# Combining Intra- and Inter-cellular Dynamics to Investigate Intestinal Homeostasis*

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**Abstract.** This paper reports on the multi-scale modelling of an intestinal crypt cellular structure coupled with Wnt signalling. Using formal modelling techniques based on the stochastic $\pi$-calculus, which supports ambients needed for compartments, we develop a collection of cell and molecular level models. The focus of our study is the role of Wnt in the control of cell division and differentiation. Using the BioSPI simulation platform, we analysed the model and reveal a plausible explanation for a mechanism that ensures robustness of cell fate determination.

## 1 Introduction

Analysis of signal transduction has uncovered a remarkable complexity of signalling networks in terms of their structure and dynamics. Despite the availability of a vast amount of data on the properties of individual molecules, the understanding of the function performed by a particular molecular network as a whole is still lacking. The complexity of signalling architecture can be explained by the need to optimize cellular response to the information it receives about environmental and internal conditions. In particular, a significant degree of this complexity can be attributed to ensuring robustness of the response despite varying environmental conditions.

Numerous experimental data are known that implicate the Wnt pathway in the control of different aspects of homeostasis [1,2,3,4,5]. It has been proposed that Wnt signalling regulates cell proliferation and renewal in the large intestinal epithelium. The large intestinal tract is built of geometrical tubular structures, called crypts. Intestinal homeostasis involves cell generation by division at the crypt base, progressive cell differentiation while they migrate to the top of the crypt, and cell death followed by extrusion when they reach the top. Stem cells, believed to reside at the crypt bottom, have the unique ability to maintain the entire epithelium. As they divide and move up, stem cells must constantly adjust their behaviour by entering partially differentiated population (called transit) prior to terminally differentiating. Simultaneously, the proliferative capability of transit cells is the highest and decreasing as cells move upwards. The question

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that arises is which factors control the ability of intestinal cells to keep a fine-tuned balance between cell division and differentiation.

In this paper, we test the feasibility of different biological hypotheses about the influence of Wnt signalling on the cell fate and the emergence of the robust regulation of cell numbers in the tissue. The function of Wnt cannot be measured experimentally; rather, only the average behaviour of the collection of cells in response to Wnt factors can be observed. We therefore employ computational modelling to examine if the specific properties of the Wnt pathway architecture can provide the conditions for the emergence of the robust regulation mechanism that ensures homeostasis in the intestine.

We base our approach on a conceptual extension of the stochastic $\pi$-calculus for spanning multiple scales. We describe in detail how to build a multi-scale model that couples signal transduction network to cellular decisions to proliferate and differentiate. We then analyze the model to reveal how a population of cells interacts and develops into a tissue under the influence of the environment.

## 2 Related Work

Several modelling approaches for studying the self-renewal process in the intestine exist [7,8,9]. A recent model [7] is representative of the class of deterministic spatially-uniform models. The authors describe the evolution of cell numbers in stem, transit, and differentiated compartments, assuming the constant compartment-dependent rates of renewal, differentiation and death. The model is shown to be very sensitive to changes in these macroscopic rate constants. The authors subsequently investigate the impact of the hypothetical negative feedback mechanism that, based on regulation of the rate at which cells differentiate, allows the crypt to maintain the equilibrium in cell numbers.

In a similar compartment-based but stochastic approach [8], crypt growth is described by a Markov process that models a stem cell population in which each stem cell produces zero, one, or two stem cells, according to a fixed probability distribution that does not vary from cell to cell. In the same manner as [7], the probability of self-renewal vs differentiation is assumed to be pre-programmed and independent of the conditions, except in case of stem cells knowing their number. Both models, however, do not indicate how the knowledge of stem cell numbers is acquired and propagated between physically separated cells. No experimental evidence exists that supports this assumption.

The incorporation of spatial cell fate control mechanism is achieved in [9], where deterministic model for crypt proliferation regulated by diffusible growth factor is presented. Epithelium is modelled as a one-dimensional array of cells. Each cell enters a cell cycle only if the growth factor concentration in the respective cell exceeds a certain threshold. Growth factor is spread by diffusion starting from the bottom of the crypt, but the concentration of growth factor in the tissue is constant. The model mechanism ensures the dynamic regulation of cell proliferation without the need to impose a static type-dependent program executed by every cell. However, under more realistic conditions of stochastic

time-varying growth factor field, the accuracy of this mechanism would collapse, resulting in high variability in the numbers of proliferative cells and crypt size.

