Growth, homeostatic regulation and stem cell dynamics in tissues

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The regulation of cell growth in animal tissues is a question of critical importance: most tissues contain different types of cells in interconversion and the fraction of each type has to be controlled in a precise way, by mechanisms that remain unclear. Here, we provide a theoretical framework for the homeostasis of stem-cell-containing epithelial tissues using mechanical equations, which describe the size of the tissue and kinetic equations, which describe the interconversions of the cell populations. We show that several features, such as the evolution of stem cell fractions during intestinal development, the shape of a developing intestinal wall, as well as the increase in the proliferative compartment in cancer initiation, can be studied and understood from generic modelling which does not rely on a particular regulatory mechanism. Finally, inspired by recent experiments, we propose a model where cell division rates are regulated by the mechanical stresses in the epithelial sheet. We show that pressure-controlled growth can, in addition to the previous features, also explain with few parameters the formation of stem cell compartments as well as the morphologies observed when a colonic crypt becomes cancerous. We also discuss optimal strategies of wound healing, in connection with experiments on the cornea.

1. Introduction

Homeostasis of tissues requires a complex balance between cell division and cell loss. Stem cells, which often reside in a specialized niche [1] of an epithelium, have two contradictory tasks: replenishing the tissue, which implies a high rate of division and maintaining genetic integrity despite these frequent divisions. In the intestine, it is now recognized that there is a well-defined population of stem cells, which has the ability to repopulate the whole epithelium, and can be implicated in cancer [2]. Stem (S) cells are located mainly at the bottom of small tubular foldings called crypts. In order to be more efficient, stem cells give rise to partially differentiated, transit-amplifying (T) cells, which divide rapidly for a few rounds before becoming fully differentiated (F) cells, which do not divide [3]. The disruption of this balance leads to tumours [4], which to a certain degree retain the organization of the original tissue [5]. It has recently been shown that homeostasis is characterized by the neutral competition of symmetrically dividing stem cells [6,7]. Nevertheless, much less is known about the mechanisms to maintain this homeostasis, about the control of excessive proliferation or about the dynamical reaction of a crypt to a wound [8,9]. It is also increasingly acknowledged that mechanics play an important role in organ morphogenesis [10,11], cell differentiation [12], tissue homeostasis [13,14] as well as in cancer initiation [15]. A growing epithelial tissue exerts pressure, which shapes its environment [16].

Several models [17–19] have studied the renewal dynamics in the crypts, taking the three types of cells (S, T, F) into account. Nevertheless, the mechanism leading to the maintenance of a constant number of stem cells which is of crucial importance remains unclear. It has been proposed that differentiation rates should depend on the fraction of each type of cell [18] but this lacks a clear biological basis. Contact with nursing (Paneth) cells has been also invoked [20] as a determinant for stem cell fate in the intestine, and it has been shown...
that intestinal adenoma proceeds with a concomitant increase in stem cell and Paneth cell numbers [21].

Nevertheless, this only displaces the issue to how the Paneth cell number is itself regulated. Indeed, we show (see electronic supplementary material, S1) that such a contact mechanism only regulates the ratio of stem and Paneth cells but does not convey any global information, which could set the size of the proliferative compartment. Moreover, stem cell number saturation precedes Paneth cell appearance in developing crypts [8], suggesting an additional regulatory mechanism.

On the other hand, it was shown that division rates of stem cells decrease significantly as cell density increases during crypt formation [8]. We therefore assume that cells divide symmetrically and that the cell division rates alone depend on cell densities, a signalling phenomenon known as contact inhibition, and we couple the stem cell dynamics to the mechanical equations describing crypt size.

We show that this mechanism yields a robust homeostatic state and that several features of crypt development, homeostasis and carcinogenesis can be described within a simple theoretical framework, with a few important parameters. The crucial parameters of the model are the degree of coupling between the division rates and the fractions of respective cells.

This paper is organized as follows. We first discuss the growth scenarios observed in developing crypts. We then introduce the generic equations of our model, not assuming at first any detailed mechanism for contact inhibition and apply these equations to discuss the stability of homeostatic regulation and cancer initiation. Finally, to go further and try to quantitatively understand crypt development as well as compartment formation in mature crypts, we make the hypothesis that pressure is the main regulatory mechanism of cell growth, which would explain all the above observations using a single scalar quantity, capable of transmitting global cell-number information locally to each cell. This hypothesis could be tested by measuring pressure in the cell sheet, for example through laser-ablation experiments, and observe how this quantity evolves during development [22].

