A multiscale model of intestinal crypts dynamics

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Abstract. Intestinal crypts are multicellular structures the properties of which have been partially characterized, both in the “normal” and in the transformed development, i.e. under specific mutations or pathway alterations eventually leading to the appearance of colorectal cancer. Only in the last years there has been an increasing interest in using mathematical and computational models to achieve new insights from a “systems point-of-view”. However, the overall picture lacks of a general model covering all the key distinct processes and phenomena involved in the activity of the crypt and, hence, a holistic and system-based picture of the overall dynamics is still missing. In this paper we propose a new multiscale model of crypt dynamics combining Gene Regulatory Networks at the intra-cellular level with a morphological model comprising spatial patterning, cell migration and crypt homeostasis at the inter-cellular level. The intra-cellular model is a Noisy Random Boolean Network ruling cell growth, division rate and lineage commitment in terms of emergent properties. The inter-cellular spatial dynamics is an extension of the Cellular Potts Model, a statistical mechanics model in which cells are represented as lattice sites in a 2D cellular automaton successfully used to model homeostasis in the crypts.

1 Introduction

The idea that complex biological systems can be described and analyzed by means of mathematical and computational models has increasingly gained appeal and regard in the scientific community, at least after that the foundations of (computational) systems biology were set about one decade ago [41,42]. Since then, researchers directed their efforts in developing effective and powerful tools to explore and depict the complex interplay occurring at various levels in biological systems, hence giving rise to the so-called “-omics” sciences [67,47,12,33]. At the center of the attention is the in-depth investigation of the many relations between the structural and the dynamical features of specific biological systems. As a consequence, one of the key concepts turns out to be that of network [5].
A relatively recent declination of this approach, termed complex systems biology [35], consists in using mathematical concepts and methods provided by the science of complex systems to study (dynamical) biological systems, focusing on the description of the so-called generic (or universal) properties rather than on the characterization of specific ones.

Within this wide research area we focus on the modeling of multicellular systems and biological tissues, with particular regard to the intestinal crypts. These multicellular structures are of great interest, mostly because some of their key structural and dynamical features have been quite well characterized, both in the “normal” and in the transformed development. In the latter some specific mutations or pathway alterations eventually lead to the appearance of colorectal cancer (CRC), one of the current major causes of deaths in adults [32]. A description of the overall biology of the intestinal crypts is provided in Sect. 2. Despite numerous biochemical and clinical studies in CRC accomplished outstanding successes in the description of both general phenomena and specific processes involved in the crypt activity, only in the last years there has been an increasing number of attempts to use mathematical and computational models to achieve new insights from a “systems point-of-view”.

In this regard, in [11] we reviewed the existing computational models designed for the description of the morphology and the morphogenesis of the intestinal crypts, with a particular focus on the development of the CRC. In brief, the review highlighted the need of a general model covering all the key distinct processes and phenomena involved in the activity of the crypt and, hence, a holistic and system-based picture of the overall dynamics is still missing.

To try to fill this gap, we adopted a multiscale modeling methodology to describe the crypts. Multiscale modeling is intended to deal with the separation of the spatial and temporal scales entailed in the distinct cellular and intercellular processes, at different abstraction levels. Thus, the notion of hierarchy becomes fundamental to describe all the levels of the crypt organization, from the intracellular (e.g. gene regulation, intra-cellular communication), to the inter-cellular (e.g. signaling pathways, inter-cellular communication, cell-environment communication) and the tissue level (e.g. spatial patterning, movement and migration, crypt homeostasis). This separation is useful and it allows for the partitioning of the model implementation on parallel or distributed computer architectures (see, for instance, the Mapper project [49] and MML proposal [16]).

Setting on these premises, the aim of this paper is to propose a new multiscale computational model integrating a general model of gene regulatory network (GRN) with a spatial/morphological model of crypt dynamics. The model should be capable of covering a broad range of experimentally observed phenomena, both from the qualitative and the quantitative point of view. Up to now our contribution, which is developed within the RETRONET project (see Acknowledgments), is the model construction. Its implementation and the subsequent analyses are the next expected steps for the same project.

In brief, Noisy Random Boolean Networks (NRBNs) [53,61] are used to model the internal dynamics of the cells in the crypt, with a special focus on the most
relevant features of the differentiation process, as proposed in [68]. In particular, some key cellular processes such as cell growth, division rate and lineage commitment will be ruled by the emerging dynamical behaviour of ensembles of NRBNs. Since NRBNs are stochastic, a generative approach is required to search for those NRBNs which exhibit the desired (i.e. biologically well-founded) emergent properties. In fact, the only structural assumption we require on these networks is that they are designed on the basis of the known available data on GRNs (e.g. [45,23,57,17,7,48,60,26]).

The high-level spatial dynamics of the crypt is instead modeled by an extension of the Cellular Potts Model (CPM) [20,21]. CPM is a statistical mechanics model in which cells are represented as lattice sites in a 2D cellular automaton. The changes in cell shapes are induced by the stochastic motion ruled by changes in the overall system energy. In [70] CPM was successfully used to model homeostasis in the crypts.

By combining low and high-level dynamics we expect that our model permits a better understanding of key dynamical phenomena such as cell sorting, migration, niche maintenance and general homeostasis. Furthermore, we think that the model should permit to investigate the repercussion of different kinds of gene-level perturbations on the overall dynamical behaviour, with a obvious reference to the emergence of cancer.

In Section 2 a brief overview of the biology of the crypts is presented. In Section 3 the internal model of gene regulatory network is described, while in Section 4 the spatial/morphological model of intestinal crypt is depicted. In Section 5 the criteria according to which the different levels of the model are connected are introduced. Finally, Section 6 contains some comments on the current developments of the model.

2 The biology of the intestine

In this section we describe the most important features of the gut biology which are considered in our model, i.e. spatial morphology, cell lineage and cell turnover [2,8,52]. For a more detailed description of the main biological phenomena involved in the crypt, e.g. the signaling pathways and the development of the colorectal cancer we refer the reader to [11] and to the further references therein.