## 3 Using π-calculus for Modelling Intracellular Dynamics

The process-algebra approaches, originally developed in computer science for describing and executing networks of concurrent components, have since been successfully applied for analyzing molecular and genetic systems [10,11,12]. Once a biological system has been modelled using basic components of the process algebra, the model can be stochastically simulated to derive the properties under study over time. Stochastic π-calculus [6] is one type of process algebras where interactions are assigned rates controlled by exponential distributions. In this paper, we use the BioSPI [11] as the platform which performs simulations of the π-calculus code using an adaptation of the Gillespie algorithm [13].

In this section, we describe how stochastic π-calculus can be applied to modelling and analyzing molecular interactions and transient changes occurring within cell. At the intracellular level, molecules are modelled as independent agents, governed by discrete reaction rules. Concurrent molecular agents are interconnected to describe dynamic changes of intracellular state in response to internal and external changes.

### 3.1 Molecules as Mobile Processes

A model in the stochastic π-calculus is a composition of concurrent components (called processes), each of which operates as a state machine describing the possible behaviours of the component. Processes communicate by sending data on channels, which they can dynamically create and destroy. Probabilistic choice, parallel composition, and scope restriction are among the built-in primitives of the π-calculus.

A process $X$ defined as a choice

$$X ::= \pi_1, X_1 + ... + \pi_n, X_n$$

may evolve as either of $X_i$, depending on which of the operations $\pi_i$ (explained below) is the first one to complete in the current context, thus representing a race condition between a set of processes. A process $X$ given by

$$X ::= X_1|...|X_n$$

denotes a composition of processes $X_1$, ..., $X_n$ running in parallel. The creation of the private channel $r$ within the scope of the given process $X$ is achieved by the operator

$$(new\ r(c))\ X$$

where channel $r$ with rate $c$ is bound to process $X$. Only processes that share a private channel may interact using that channel. Timing is incorporated into

$\pi$-calculus models by associating each channel $r$ with the rate governed by the exponential distribution with the mean $1/c$.

Another basic operation of the $\pi$-calculus is synchronous communication of a pair of processes over a channel. Output $r!$ and input $r?$ prefixes, where $r$ is a channel name, are elementary constituents of communication capabilities. The result of communication between process $X ::= r!\{y\}, X'$, containing an output capability $r!\{y\}$, and process $Y ::= r?\{z\}, Y'$, containing an input capability $r?\{z\}$, follows from the central reduction rule:

$$r!\{y\}, X' | r?\{z\}, Y' \longrightarrow X' | Y'[y/z],$$

where $y$ is substituted for $z$ in $Y'$. Communication between processes may carry information that further changes their interaction capabilities.

It is possible to represent $\pi$-calculus processes using a graphical notation. In graphical $\pi$-calculus, a model is a graph whose nodes correspond to processes and edges correspond to state transitions. Fig. 1 illustrates how basic operations are represented graphically.

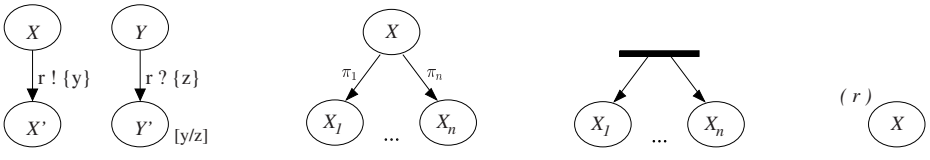


**Fig. 1.** Communication, choice, parallel composition, and scope restriction primitives of the stochastic $\pi$-calculus

A translation scheme that maps molecular reaction networks into $\pi$-calculus programs was the subject of previous publications [10,11]. Molecular entities can be coded in the $\pi$-calculus as processes that participate in reactions by communicating over channels. State transitions resulting from process communication correspond to covalent modification, association/dissociation, or degradation of signalling molecules. Molecules with several independent functional domains are represented as a parallel composition of $\pi$-calculus processes.

### 3.2 The Model of the Wnt Signalling Pathway

Wnt signalling induces a great variety of cell responses, spanning from morphogenesis and adult tissue homeostasis, to cancer formation. The main event of Wnt signalling is the accumulation of $\beta$-catenin which sends a signal into the nucleus for further processing. In the absence of the signal, a cytoplasmic degradation complex consisting of proteins Axin and APC, and kinase GSK3, rapidly degrades $\beta$-catenin. To activate the pathway, extracellular Wnt binds to the membrane receptor complex which generates the signal by inhibiting the degradation complex. This reduces the degradation rate of $\beta$-catenin, leading to its accumulation in the nucleus [14].

