Colorectal Cancer Through Simulation and Experiment

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Abstract

Colorectal cancer has formed a canonical example of tumourigenesis ever since its use in Fearon and Vogelstein's linear model of genetic mutation, and continues to generate a huge amount of research interest. Over time, the field has witnessed a transition from solely experimental work to the inclusion of mathematical and computational modelling. The fusion of these disciplines has the potential to provide valuable insights into oncologic processes, but also presents the challenge of uniting many diverse perspectives. Furthermore, the cancer cell phenotype defined by the 'Hallmarks of Cancer' has been extended in recent times and provides an excellent basis for future research. We present a timely summary of the literature relating to colorectal cancer, addressing the traditional experimental findings, summarising the key mathematical and computational approaches, and emphasising the role of the Hallmarks in current and future developments. We conclude with a discussion of interdisciplinary work, outlining areas of experimental interest which would benefit from the insight that theoretical modelling can provide.

1 Introduction

An intricate landscape of geometry and function, the colonic epithelium forms a highly invaginated tissue, endowed with glandular structures and mucus-secreting goblet cells. It comprises millions of test-tube shaped structures called *crypts*, each consisting of up to 700 cells in mice [1] and 2000 in humans [2]. These structures feature in both the large and small intestine[1]. Crypt size varies greatly according to colonic site, with the longest crypts occurring in the transverse (middle) colon; here murine crypts are typically 35 cells in height, compared to 19 cells in the ascending (early) colon [3].

A schematic of a typical crypt is provided in Figure 1, which demonstrates the arrangement of the epithelial lining into a finger-shaped projection extending into the gut wall. The structure and

[1]Given our interest in colorectal cancer, we shall focus upon the large rather than small intestine for the majority of this review.

function of the intestinal epithelium has long been a subject of interest [4] and the attainment and maintenance of homeostasis in this tissue reflects a nuanced coordination of cell birth, migration and death. This rich complexity raises many questions about the biochemical workings of intestinal epithelial cells and their integration into a coherent physical structure which unites form and function. It is this same epithelium that forms the site for colorectal cancer (CRC), in which disruption to the genetic profile or subcellular function of crypt cells can lead to polyp-like outgrowths of the intestinal tract which develop into larger tumours. Our understanding of how normal cell processes falter during tumourigenesis is far from complete and thus invites further investigation, by both the experimental biologist and the applied mathematician.

Although cancer cells are known to exhibit wide variability in genetic composition, even within the same tumour [5], they nonetheless share certain common behaviour patterns. Hanahan and Weinberg's seminal work on the Hallmarks of Cancer [6] attempted to summarise this cancer cell phenotype, outlining six principal facets of cancer cell behaviour, namely: limitless proliferative potential; apoptotic resistance; rewiring of signalling pathways; induction of tissue invasion; sustained angiogenesis; and insensitivity to anti-growth signals. A recent update to the Hallmarks has added two 'enabling characteristics' and two 'emerging Hallmarks' [7]: genome instability and mutation; tumour-promoting inflammation; reprogramming energy metabolism; and evading immune destruction. All ten Hallmarks are depicted in the diagram of Figure 2.

These additions represent important aspects of cancer cell behaviour and may influence the direction of future research. Although Hanahan and Weinberg's phenotype addressed cancer in a general sense, the 'Hallmarks' provide an excellent basis for further investigation, whether experimental, theoretical, or mathematical. We shall therefore refer to these 'Hallmarks' throughout this review, aiming to show not only how existing findings in the CRC literature have elucidated these, but also to identify how they may be investigated further through wet-lab experiments, mathematical modelling, or a combination of both.

This review is structured as follows. Section 2 presents the current level of biological understanding of the intestinal epithelium in healthy and cancerous form. Section 3 discusses existing mathematical and computational models of the crypt, starting with compartment models and progressing to continuum and discrete frameworks. The subcellular reaction networks governing the biochemical processes of the colon are detailed in Section 4, along with mathematical representations for some of these key reaction systems. Section 5 unites the cellular and subcellular scales of interest with its examination of multiscale models for CRC. Possible directions for future research are identified in the concluding Section 6. Throughout we highlight the relevance of the Hallmarks of Cancer to current developments in the field.