Roughly, the main tasks of the human intestine are essentially classifiable in (a) food digestion and (b) the absorption of the nutrients, while several other minor processes are linked to the general homeostasis of the system and to the immune system mechanisms. In particular, the distinct compartments of the intestine are composed by muscular, stromal and cuboidal epithelial cell. The lining of the small intestine is composed by a single-layer epithelium that covers the villi and the crypt of Lieberkhun, i.e. invagination in the connective tissue, which are the object of our model. Notice that in the large intestine there are not villi, but only crypts.

Molecular biology techniques permitted to identify 5 principal types of cells in the crypts [2], which are the following:
- Stem cells, which give rise to multi-lineage progenies that undergo different post-mitotic differentiation events [54,15];
- Enterocytes, which accomplish both absorptive and digestive tasks;
- Goblet cells, which produce the mucus fundamental for preventing the cells from being digested;
- Paneth cells, which perform several defensive tasks;
- Enteroendocrine, which are entailed in different processes and key signaling pathways.

One of the most distinctive features of the intestine morphology is that cell populations are segregated and sorted in well-defined compartments and the (fast) turnover process is accomplished through a very complex coordinate migration process. Understanding the basic mechanisms and rules at the base of this complex phenomenon is our major goal.

In particular, stem cells are positioned in the lower part of the crypt, within a specific niche, intermingled with Paneth cells, and the other types of cells reside in the upper portion of the crypt. The overall dynamics can be summarized as a coordinate upward migration of cells in transit amplifying stage, the intermediate state between stem and fully differentiated stages, moving from the stem cell niche toward the top of the crypt. These cells continue dividing and differentiating as long as they migrate, up to their final differentiated stage, which will be either enterocyte, Goblet or enteroendocrine, and they will be eventually shed into the lumen. Only Paneth cells move downward from the stem cell niche. It is important to remark that the positioning, proliferation, differentiation and migration processes are clearly fundamental for the maintenance of the general homeostasis and functionality and that the turnover process is very fast [50,59,56,18,8]. A deeply schematized representation of the lineage commitment tree, also referred to as the differentiation tree, is shown in Fig. 1.

We only mention the major importance of the many signaling pathways (e.g. Wnt, Notch and Eph/ephrins) throughout the epithelial cells and between the epithelium and the mesenchyme, mostly in relation with the regulation of various aspects of the cellular activity such as spatial patterning, proliferation in transit-amplifying compartments, commitment to specific lineages and apoptosis[59]. A deeply schematized representation of the crypt morphology and of the main pathways involved in its dynamics is shown in Figure 2.

3 Modeling the internal dynamics as NRBNs

The internal dynamics of each cell is modeled with a Noisy Random Boolean Network (NRBN) [53,61], a generalization of classical RBNs [38,37,39], a highly abstract and general model of gene regulatory network, which was proven to reproduce several biological properties of real networks [63,64,62]. As proposed by Villani et al. [68], NRBNs are particularly effective in describing some of the most relevant features of the differentiation process, in detail:
Fig. 1. Schematic representation of the crypt differentiation tree. Stem cells (St) are the root of the tree, while the 4 differentiated cell types (i.e. Paneth (Pa), Goblet (Go), enteroendocrine (Ee), absorptive or enterocyte (Ec)) are the leaves. TA stands for transit amplifying stage, which is known to be the intermediate state between stem and fully differentiated stages.

- the definition of a specific stem cell type which can generate any other type of cell (totipotent) or a subset of them (pluripotent) [2], usually through transit amplifying stages;
- the presence of a (recursive) stochastic differentiation process [31,10,29] according to which a population of identical stem cells generate different cell types, which in turn generate more differentiated cell types and the process repeats;
- the deterministic differentiation [69], according to which there exist specific signals triggering, through the mutation of specific genes, the development of a stem cell along a specific differentiation pathway.

The background about Noisy Random Boolean Networks is now recalled.

3.1 Background: Classical and Noisy RBNs

Classical Random Boolean Networks. Classical RBNs are directed graphs in which nodes represent genes and their Boolean value stands for the corresponding activation (i.e. production of a specific protein or RNA) or inactivation, while the edges symbolize the paths of regulation. A boolean updating function is associated to each node and the update occurs synchronously at discrete time step for each node of the network, according to the value of the inputs nodes at the previous time step. In the so called quenched model [39] both the topology and the boolean functions do not change in time. Since the network dynamics is dis-
Fig. 2. Representation of the crypt morphology, with specific regard to the migration directions and the signaling pathways involved in its dynamics. Four types of cells are represented: Paneth cells (yellow), stem cells (green), cells in transit amplifying stage (orange) and differentiated (Goblet, enteroendocrine and enterocyte) cells (light blue). All cells but stem and Paneth migrate upward. The three major signaling pathways involved in the crypt activity are the Wnt, the Notch and the Eph/ephrins pathways. The complex interplay among them, with reference to the repercussions on the various phenomena in the crypt, is schematized in this figure, taken from [11].

crete, synchronous and deterministic the only asymptotic states (i.e. attractors) of the system are cycles (and fixed points); some definitions follow.

Formally, a RBN is a network composed of a set of $n$ boolean nodes with associated variables $\sigma_i \in \{0, 1\}$ for $i = 1, 2, \ldots, n$ representing the boolean expression of a gene: if $\sigma_i = 1$ the $i$-th gene synthesizes its product (i.e. proteins or RNAs), otherwise it is inactive. Each node is connected to $k_i \in \{0, n - 1\}$ input nodes $\sigma_{j_1}, \sigma_{j_2}, \ldots, \sigma_{j_{k_i}}$. The values of the variables change at discrete time steps according to the deterministic time evolution of the RBN. The value of the node $\sigma_i$ at time $t + 1$, denoted $\sigma_i(t + 1)$, is determined according to a specific boolean function $f_i$ depending on the input nodes at time $t$, that is

$$\sigma_i(t + 1) \overset{\text{def}}{=} f_i(\sigma_{j_1}(t), \sigma_{j_2}(t), \ldots, \sigma_{j_{k_i}}(t)).$$

(1)