We assume that stem cells either divide symmetrically or differentiate into T cells. Just after the division of a stem cell $S_0$ into two daughter stem cells $S_1$ and $S_2$, if the cell $S_1$ then divides symmetrically while the other cell $S_2$ differentiates into a T cell before being able to divide the outcome of $S_0$ division appears as asymmetric when looking at discrete time frames. We define this event as an ‘asymmetric outcome’.

Thus, following stem cell lineages at discrete time frames, as performed in the experiments cited above, can lead to incorrectly interpret as an intrinsically asymmetric stem cell division what is simply a symmetric division followed by differentiation. This issue can only be resolved through live-imaging analysis.

In the following paragraph, we explore quantitatively how the probability of asymmetric outcomes depends on the probabilities of cell division and differentiation.

We assume that cells behave stochastically and have a probability $P_{\text{div}}(t)$ to divide symmetrically after a time $t$ and a probability $P_{\text{diff}}(t)$ to partially differentiate into a transit-amplifying cell.

The probability that a stem cell differentiates before dividing again is

$$
\mu = \int_0^\infty \mathrm{d}t' P_{\text{div}}(t') \int_0^t \mathrm{d}t'' P_{\text{diff}}(t'').
$$

(2.1)

When a division occurs, its outcome appears symmetric (i.e. the two daughter cells divide again before differentiating) with probability $P_{\text{SS}} = (1 - \mu)^2$ and asymmetric with probability $P_{\text{ST}} = 2\mu(1 - \mu)$. There is also a probability of double differentiation $P_{\text{TT}} = \mu^2$. At homeostasis, there is a constant average number of stem cells so that $P_{\text{SS}} = P_{\text{TT}}$, leading to $\mu = 1/2$ and $P_{\text{SS}} = 25\%$. As the first example, we assume that all cells divide after a well-defined time $T$ so that $P_{\text{div}} = \delta(t - T)$ and that differentiation is a random Poisson process, with a probability to differentiate after a time $t$ after the previous division $P_{\text{diff}}(t) = k_d e^{-k_d t}$. The differentiation and division rates are then $k_d$ and $k = \ln(2)/T$, respectively, and one finds

$$
\mu = 1 - 2^{-k_d/k}.
$$

(2.2)

Thus, mostly asymmetric outcomes are expected at homeostasis ($k = k_d$) because $P_{\text{SS}} = 50\%$. This predicts a significant proportion ($P_{\text{SS}} = 25\%$) of symmetric outcomes at homeostasis, matching the value observed in several experiments [9,25]. Moreover, during development, the stem cell division rates can be as large as three times their homeostatic value. In this case, mostly symmetric outcomes are expected: $P_{\text{SS}} \approx 63\%$ and $P_{\text{ST}} \approx 33\%$. Double differentiations are here almost non-existent ($P_{\text{TT}} \approx 4\%$). This is in good agreement with the experiments of [9], where the Drosophila midgut displays at homeostasis ($k = k_d$) and during growth ($k \approx 3k_d$), respectively, $31\%$ and $68\%$ of symmetric outcomes. This enhancement of stem-cell production during development is even stronger if one also assumes a bell-shaped law for differentiation (see electronic supplementary material, figures S2 and S3). Figure 1 shows, for various choices of the differentiation probability $P_{\text{diff}}$ a numerical integration for the percentage of symmetric outcomes $(1 - \mu)^2$ as a function of the division rate $k$, $1/k_d$ being the mean differentiation rate.

Thus, even considering only purely symmetric divisions relative changes in the division ($k$) and differentiation ($k_d$) rates result in a changing ratio of symmetric/asymmetric outcomes. Early in development, division rates are larger than...
differentiation rates, which lead to a higher proportion of apparent symmetric divisions $P_{sym}$. Obviously, this does not exclude the existence of intrinsically asymmetric cell divisions.

So far, our arguments only consider stem cell divisions. We show in the next sections that considering the dynamics of other cell types yields several non-trivial phenomena, such as cell competition and stem cell number overshoot.