2 Biology of the Intestinal Epithelium

2.1 Crypt Homeostasis

The upward migration of cells known to occur in the crypt – Paneth cells excepted – was first identified by Friedman [8] in irradiative studies of rat intestine. Later experiments identified a population of putative stem cells in the lower regions of the crypt, displaying elevated proliferation rates [9]. Division of the stem cells yields transit-amplifying cells which occupy the middle regions, whose proliferative potential reduces on ascending the crypt. Ultimately cells adopt a terminally differentiated fate in the upper crypt, irreversibly specialised as either absorptive enterocytes or as mucus-secreting goblet cells [10]. The uppermost third of the crypt is thus devoid of mitotic activity [11]; here cells die and are sloughed into the gut lumen. Paneth cells are a special type of differentiated cell; only present in the small intestine, they migrate down to the crypt base and are thought to assist in crypt development and defence against microbes [12]. Location and function consequently hold a close association in intestinal tissue; an enumeration convention for cell location was therefore established by Cairnie *et al.* [11]. Under this system, a cell is said to be in the '$+n$

position' if it sits atop $n-1$ other cells, counting columnwise from the crypt base[2].

What induces the abrogation of mitosis in the upper crypt? What is meant by a 'stem cell' and how is this subpopulation maintained? The 'slow cut-off' model suggests that cells in the middle of the crypt experience a progressive decline in the probability of producing proliferative cells, owing to a gradient of extracellular factors along the length of the crypt, or an accumulation of intracellular components that arrests mitosis above some threshold value [13]. This spatial formulation is closely related to the idea of a *stem cell niche*, in which the local cell environment around the crypt base supplies growth factors which induce stemlike behaviour. Away from this niche, cells have reduced exposure to such factors and do not show stemlike behaviour [14]. Under the niche model, 'stemness' arises from external cues associated with cell location. Conversely, the '*generational*' or '*pedigree*' model assumes that cells progress through a strict hierarchy, such that stem cells give rise to transit cells endowed with a fixed number of divisions, prior to terminal differentiation. This approach accompanies the *divisional* model of stem cell proliferation, in which the size of the stem cell population is maintained through asymmetric division (yielding one transit and one stem cell) or symmetric division (yielding either two stem or two transit daughters with equal probability) [15]. These alternative models of stem cell maintenance are an important feature of the mathematical modelling work described in Section 3, whether niche-based [16, 17, 18] or divisional [19, 20].

Current definitions of stemness require an intestinal stem cell to exhibit *pluripotency* (the potential to develop into several distinct cell types) and the capacity to sustain epithelial regeneration in the crypt [21]. Barker *et al.* identified the putative stem cell marker Lgr5 (also known as Gpr49) and observed actively cycling Lgr5+ cells near the base of the colonic crypt, displaying a clonal capacity to populate the entire crypt in lineage tracing experiments [21].

Successful *in vitro* crypt synthesis demonstrates the ability of Lgr5+ stem cells to generate the full spectrum of crypt cell types [22, 23]. The progeny of the Lgr5+ cells in these experiments spontaneously assemble into crypt geometries; furthermore, the resulting spheroidal megacolonies display a contractility reminiscent of *in vivo* peristalsis [23], apparently recapitulating crypt function as well as form. Much of this work supports the stem cell niche hypothesis; colony growth responds well to external proliferative cues (such as the mitotic upregulator R-Spondin1, discussed in Section 4 and shown in Figure 5). Sato *et al.* suggest that differential response to external cues, rather than differential exposure, is more important in cell proliferation [22].

Although the Lgr5 marker seems a good candidate for stem cell identification, it remains uncertain whether all such cells are functionally equivalent [24]. Studies point to *Bmi1* as an alternative marker in the small intestine [25], although it may delineate a subpopulation of less potent stem cells near the +4 position, rather than those at the crypt base. The overexpression of *Bmi1* in CRC cells is well documented [26, 27].

