals (individual-based model (IBM)), while the VEGF concentration was modeled as a continuum. The cells were modeled as non-overlapping circular disks that move by randomly sampling the VEGF concentration at a certain number of points a fixed distance away from their center in randomly chosen directions (Figure 2) and then moving towards higher levels of VEGF. This is a simple way to model cells sending out filopodia to sense the VEGF gradient. The elongation of the domain was prescribed, based on empirical measurements.[36] McLennan et al.[36] assumed that VEGF is consumed and/or degraded by the cells, setting up a gradient to which cells respond by becoming polarized and moving up the gradient. In their model for VEGF dynamics, they included VEGF diffusion, production, uptake by cells and dilution due to domain growth. This led to a reaction-diffusion equation on a growing domain. One of the key issues was to choose appropriate boundary conditions. The initial studies assumed that the VEGF concentration was fixed at zero on the lateral boundaries creating an inward pointing VEGF gradient to ensure that the cells do not leave the domain.[36] Later studies used zero flux boundary conditions for VEGF, and the key results of the model were independent of the choice of these two different sets of boundary conditions.[35,37,38]

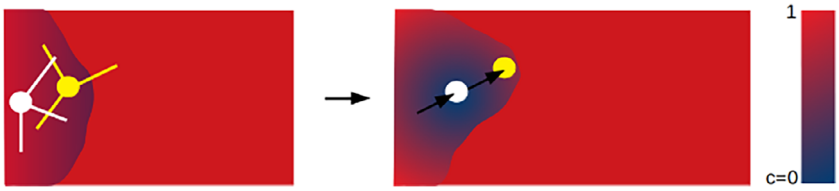

FIGURE 2 McLennan et al.[36] model schematic. The yellow circle corresponds to a leader cell, the white circle to a follower. The white and yellow short cell protrusions represent filopodia and c is the VEGF concentration. The elongation of the rectangle corresponds to domain growth. The leader cell senses the gradient in c and moves forward, while the follower cell senses the leader and adopts its direction of motion

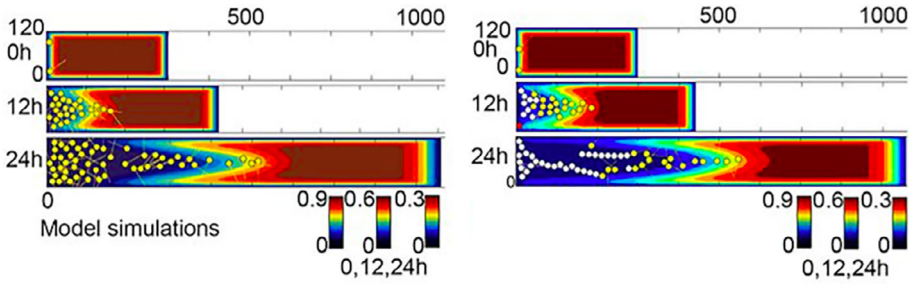

FIGURE 3 McLennan et al.'s [36] model simulations: left computational model with one cell phenotype; right computational model with two cell phenotypes. Yellow—leader cells, white—follower cells, chemoattractant concentration represented by color bar. For each case, the left-hand boundary of the domain is the neural tube, and the right-hand boundary is the end of the domain which corresponds to ba2. It can be seen that in the left panel (in which there is only one cell phenotype) cells are stranded at the neural tube, while in the right panel (with two cell phenotypes) they invade. The length units are μm. Figure reproduced with permission from McLennan et al.[36]

Initially, McLennan et al.[36] assumed that all the cells were homogeneous in their behavior. Their computational results revealed that, in this case, the cell stream breaks, meaning that there are large gaps in the stream. This behavior is due to the fact that, for biologically realistic parameter values, the VEGF gradient is highly localized, moving with the leading cells and away from those cells newly entering the domain (Figure 3, left). Although the model predicted that some cells reach ba2, the experimentally observed intact streams could not be generated to allow a sufficient number of cells to reach their target. Hence, the hypothesis that effective collective cell invasion can result from a cell-induced gradient is not consistent with experimental observations. However, on implementation of a new hypothesis, namely that there are two NC cell phenotypes: "leaders" that generate and move up the gradient of chemoattractant and "followers", that follow (unspecified) signals from the leaders, the model simulations suggested that successful streaming migration is possible (Figure 3, right).