2.2. Stem cell kinetics and homeostasis regulation

In the simplest model of symmetric stem cell division, shown in figure 2, stem cells (S) can divide symmetrically at a rate $k$ into two stem cells or partially differentiate at a rate $k_d$ into one transit-amplifying (T) cell. T cells divide more rapidly at a rate $K$ into two T cells (typically stem cells divide every 1–2 day, T cells every 12 h [11]), or fully differentiate at a rate $K_d$ into an F cell. Fully differentiated cells do not divide and are lost at a rate $k_p$. We do not include here the possibility of dedifferentiation of T and F cells into stem cells following crypt damage [26,27], which will be examined in future work.

We call $S$, $T$ and $F$ the total numbers of $S$, $T$ and $F$ cells, respectively, in one crypt, $N = S + T + F$ the total number of cells and $A$ the available area. The cell concentrations are, respectively, $c_s = S/A$, $c_t = T/A$ and $c_f = F/A$. The total concentration of cells is $n = N/A$. Provided that division and differentiation are random processes, the dynamics of cell renewal are captured by the following kinetic equations:

$$\begin{align*}
\frac{dS}{dt} &= kS - k_d S, \\
\frac{dT}{dt} &= k_d S - (K_d - K) T, \\
\frac{dF}{dt} &= K_d T - k_p F.
\end{align*}$$

(2.3)

It can readily be seen from equation (2.3) that a feedback term on stem cell growth is crucial otherwise, the population of $S$ cells would almost always be unstable. Homeostasis can be achieved because cells react to the surrounding pressure and density of other cells, as shown by in vitro experiments [25]. Generically, we thus write the division rates $k(c_s, c_t, c_f)$ and $K(c_s, c_t, c_f)$ as decreasing functions of the various concentrations.

We also included Paneth cells in the model (see electronic supplementary material, S1), a modification that does not change the qualitative results at homeostasis, but significantly slows down the dynamics of the system, because Paneth cells are long lived (estimated life time of 30 days) [28]. We explore several possible hypothesis on Paneth cell regulation (feedbacks on stem cell division or differentiation rates), and show that they give rise to qualitatively different dynamics (see electronic supplementary material, figure S1), which could be compared to future experiments.

The kinetic equations lead to two competing homeostatic states, $S = 0$ or $k = k_d$. At the homeostatic state $k = k_d$, $S \neq 0$, all three types of cells are present. This fixed point only exists if the division rate of $T$ cells in these conditions is such that $K < K_d$. Otherwise, the dynamics of $T$ cells become explosive and the only homeostatic state is $S = 0$, with a homeostasis condition $K = K_d$. Stem cells are eliminated, and as $T$ cells can only undergo a finite number of divisions, the tissue would eventually die out, although this is not captured by these equations. We refer to the stem-cell-containing tissue as the reference homeostatic state.

The homeostatic state $S = 0$ is always stable, provided that the division rates decrease with increasing concentrations. We now discuss the stability of the reference homeostatic state $S \neq 0$.

The reference homeostatic state is a crypt of density $n_0 = N_0 / A$ and concentrations $c_{s0}$, $c_{t0}$ and $c_{f0}$. In this state, the division rates $k$ and $K$ are equal to their homeostatic values $k_h = k_d$ and $K_h$. In the vicinity of the reference homeostatic state, we expand the division rates around the homeostatic values of the concentrations $c_{s0}$, $c_{t0}$ and $c_{f0}$ at linear order.

$$\begin{align*}
k - k_h &= -\alpha_1 (c_s - c_{s0}) - \alpha_2 (c_t - c_{t0}) - \alpha_3 (c_f - c_{f0}) \\
K - K_h &= -\beta_1 (c_s - c_{s0}) - \beta_2 (c_t - c_{t0}) - \beta_3 (c_f - c_{f0}).
\end{align*}$$