Intestinal stem cells populate and sustain the crypt by a process of 'Neutral Drift Dynamics' (NDD), in which a cell lineage exhibits randomised expansion or contraction over a period of time up to a point of universal dominance (crypt monoclonality) or extinction. If a stem cell dies, a neighbouring cell from the local population of equipotent stem cells will expand to compensate for this loss. Lopez-Garcia *et al.* [28] identify NDD behaviour within intestinal crypts, outlining NDD and symmetric stem cell division as the chief means of realising a stable, dynamic equilibrium across the stem cell population. Snippert *et al.* corroborate this experimentally through striking use of multicoloured Cre-reporter fate mapping of Lgr5+ stem cells [29], suggesting that a stochastic mechanism of stem cell expansion can yield monoclonality in the long term.

Homeostasis owes its maintenance as much to cell death as it does to mitosis. Programmed cell death (apoptosis), in which the cytoplasm shrinks prior to disintegration of the cell, is predominantly restricted to upper crypt regions in colonic tissue [30, 31]. However, apoptosis is seen to occur at positions +4 to +5 of the small intestine. This is purported to act as a regulatory mechanism for the stem cell population and is not observed in crypts of the large intestine [31]. A specific form of apoptosis known as *anoikis* occurs in the uppermost regions of the crypt, when senescent cells detach

[2]Longitudinal enumeration introduces greater uncertainty for later-numbered cells: the selection of the crypt base as the region of interest influenced the counting direction in this case, permitting more accurate assignment of location number in the proliferating regions.

from the basement membrane [32, 33]. Resulting dead cells are thus sloughed from the epithelium into the lumen of the gut. Anchoring of stem cells to the basement membrane *in vivo* serves a vital purpose, since such cells have been shown to exhibit particular susceptibility to anoikis *in vitro* [33].

Migration also plays a role in homeostasis. Transit from crypt base to surface in the murine colon takes approximately 2 days [34], prior to desquamation into the lumen of the gut. Cell velocity increases linearly with crypt height before levelling out [11, 35, 36] and exhibits circadian variation [37]. Some theories suggest a coordinated movement amongst the crypt cells, whereby cell-cell connections induce unified migration of an epithelial layer [10]. Experiments measuring cell velocity have proven crucial in the development of spatially resolved mathematical and computational approaches (see Section 3), by providing data with which to parametrise the models.

Analysis of proliferative and migratory processes typically involves a cell labelling procedure, followed by longitudinal sectioning of the crypt. The angling of crypts within the tissue, along with the hexagonal packing arrangement of cells, can often lead to overestimation of crypt height [15]. Stathmokinetic (mitosis-inhibiting) studies provide an insight into mitosis and migration in the intestinal epithelium, whether through use of cytotoxic agents [38, 39, 40], irradiation [39, 41], or both [41]. Cell movement is still observed during mitotic arrest; the authors of [41] therefore suggest that mitotic pressure may not be the sole cause of migration. Results from application of the protein synthesis inhibitor puromycin suggest that active mechanisms also contribute to cell movement up the crypt [38].

Cells of the intestinal crypt exhibit asynchronous cycling, with stochastic variation in cycle length [9], although some patches of apparent synchrony have been observed [42]. In healthy colonic tissue, stem cells reside at positions +1 to +2 [31]. At homeostasis the number of stemlike cells is thought to remain approximately constant; this could be achieved via maintenance of asymmetric division by each individual stem cell, or by a collective behaviour pattern across the population of stem cells [43].