These model driven hypotheses were verified experimentally using qPCR gene profiling and multiplexed imaging of mRNA expression using a fluorescent hybridization chain reaction method.[36] It was observed that the gene expression profiles of cells at the front of the stream (leaders) differed significantly from the profiles of the cells at the back of the stream (followers). The characteristics of followers were verified by showing that in vivo knock-down of VEGF did not affect their migration. These findings were further validated by subsequent investigations: for example, the model was used to make predictions on the effects of (i) removing followers, and (ii) transplanting leaders from a donor to the back of the stream in a host. In parallel with the simulations, biological experiments were carried out and their results validated the model predictions. However, model predictions for a third experiment – implanting followers from a donor ahead of the host leaders and profiling the cells at a later stage – did not agree with experimental results. The conclusion of this experiment was that cell phenotype is not fixed and that microenvironmental signals, including VEGF, regulate leader/follower cell behaviors and gene expression cell phenotype (see Section 5). To summarize, this interdisciplinary study showed that the hypothesis of a cell-induced gradient, while verbally elegant and persuasive, was not wholly correct. Instead, the computational model predicted that, in addition, cell phenotype heterogeneity is required, and this prediction was validated experimentally. This new hypothesis leads to several further questions, some of which we now discuss.

4 | HOW MANY LEADERS ARE NECESSARY FOR SUCCESSFUL INVASION?

Having established that there appear to be specialized cells (leaders) at the front of the invading stream, a natural question to ask is how many leaders are necessary for successful invasion. However, McLennan et al.'s[36] model is not sufficiently detailed to answer such a question, as experimental data are not yet at the level of detail required to allow very accurate identification of parameter values. Further, experimental photoablation methods to delete leader cells are difficult to interpret due to collateral damage of surrounding tissue within the cell migratory pathway.[39] Therefore, the model is more appropriate for making qualitative predictions, and McLennan et al.[38] used their model to investigate how invasion depended on the proportion of cells that are leaders. The model predicted that it is possible for cells to invade ba2 for a wide range of leader cell numbers, but that beyond a certain (small) number of leaders, the total number of cells in a stream will decrease (Figure 4).

To test these predictions, McLennan et al.[38] performed single-cell gene expression profiling using real-time qPCR. They discovered regional differences in gene expression and identified a unique stable molecular signature of the cells at

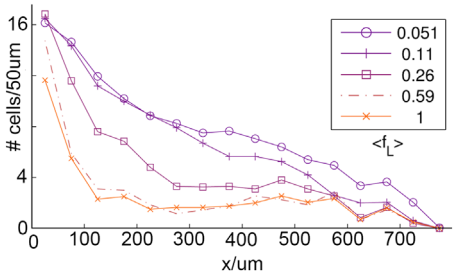

FIGURE 4 Cell counts versus distance migrated for five different leader fractions (f_L). It can be seen that in all cases cells invade, but that a small number of leaders ensures higher cell density all along the stream. Figure reproduced with permission from McLennan et al.[35]

the front of the migrating NC stream. HAND2 and BAMBI, both known for their importance in development,[40,41] were the two genes highly expressed by the cells within the most invasive front of the stream, and their overexpression resulted in alterations of migratory patterns, consistent with the predictions of the model.

The role of leaders and followers, as well as the optimal number of leaders in a group, has been investigated in a number of biological systems, for example, lateral line in zebrafish embryos,[42] angiogenesis (tip and stalk cells as leader and followers, respectively),[43] border cells,[44] and bees,[45] to name only a few.

5 | IS CELL PHENPOTYPE PREDETERMINED?

As mentioned in Section 3, the experiments of McLennan et al.[36] suggested that cells may switch their phenotype in response to VEGF. This finding was further investigated in McLennan et al.[35] where the model was updated to include phenotype switching. They assumed that cell phenotype is determined by the gradient of VEGF, with cells that are exposed to gradients above a threshold value for a certain time becoming leaders while those in low gradients become followers. A natural question to ask when implementing such a mechanism is whether the timescale of cell phenotype switching has a significant effect on migration efficiency. Computational modeling predicted that switching times from leader to follower and vice versa should be similar to each other to ensure optimal migration efficiency.