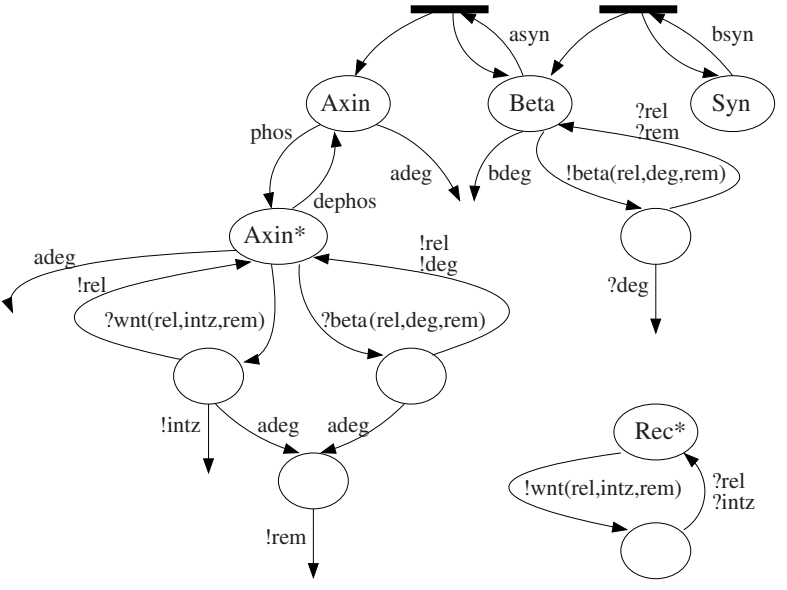


**Fig. 2.** $\pi$-calculus model of the Wnt pathway

The detailed model of the Wnt signalling pathway has been described elsewhere [15,16]. We further experiment with the model by testing additional mechanisms with respect to their capacity to enhance system robustness and adaptability. Altering the number of intracellular components, for example, is expected to alter cellular response, but if the system is robust, the extent of this alteration will be minimized. The system has, however, to sense and adapt to changes of the environmental conditions. We find that the addition of feedback loops [14] has a significant effect on promoting system robustness to parameter variation, a characteristic crucial for reliable performance of many biochemical networks.

Fig. 2 describes the $\pi$-calculus implementation of the revised Wnt pathway model. The process $Beta$, a $\pi$-calculus abstraction of $\beta$-catenin, interacts on channel *beta* with the process $Axin^*$ used to represent an activated state of the destruction complex. After interaction is completed, the resulting process can use channel *rel* to break the binding and return to its original state $Beta$, or can be degraded (transition marked *deg*). $Axin$ can transit between inactive and active states with delay times specified by *phos* and *dephos*. Active $Axin$ contains a complementary interaction capability ?*beta* that allows it to interact with $Beta$. Using channel *wnt*, $Axin^*$ can bind and subsequently be inhibited by the activated receptor complex $Rec^*$. A negative feedback loop is created by $Beta$ which spawns further instances of the process $Axin$ (at the rate specified by *asyn*).

In Fig. 3 we plot model outputs obtained from a single simulation run. Depending on the strength of the incoming stimulus Wnt (represented as a fraction of activated receptors), the system allows for different dynamic regimes. At low levels of Wnt, the model predicts stochastic outbreaks of $\beta$-catenin activity

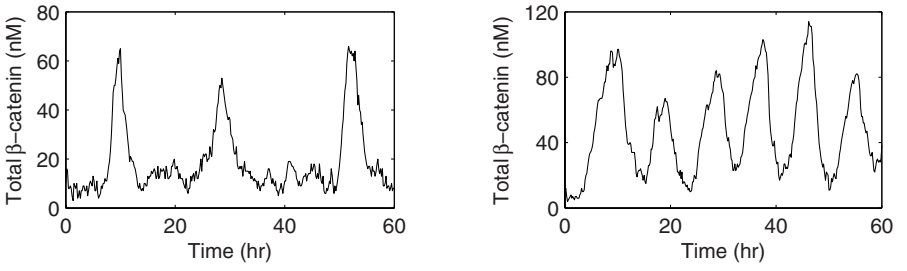


**Fig. 3.** Steady state value of $\beta$-catenin for different levels of the incoming Wnt: 25% (left) and 100% (right) of the receptors are activated