2.2 Colorectal Cancer and the Stem Cell Hypothesis

Colorectal cancer has long been considered the canonical example of tumourigenesis under the traditional model of sequential mutation [44]. Nowadays this theory has largely been supplanted by the Cancer Stem Cell (CSC) hypothesis, in which the tumour is generated by a small group of malignant stem cells which account for as little as 1% of the tumour mass [45]. It remains unclear whether such cells derive from stem cell mutations, or whether genetic hits occur in progenitor cells which subsequently regress to a stemlike phenotype. Nonetheless, CSCs seem to mirror the capabilities of their healthy counterparts in originating, maintaining and in some cases regenerating (in this case, cancerous) crypt tissue [46]. CSCs also show an improved ability for correcting DNA errors and experience a longer cell cycle than normal stem cells [45], even if cancer cells collectively exhibit a higher than average birth rate [47].

If the CSC hypothesis is valid, it holds considerable implications for the treatment of colorectal cancer, requiring the eradication of malignant stem cells that have few obvious morphological identifiers. Investigation of CSC lines has started to associate some Hallmarks with particular markers. Dalerba *et al.* identify CD44+ CRC cells expressing the cell adhesion molecule $EpCAM^{high}$ [48] and apoptotic resistance in colon cancer stem cells has been observed *in vitro* in CD133+ cell lines [49].

The CSC hypothesis offers an intriguing cellular perspective on CRC: however, we are also interested in the maintenance of the entire tissue in CRC and the means by which it evolves.

2.3 Dynamic Evolution of Healthy and Abnormal Tissue

Disruption to mitotic processes frequently signals the onset of oncogenesis. Such considerations focus primarily on the cellular scale of interest; what can be said about how the appearance of the tissue as a whole changes during abnormal development?

Just as kinetic behaviour of epithelial cells varies throughout the healthy intestine, so it does in abnormally proliferating tissue. Roncucci *et al.* observe considerable site-dependent variation in the labelling index of crypt cells throughout the colon and rectum in human patients presenting with advanced polyp formation [50]. An increase in cell proliferation is particularly evident in the left colon and the rectum, with general expansion of the proliferative compartment featuring heavily in the adenomatous tissue under study.

Mutability of the proliferative response is not solely confined to hyperproliferative tissue. Far from being a static structure, the intestinal epithelium behaves as a dynamically evolving tissue [51]. Crypt production is maintained throughout life via a process of crypt branching or fission, the frequency of such occurrences reducing after birth and eventually falling to a low level in adulthood. Cairnie and Millen [52], through irradiative studies of crypt fission in mice, propose a model in which crypt size and number are maintained through feedback control and further support a spatial approach in commenting that '*every proliferating cell is a potential stem cell*' (the latter a development of the ideas of [53, 54]).

It has been conjectured that intestinal polyps initiate from abnormal crypt fusion, followed by a series of crypt fission events. This behaviour is captured by the striking microscopy work of Araki *et al.* [55]. Heightened cell turnover in a polyp region appears to be confined to individual crypts: a hyperplastic crypt does not populate any neighbouring crypt structures. Such theories are consistent with observations of crypt fission events in murine small intestine [52] and rat colon [56]. Crypt fission is considered a key mechanism in the formation and regeneration of the intestinal epithelium. An elevated rate of crypt production has been observed in tissue samples from patients with intestinal disorders such as Crohn's disease, ulcerative colitis or multiple polyposis [51] and the existence of a crypt cycle is suggested, in which all healthy crypts (asynchronously) fissure every 9-18 years. Control mechanisms such as chemical or electrical feedback from crypt cell gap junctions have been proposed as a means of inducing plasticity in the crypt cycle [57].

Given the geometrically intricate and dynamically changing nature of the intestinal epithelium, accurate visualisation of crypt structures within the tissue has proved challenging. The electron microscopy work of [55] obtains clear images however, as do the stained tissue sections of [58], both these cases also capturing the process of crypt fission. Nonetheless, the majority of studies infer three dimensional structure from two dimensional tissue sections: quite how representative such analysis can be is debatable. Advances in multiphoton microscopy and tissue preparation techniques have begun to remedy this, evincing the striking architecture of intestinal crypt structures and enabling the simultaneous imaging of up to 50 intact crypts [59].