To test the hypothesis of the plasticity of gene expression, McLennan et al.[35] performed experiments in culture to test the effect of the addition and removal of VEGF. They observed rapid (of the order of a few minutes) changes in relevant gene expression. This result validated the modeling assumption that cell phenotype is determined by VEGF concentration. Further, previous studies have shown the plasticity of lead NC cells to reroute to a head target in response to either physical barriers placed in front of a migratory stream[46] or laser ablation of leader cells.[47]

The model was then used to predict the outcome of an in vivo experiment in which cells behind the front were exposed to VEGF. The prediction was that these cells would become leaders, break away from the initial stream, and move towards the new source of chemoattractant. This prediction was validated experimentally.[35] Note that these observations differ from the CiL and CoA model that proposes no role for chemoattractant in the collective migration of NC cells,[19,20] but require a constrained 2D boundary in order for cells to propagate in a forward directed manner.[21]

6 | WHAT INFLUENCES NEURAL CREST CELL SPEED?

The studies described above help us to understand how a number of different factors conspire to play key roles in NC cell migration. One issue with the model is that it is possible for some leader cells to invade too far ahead of the stream and therefore not be able to signal to followers, causing stream break up (leader cells moving too fast might eventually leave followers behind, Figure 3, right). This observation suggests that cell speed needs to be tightly controlled. McLennan et al.[37] observed that, in the early stages of invasion, DAN, a BMP inhibitor, is expressed within the paraxial mesoderm adjacent to the dorsal neural tube and extending in the antero-posterior direction along the hindbrain. As the NC cells move through the migratory domain, DAN expression decreases where NC cells have migrated through. In vitro experiments showed that cells tended to be inhibited from moving on stripes of a matrix on which DAN protein was present. A detailed study showed that knockdown of DAN led to enhanced cell speed and directionality, while overexpression of DAN significantly reduced the number of invading cells. From these observations, McLennan et al.[37] concluded that DAN may act to reduce cell speed close to the neural tube, in order to maintain better stream cohesion.

To test the validity of this hypothesis using the model, McLennan et al.[37] simulated scenarios with changes in DAN expression incorporated as changes in cell speed in the region adjacent to the neural tube. The results were consistent with experimental observations and, indeed, reduction in cell speed corresponding to overexpression of DAN led to inhibition of cell invasion, whereas downregulation led to increased distances travelled by a few leader cells, but the stream broke more frequently (Figure 5).

As mentioned in the Introduction, NC cells serve as an important paradigm for cell invasion. In particular, NC cells are the precursors of many of the most invasive cancers,

