An Interaction with Biologists: Insights into Development and Disease

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Abstract The enteric nervous system (ENS) in our gastrointestinal tract is responsible for normal gut function and peristaltic contraction. Embryonic development of the ENS involves the colonisation of the gut wall from one end to the other by a growing population of motile neural crest cells. The colonisation wave is strictly timetabled and predictable, but individual neural crest cell movement is unpredictable in speed and direction. Failure of these cells to invade the whole gut results in the relatively common, potentially fatal birth defect. Continuum models of the population-level behaviour, based on the Fisher equation, are highly predictable. Discrete agent-based models, governed by agent probabilities, reproduce the population-level behaviour of the Fisher equation. However, individual agent contributions to the total population, measured by agent lineage, are highly variable. Both behaviours have been verified in a developmental invasion system. This work is the result of a rewarding long-standing and on-going collaboration between applied mathematicians and developmental biologists.

Keywords Mathematical biology · Cell invasion · Diffusion · Proliferation · Lineage

1 Introduction

The enteric nervous system in our gastrointestinal tract is responsible for normal gut function and peristaltic contraction. Embryonic development of the enteric nervous system involves the colonisation of the gut wall from one end to the other by a growing population of motile neural crest (NC) cells. Failure of these cells to invade the whole gut results in the relatively common, potentially fatal condition known as Hirschsprung disease.

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During this colonisation process, there is a huge increase in NC cell numbers through cell division (proliferation) up to a preferred density, called the carrying capacity. At the same time, the cells making up the gut tissue also undergo cell division, resulting in massive elongation of the intestine. However, two styles of experiments on gut explants can be performed—one where the gut undergoes no elongation and one where the gut undergoes significant elongation.

Observations using time-lapse imaging in bird and mouse guts have emphasised that the speed and direction of movement of individual NC cells is unpredictable. In contrast, the wave-like spread of the entire population is predictable [11].

Here we discuss modelling and simulation of diffusive processes, both continuum and discrete, which have been used to provide insight into the key biological processes required for complete colonisation. Moreover, the modelling work has also generated experimentally testable predictions.

This modelling work was initiated by an approach by Dr. Donald F. Newgreen, a developmental biologist who heads an Embryology Laboratory at Murdoch Childrens Research Institute in Melbourne, Australia. He thought that mathematical modelling might help with understanding the colonisation process and the cause of Hirschsprung disease. Our research groups have collaborated for over ten years, addressing various aspects of ENS development and disease.

2 Colonisation Models

We have developed both macroscopic population-level models (partial differential equation models) based on diffusive processes and microscopic cellular-level stochastic models (cellular automata (CA)/agent-based models) based on random walks. These approaches are complementary, but the microscopic models include the variability inherent in biological phenomena. In the conclusions, we make some comments about interesting theoretical questions that arise in connecting the two approaches.

It is worth noting that there is no overriding directional signal in the gut. Placement of the NC cells at the anal end of the gut results in reverse-direction migration [2]. In the models discussed here, no directional signal is imposed. Colonisation proceeds from left to right only because the initial cell population is placed at the left-hand end.

In the population-level models, the cell density changes in time due to a flux term (e.g. linear diffusion due to NC cell random motion) and a logistic growth term (due to cell proliferation up to a preferred density). The Fisher equation [4, 7] is the typical continuum description of an invading population. Here we modify that equation by including an extra advective term to describe domain growth. The invading NC cell population is restricted to a cylindrical surface, so a one-dimensional spatial description in terms of the cylindrical axis is appropriate. Let $u(x, t)$ be the density as a function of longitudinal distance x and time t. Then the continuum model is

$$\frac{\partial u}{\partial t} = \mathscr{D} \frac{\partial^2 u}{\partial x^2} + \lambda u (1 - u) - \frac{\partial}{\partial x} (v(x, t)u), \tag{1}$$

where $\mathscr{D}$ is the diffusivity, λ is the proliferation rate, unity is the normalised carrying capacity, and $v(x, t)$ is the advective velocity due to the underlying domain growth induced by the elongating tissue. For uniform growth