(Fig. 3 (left)) (note that the deterministic counterpart of the model reaches a steady state with low levels of $\beta$-catenin, for details see supporting website [25]). As the stimulus increases, the oscillations become regular and are most coherent in the deterministic limit when the number of molecules is very large, thus corresponding to the limit cycle behaviour of the deterministic system. These results agree with experimental evidence that high-intensity staining for $\beta$-catenin is observed only in a few cells at the bottom of the crypt, where Wnt signal is the strongest. Transient $\beta$-catenin is observed throughout the proliferative compartment in the lower 2/3 of the crypt. Indeed, we validated model predictions of oscillatory pathway dynamics in human cell cultures (a manuscript in preparation).

## 4 Extending a Framework to Model Cells

We are interested in testing possible hypotheses about Wnt-based control of cell proliferation and differentiation in the intestine. To test the feasibility of the mechanisms proposed by different research groups, we build a model that couples cellular decisions with the state of the Wnt signalling network embedded in every cell. Next, we explain how an extension of the $\pi$-calculus can be used for spanning different scales of the biological system.

### 4.1 Cells as Mobile Ambients

In order to extend the model with the cell-level dynamics, we first acquire a mechanism of embedding molecules into cells. We use ambients [19] to define a bounded place where interactions between agents happen. Enrichment of the stochastic $\pi$-calculus with ambients, introduced in [17], provides an ability to specify communication between $\pi$-calculus processes based on their location within a common boundary.

An ambient is a location where communication happens: $cell[X]$ stands for the process $X$ running at the location $cell$ (i.e., in ambient $cell$). Locations may reside within locations: in $cell[mol[A] \mid mol[B]]$ two ambients $mol$ are incorporated into

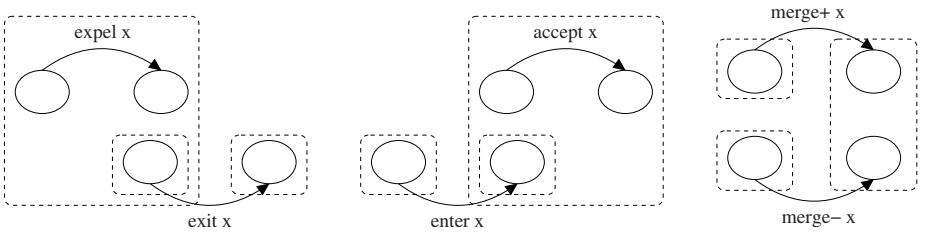


**Fig. 4.** Ambient capabilities

the ambient *cell*. Computation may contain the reconfiguration of a hierarchy of locations. In the following, we graphically represent an ambient as a dashed rectangle around the processes and sub-ambients it contains, possibly labelled with the ambient name. The derived models are simulated using the BioSPI platform [11] which supports ambients without modifying the semantics of the stochastic simulation [13].

Spatial configuration of the ambient system can be changed using capabilities such as *exit/expel* from the ambient, *accept/enter* into the ambient or *merge* with the ambient (Fig. 4). Communication abstraction is extended to represent compartment restriction on interactions based on their locations. Three types of communication restrictions are *local* (between processes in the same ambient), *s2s* (between processes in sibling ambients) and *p2c/c2p* (between processes in parent and child ambients) (Fig. 5).

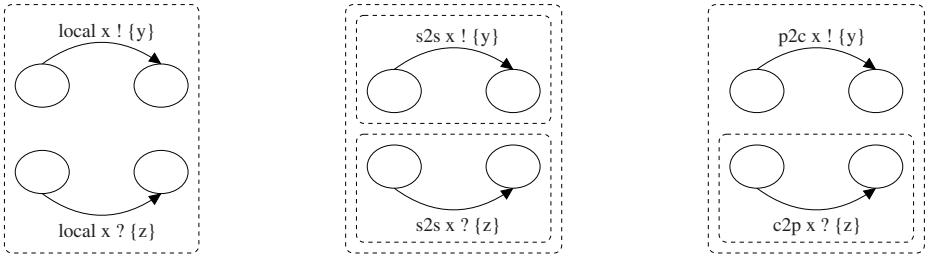


**Fig. 5.** Communication directions between ambients

To represent cells, allowing molecules to be assigned and re-assigned to specific cells, we abstract cells as ambients. Consequently, molecular communication within one cell is abstracted by the *s2s* communication direction. For communicating the state of the intracellular molecular network to the cell decision-making process, we use the *p2c/c2p* direction.