Here, $f_i$ is a boolean function with arity $k_i$ associated with $\sigma_i$. Summarizing, a RBN is fully determined by the set of $n$ boolean variables $\Sigma = \{\sigma_i \mid i = 1, \ldots, n\}$ and by the set of $n$ boolean functions $F = \{f_i : \{0, 1\}^{k_i} \to \{0, 1\} \mid i = 1, \ldots, n\}$. For any node $\sigma_i$ of the RBN the set $\{j_{i_1}, \ldots, j_{i_{k_i}}\}$ determines the topology of the network for that node, and consequently for the overall RBN. Describing the global state of the RBN at time $t$ with the vector

$$\sigma(t) = [\sigma_1(t), \ldots, \sigma_n(t)]$$
the evolution of the system is determined by the parallel update of all the elements at any discrete time step, that is
\[ \sigma(t+1) \overset{\text{def}}{=} [\sigma_1(t+1), \ldots, \sigma_n(t+1)] \]
where \( \sigma_i(t+1) \) is determined according to Equation (1). An execution of an RBN is a series of steps \( \sigma(0) \rightarrow \sigma(1) \rightarrow \ldots \) where \( \sigma(t) \) is the states of the network. Given that the RBN has a finite state-space (i.e. there exist at most \( 2^n \) vectors in \( \{0, 1\}^n \)) and the dynamics is fully deterministic, starting from any initial state \( \sigma(0) = [\sigma_1(0), \ldots, \sigma_n(0)] \) in at most \( z \leq 2^n \) steps the RBN will encounter an already visited state \( \sigma(z) \), entering a limit cycle. We term attractor of the RBN the loop starting from \( \sigma(z) \) and the sequence of steps from \( \sigma(0) \) to \( \sigma(z) \) the transient of the attractor, that is
\[
\sigma(0) \rightarrow \ldots \rightarrow \sigma(z-1) \rightarrow \sigma(z) \rightarrow \ldots \rightarrow \sigma(w) \rightarrow \ldots
\]
where \( \sigma(w) = \sigma(z) \) and \( \sigma(j) \neq \sigma(z) \) for \( j < z \). The length of the attractor is the number of steps required to perform a complete loop, i.e. \( w - z \). The set of initial condition that end up in a specific attractor \( \sigma(z) \) is its basin of attraction
\[
B(z) = \{ \sigma(0) \mid \sigma(z) \text{ is an attractor for } \sigma(0) \}.
\]
By varying the parameters of the network (i.e. topology, set of Boolean functions, etc.) it is possible to distinguish different dynamical regimes [3]. In the ordered regime small perturbations are usually absorbed by the system and, on the average, the number and the length of the attractors is small, while, in disordered RBNs perturbations spread through the system and both the number and the length of the attractors tend to be considerably larger (see [13] for a review on the topic). The RBNs in which the structural parameters are intermediate are defined as dynamically “critical” and present behaviours of particular interest, with specific regard to robustness and adaptiveness properties. Recent experimental results [62,4] seem to confirm the intriguing hypothesis that real genetic networks lay in the critical dynamical region, sometimes referred to as the “edge of chaos” [44].

Noisy RBNs. The rationale at the base of the development of the Noisy Random Boolean Networks model (NRBN) is that noise plays a major role in numerous cellular phenomena [51,66,9,55,46,14] and, above all, it is supposed to drive the differentiation process [43,30,27]. Classical RBN are fully deterministic and, hence, they do not properly take this aspect into account.

In the classical version of the RBN model cell types are associated to attractors, given that all the different cells of an organism share the same gene network and the differences in the cell types can be interpreted as different gene activation patterns. Nevertheless, attractors in RBNs are, in general, deeply sensitive to the introduction of noise, intended as perturbations of the value of one or more nodes. To this end, Ribeiro and Kauffman proposed a more sound approach to
connect attractors and cell types \cite{58} and, successively, Villani et al. developed a further refinement of the idea \cite{68}, which is described in the following.

Given a specific RBN, a temporary flip\footnote{When a flip in the $i$–th gene is performed the value $\sigma_i$ is complemented, the flip lasts 1 time step in the time-evolution of the network. This is indeed the smallest perturbation which can affect a boolean network.} is performed for each node in each state of each attractors, detecting all the possible transitions from one attractor to another one and, consequently, drawing the so-called attractor transition network (ATN). In this sense, the ATN resembles a stability matrix of the system if its entries are used to determine a probability of switching from one attractor to another. NRBNs rely on the assumption that the level of noise is sufficiently low to allow the system to reach its (new or old) attractor before another flip occurs. This assumption is endorsed by simulations showing that the number of steps to skip from one attractor to another one is usually small \cite{68}. In this way it is possible to make use of the deterministic definition of attractor, avoiding to use notion of loose attractors, which is generally used when dealing with stochastic dynamical systems \cite{24}. Notice that even though a temporary single flip is the smallest possible random fluctuation of this particular dynamical system, it represents a rather intense one, given that it abstracts from the superimposed activation or inactivation of a gene (therefore, much more intense than a molecular-scale fluctuation). Moreover, in \cite{61} it was also shown that the transition probability decreases sub-linearly with respect to the network size and, therefore, the overall number of transitions is an increasing function of $n$.

A threshold is then introduced to remove from the ATN those transitions that are considered too rare to occur, i.e. it is reasonable to think that some “jumps” are too rare to happen with a significant probability within the lifetime of the cell\footnote{Notice that in this case we do not account for multiple flips, which would be even more rare than single ones.} and, therefore, we consider threshold-dependent ATNs. Accordingly, a Threshold Ergodic Set (TES in brief or TES$_\delta$ when $\delta \in [0, 1]$ is the threshold) is a set of attractors in which the dynamics of the system continue to transit, in the long run, due to random flips (i.e. noise) or, using the graph theory terminology, a strongly connected component (SCC) in the threshold-dependent ATN.