3 Mathematical Models for Crypt Tissue

Insight into biological processes may be gained by constructing mathematical and computational models of the system. Armitage and Doll presented one of the earliest mathematical studies of cancer in the 1950s, with a statistical analysis of the age distribution of mutations in different organ systems [60, 61]. Ultimately this led to the linear model of carcinogenesis, involving sequential acquisition of mutations over an extended time period (discussed further in Section 4). These statistical approaches yielded estimates for the mutation rates in CRC, but could not explain its propagation throughout the colonic epithelium, owing to the absence of biophysical mechanisms. This created a demand for models of healthy crypt tissue, which could then be manipulated to address the problem of tumourigenesis.

Developments in modelling require an appropriate balance between computational tractability and biological accuracy; experimental progress often instigates a wave of new mathematical and computational approaches. For example, discrete cell-based modelling of the intestine initially arose from *in vivo* crypt labelling experiments [42]. Prior to the turn of the century, the notion of hardwired cell functionality dominated experimental opinion and is reflected in many computational studies of the time, while more recent approaches account for the 'spatial' concept of cell typing, where proliferative phenotype derives from a cell's location. Such interrelation of simulation and experiment is borne out by the chronology presented in Figure 3.

Mathematical crypt models can be classified into one of three types. *Compartment models* examine the behaviour of interdependent cell populations in the crypt but do not anchor these in a spatial domain. *Continuum models* generally use systems of partial differential equations to characterise the quantities of interest at all points in the tissue region. *Discrete models*, by contrast, rely upon the representation of individual cells: this assumes that the dynamics of cell movement can be adequately described by a finite number of forces, protein concentrations and other quantities evaluated at a set of points in the domain. Distinct modelling approaches may also yield different insights: not every model is appropriate for a given scenario. To this end we now outline the array of existing crypt models, their methods and applications, indicating which 'Hallmarks' each has explored.

3.1 Crypt Tissue as Distinct Populations

The compartment model decomposes the crypt into populations of stem, intermediate and differentiated cells [62]. The latter is given a constant death rate, while the other compartments are assigned fixed rates of death, differentiation and renewal. In [62], recurrence relations then track the cell count in each compartment over time. This approach characterises the cell populations under an assumption that each compartment is 'well-mixed' and ignores spatial information regarding their relative positions in the crypt. A 'healthy' homeostatic equilibrium can be attained when the stem cell population exactly renews itself and intermediate proliferation is calibrated to achieve self-renewal relative to the stem cells. Compartment perspectives focus on the change in size of the three cell populations and therefore facilitate study of the 'big three' Hallmarks: *sustained proliferation*, *apoptotic resistance* and *replicative immortality*. Mutation is simulated through a change in the rate parameters. In the model of [62] this yields either exponential growth (as with a malignant tumour) or an increase in cell numbers to a new, higher equilibrium (e.g. formation of benign lesions). An increase in stem cell proliferation can induce tumourigenic growth irrespective of conditions in other compartments.

Model extensions of note (see Figure 3) examine the effect of transition rates on the emergence of exponential growth or the persistence of a stable equilibrium. D'Onofrio and Tomlinson [63] introduce variable kinetic rates, dependent on the size of the intermediate compartment, and also consider stochastic fluctuation of the stem cell renewal parameter. Exponential growth is more likely if renewal exceeds a critical threshold for sustained periods; this motivates interest in controlling the stem cell population during early-stage cancer treatment. Crucially, an apparent plateau phase can also erupt into exponential growth, suggesting that tumour size is not necessarily an appropriate predictor of severity. Johnston *et al.* extend this work to investigate the ability of feedback to stabilise the crypt against mutations [64]. They also relax the assumption of synchronous cell division in [62] to allow for asynchrony. If the differentiation parameters include feedback from various compartments, the cell population can transition through a series of progressively higher steady states until exponential growth is attained, akin to multistage carcinogenesis.