### 4.2 Modelling Cell Division and Movement

Here we present the spatial abstraction that describes the diffusion of the extracellular morphogene in one direction in the tissue. The pressure exerted by cell

division due to higher amounts of the morphogene directs the cell to move away from the morphogene source. This would accommodate the scenario of Wnt morphogene distribution along the crypt length and cell movement to the top of the crypt. Analogous extension of the $\pi$-calculus framework with spatial information is necessary when the desired objective is to simulate diffusion of extracellular growth or inhibitory factors, competition for space between different cells, or cell adhesion.

To model spatial abstraction, we define a neighbourhood relationship between cells. Two cells are neighbours if they share a private channel which is used to send instructions from one neighbour to another. In one-dimensional space, it is sufficient for each cell to keep the reference to its upper neighbour (channel *next* in Fig. 6). Extracellular signal and cell movement are functions of the neighbourhood. Following cell division, the upper neighbour is requested to free its position by moving upwards. Another instance of a cell is created and is inserted in the neighbouring position by updating its references to the neighbourhood, as illustrated in Fig. 6.

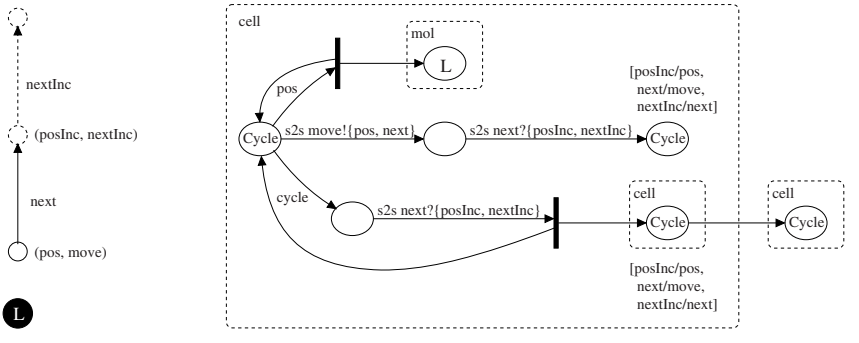


**Fig. 6.** Cell organization: linear array of cells referencing upper neighbours (left); and lattice representation in $\pi$-calculus (right)

Diffusion of the external morphogene is simulated by calculating the concentration of the external factor field at each cell position rather than simulating the movement of factor molecules within the spatial lattice. The channel *pos* with appropriate rate is carried by each cell to indicate its distance to the morphogene source.

## 5 A Model of Intra- and Inter-cellular Dynamics of the Crypt

Numerous and often inconsistent evidence exists suggesting that Wnt signalling controls the balance between cell proliferation and differentiation in the intestinal crypts and other tissues. Wnt is suggested to influence cell advance or withdrawal from the cell cycle [1,3,4], and cell ability to maintain its stem-cell phenotype

or to differentiate [2,5,21]. To test different hypotheses about the Wnt-based regulatory mechanisms involved in the intestinal homeostasis, we build a multi-scale model that couples the state of the intracellular network based on the previously described Wnt pathway to different decisions that the cell might make. The extracellular diffusible Wnt triggers changes in the intracellular state and thus influences cellular behaviour. We examine how these mechanisms influence the robust turnover of cells in the intestinal crypt and its disregulation in cancer.

### 5.1 Proliferative and Differentiated Cell Fate

In our model, we adopt two threshold mechanisms to decide whether the cell undergoes proliferation, differentiation, or stays quiescent. Increased $\beta$-catenin activity influences the initiation of a new cell cycle. The time to complete the cycle is assumed to follow an exponential distribution. Variability of the cycle length is thus incorporated into a delay needed for the cell to make a decision to proliferate.

In addition, $\beta$-catenin expression is linked to the ability of a stem cell to preserve its phenotype. We assume that once the cell starts expressing differentiation markers, differentiation is irreversible. While stem cell divides to produce two cells with an equal stem-cell capability, differentiated cell divides to produce two identical differentiated cells. Differentiated cells are also assumed to have a limited life span, as opposed to stem cells which are subjected to only a low-level apoptosis.