Formally, let $\mathcal{A} = \{\alpha_1, \alpha_2, \ldots\}$ be the finite set of attractors of a NRBN. By performing for each attractor and gene a single flip\footnote{When a flip in the $i$–th gene is performed the value $\sigma_i$ is complemented, the flip lasts 1 time step in the time-evolution of the network. This is indeed the smallest perturbation which can affect a boolean network.}, and by observing which potentially attractor is reached, it is possible to define a weighed transition graph among attractors. By using the normalized frequency of such switches it is possible to determine a stochastic matrix $P_{\mathcal{A}} \subseteq [0, 1]^{|\mathcal{A}| \times |\mathcal{A}|}$ where $p_{i,j}$ determines the probability of switching from attractor $\alpha_i$ to attractor $\alpha_j$, and $\sum_{j=1}^{|\mathcal{A}|} p_{i,j} = 1$ for $i = 1, \ldots, |\mathcal{A}|$. Given a threshold $\delta$ an attractor $\alpha_j$ is $\delta$-reachable from another attractor $\alpha_i$ if $p_{i,j} \leq \delta$. Besides, $\alpha_j$ is $\delta$-reachable from $\alpha_i$ if there exist a path connecting $\alpha_i$ to $\alpha_j$ through transitions between pairs of $\delta$-reachable attractors. A set $\theta \subseteq \mathcal{A}$ is a TES$_\delta$ if (i) any $\alpha_i \in \theta$ is $\delta$-reachable from any other member of the TES. When the threshold is 0 a unique TES, which is indeed an Ergodic Sets in the sense of \cite{58}, is usually found. When the threshold
Fig. 3. An example of the threshold-dependent ATN and the corresponding tree-like TES landscape. The circle nodes are attractors of an example NRBN, the edges represent the relative frequency of transitions from one attractor to another one, after a 1 time step-flip of a random node in a random state of the attractor (performed an elevated number of times). In this case we show three different values of threshold, i.e.: $\delta = 0$, $\delta = 0.15$ and $\delta = 1$. TESs, i.e. strongly connected components in the threshold-dependent ATN are represented through dotted lines and the relative threshold is indicated in the subscripted index. In the right diagram it is shown the tree-like representation of the TES landscape.

is smoothly increased the TESs divide into smaller TESs, i.e. composed by less attractors, up to the point in which the TESs are indeed the attractors.

3.2 The model

*The biological metaphor.* Here we assume that each TES of a NRBN represents a specific cell type characterized by a peculiar noise resistance, as indicated by the relative threshold. The degree of differentiation (i.e. highly differentiated against less differentiated) relates to the possibility for the cell in its attractor to roam in a wider or smaller portion of the phase space (i.e. the size of the TESs which decreases as the threshold increases).

At the best of our knowledge, in fact, less differentiated cells (e.g. stem cells) show fewer control mechanisms against noise (e.g. copy errors) and, thus, we characterize them by a smaller threshold allowing them for roaming in a wider portion of the phase space. On the opposite, cells in a more differentiated state
present more refined control mechanisms and, consequently, are associated to higher thresholds which actually prevent random fluctuations [46]. We remark that the association of differentiation to biological noise has been experimentally validated [36,27,34] and the fact that the level of noise in undifferentiated cells is higher was shown in [28,25,19].

Parameters setting. In the construction of a suitable NRBNs to be used in the model, we impose some constraints based on the current biological knowledge of real genetic networks. We remark that, in line with the complex systems biology approach, it is not our objective to describe any specific GRN, also considered that the human GRN has not been completely deciphered and only some of its general features, as well as only some sub-portions of the network, have been exhaustively described. Instead, we want to statistically analyze ensembles of structurally analogous NRBNs, in order to investigate their emerging and generic dynamical properties, mostly in relation with the overall dynamical behaviour of the multi-scale model.

The main constraints we impose concern the topology and the choice of the updating boolean functions. In regard to the former, we design NRBNs with \( n \) genes and scale-free topology [5]. A scale-free NRBN is a network in which the degree distribution (i.e. the distribution of the values \( k_i \) in Section 3) follows a power-law. More precisely, the fraction of nodes in the network having \( k \) connections to other nodes follows \( c k^{-\gamma} \), for large values of \( k \), where \( c \) is a normalization constant and \( \gamma \approx 2.3 \div 2.5 \) is a parameter whose value relates to the structure of many biological networks, including GRNs [7].

On the other hand, we impose a constraint on the choice of the boolean functions, based on the biological plausibility of updating functions in GRN models [22]. In particular we impose that all the functions must be canalyzing. A function is canalyzing if at least the value of one of its inputs is sufficient to determine the output [40].

In regard to the remaining structural parameters we decided to concentrate on “critical” networks which, as briefly mentioned above, are supposed to be a good candidate for the description of real genetic networks.

Search of the suitable NRBNs. In our model we associate totipotent stem cells with TESs at threshold 0, cells in a pluripotent or multipotent state (i.e. transit amplifying stage or intermediate state) with TESs with a larger threshold composed by one or more attractors, while completely differentiate cells (i.e Paneth, Goblet, enteroendocrine and enterocyte) correspond to TESs with the highest threshold, usually composed by single attractors.

It should be clear at this point that an apriori choice of a specific NRBN does not guarantee the existence of the TES and thresholds corresponding to the desired differentiation tree (i.e. Figure 1). In addition, this would contradict our choice of not imposing specific detailed assumptions concerning the interaction which drive the modeled phenomena. As a consequence, we must perform a search among plausible NRBNs to match the outlined acceptability criteria. This is sometimes called model sweep and, despite being an undoubtedly costly
operation, this is done only once to instantiate our model. Once the suitable NRBNs are collected, they can be used to complete and analyze the whole model, as we shall see in the next sections.

Algorithmically, we operate as follows. We (a) generate a scale-free NRBN with canalizing boolean functions, which also are generated at random. Then, we (b) sample a possibly exhaustive number of initial conditions for the network. As in [61], such a search can be exhaustive only for small number of genes, e.g. \( n \approx 20 \) genes. We (c) find a subset of its attractors by flipping the genes\(^6\), i.e. a subset of \( \mathcal{A} \) which is dependent on the number of different sampled initial configurations, and we define the matrix \( P_A \) describing the stability of all the attractors. For a \( \delta \in [0,1] \) we define the prune of \( P_A \) to be the matrix \( P_{\geq \delta} \) whose elements \( p'_{i,j} \) are equal to the corresponding \( p_{i,j} \), the elements of \( P_A \), if \( p_{i,j} > \delta \), and \( p'_{i,j} = 0 \) otherwise. The prune completes once we re-normalize the matrix, since otherwise it does not represent a correct stochastic matrix, which is actually required in the next part of the model, as we shall see later. As expected, different TESs are determined by different values of \( \delta \) and \( P_{\geq 0} = P_A \) and \( P_{\geq 1} \) is the zero matrix. We denote with \( \Theta_{\geq \delta} \) the set of TESs for matrix \( P_{\geq \delta} \).