$$v(x, t) = \frac{dL}{dt} \frac{x}{L}. \tag{2}$$

If there are host $H(x, t)$ and donor $D(x, t)$ NC cells, with the same properties, then the host and donor populations are governed by

$$\frac{\partial H}{\partial t} = \mathscr{D} \frac{\partial^2 H}{\partial x^2} + \lambda H \Big(1 - (H + D)\Big) - \frac{\partial}{\partial x} (v(x, t)H), \tag{3}$$

$$\frac{\partial D}{\partial t} = \mathscr{D} \frac{\partial^2 H}{\partial x^2} + \lambda H \Big(1 - (H + D)\Big) - \frac{\partial}{\partial x} (v(x, t)D), \tag{4}$$

respectfully.

In the CA model, a two-dimensional square lattice is used. At each time step, randomly chosen agents are offered opportunities to move and proliferate, governed by local rules and probabilities. These are illustrated in Fig. 1b, c. Each lattice site can be occupied by at most one agent, representing a NC cell, due to volume exclusion. Movement or proliferation events which attempt to place a NC agent on an already occupied site are aborted.

Fig. 1 CA mechanisms for domain agent (*yellow*) proliferation and NC agent (*blue*) motility and proliferation. **a** Domain agent proliferation rule produces a growing domain. **b** A NC agent can move to one of the four configurations shown with equal probability. **c** A NC agent can divide into two daughter agents, placed at two possible configurations (to replicate experimental observations) with equal probability
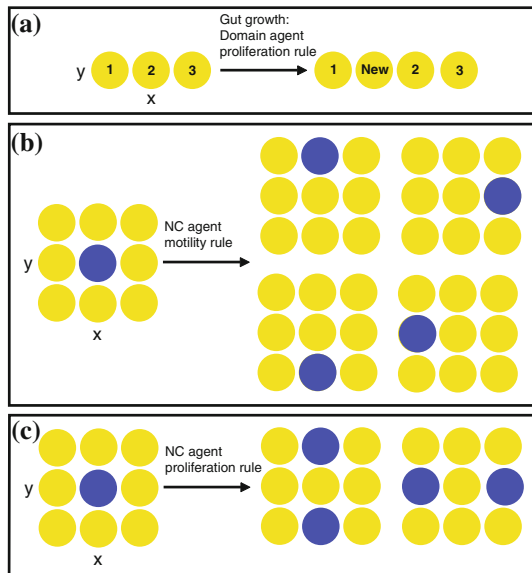
We developed a discrete growing domain model [1], where the underlying lattice sites represent the gut tissue cells. To model gut elongation, new lattice sites are randomly inserted into each row of the lattice, thus growing the lattice, as illustrated in Fig. 1a.

The domain has periodic boundary conditions on the two horizontal boundaries, and there will be no-flux conditions on the vertical boundaries.

2.1 No Gut Growth

In the absence of gut growth, we can determine experimentally how NC cells behave in different parts of the colonisation wave using quail and chick NC cells, since they are functionally equivalent but label differently using antibodies. We suggested experiments to test against our models.

In tissue culture where there is no gut growth, a segment of chick host gut tissue is removed and replaced with quail donor tissue containing NC cells. Three days later the resulting host and donor cells are identified. Donor cells can be placed at or behind the wavefront (Fig. 2a, b).
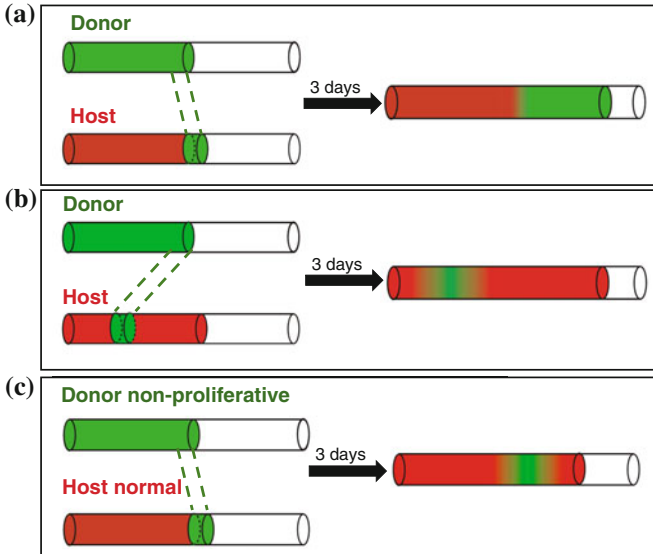