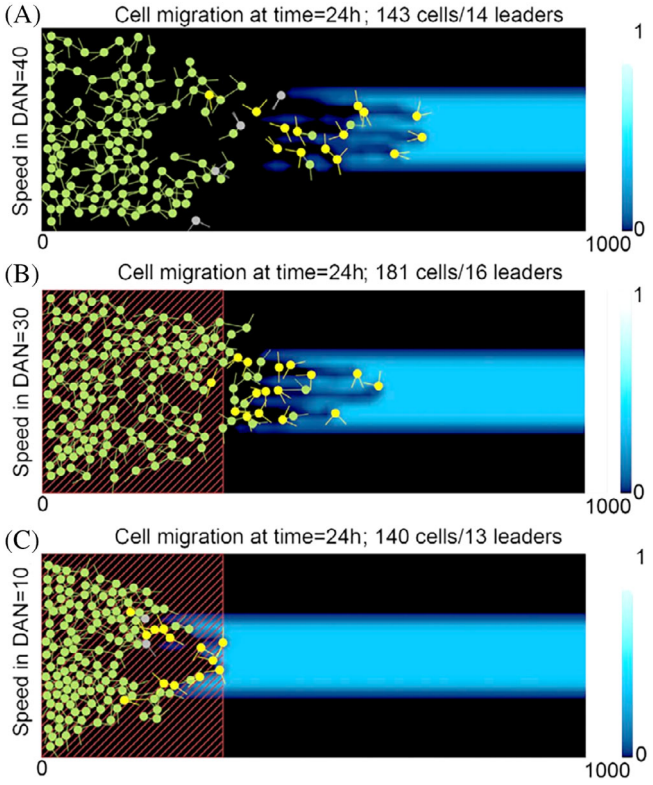

FIGURE 5 McLennan et al.'s [37] model simulations. Collective NC cell migration can be facilitated by DAN expression through regulation of cell speed within range of the dorsal neural tube. (A) No reduction in speed: the model simulated is as in McLennan et al.[35,38] on a widened domain with cell speed 40 μm/h. Leader NC cells are in yellow, follower cells are in green, and the background blue color shows the VEGF chemoattractant concentration. (B) Moderate reduction in speed: inside the area representing DAN expression (red dashed rectangle), cell speed is reduced in proportion to DAN concentration. The minimum cell speed is 30 μm/h. (C) Modelling increased DAN: the simulation is set up as in (B), but the cell speed is now reduced down to 10 μm/hour (at peak concentration of DAN). Figure reproduced with permission from McLennan et al.[37]

such as melanoma. McLennan et al.[37] confronted melanoma cells with DAN in vitro, and observed that they avoided the DAN-rich regions. From this observation, they concluded that DAN could be a candidate inhibitor of human metastatic melanoma. This study provides an example of where a deeper understanding of what controls and regulates cell behavior in developmental biology could have a significant impact on our understanding of certain diseases.

Maiuri et al.[48] have demonstrated using an interdisciplinary approach that cell persistence is robustly coupled to cell migration speed via actin flows in many different cell types, such as pigment epithelial or dendritic cells, and propose this as a universal mechanism. Our recent studies have revealed that a water channel protein AQP-1 also regulates NC cell speed and affects invasive abilities,[49] further supporting a critical role of leader cells.

7 | DISCUSSION

In Alan Turing's seminal 1952 paper[50] he states of his model, "This model will be a simplification and an idealization, and consequently a falsification. It is to be hoped that the features retained for discussion are those of greatest importance in the present state of knowledge." A corollary of this statement is that if a model is successful, it will change the present state of knowledge, thus requiring us to re-evaluate and modify the model with this new knowledge – a process now commonly known as "predict-test-refine-predict". In this Commentary, we have showcased this process in the context of cranial NC cell migration. We have shown how the integration of experiment with theory can lead to new insights into fundamental processes in biology, with mathematical modeling used to test experimental hypotheses, generate new hypotheses and make predictions that can again be tested experimentally. Crucial to this collaboration (see Figure 6 for more information about our collaborative group) is the identification of well-defined problems that can be investigated by both modeling approaches, namely experimental models (in this case, chick) and theoretical models (in this case, a hybrid computational model). Having defined such a problem, the computational model simulation study and the biological experimental study are carried out in parallel, but with no communication during this process to ensure complete independence between each study. Only when the studies have been completed are they compared with each other. Using this approach, we showed how a mathematical model was able to refute the experimental hypothesis that successful cranial NC cell invasion is the result of a single cell type responding to a cell-induced chemotactic gradient but, instead, that heterogeneity in cell response (phenotype) is required. This new mathematical model led to predictions that were invalidated experimentally, suggesting that cell phenotype was not fixed. The resultant modified model gave rise to predictions that were then consistent with experimental results that manipulated the chemoattractant gradient. Furthermore, the model was able to make correct predictions on the results of manipulating cell phenotype, and suggested a role for the BMP inhibitor DAN (to slow leader cells) that is expressed within the initial subregion of the cranial NC cell migratory pathway. Current computational models, including the one we discussed in this review, are mainly used to produce qualitative predictions. Faster and more powerful quantitative techniques in both biological data acquisition and data storage, manipulation, and presentation are likely to lead to more refined models that could be used to make more accurate quantitative predictions. To illustrate this paradigm, we use the example of the insight that distinct cell phenotypes are required for collective cell migration, as