(2.4)
An approximate analytical criterion for the stability of the homeostatic state can be obtained by considering that the timescale for the kinetics of S cells is slow compared with the renewal times of T and F cells ($k_s \ll K_s, K_f$). Expanding around the homeostatic value, $c_S = c_{S_0} + \delta c$, one gets the dynamical equation

$$\frac{d\delta c}{dt} = -\alpha c_S \delta c \left( 1 - \frac{\alpha_2 + \alpha_3 (K_f / K_s)}{\alpha_1} \right) \times \frac{c_{T_0} (S_0 + T_0 / C_0)}{c_{T_0} (S_0 + T_0 / C_0) + k_d (c_S / c_{S_0})} \right).$$

In the limit $\beta_3 / \alpha_1 (\alpha_2 + \alpha_3 (K_f / K_s)) - (\beta_2 + \beta_3 (K_f / K_s)) > 0$, the homeostatic state can become unstable. Keeping all other quantities constant, this happens if the concentration of stem cells $c_S$ is smaller than a critical value $c_{S_c}$ (see electronic supplementary material, figure S4). The threshold $c_{S_c}$ is either negative or increases monotonically with $c_{T_0}$.

This condition for the stability of the reference homeostatic state may shed light on the large number of stem cells in a crypt. As colorectal cancer originates from stem cells [2], one may think that minimizing their number, relative to T cells, would have been favoured by evolution. Our analysis indicates that too few stem cells might be detrimental and evolutionarily unfavoured because they are in competition with transit-amplifying T cells, which can cause the extinction of the stem cell pool in the crypt. For instance, a mutation causing the deletion of $\beta$-catenin would increase $k_d$, causing an increase in T cells relative to S cells. If the stem cell number falls below the stability threshold, this would cause an abrupt extinction of the stem cell population, in agreement with the observations of Fevr et al. [29].

So far, our arguments do not depend on the mechanism at work to regulate symmetric division and/or differentiation, which can depend on pressure, chemical gradients, contact inhibition, etc. We now wish to apply these equations first to crypt development. Crypts form through the invagination of the dividing cells. Knowing the pressure exerted by the tissue may think that minimizing their number, relative to T cells, is important, as it dictates the depth of the invagination in the crypt. Moreover, recent experiments on colon carcinoma cells in three dimensions have shown that exerting mechanical pressure on an aggregate lowers its division rate in a significant and predictable way [32]. Therefore, in the following section, we make the additional assumptions that pressure-based growth is sufficient to regulate the first stages of crypt development ($k - k_d \propto (I - I_k)$) and that the regulation of cell division rate is proportional to the fraction of the proliferative compartment which we label $\phi = c_S / c_T$.

Then, $k - k_d = -\alpha (\phi - \phi_0)$, where $\alpha = \alpha_1 = \alpha_2$. Measurements show that in intestinal crypts, $K$ is independent of crypt size and density [8], which translates in our model as $\beta_1 = \beta_2 = \beta_3 = 0$. At initial, low densities, we make the strong assumption that we can still use the expression of the pressure expanded around the homeostatic state i.e. $P = p(S + T) / A - p_{\phi_0} + I_{H_0}$ and $P_{H_0} = p_{\phi_0}$ knowing that $I = 0$ for $\phi = 0$. The kinetic equation for the stem cell number $S$ then reads $dS / dt = \alpha (\phi - (S + T) / A)$. If the area accessible to the cells were constant, $A = A_0$, the system of equations would be closed. Nevertheless, this misses the essential point that a crypt develops not only through an increase in cell density, but also through the formation of folded structures, which enable cells to increase the available area.

If the area $A$ is not fixed, another mechanical equation is needed. In general, the available area is a non-trivial function of the cell pressure $A = f (I)$. Here, we model a crypt as a cylinder, with an elongation driven by cell pressure and limited by the surrounding elastic membrane of effective modulus $E$. The area $A$ obeys $E (A - A_0 / A_0) = A$. As $I = p(S + T) / A$, one can express $A$ as a function of $S + T$. For large enough deformations ($A \gg A_0$), we use the approximate expression $A / A_0 \approx (p / E) (S + T) / 12$. We rewrite the kinetic equation for $S$ as

$$\frac{dS}{dt} = k_d A \left( 1 - \left( \frac{S + T}{S_0 + T_0} \right)^{1/2} \right),$$

where $A_1 = \alpha (\phi_0 / K_s)$ is the excess division rate at very low densities compared with the division rate at homeostasis and $S_0$ and $T_0$ are the number of stem cells of each type at homeostasis.