Last step (d) requires to finding a set of suitable thresholds with respect to the differentiation tree shown in Figure 1, i.e. a set of 4 thresholds \( \delta_0 < \delta_1 < \delta_2 < \delta_3 \) with \( \delta_0 = 0 \) since the tree has depth 3. This is done by accepting the NRBN only if the following conditions hold:

(i) the overall number of attractors, i.e. \( |\mathcal{A}| \), must be congruent with the number of differentiated types, i.e. 4 to resemble the considered cell types Goblet (Go), Paneth (Pa), enterocytes (Ee) and enteroendocrine (Ec);

(ii) there must exist a unique TES \( \theta_{ST} \) at threshold \( \delta_0 = 0 \), i.e.

\[
\Theta_{\geq 0} = \{ \theta_{ST} \}
\]

comprising all the different attractors, representing the stem cell type (St);

(iii) with threshold \( \delta_1 \) the matrix \( P_{\geq \delta_1} \) must induce two TESs \( \theta_{PA} \) and \( \theta_{TA1} \), i.e.

\[
\Theta_{\geq \delta_1} = \{ \theta_{PA}, \theta_{TA1} \}
\]

which are associated to the Paneth and TA1 types in the tree. The fact that Paneth cells are already differentiated immediately imposes the further constraint that the TES \( \theta_{PA} \) is robust (i.e. it is not split in smaller TESs) when the next thresholds are considered, i.e. \( \theta_{PA} \in \Theta_{\geq \delta_2} \cap \Theta_{\geq \delta_3} \) to resemble that such cells are already fully differentiated;

(iv) with threshold \( \delta_2 \) the matrix \( P_{\geq \delta_2} \) must induce two new TESs \( \theta_{TA2-A} \) and \( \theta_{TA2-B} \) beyond \( \theta_{PA} \), i.e.

\[
\Theta_{\geq \delta_2} = \{ \theta_{PA}, \theta_{TA2-A}, \theta_{TA2-B} \}
\]

\(^5\) We use the “preferential attachment” algorithm [6] to generate the NRBN topology.

\(^6\) To draw the exact ATN all the possible flips of all the nodes in all the states of all the attractors should be performed [68]. Since even relatively small networks have huge state spaces, an exact sampling is practically unfeasible. Nonetheless, it is possible to draw the ATN up to a desired precision.
which are associated to the TA2-A and TA2-B types in the tree;

\( (v) \) with threshold \( \delta_3 \) the matrix \( P_{\geq \delta_i} \) must induce two new TESs \( \theta_{\text{Go}} \) and \( \theta_{\text{Ec}} \) beyond \( \theta_{\text{Pa}} \) and \( \theta_{\text{TA2-B}} \). Notice that the fact that absorptive (or enterocyte) cells are the unique differentiated type after TA2-B (i.e. deterministic differentiation) means that the TES \( \theta_{\text{TA2-B}} \) is required to be a TES for \( \delta_3 \). However, we impose that (at least one of) the entries in \( P_{\geq \delta_i} \) used to determine such a TES differ from those in \( P_{\geq \delta_2} \). This yields that the TES \( \theta_{\text{TA2-B}} \) at this level is an equivalent TES \( \theta_{\text{Ec}} = \theta_{\text{TA2-B}} \), with different probabilities. Although this might look cumbersome, this is actually necessary to resemble that the two cells have different robustness with respect to noise (i.e. TA2-B is less robust than absorptive), in fact even though the TES in which they move is the same, with this further constraint we have have that they jump within the TES with different probabilities. We have than

\[
\Theta_{\geq \delta_3} = \{ \theta_{\text{Pa}}, \theta_{\text{Go}}, \theta_{\text{Ec}}, \theta_{\text{Ec}} \}.
\]

Notice that this approach can be mechanized to a generic differentiation tree and then can be used for further refinement of this or other models. At the end of this process the TES landscape and all the distinct threshold-dependent ATNs are defined. Once an NRBN with these peculiar features has been determined, this internal model can be used to rule several key cellular processes involved in cell division and differentiation.

The NRBN dynamics. As far as the NRBN dynamics is concerned, when the simulation starts a random attractor of the specific TES is assigned to every cell, according to its type. We remark that all the cells are characterized by a structurally identical NRBN (i.e. same genome), even if their state can belong to different portions of the state space. While time advances, if no NRBN-level perturbations are performed the cell potentially resides in the same attractor, unless higher-level events which shall be discussed in the next sections happen. At this level noise is accounted as the probability \( p \) that each node has to perform a transient flip at any time step of the dynamics. We remark that this (stochastic) phenomenon is essential mostly in regard to the division and differentiation processes, which will be described in the next sections.

The advantage of having drawn all the pre-computation about the NRBN comes into play at this stage. In fact, instead of running the step-by-step NRBN dynamics we make use of the already mapped TES landscape and of the relative frequency of transition among the attractors. In this way NRBNs can jump from one attractor to another one within their own TES, according to the peculiar level of noise, in the course of the simulation time. In this sense, if a cell is at level \( \delta_i \), each time a perturbation is performed it jumps according to the matrix \( P_{\geq \delta_i} \), which is interpreted as a Discrete-Time Markov Chain.

4 Modeling the spatial/morphological dynamics

As before, we introduce basic background, and then we discuss our model.
4.1 Background: the Cellular Pott’s Model

The original Cellular Pott’s Model (CPM) \([20,21]\), a more complex version of the 2-spin Ising model, considers a population of \(k\) cells of a unique type disposed over a 2-D lattice of positions \(L \in \{1, \ldots, k\}^{n \times m}\), i.e. a cellular automaton, where \(l_{i,j} = w\) if the lattice position \((i, j)\) is occupied by cell \(w\). Cells are delimited by connected domains, namely cell \(w\) is determined by the positions \(C(w) \equiv \{l_{i,j} = w \mid (i, j) \in L\}\).