FIGURE 6 This decade-long interdisciplinary collaboration began through discussions, lasting several months, between the experimental group (Rebecca McLennan and Paul Kulesa, main picture, left) and the theoreticians Philip Maini and Ruth Baker (main picture, right). The mathematical modelling has been carried out by three students who were/are part of a special interdisciplinary doctoral training program that exposes students from a variety of scientific backgrounds to a wide range of areas in the biological and mathematical sciences before they chose the appropriate department in which to do their doctorate. Louise Dyson (top right) and Rasa Giniūnaitė (bottom right) were trained as mathematicians, while Linus Schumacher is a physicist. All three students spent time in the laboratory at the Stowers. Louise's thesis developed the prototype agent-based model and also carried out detailed mathematical analysis of the system. She is now a permanent faculty member with a joint appointment at the Mathematical Institute, and the School of Life Sciences, at the University of Warwick. Linus extended the model and also carried out gene expression analysis and more detailed studies of collective cell behavior. He is now a Chancellor's Fellow at the MRC Centre for Regenerative Medicine, University of Edinburgh. Rasa is presently doing her doctorate, focusing on extending the study on domain growth. The group photograph was taken during the annual visit that the experimental group makes to Oxford and it is at these meetings that we compare experimental results with model predictions and discuss/design new experiments that can be done with the theoretical model and also in the lab—the results of these are then compared at the next meeting with no discussion in between in case it compromises the comparison (of course we continue to discuss other parts of the collaboration)

discussed in Section 3. The necessity of distinct leader and follower phenotypes is a qualitative prediction, and detailed gene expression analysis has to be performed to investigate phenotypic differences across cells. Recent measurements of the whole transcriptome suggest a continuum of cell states, from the most leader-like cells to the most follower-like cells. Schumacher's[51] computational model supported the continuum of cell states hypothesis. This study is a promising example of how refined biological data can be used to clarify modeling assumptions to ensure more quantitative predictions.

7.1 | Future directions

There are many questions that still remain to be answered and we discuss some of these here.

7.1.1 | Results of stream manipulations

With the present model described in this Commentary, there are still a number of tests that can be performed. For example, our mathematical model, based on McLennan et al.'s[35,37,38] model, can make predictions on what would happen if other manipulations were carried out on the streaming cells. We illustrate this with two cases. Firstly, we use the model to predict what would happen if, after 15 hours, cells were restricted from entering the domain. Figure 7 shows the model prediction, that the restriction of cell influx does not impede the cells at the front, which continue to invade the domain as in the control case. This observation suggests that crowding at the back of the stream is not significant for a successful invasion by the cells at the front of the stream.

Let us now consider what would happen were we to ablate cells from the middle of the stream. Figure 8 shows the model prediction after removal of the cells in the middle of the stream (at a distance more than 150μm but less than 200μm from the neural tube, after 15 hours). In this case, we observe that the proximal segment of followers do not manage to catch up with the followers ahead of them, and remain moving randomly close to the neural tube. The follower cells in the most distal subregion of the abandoned segment cannot switch phenotype to leaders because of the lack of a

VEGF signal. The stream breaks and collective invasion is entirely disturbed.

The above examples are purely qualitative predictions of the outcomes of manipulations made within the stream. In order to reach rigorous conclusions on the effects of these alterations, more detailed computational studies have to be performed; for example, the restriction of cell influx at different times or the removal of a different number of cells. However, we present them to highlight computationally tractable alterations of the system that can potentially shed light upon the underlying mechanisms crucial for successful invasion.

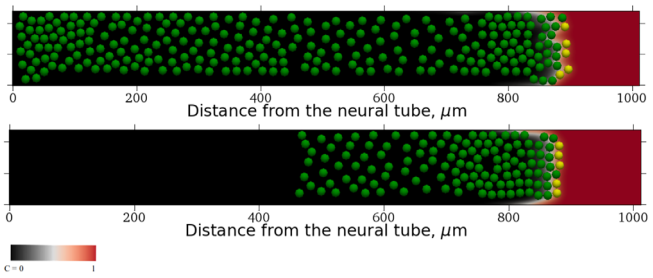

FIGURE 7 Snapshot of the simulations at $t = 24$ hours of the model developed based on McLennan et al.'s[35,37,38] model and described in the first part of Section 3. Top: Control case. Bottom: Limited cell influx. Yellow circles correspond to leader cells, green circles correspond to follower cells, and c is the concentration of chemoattractant. We restrict the influx of cells, so that no cells enter the domain after 15 hours