Our kinetic equations involve six parameters that all can be deduced from experimental measurements of Itzkovitz et al. [8]; the rates $k_s$, $K_s$, and $A_1$ are measured, at homeostasis $K_s = 1/23 h^{-1}$ (about one division per day). $K_s = 1/16 h^{-1}$, and we adjust $A_1$ such that the mean division rate for S cells in developing crypts is the observed value of 1/15 h$^{-1}$. Moreover, the three parameters $k_s$, $K_s$ and $S_0 + T_0$ are set by the experimental steady-state number of each type of cell: $S_0 = 10$, $T_0 = 25$. Although only the number $T_0 + F_0$ is provided in [8], it is known that T cells represent around half of this total [33], so we set $T_0 = 13$ and $F_0 = 12$.

The dynamical evolution of the cell numbers (figure 3b) fits well the experimental data, even though all the parameters are obtained from steady-state quantities or measured rates. This shows that the change in the symmetric stem cell division rate is sufficient to explain in a satisfactory way the observed curves and that there may be no need to invoke more complicated explanations, such as the presence of asymmetric divisions or varying differentiation rates. When the crypt is small, stem cells divide quickly, in agreement with [8], and many stem cells are created. Then, the density increases and differentiation takes over, filling the compartment with non-stem cells.

We show in the electronic supplementary material, figures S5 and S6 that different parameter choices yield very different curves, which would provide a strong test for our model. We also make quantitative predictions, which can be verified experimentally on the dynamic evolution of the division rate and crypt area (figure 3b,c).

Even without assuming pressure-based growth, it is still possible to discriminate between our model of regulated, symmetric stem cell growth and models involving transitions from symmetric to asymmetric divisions. In the following section, we show that for any type of symmetric division
rate regulation, the stem cell number can overshoot its homeostatic values for a wide range of parameters, which would not be expected otherwise. This behaviour has been observed in the development of neocortical neurons [34].

As the concentrations \( c_{S_1}, c_{T_1} \) are fixed at steady state, if the build-up of S cells is much faster than that of T cells, stem cell number overshoots at a concentration \( c_S = c_{S_0} + c_{T_0} \), before decreasing owing to the build-up of T cells.

### 2.3. Overshooting of stem cell numbers

We now assume, as a toy model, the presence of only S and T cells at a constant area. This assumption of constant area simplifies the problem: we can forget about the more restrictive hypothesis of pressure-based growth and go back to a generic dependency of growth on cell densities. We also do not restrain ourselves to linear approximations and use a more generic expression for the division rates \( k - k_d = -\alpha((c_S + c_T)^i - \phi_i) \), where \( i \) is a positive exponent. For \( i < 1 \), contact inhibition of growth is high even for low densities, while for \( i > 1 \), the regime of high growth rate persists until high densities. There are two relevant parameters that we call \( A_1 \) and \( A_2 \): \( A_1 = \alpha \phi_{i0}/k_d \), the excess division rate at low densities and \( A_2 = K_d - K/k_d \), the decay rate of T cells. The total number of cells at homeostasis \( N_h \) is maintained constant. The kinetic equations are thus

\[
\frac{dS}{dt} = k_d A_1 \left( 1 - \frac{(S + T)}{N_h} \right) S
\]

and

\[
\frac{dT}{dt} = k_d (S - A_2 T).
\]

If \( A_1 \) is larger than a critical value, \( A_{1c} \), the number of stem cells S overshoots transiently before reaching its steady-state value. The threshold is \((1 + iA_1 + A_2)/(1 + A_2)\sqrt{(A_2/A_1)(1/2i)} > 1\). We also performed numerical integrations of the equations starting from a single stem cell, to create the phase diagram of overshooting, shown in figure 4a, which agrees with this analytical criterion.

Moreover, the domain of overshooting gets larger when the exponent \( i \) increases (figure 4b), because S cells start feeling their neighbours only at higher densities.