The alternative hypothesized scenarios of cell-fate decisions which we compare are:

**Hypothesis 1.** Transient activation of $\beta$-catenin in the cell triggers initiation of a new cell cycle. High levels of $\beta$-catenin are required to preserve stem cell properties.

**Hypothesis 2.** Transient activation of $\beta$-catenin is sufficient to push the cell into a new cycle while prolonged $\beta$-catenin signalling causes the stem cell to start expressing differentiation markers.

Each threshold mechanism is associated with a $\pi$-calculus channel which transmits a signal to the cell once the level of the intracellular $\beta$-catenin exceeds a specified threshold. In Fig. 7, channel `molcycle` is used to instruct the cell to enter a new cycle. Another threshold `moldiff` blocks (Hypothesis 1) or triggers (Hypothesis 2) cell differentiation (Fig. 7).

### 5.2 Wnt Gradient in the Tissue

Because Wnt targets are generally expressed in stem and proliferative cell compartment, it is widely accepted that Wnt factors are produced at the bottom of the crypt and are then transported by diffusion [20]. However, it has recently been suggested that Wnt gradient follows a more complex pattern due to surprisingly strong expression of the extracellular Wnt inhibitors at the bottom of the crypt [24]. We approximate this by additionally decreasing the rate at which Wnt is received by cells located at the bottom of the crypt spatial lattice (channel *pos* in Fig. 7).

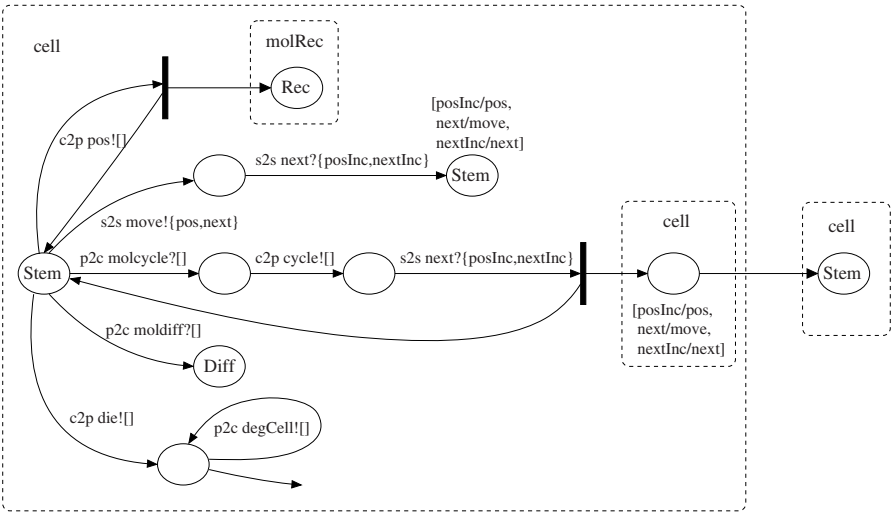


**Fig. 7.** Stem cell evolution: a cell undergoes proliferation, differentiation, or death. Additionally, the cell is constantly receiving information about the environment, and adjusts its position within the spatial lattice to accommodate newly born cells.

## 6 Robust Cell Fate Determination by Wnt Signalling

Using the BioSPI platform, we perform extensive simulations of the described scenarios in order to derive the properties of the multi-scale cellular system whose regulatory control is the extracellular diffusible factor Wnt. The derived models are subsequently analyzed with respect to the number of cell divisions as a function of cell position along the crypt vertical axis (i.e., distance to the Wnt source), the total number of cells in the crypt, and influence of stochasticity and random parameter perturbations on the tissue response (details about model parameters used in simulations and full model implementation are available at the website [25]).

The first family of models implement cellular decision mechanism described by Hypothesis 1. Our analysis shows that under these assumptions the fate that the cell assumes is very sensitive to the level of Wnt it is exposed to. The distribution of proliferating cells mimics the distribution of the Wnt factors along the crypt axis. The result is a high variability of the size of proliferative cell compartment and crypt size, which is inconsistent with the experimental observations. Moreover, activating mutations in the Wnt pathway, which increase the level of intracellular $\beta$-catenin, lead to significant expansion of the stem cell compartment. Consequently, the number of cells in the crypt becomes unstable and starts growing exponentially. We conclude that Hypothesis 1 is unable to reproduce the tissue response observed experimentally.