Several other compartment models identify stem cell overproduction as a causal factor in CRC [65, 66], a theme which has inspired many variations on the standard approach. These include [18], based on a generational division model which includes multiple transit stages with stochastically varying cell cycle lengths and a special pool of transit cells which can mature into differentiated cells, or regress to stem type. The model can compensate for cell loss after simulated irradiation of the stem compartment; this recovery compares favourably with *in vivo* data from murine crypt tissue. The results support the notion of a stem cell niche which is regulated by either long-range feedback or short-range chemical regulation. Some models have focused on the transit population instead [67]. The model of Boman *et al.* involves 81 compartments, each representing a separate cell generation and composed of subcompartments of cells in particular phases of the cell cycle [68]. S phase transition rates (chosen to match existing experimental data) vary with crypt height; mitosis is asymmetric, a cell of generation g dividing to produce two daughter cells of generations g and $g + 1$. This framework is equipped to evaluate the contribution of overpopulation and cell cycle phase shortening to early-stage tumourigenesis. Ultimately, dysregulation of S-phase mechanisms

in the stem compartment is suggested as a key factor in tumour initiation.

Compartment approaches allow us to explore questions about highly generalised crypt populations. Their focus on birth and death mechanisms provides useful insights into the Hallmarks of *proliferative signalling* and *resisting apoptosis*. However, they cannot incorporate the spatial information needed to study processes such as *tissue invasion and metastasis*; this motivates a progression to continuum models. We also note that further details of spatial crypt models can be found in the useful review [69].

3.2 Crypt Tissue as a Continuum

Continuum models usually use systems of partial differential equations to characterise quantities of interest at all points in the domain, although some one-dimensional examples use ODEs [70]. For example, in the two-dimensional continuum model [71], Darcy's law provides a constitutive relation describing cell movement down a pressure gradient, caused by cell proliferation. This, combined with a conservative mass-balance equation, characterises a pressure-driven cell flow through the domain. Proliferation is introduced via a spatially varying source term; the domain is assumed to be cylindrical and features two distinct cell populations separated by a moving boundary. Variation of the relative viscosity (i.e. the resistance to cell movement) of these two populations is found to have a strong influence on the character of the interface. Murray *et al.* construct a one-dimensional continuum model of the healthy crypt [72], an extension of [19] (see Section 5). This assumes symmetry about the longitudinal crypt axis and adopts a nonlinear diffusion equation for evolution of cell density, augmented by a proliferation source term, while proliferation ceases above some threshold height. Under particular parametrisations, the model proves a good match for existing experimental data on cell velocities. Both [71, 72] compare their continuum models with discrete analogues.

Other continuum models for the crypt include Figueiredo *et al.* [73], which maps the path of a region of epithelial cells up the crypt. A convection-diffusion equation tracks the density of the cell group, while a level set equation describes its location and shape. This technique could be used to study the path of malignant cell groups in the crypt.

Continuum approaches have proved useful in modelling the tissue mechanics of the crypt, particularly with regard to crypt buckling and fission, both of which occur during the early stages of CRC. Edwards and Chapman model the crypt epithelium as a flexible, growing beam, attached to the underlying stroma by springs [70]. In this model, buckling may arise as a consequence of increased net proliferation, expansion of the proliferative compartment, or increased stromal adhesion. Indeed the Wnt signalling pathway (see Section 3) is known to influence both proliferation and adhesion; these results support the idea that dysregulation of Wnt is a causal factor in oncogenesis. Nelson *et al.* model the epithelial cells as interconnecting linear springs resting upon an extensible, elastic beam, with frictional forces acting between the beam and the cells [74]. Compressibility of the two layers is a key factor in buckling: indeed it is material, rather than growth, properties that appear to influence the resultant shape. Furthermore, the relative stiffness of the epithelium and stroma determines the wavelength of the buckling instability.

Continuum models may prove useful in the limit of large cell numbers. Nonetheless, specific scenarios relating to individual cells may call for greater detail, as with the study of mutant cells, cell lineages or contact-dependent cell signalling. Such problems are intrinsic to the study of CRC, which is often regarded as developing from a single rogue cell (or indeed a group of such cells), through the Hallmark of *genome instability and mutation*. This motivates the requirement for cell-based models of the colorectal crypt, which represent the individual cells forming the tissue. To this end we shall now discuss cell-based modelling, a broad term encompassing a wide range of different approaches.