In addition to this purely analytical criterion, we performed particle-based simulations of a tissue containing stem cells. The simulation technique is detailed in Basan et al. [35]. The area is fixed, and cells are seeded at low densities. The rules for symmetric division and differentiation are the same as before, and a cell is modelled as two elastic spheres, needing to push their surroundings to expand and divide. Thus, the sensitivity of cell division to the density is modulated by the adhesion–repulsion potential implemented in the simulation. For very cohesive tissues (similar to \( i \leq 1 \) in the analytics), we get non-overshooting crypt dynamics, which again reproduce qualitatively the experimental data (figure 4c,d). For weakly cohesive tissues (similar to \( i \gg 1 \)), stem cells grow at an exponential rate before feeling their neighbours only at very high densities, thus overshooting. This may be relevant to explain the overshooting of stem cell number in neocortical neurons, as neural tissues are known to be both less cohesive and less rigid. For each graph, we performed 5000 simulations and averaged the results.

### 2.4. Determinants of cancer initiation

Until now, we have studied how a crypt reaches homeostasis, and how homeostasis is maintained. During cancer initiation, mutations occur [2], which change the kinetic rates of interconversion or could change the homeostatic densities. Nevertheless, the adenoma still retains to a certain degree...
the same organization and dynamics of renewal as the normal tissue [21]. We thus examine how sensitive the homeostatic state is to changes in various relevant parameters, again not assuming a specific form of homeostatic regulation. The concentration of fast-dividing cells $f$ is an observable of particular interest because it relates experimentally to the aggressiveness of the adenoma [36]. Moreover, measurements of cell dynamics in intestinal tumours have suggested that tumour initiation comes from an expansion in the size of the stem cell niche [21]. An early sign of colorectal cancer is the presence of adenomous crypt foci [37]: crypts appear enlarged, and then form convoluted structures instead of flat tubes. In our framework, such crypt expansion and buckling would be caused by an increase in pressure, leading to a negative surface tension (figure 5) [30,38]. Therefore, as the tube destabilizes to undulated shapes, the area available to cells, and thus the cell number, increases sharply, which means that additional stochastic mutations could occur more often.

We therefore examine the change in the concentration of fast-dividing cells $f$ as a function of the kinetic parameters of interconversion around the homeostatic state. In the following, the value of these parameters for a normal crypt is indexed in the absence of mutations with the subscript $h$ and after mutation with the subscript $m$ (for instance, the fraction of proliferative cells after mutation $f^m$). This fraction $f^m$ can be expressed from equations (2.3)
and (2.4) at steady state:

\[
\phi_m = \phi_h \frac{G_m}{G_m} + \frac{k_d - k_{dm}}{G_m} \tag{2.9}
\]

We define here the parameter \( G \), which contains all the kinetic parameters of renewal. For the sake of simplicity, we assume that cell division depends solely on the global density \( n = c_5 + c_r + c_T \), stressing that it is only an assumption made in this section. We define \( \alpha = \alpha_1 = \alpha_2 = \alpha_3 \) the coefficient of the linear expansion. Then, \( G = \alpha(1 + (K_d/k_d)/(1 + ((K_d - K)/k_d)) \).

In the absence of mutations, we choose the kinetic rates to match observed division rates and proportions of cells in human colonic crypts [18]: \( K_h = 2k_d, K_{dh} = 3k_d, k_{dh} = 0.3k_d \). We define \( \phi_h = 1 \) as the unit concentration, and \( 1/\alpha = 1 \) s as the unit time. Our aim is to understand the nature of the
mutations or the parameters of the model that can drive a crypt towards an invasive phenotype, which in our description corresponds to a larger value of the concentration of the proliferative compartment $\phi$.

We first discuss mutations of non-stem cells, with $k_d = k_b$ (figure 5a–d). Quite surprisingly, an increase in the loss rate $k_b$ of fully differentiated F cells (for example, through apoptosis), or a decrease in the division rate $K$ of T cells, increase the fraction of dividing cells, as seen from equation (2.9). The importance of the apoptotic rate in carcinogenesis is increasingly being recognized: apoptotic rates seem to increase for colorectal cancerous epithelium, although this remains a matter of debate [39,40]. In our model, an increase in the loss rate $k_b$ of F cells favours hyperplasia. It decreases the concentration of F cells that are then replaced by S and T cells, therefore increasing the malignant potential of the tumour. This is fully in line with the idea of cell competition as a precursor to cancer [41]: the first step of carcinogenesis occurs at constant cell number, while fast-dividing cells are replacing regular cells.