In CPM cells are expected to rearrange (i.e. via cell sorting) and change shape according to an energy gradient. Potts assigned to each lattice configuration \(L\) a hamiltonian energy \(H : \mathbb{R}^{n \times m} \rightarrow \mathbb{R}\) denoted \(H(L)\). In practice, we arbitrarily define \(H\) as far as we can describe a process in terms of a real or effective potential energy. So, for instance, the original CPM hamiltonian included adhesion energies,

\[
H(L) \equiv J \sum_{(i,j) \in L} \left( \sum_{(i',j') \in N(i,j)} 1 - \delta(l_{i,j}, l_{i',j'}) \right)
\]

where \(N(i,j)\) denotes the neighborhood of position \((i, j)\) according to some distance metrics (e.g. Von Neumann neighborhood) and \(J\) is the spin-spin coupling energy constant. Notice that the use of the delta function (i.e. Kronecker symbol) ensures that only the surface sites between different cells contribute to the adhesion energy. Pott’s model describes the time-evolution of a lattice through a series of flips. Given a lattice \(L\), we define the flip of a position \((x, y)\) to a cell \(w\) to be the new lattice \(L'\), that is

\[
L' \equiv L[w \leftarrow (x, y)] \quad \text{where} \quad l'_{i,j} = \begin{cases} w & \text{if} \ (i, j) = (x, y) \\ l_{i,j} & \text{otherwise} \end{cases}
\]

where \(l'_{i,j}\) and \(l_{i,j}\) are the same position in \(L'\) and \(L\), respectively.

The simulation of the CPM works according to a Metropolis-like algorithm. In the following, we denote with \(L(t)\) the lattice at time \(t\). Given the \(n \times m\) lattice \(L(t)\), at each step a position \((i, j)\) is chosen with uniform probability, i.e. \(i \sim U[1, n]\) and \(j \sim U[1, m]\). Moreover, a neighbour \(w \in N(i, j)\), uniformly distributed on the set \(N(i, j)\) is chosen and is used as a candidate flip. The algorithm probabilistically accepts or rejects the flip, i.e. \(L'(t) \equiv L[w \leftarrow (i, j)]\), according to the hamiltonian energy evaluated in both lattices. Formally, \(L'(t)\) is accepted or rejected according to the probability distribution \(P(L'(t))\) where

\[
P(L'(t)) \equiv \min \left\{ 1, \exp \left( -\frac{\Delta H}{k_B T} \right) \right\}
\]

for \(k_B T > 0\), and

\[
P(L'(t)) \equiv \begin{cases} 0 & \text{if} \ \Delta H > 0 \\ \frac{1}{2} & \text{if} \ \Delta H = 0 \\ 1 & \text{if} \ \Delta H < 0 \end{cases}
\]

(5)
Fig. 4. Example CPM simulation of cell sorting using CompuCell3D [1], originally appearing in [11]. The crypt is represented on a 50 × 100 lattice with periodic boundary condition on the x-axis, the temperature is set to 30 degrees and the neighborhood order is 5. Cells are square-shaped and initially composed by 25 lattice sites. As in [70] there are 7 cell types, Paneth cells (yellow, 30 cells in the initial conditions), stem cells (green, 30 cells), cells in transit amplifying stage 1 (TA1, light orange, 30 cells), TA2 (orange, 30 cells), TA3 (dark orange, 20 cells), TA4 (red, 30 cells) and differentiated cells (blue, 30 cells). The contact energy is set as in [70], and no mitosis or differentiation processes are simulated. Time is measured in MonteCarlo steps (MCS).

for \( k_B T = 0 \). These equations account for the change of energy induced by the flip, the temperature \( T \) and the Boltzmann constant \( k_B \).

Intuitively, the goal is to minimize the energy of the lattice by re-ordering the cells. A computation starting at time \( t_s \) and ending at time \( t_e \) performs \( t_e - t_s \) MonteCarlo steps (MCSs) each one attempting \( nmk \) random flips, where \( k \in \mathbb{I} \) is an arbitrary constant. Once all the attempts of flips are finished, the new lattice \( L(t + 1) \) is determined as a result of all the accepted flips. A stochastic process \( \{L(t) \mid t = 0, 1, \ldots\} \) whose states are the lattice configurations, underlies the CPM. Formally, such process is a Discrete Time Markov Chain.

The extension of CPM to model intestinal crypts. Wong et al. [70] extended the original CPM model to a 2-D lattice with periodic boundary conditions, representing the (unrolled) surface of the crypt with a certain degree of approximation, i.e. the 3-D structure of the crypt is approximated with a open cylinder.

The Hamiltonian in Equation (3) is integrated with two terms regarding the differences in cell types and a specific target size, which we now discuss. When a finite set of cell types \( T \) is considered, a lattice site is naturally extended to \( l_{i,j} = (w, \tau) \) if the position is occupied by cell \( w \), and cell \( w \) has type \( \tau \in T \). The spin-spin coupling energy constant is extended to a function \( J : T \times T \to \mathbb{R} \) where \( J(\tau_1, \tau_2) \) denotes the energy bond required by cell of type \( \tau_1 \) to adhere one of type \( \tau_2 \). The energy constants are then defined by the symmetric matrix \( J \) given, for instance, in [70]. The entries of the matrix are related to the spatial
gradients of specific signals and receptors (i.e. Eph receptors and ephrin ligands) which are supposed to regulate the adhesion properties of the cell populations in the crypt (see [11] for a description of the process). The current area of a cell \( w \) in a lattice is defined as

\[
a(w) \equiv \sum_{c(w)} a = |C(w)|a
\]

where \( a \) is a basic quantity of area assigned to a single lattice position. We remark that this quantity dynamically evolves in the lattice according to the performed flips. Therefore, the above energy \( \mathcal{H}(L) \) reads as

\[
\mathcal{H}(L) \equiv \sum_{(i,j) \in L} \left( \sum_{(i',j') \in N(i,j)} J(\tau, \tau') (1 - \delta(w, w')) \right) + \lambda \sum_{\tau \in T} [a(w) - A(\tau, w)]^2 \text{Heav}(A(\tau, w))
\]

if \( l_{i,j} = (w, \tau) \) and \( l_{i',j'} = (w', \tau') \). Here, \( \lambda \) is the strength of size constraint which is proportional to the capacity to deform the cells membrane, \( J(\tau, \tau') \) is the surface energy between cells of type \( \tau \) and \( \tau' \), \( a(w) \) the current area of cell \( w \) and \( A(\tau, w) \) the target area for cells of type \( \tau \), i.e. a parameter representing the area of any newborn cell of type \( \tau \). Moreover, biological aggregates are also surrounded by a hosting fluid (ECM) which is usually marked with a special cell type which has unconstrained area. Therefore, the medium target area is set to be negative and the corresponding area constraint is suppressed by including the Heaviside function, i.e. \( \text{Heav}(A(\tau, w)) \).