Fig. 2 Schematic profiles of chick-quail graft experiments. Host cells (*red*), donor cells (*green*). **a** Grafting donor cells into the wavefront, **b** grafting donor cells behind the wavefront. In (**a**, **b**), cells at the wavefront are responsible for driving further colonisation, called frontal expansion. **c** Grafting donor cells with no proliferative ability into the wavefront. Host cells overtake the non-proliferative donor cells and then have proliferative opportunity to drive further colonisation [9]. (*Note* Variants were experimentally tested where the donor tissue is removed from behind the wavefront instead of at the wavefront as shown. The outcomes were the same)

Both models (Eqs. (3) and (4) with appropriate initial conditions [9] and discrete CA) and experiments give the same results. Cells at the wavefront are responsible for an increase in cell numbers into unoccupied regions, thereby driving the colonisation wave. We call this frontal expansion. We conclude that proliferation at the wavefront is the driver of colonisation [9, 10].

What happens if NC cell proliferation is eliminated at the wavefront (Fig. 2c)? Donor cells can be treated to eliminate cell division but preserving cell motility. The experimentalists thought there were two possible outcomes. Either nothing happens (stall) or the host cells proliferate and push the donor cells forward (shunting). In contrast, our models predict that the donor cells diffuse, leave space for the host cells to move into and eventually overtake the donor cells, proliferate at the wavefront and drive further colonisation. The experimentalists were surprised at their results—they matched the model predictions [10].

2.2 With Gut Growth

The models predict that colonisation again proceeds by frontal expansion; however, now there is also proliferation behind the wavefront due to additional space produced by domain growth. Successful colonisation only occurs if the NC cell proliferation rate is sufficiently large, compared with the domain growth rate (Fig. 3). The predictions were confirmed experimentally [12]. Incomplete colonisation corresponds to Hirschprung disease.

For the deterministic PDE models, there is a critical value of the NC cell proliferation rate that determines whether colonisation is complete or not. Therefore, if the
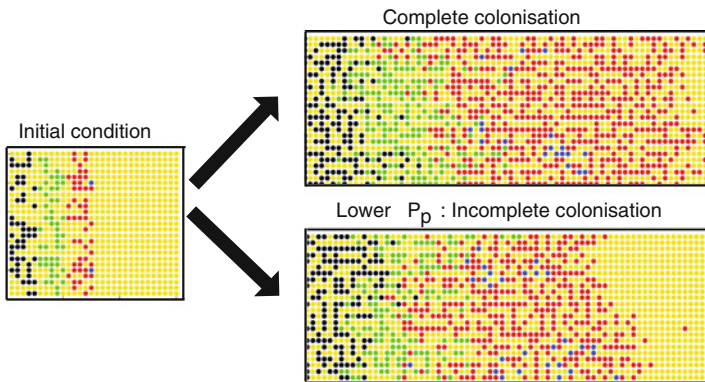

Fig. 3 With gut growth. Two realisations of the CA algorithm for two proliferation rates P_p. The subpopulations mix more than they do in the non-growing case. If the proliferation rate is sufficiently high, then there is complete colonisation. If the proliferation rate is sufficiently small, then there is incomplete colonisation, which emulates Hirschprung disease
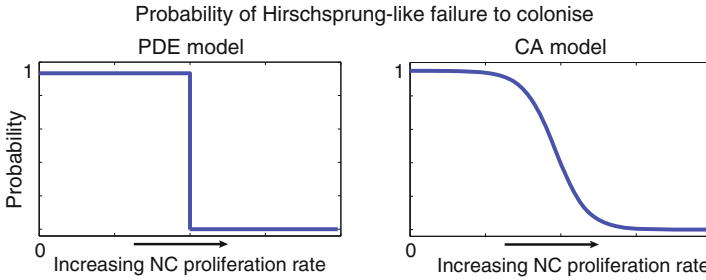
Probability of Hirschsprung-like failure to colonise

Fig. 4 The probability of Hirschsprung-like failure to complete colonisation for the PDE (deterministic) and CA (stochastic) models

proliferation rate is below the critical value, then a Hirschsprung-like failure occurs (Fig. 4). However, the CA approach is stochastic. This means that there is a range of NC cell proliferation rates where the probability of incomplete colonisation is nonzero, but not unity (Fig. 4). In particular, there exists a value of the proliferation rate where there is a 50 % chance of a Hirschsprung-like failure.