7.1.2 | Within stream signaling

In our computational model, we assume that follower cells are guided by the leader cells forming chains with leaders at the front. The cells in a chain take on the direction of a leader at the front of that chain. However, we do not know the type of signal to which these follower cells respond. For example, are leader cells producing a chemical signal to which follower cells respond? If so, what is the signal and how is it relayed down the stream? Alternatively, leader cells could be boring a tunnel through, or remodeling, the extracellular matrix making a path for the followers. There is experimental evidence for this[49] but there is the question of how polarity is determined along this tunnel. Leaders could also align the extracellular matrix or produce components of it. Alternatively, the leaders may set up mechanical guidance cues to which the followers respond, a mechanism which is present in other collective cell migration phenomena, such as angiogenesis.[52,53] It has been shown that enteric NC mesoderm stiffens during the period of colonization[54] and that in *Xenopus*, tissue stiffening triggers NC cell migration.[55] The present model cannot distinguish between different signaling possibilities, and so further model refinement which accounts explicitly for other chemical signals or extracellular matrix mechanical cues is required to investigate these questions. This should be accompanied by a parallel experimental study to investigate whether, for example, followers express receptors for possible signals from the leaders. How does cell response to guidance cues change along the stream?

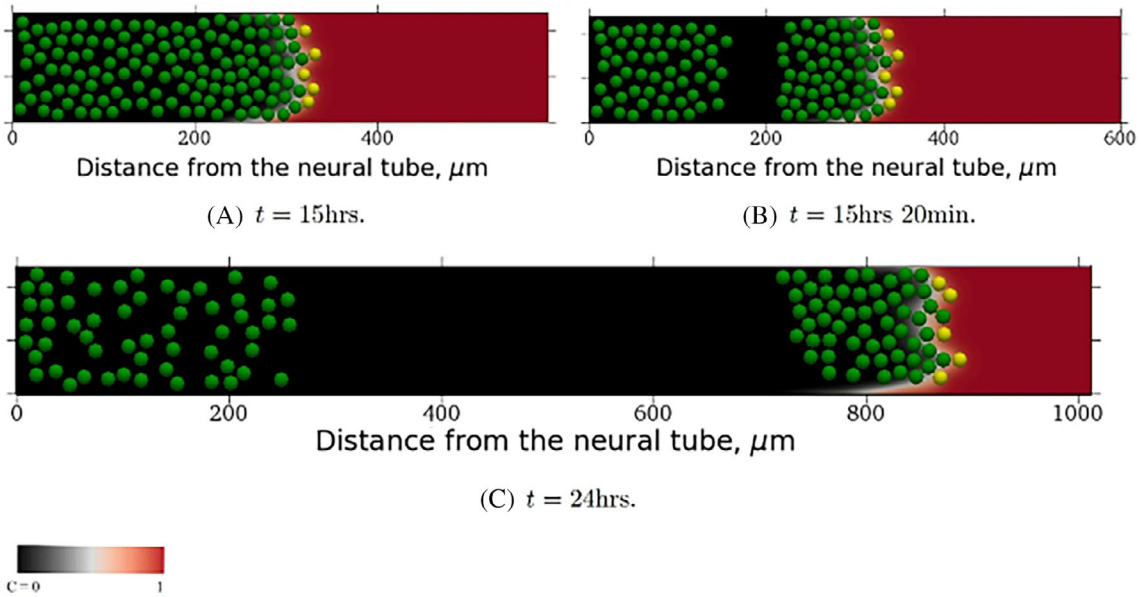

FIGURE 8 Snapshots of simulations of the model developed based on McLennan et al.'s[35,37,38] model and described in the first part of Section 3. Yellow circles correspond to leader cells, green circles correspond to follower cells, and c is the concentration of chemoattractant. The cells in the middle of the stream (at distance more than 150μm but less than 200μm from the neural tube) are removed after 15 hours. Follower cells do not manage to catch up with the leaders and the stream breaks

McLennan et al.[35,38] assumed that cells behave differently in their response to signaling cues, depending on VEGF concentration, and hypothesized two cell phenotypes. They imposed a generic within stream signaling mechanism which could originate from chemical or mechanical gradients. However, their model is too coarse-grained to be able to distinguish between these possibilities and it is most likely that cells have the ability to respond to multiple signaling cues. Moreover, as mentioned above, Schumacher[51] has shown that a model comprising a continuum of phenotypic states is consistent with the experimental results described in the Morrison et al.[56] paper. Further biomarkers that identify distinct cell states within the stream and the functional analysis of cell behaviours after gene manipulation are required to understand the importance of heterogeneity in successful invasion.