This new equation for the hamiltonian energy accounts for the so-called differential adhesion hypothesis (DAH), according to which cell sorting could be due to cell motility combined with differences in intercellular adhesiveness [65]. Different studies ([11] and references therein) in fact, proved that differential adhesion suffices to drive cell sorting and patterning. In practice, when cells with different adhesion properties get in contact, cells with weaker binding properties tend to be displaced from those with higher adhesiveness. In addition, the second term, i.e. an elastic area term, imposes a constraint on the spatial evolution of cells which, according to their type, have a generally fixed range of sizes.

Cells perform their cell-cycle and, when they complete it, \( A(\tau, w) \) is instantaneously doubled and the area elastic constraint in Equation (6) causes the current area \( a(w) \) to adapt to the new target size (i.e. \( 2A(\tau, w) \)) quasi-istantaneously, accounting for cell growth. Subsequently, the cell divides by mitosis into two daughter cells, which are characterized by specific target areas, one of the two keeping the type of the progenitor, the other changing type according to the prefixed differentiation evolution. Finally, when a cell reaches the top of the lattice because of the mitotic pressure it is eventually expelled from the system, simulating the shedding into the lumen of fully differentiated cells.


\[
\begin{pmatrix}
J & \text{St} & \text{TA1} & \text{TA2-A} & \text{TA2-B} & \text{Pa} & \text{Go} & \text{Ec} & \text{Ee} \\
\text{St} & 2 & & & & & & & \\
\text{TA1} & 12 & 5 & & & & & & \\
\text{TA2-A} & 35 & 30 & 15 & & & & & \\
\text{TA2-B} & 35 & 30 & 15 & 15 & & & & \\
\text{Pa} & 8 & 20 & 40 & 40 & 2 & & & \\
\text{Go} & 45 & 40 & 30 & 30 & 50 & 5 & & \\
\text{Ec} & 45 & 40 & 30 & 30 & 50 & 5 & 5 & \\
\text{Ee} & 45 & 40 & 30 & 30 & 50 & 5 & 5 & 5 \\
\end{pmatrix}
\]

Fig. 5. The energy contact matrix $J$ which considered any combination of different cell types among those considered. The matrix is based on the work by [70] where possible, and is refined in order to comprehend the different cell types considered here.

4.2 The model

Our model is inspired by Wong’s extension of CPM to model crypts, in which we introduce minor modifications. In particular, we consider a slightly different set of cell types $T = \{\text{St, TA1, TA2-A, TA2-B, Pa, Go, Ec, Ee}\}$ which indeed corresponds to the differentiation tree presented in Figure 1. The energy constants are defined by the symmetric matrix given in Figure 5, which is inspired by the results of the work by Wong et al. [70], but is refined to account for the different cell types.

Besides, while in the original model the growth and division dynamics are prefixed and occur as described above, in the multi-scale model they are strictly interconnected with the low-level dynamics, i.e. thus driven by the NRBN, as it will be explained in the next section.

5 Linking NRBNs and CPM: the multi-scale model

We recall the idea at the basis of the model: the spatial features of each cell of the crypt are represented on a cellular automaton, and the changes in their size and position are ruled by the CPM, according to the energy relationships that depend on the specific position, size and type of the cells. Besides, each cell has its own gene regulatory network, which is modeled through a specific NRBN. We remark that, since all the cells of an organism share the same genome, all the cells in our model are characterized by structurally identical NRBN (i.e. identical in terms of topology and boolean functions associated to the nodes). In fact, the differences among cells lays in the fact of being in different positions and following different dynamical trajectories in the phase space of the NRBN.

In detail, three major processes of the cellular activity are ruled by the internal dynamics of the NRBNs, hence influencing the CPM spatial dynamics:

(i) the length of the cell cycle, which is tied to the weighted length of the attractors belonging to the corresponding TES, thus being an emergent property;
the growth rate of the size of the cell, which is assumed to linearly increase up to the doubling of the original size, so that when the size is doubled (at the end of the cycle) the cell divides and differentiates;

(iii) the lineage commitment tree, which depends on the structure of the landscape of the attractors and, consequently, of the TESs so that the differentiation bifurcations consequently depend on the position in which the dynamical trajectory of the system is when the cell divides (i.e. stochastic differentiation).

We remark that, in a non-multiscale model as the one by Wong .., the length of the cell cycle is a CPM parameter and cells grow instantaneously. By considering the internal dynamics we can, instead, define such values as emergent properties at the genetic-level. For the sake of clarity we present now such features.

**Cell cycle length.** In regard to the length of the cell cycle, for a cell of type $\tau_i$, whose NRBN threshold is $\delta_i$, we consider its biggest TES in $\Theta_{\geq \delta_i}$ and the stochastic matrix $P_{\geq \delta_i}$ restricted to the states and the transitions regarding only the attractors in the TES and, with abuse of notation, we still denote it as $P_{\geq \delta_i}$. As we said, $P_{\geq \delta_i}$ is a Discrete-Time Markov Chain (DTMC). By standard DTMC theory if it is possible to go from each state, in any number of steps, to every other state, then the chain is *ergodic* and, obviously, this is the case by the definition of TESs. This implies also the *irreducibility* of $P_{\geq \delta_i}$. It holds that the stationary probability distribution $\pi_i$ of an irreducible DTMC in an ergodic set of states is unique and is the fixed point of

$$\pi_i P_{\geq \delta_i} = \pi_i$$

where $P_{\geq \delta_i}$ is the stochastic matrix for the considered TES. For the cell of type $\tau_w$ we determine the length $\ell_w$ of its cell cycle as

$$\ell_w \overset{\text{def}}{=} \sum_{\alpha_j \in \Theta} \eta_j \pi_i(\alpha_j)$$

where $\eta_j$ is the length of attractor $\alpha_j$. Notice that we are “weighting” the length of the attractors by using the stationary distribution. The length of the cell cycle in CPM is then an emergent property of the NRBN dynamics. This requires the conversion between the time-scales of the internal and external models which, at the best of our knowledge, is a new result.