These results have important implications for Hirschsprung disease. We now understand Hirschsprung disease to be in most cases a NC cell proliferation defect and not, as thought, a NC cell motility defect. Furthermore, we have identified proliferation as the common link between the known Hirschsprung disease genes. In particular, stochastic effects can determine success or failure of the colonisation process for certain range of NC cell proliferation rates. This may explain the existence of identical twins where only one of the pair has Hirschsprung disease [6].

3 Cell Lineage

We know investigate the contribution of individual agents/cells to the colonisation wave. A lineage tree can be constructed for each agent. Agents either divide into two at a successive generation, or do not divide, as illustrated in Fig. 5.

Fig. 5 Schematic diagram representing the lineage of a single agent at some time t. The total number of filled discs represents the agent lineage tracing, while an empty disc represents a division event
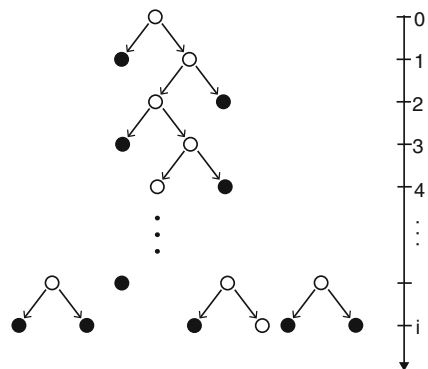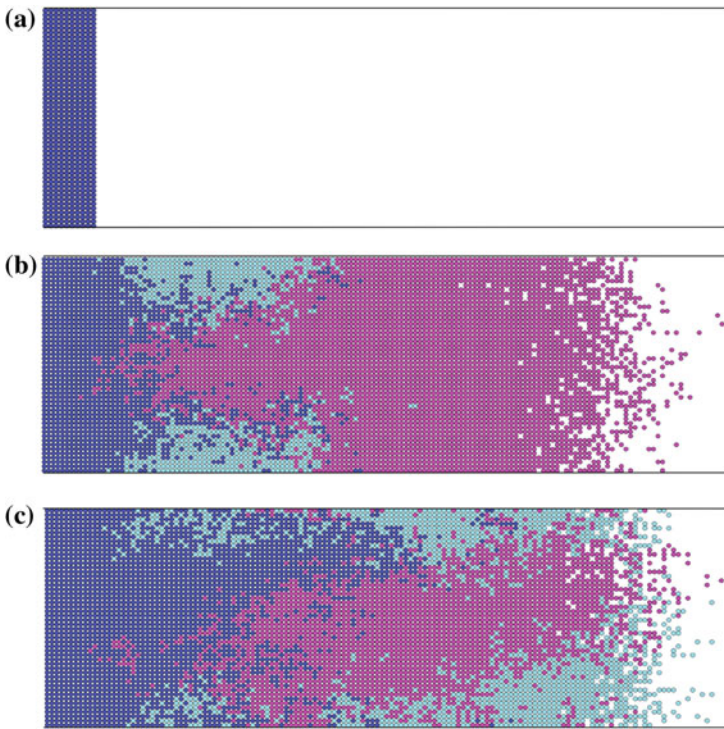
Fig. 6 Invasion wave and spatial distribution of agent tracings in two realisations for no gut growth case. **a** Initial condition for all simulations. **b**, **c** Two realisations of the travelling wave that moves progressively to the *right*. The largest and second largest single agent lineage tracing (*pink* and *turquoise* respectively) and the 498 other agent lineage tracings (all collected together in *blue*) are illustrated. In **(b)** there are significant differences in the agent numbers between the two largest tracings, while in **(c)** the two largest tracings have a similar number of agent numbers