Alternatively, a mutation that increases the division rate $K$ of T cells decreases the concentration of dividing cells. This stems directly from the homeostatic regulation of the crypt, increasing $K$ increases the number of T cells but decreases even more dramatically the number of stem cells. It therefore cannot be a cause of overproliferation. A change in the differentiation rate of T cells $K_d$ has a more subtle effect. If $K_d > K$, increasing $K_d$ decreases $\phi$, if $K_d < K$, the opposite occurs. This is again a non-trivial effect stemming from the competition for space between S and T cells.

We now study the effect of a mutation on stem cells (figure 5e,f). As expected, decreasing the differentiation rate of stem cells $K_d$ also increases $\phi$. If we now assume that T cell division depends slightly on the density $K = K_0 - \beta (\phi - \phi_0)$, this also changes the equilibrium of T cells so that their division rate is reduced (the stem cells are imposing a higher equilibrium density). This echoes another surprising observation of colonic cancer: the first stage of carcinogenesis is characterized by a decreased division rate [40], although the proliferative compartment is larger.

### 2.5. Model of compartment formation

In the preceding sections, we have made the important assumption that the cell concentrations are spatially uniform. Nevertheless, in many tissues, stem cells are compartmentalized within a niche. The differentiated cells produced in this niche flow out and undergo extrusion in a different region. We therefore need to refine our model to take the spatial variation of concentrations of each of the cell type into account.

The model of pressure-controlled growth predicts in a natural manner the presence of cell compartments: there is a pressure gradient from regions that are dense in S and T cells, as these cells would divide and create high pressure, to regions dense in F cells. This creates a flow, advecting cells out of the crypts, driven by the cell pressure itself. Moreover, as we wish again to remain in the same framework as before this model introduces only one new parameter, the friction between the cells and the substrate during cell migration out of the niche.

We write a local version of the previous kinetic equations for the concentrations $c_S(x, t)$, $c_T(x, t)$ and $c_F(x, t)$ that depend on the position $x$ from the bottom of the crypt. A crucial feature of crypts is the presence of a gradient of Wnt/β-catenin signalling, which restricts stem cell fate at the bottom of the crypt. This can be modelled simply by considering that the differentiation rate of stem cells $k_d(x)$ is an increasing function of $x$.

We use here the hydrodynamic description of tissues of Ranft et al. [42] in which, on timescales larger than the division rate, the epithelial monolayer can be considered as a fluid of viscosity $\eta$. The cell velocity $v$ satisfies then a Stokes equation

$$0 = \eta \Delta v - g v - \nabla P, \quad (2.10)$$

where $P$ is the pressure driving the cell flow. As in the previous section, we write the equation of state of the tissue as $P = c_S + c_T + c_F$. We have included in the Stokes equation a friction force between the cells and the basement membrane with a friction coefficient per unit area $\xi$. As discussed in the electronic supplementary material, S5, the dissipation is dominated by friction and we ignore the viscous stress in the following.

The Stokes equation must be solved together with a local version of the conservation equations for the three concentrations equation (2.3) that includes the convective fluxes owing to the cell flow, i.e. where the time derivative $\partial_t c_i$ is replaced by $\partial_t c_i + \nabla (v c_i)$. Note that this neglects cell diffusion.

The differentiation rate can depend on the chemical gradient, mechanical stress and local curvature. A precise discussion goes beyond the scope of this work. We only suppose here that it increases with the distance from the bottom of the crypt. For simplicity, we use a linear variation $k_d(x) = k_0 x / \lambda$, although the real profile is likely to be more complex. Nevertheless, the results of compartment formation described below do not depend qualitatively on the shape of this profile.

Figure 6 gives the three concentration profiles at steady state obtained by numerical integration for decreasing values of the friction constant $\xi$. If the friction $\xi$ is very large (figure 6a), the flow is negligible and no distinct compartment can be seen for T and F cells, as equation (2.3) imposes that their concentrations are proportional. We set $\lambda = 1$, which defines the length scale of the crypt. As the friction is lowered in figure 6b,c, the amplitude of the flow increases and cells are advected by the flow. This causes the formation of distinct compartments of S, T and F cells, with concentration profiles similar to those measured experimentally in [8].