7.1.3 | What constricts cells to the stream?

It is observed that NC cells primarily move within a "corridor" (see, for example, the review by Kulesa et al.[57]). It has been shown that in *Xenopus*, where cranial NC cell migratory streams are adjacent to one another, the cells are confined in streams by cell-cell interactions through Eph/ephrin signaling[58] and cell-tissue interactions through Versican.[21] Recently, we have discovered several membrane-bound and secreted factors expressed distinctly within the chick cranial paraxial mesoderm in NC cell exclusion zones that, in preliminary results, inhibit NC cell movements, suggesting redundant mechanisms to prevent stream mixing. Computational modeling could be used to demonstrate whether stream confinement is regulated by external factors, or by the ability of the NC cells to remain within a confined group.

7.1.4 | The role of domain growth?

In the model we presented, domain growth was assumed to be spatially uniform but changing in time (growing exponentially initially before tapering off). This was motivated by measurements of the total length of the domain.[36] However, our recent experimental findings suggest that domain growth is not spatially uniform, but has a spatial pattern.[59] Naturally, we question whether this makes migration more robust. Could there be a feedback loop between the migrating NC cells and the microenvironment, in which the former controls domain growth? Alternatively, is spatially non-uniform domain growth necessary for other processes occurring in development, and so must NC cell migration be robust to such non-uniformities? Due to the challenges in manipulating domain growth experimentally, computational modeling provides a natural means to directly investigate the effect of changes in domain growth profiles on the migration of cells.

7.1.5 | The role of geometry?

The above model has considered a flat two-dimensional domain, but in reality, cells are moving through a three-dimensional curved domain. A natural question to ask is whether the above model is a good approximation to that. This is a question that is amenable to computational analysis but has not yet been investigated.

7.1.6 | How do we integrate bioinformatics with mechanism?

Typically, in mathematical biology, gene expression data are derived and theoreticians are asked to then determine meaning and mechanism. In the study of McLennan et al.[36] this process was reversed—mechanistic modeling predicted gene expression variation, and this was verified experimentally. A key challenge in all areas of biology is how to integrate data from across spatial and temporal scales to inform models, to generate predictions and drive new experiments to allow us to gain further mechanistic insights.

7.1.7 | Is there a unifying theory for collective cell migration?

As stated in the Introduction, collective cell migration is ubiquitous in biology. In the NC system, there is a wide variety of stream behaviors depending on the axial level of origin (e.g. cranial, vagal, or trunk), and on the model organism. A natural question to ask is: are there certain universal principles that must be obeyed for successful collective cell invasion, and do these variations simply correspond to different biological ways to satisfy these principles? There is the need for directed cell motion to invade distal targets collectively – this can arise from some polarizing agent, be it chemical or mechanical. Then, there has to be signaling between cells to ensure collective movement. Cells may also need some external signals to confine them to a certain region. In the case of the NC, where there is also domain growth, does the growth have to be regulated by the migrating cells in some way? Are there other hallmarks of collective cell migration?

It should be possible to understand the different ways in which biology performs these tasks (see, for example, the reviews[13-16]). From a mathematical point of view, the challenge is to develop a generalized model that encompasses all these features. This would then allow us to see how the different modes of migration we observe are related to each

other, and may also predict other modes of motion yet to be seen.

The challenge of modeling collective behavior in general (including ecology and human interactions) has also led to significant mathematical advances in multiscale modeling and the analyses of these models, ranging from cellular automaton to partial differential equation models (see, for example, the review by Giniūnaitė et al.,[60] and collected articles in the special issues[61,62]).

In summary, we have shown how mathematical modeling, combined with custom-designed experiments, can lead to new biological insights. The recent advances in imaging techniques offer the real possibility of further tight integration of modeling with experiment, with the exciting potential of allowing us to fully exploit these data and accelerate biological discovery.

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