Simulations of the model based on Hypothesis 2 reveal that this combination of intracellular and cellular dynamics ensures robust tissue response mediated by

Wnt (Fig. 8 (left)). Rather than being scattered throughout crypt length, proliferative cells are confined to the restricted compartment at the bottom of the crypt. This is consistent with the experimental data ([18,22] and Fig. 8 (right)). The number of proliferative cells as well as the total number of cells in the crypt shows little variability, despite random noise and stochastic perturbations present in the model. This is consistent with the reports of a surprisingly narrow distribution of crypt sizes, the fact that has not yet been reproduced in modelling studies.

We next investigate the effects of the mutations in the Wnt pathway which were identified in concrete cancer models: Familial Adenomatous Polyposis [18], hyperplastic and adenomatous polyps [22,23]. To simulate the effect of mutations, we decrease the rate of the $\beta$-catenin inhibition by the active APC/Axin destruction complex (channel *beta* in Fig. 2). Up to 5-fold decrease of the $\beta$-catenin inhibition rate results primarily in a shift of the proliferative cells toward the top of the crypt (Fig. 8 (left)). The size of the crypt is increased only slightly. These predictions are in good agreement with the experimental evidence [18,22]. Fig. 8 (right), adapted from [18], shows experimental evidence of changes in the structure of the proliferative compartment resulting from mutations that decrease the activity of $\beta$-catenin inhibitor complex.

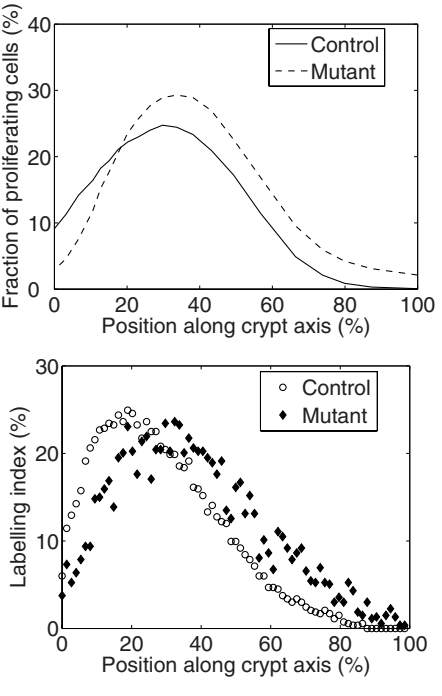


**Fig. 8.** Cell fate control by the Wnt pathway: model predictions (left) of the proliferative cell distribution in both healthy and mutant tissues agree well with the experimental data (right)

Further inhibition of the destruction complex leads to more advanced forms of intestinal cancer: colorectal adenomas [22,23]. While cell proliferation shifts upwards at the initial stage, the model predicts the break up of the mechanism that confines proliferative cells to the bottom of the crypt. This is consistent with the experimental observations of proliferation in adenomas being almost evenly distributed throughout the crypt length [22,23].

Our model provides an explanation to the observed phenomena. Cell proliferation is triggered by even modest increase of the Wnt levels which is sufficient to upregulate $\beta$-catenin to high amplitude. As Wnt increases, stochastic oscillations in $\beta$-catenin expression become deterministic and their frequency increases along with the cell proliferation rate. Analysis of the model also shows that under Hypothesis 2, which links stem cell fate to the region of rare stochastic oscillations of $\beta$-catenin activity, stem cells are limited to low Wnt region and decrease in numbers under mutant conditions. Thus, the model is not only consistent with the reports of low $\beta$-catenin activity in stem cells [2,5] and the reduced proliferation rate of stem cells caused by rare outbreaks of $\beta$-catenin, but also suggests the protection mechanism against the stem cell expansion that would immediately lead to the exponential growth of tumours [7].

## 7 Conclusions

In this paper, we employed formal modelling techniques based on the stochastic $\pi$-calculus to examine different hypotheses about the influence of the Wnt pathway on homeostasis of the intestinal epithelium, and its role in tumourigenesis. We proposed that possible function of Wnt is to ensure the robust cell fate determination. The model of the Wnt signalling pathway was subsequently coupled to the cellular behaviour and the environment to test its role in maintaining a fine-tuned balance between cell division and differentiation. The result of the model is consistent with different properties of the distribution of cells in the crypt. The model can explain both the stability of the healthy regulation and the changes seen in mutant phenotypes. The model also suggests which characteristics of tissue architecture can protect it from unbounded growth.

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