Moreover, the pressure difference that drives the flow can be estimated, using the speed of cell flow measured in vivo, through the relation $\Delta P = 2 \xi v L / h$. In this relationship, we neglected the viscosity and assumed a colonic crypt of depth $L \approx 200 \mu m$, a cell thickness $h = 10 \mu m$, a cell flow out of the crypt with a characteristic speed of $v \approx 10 \mu m \ times^{-1}$ and a typical friction $\xi \approx 10^{10} \ Pa \ m s^{-1}$ [43]. Then, equation (2.10) yields a pressure difference: $\Delta P = 2 \xi v L / h \approx 500 Pa$. Of course, one could also inverse the present relationship to predict the migration rate one would get from a measured pressure gradient.

### 3. Discussion

We have presented in this paper a generic model for homeostasis in stem-cell-containing tissues. The key ingredient of the model is the dependence of the stem cell division rate on both mechanical and chemical cues. This provides a
natural mechanism for reaching a steady homeostatic state where the division rate is equal to the differentiation rate. This mechanical coupling obviously does not rule out other modes of regulation, for example the variation of the differentiation rates. Nevertheless, it is known for example that division rates do vary throughout development, and our modelling is generic enough that its validity domain goes beyond the strict framework described here.

On more general grounds, mechanical pressure is a global property of the tissue, transmitted over large distances, and can act as an elegant and extremely generic regulator not only of growth rates but also of organ size and composition of the tissue. We show that with this hypothesis, we can quantitatively account for many experimental observations. For example, our predictions are in agreement with the observed mechanical regulation of growth in simple in vitro tissues of MDCK cells [14,25]. Moreover, supposing that stem and transit-amplifying cells exert a higher pressure, through division, than fully differentiated cells, we explain within the same framework the formation of compartments of distinct cell types, and the cell flow from the crypts to the villi in the intestine.

One of the main outputs of our model, which does not depend on the pressure hypothesis, concerns the competition between different cell types in interconversion, which shows a very rich variety of behaviours. A very generic effect of the competition between cell types is a brutal breaking of homeostasis, which can occur if a genetic mutation causes the loss of stability of the homeostatic fixed point. We also obtained non-trivial predictions about the factors that drive a colonic crypt towards a cancerous, invasive phenotype. These predictions can be tested through in vitro experiments on artificial crypts. Novel phenomena are also predicted, such as the overshooting of the stem cell population if division rates of S cells are increased, or the loss rates of T and F cells decreased.

These examples highlight the important differences between subconfluent epithelia that have not reached homeostasis and confluent epithelia at homeostasis. For intestinal crypts at homeostasis, an increase in the division rate of T cells increases the size of the tissue, because their division is not in competition with the division of stem cells. Moreover, observations on the retina [44] show that tissues respond to a wound through three mechanisms: faster stem cell division, faster T cell division and also larger number of T cell generations, i.e. divisions before final differentiation. We show that these characteristics are also explained by our model. As observed during development, a higher division rate of T cells, while the differentiation rate remains constant, increases transiently the number of T cell generations before final differentiation.

Finally, we also show that the convoluted shapes of a cancerous crypt are a natural result of the buckling instability of cells growing in a constrained environment. Nevertheless, if cancer cells are able to degrade the surrounding stroma, this mechanical constraint no longer exists and one expects exponential, unbound growth of the intestinal tissue, as observed in the transition from benign colorectal adenoma to carcinomas [37].

Recently, it was discovered that intestine-like organoids can be cultivated in vitro from single intestinal stem cells [45]. These spherical organoids have the same crypt architecture, with minimal physiological environment, suggesting that the organization of the crypt structure can be understood from purely mechanical principles. Spherical organoids allow for controlled experiments on which one could test these theories, following cells in real time, and exerting pressure on them, similarly to [32], in order to test the effect on growth and stem cell dynamics.

In this paper, we mainly restricted ourselves to linear expansions of the pressure and division rates, in the vicinity of the homeostatic state. This approximation allows us to give simple analytical results describing qualitatively the growing tissue. More work is clearly needed to address and measure the functional dependence of the division rates on pressure and on the cell concentrations to obtain the equation of state of the tissue as well as to characterize the rheological properties of tissues.

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