Starting with a localised number of agents at maximum density at the left-hand end of the lattice (Fig. 6a), the agent density evolves and moves progressively to the right as illustrated in the two realisations presented in Fig. 6b, c. Stochastic competition for resources (space, growth factor, nutrients) between agents can be visualised through the spatial distribution of the single progeny lineage within the invasion wave. Realisations with the largest and second largest lineage tracing (pink and turquoise respectively), together with all progeny from the remaining initial agents (blue) are illustrated in Fig. 6b, c. These two realisations illustrate the large individual variability of the spatial distribution of single agent lineage. Furthermore, in every realisation a small proportion of identical initial agents accounts for a substantial proportion of the total final agent population. We term these individuals superstars. A full analysis of the complete set of lineage tracings can be found in Cheeseman et al. [3]. (Note, that the proliferation rule is slightly modified here. Instead of the two daughters being placed in opposing nearest-neighbour sites as in Fig. 1c, the two

daughters are placed in nearest neighbour sites. The results are qualitatively the same for both cases.)

In the two realisations, the total population has advanced approximately the same extent and is therefore highly predictable at this level. When lattice-site occupancy of the agents is column-averaged for a single realisation and averaged over many identically prepared realisations, a smooth density wave evolves into a travelling wave moving from left to right with a constant speed, which is dependent on the probabilities associated with agent motility and proliferation. This travelling wave emulates the Fisher wave [7].

Experiments were performed where a single starting NC cell is labelled green (green fluorescent protein GFP labelled cell) is placed within a starting population of red labelled cells. GFP+ve progeny inherit the green label, while all other NC cells label red.

After several days the whole gut is full of NC cells (thousands). However in numerous experiments, the number of GFP+ve progeny is very low (many <10, almost all <100). We found experimental evidence of a superstar, with one GFP+ve cell lineage tracing being of disproportionately huge number.

Unfortunately, there are insufficient cell-labelling colours to uniquely identify every starting NC cell and therefore uniquely determine the progeny of every starting cell (if indeed all the hues are inheritable), as can be performed with the CA model. However, the modelling results explain why most of the determined lineages had few progeny, and contribute very little to the total final population. The models support the fact that the final population is dominated by the progeny of a few superstars.

This suggests that stochastic competition for resources, e.g. space, is the source of the large variability and therefore inequality to the individual lineages. All agents/cells are identical but only a very few contribute significantly, due to most progeny being unable to contribute further progeny because neighbouring sites are already occupied. Note that for a growing domain, the contribution of superstars is not as large as for a non-growing domain, as all agents experience new empty lattice sites/tissue, as it is uniformly growing [3].

4 Conclusions

The model and experimental results imply that Hirschsprung disease is chiefly a NC cell proliferation defect and not, as previously thought, a NC cell motility defect [9, 12]. Furthermore, we have identified proliferation as the common link between the known Hirschsprung disease genes.

Cell lineage tracing is a powerful tool for understanding how proliferation of individual cells contribute to population behaviour. Single neural crest cells labelled to follow progeny in the intestine reveal extraordinary and unpredictable variation in number and position of descendant cells, even though ENS development is highly predictable at the population level. Lattice agent-based model explains these puzzling results [3].

The experiments and model suggest that stochastic competition for resources is an important concept when understanding biological processes which feature high levels of cell proliferation. The results have implications for cell fate processes in the ENS and other developmental systems.

The ENS is a fascinating developmental system with many interesting modelling challenges, combining diffusive processes and simulation. This work has provided much insight into a real biological process.

Finally, this biological collaboration involves two mathematical approaches. An interesting mathematical problem is to investigate the connection between the two. Our agent-based CA models form an interacting particle system. A mean field approach has been successfully implemented to transform the discrete model to a continuum model. We have developed a robust framework for taking general cellular probabilistic rules and converting them into a partial differential equation (non-linear diffusion equation for single species, nonlinear advection-diffusion equations for multi-species) in a systematic way [8]. Furthermore, growing domains can also be accounted for in a proper way [5]. This is a growing era of research which combines probability theory, statistical physics and mathematical biology.

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