**Time-scales conversion.** We highlight that the difference between the time-scales of the NRBN steps with respect to the CPM steps is the key property of the multiscale model. Therefore, we link the time scale of the internal dynamics (i.e. the NRBN steps) with the time scale of the MonteCarlo simulation (i.e. the CPM steps). Along the line of Wong’s simulation experiments [70] we consider that:

(i) 10 MonteCarlo steps (MCSs) correspond to 1 hour of biological time;
(ii) the length of a cell cycle $\Delta t_{\text{cycle}}$ is in the range $12 ÷ 17$ hours, according to the different cell types, that is it takes in between $120$ and $170$ MCSs;
(iii) the natural unit for $\ell_w$ is one NRBN step of the internal dynamics.

So, we end up with the following conversion

$$1 \text{ RBN step} = \frac{\Delta t_{\text{cycle}}}{\ell_w} \text{ MCSs} = \frac{120 ÷ 170}{\ell_w} \text{ MCSs}. \quad (9)$$

Thus, as an example, if we assume that a single cell comprises around $40$ pixels (i.e. lattice sites) and that the cell cycle is $120$ MCSs, then to increment of a single site the target area will take at least $3$ MCSs.

**Cell size dynamics.** We recall that to each cell $w$ of type $\tau$ a standard area $A(\tau, w)$ is associated, representing the area of any newborn cell of that kind. In addition, we introduce a time-dependent target area $A(\tau, w, t)$ which is evolving according to the dynamics of the underlying NRBN. The target area represents the growing cell size during its cycle and as if it was \textit{mechanically isolated}, that is with no contact interaction with neighbor cells.

We now link the dynamical evolution of the target area $A(\tau, w, t)$ to the length of the cell cycle $\ell_w$ as defined in equation (8). We assume the doubling of the volume of a cell to happen after a complete cell cycle and we consider a linear growth for all the cycle. We have that, if $t_0$ is the time at which a cell starts its cycle, then

$$A(\tau, w, t) = \begin{cases} A(\tau, w), & \text{if } t = t_0 \\ A(\tau, w, t-1) + \mu \left[ \frac{A(\tau, w)}{\ell_w} \right]_{\text{int}} & \text{otherwise} \end{cases} \quad (10)$$

or, alternatively

$$A(\tau, w, t) = \begin{cases} A(\tau, w), & \text{if } t = t_0 \\ A(\tau, w) + \mu \left[ (t - t_0) \frac{A(\tau, w)}{\ell_w} \right]_{\text{int}} & \text{otherwise} \end{cases} \quad (11)$$

where $\mu = 0$ for non-dividing cells and $\mu = 1$ for proliferative cells. Notice that in (10) and (11) $[\cdot]_{\text{int}}$ denotes the nearest-integer function. This latter function comes from considering an integer number of incremental pixels in the cell area as a consequence of our lattice discretization. The Hamiltonian of Equation (6) in this case is defined by

$$\mathcal{H}(L) \overset{\text{def}}{=} \sum_{(i,j) \in L} \left( \sum_{(w',j') \in N(i,j)} J(\tau, \tau') (1 - \delta(w, w')) \right)$$

$$+ \lambda \sum_{\tau \in T} \left[ a(w) - A(\tau, w, t) \right]^2 \text{Heav}(A(\tau, w, t)) \quad (12)$$

if $l_{i,j} = (w, \tau)$ and $l_{i',j'} = (w', \tau')$. 
Cell division and differentiation dynamics. Finally, when one cell cycle is concluded (i.e. after $\ell_w$ RBN time steps) and the size of the cell has doubled its target size (i.e. $2A(\tau, w)$) we assume that the cell instantaneously divides in two on the lattice and differentiates.

For the two daughter cells the TES threshold is automatically increased to the subsequent level, which is chosen as indicated in Section 3, implying the hypothesized variation in the level of noise resistance and control [46]. The differentiation direction and the new TES to which the daughters will belong is chosen looking at the attractor in which the progenitor cell is found at the moment of division. Notice that NRBNs wander in the TES space according to the particular level of noise $p$ (i.e. the probability that a random node of the NRBN is flipped at any time step), as discussed in Section 3. In this regard, the process of stochastic differentiation is effectively depicted by the model, since the particular fate of a cell depends on the particular attractor within the TES in which the cell is found when division occurs.

We remark that, in order to preserve the existence of the stem cell niche, when stem cells divide only one of the two daughters differentiates, while the other one remains a stem cell.

6 Conclusions

The objective of this work was to present a novel model of multi-cellular system and, in particular, a new multi-scale model describing the dynamics of intestinal crypts. The main novelties of the model can be summarized as follows:

– First, the model describes in a original way phenomena and processes occurring at different spatial/temporal scales, i.e. the low-level mechanisms of gene regulation on the one hand, and the high-level phenomena regarding the crypt homeostasis and the spatial patterning of cells, on the other hand. Key cellular processes, e.g. cell growth and differentiation, are at the center of a continuous exchange of information among the levels, on the basis of the conversion of the different time scales shown in Section 5.

– Another important novelty resides on the attention casted on the concept of emerging dynamical behaviour. The dynamics of both the low- and the high-level models are strictly related to the emergence of particular gene activation patterns (i.e. attractors), which eventually determines the general activity and fate of the system. In analogous models, processes such as cell cycle or cell division are usually prefixed, depending on external parameters, and do not emerge from the dynamics.

– The comparison of statistical analyses of ensembles of NRBNs designed with biologically plausible structural parameters with experimental data concerning the overall activity of the crypt will allow to achieve new insights on the generic properties of such a system and on other possibly not expected biological constraints and mechanisms.

– The possibility of simulating various perturbations at the gene level allows innovative possibilities in investigating how biological noise and mutations
can spread through complex biological system, eventually leading to functional disorders and to the emergence of tumors and cancers.

In regard to the possible development of the model, we finally remark that in several biological processes, such as embryogenesis, differentiation is ruled not only through the stochastic process described in Section 3, but also by means of the activation of specific signals through complex pathways, which eventually contribute in modifying the gene regulative network. This deterministic differentiation can be actually modeled through NRBNs and it is our intention to integrate it with an explicit representation of the various signaling pathways involved in the crypt activity, for instance by adding reaction-diffusion equations concerning gradients of specific chemicals and/or introducing specific rules and mechanisms for the cell-cell communication.

This work is currently in progress and it will allow to provide a more exhaustive picture of the complex interplay that regulates the crypt activity and homeostasis.

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