3.3 Crypt Tissue as Individual Cells

Cell-based models fall into two main classes: lattice-based models, in which cells are confined to move within a specified grid, and off-lattice or lattice-free models, where such restrictions are lifted.

3.3.1 Lattice-Based Models

Here cells are constrained to move on a fixed lattice. Pointwise defined cells result in cellular automata (CA), where each cell occupies a single lattice site. Rules for lattice updating are user-defined and may involve such considerations as:

- defining a cell's neighbourhood and hence those nearby sites into which a cell may move; and
- defining a cell's probability of dividing or moving in a certain direction.

The use of CA to model crypt cells dates back to Meinzer and Sandblad [75], who neglected lateral migration and modelled the crypt as a one-dimensional chain of cells. Stem cells are assumed to occupy a fixed region at the crypt base and adopt an 'eternally proliferating' phenotype with asymmetric division, daughter cells dying after some stochastically varying lifetime. The model was used to compare the 'slow cut-off' model (a spatial-based hypothesis) with the 'generation cut-off' version, in which cells terminate mitosis upon reaching some predetermined cell generation [76, 77, 78]. The model is calibrated against experimental data and used to mimic mitotic labelling experiments [79]. The output provides predictions for cell cycle phase lengths in the crypt, including the existence of a relatively short G2 phase.

Loeffler *et al.* [16] proposed the first two-dimensional representation of the colorectal crypt, modifying the model of [75] such that daughter cells select their next location according to the age of their neighbours and can be placed in an adjacent column. Extension to two dimensions was necessary to accurately reproduce *in vitro* mitotic labelling data [42] (see Figure 3 for details). The model indicates that lateral displacement generates an outward force that prevents the crypt structure from collapsing inwards [80, 17].

The relative computational simplicity of lattice-based approaches also provided an attractive means of exploring hypotheses about dynamic processes within the tissue, and dominated early computational approaches (see Figure 3). By contrast, experimental procedures of the time involved fixing intestinal tissue in paraffin wax for visualisation, thus limiting observation to snapshots of dynamic processes. Frequent references to the 'number of transit cell generations' indicates the assumption of hardwired functionality that prevailed during this period, as opposed to notions of spatially-derived behaviour that are now favoured.

Paulus *et al.* incorporated goblet cells into the cell lineage, assuming randomised acquisition of goblet properties across the differentiated cell population [81]. Their results suggest that stochastic, rather than deterministic, mechanisms are more likely to explain the observed cell distribution and, furthermore, that goblet and columnar cells derive from a single type of stem cell. Later, Isele and Meinzer simulated the effect of cytotoxic drugs within a Meinzer-type 1D chain of cells by introducing distinct, stochastically determined cell-cycle phases and assuming that stem and differentiated cells respond to the drug at different rates [82]. In this model, the stem cell population is more robust to cytotoxia than cells in the rest of the crypt; however, they later demonstrate that explicit cell-cycle modelling is needed to fully understand these cytotoxic effects. Similar approaches were adopted by [83], but in a 2D domain akin to that used by [16].

In many of these models, cells only move when mitosis occurs and this process may cause a shift in an entire column of cells. This contrasts sharply with *in vitro* observations, where crypt epithelial cells move within the tissue throughout their cell cycle and where migration is also observed after the application of stathmokinetic (mitosis-arresting) substances [41]. CA models omit information relating to cell contact area, owing to their single-point representation of cells, and are therefore unsuitable for studying intercellular adhesion and contact-based signalling processes. A pointwise arrangement also neglects the polygonal packing apparent in crypt tissue, while the discrete time representation fails to acknowledge the continuous cycling processes of biological cells.

The Cellular Potts Model (CPM) represents another major subclass of lattice-based approaches and offers an adhesion-centric perspective derived from the Steinberg hypothesis [84]. Each lattice point in a fixed grid is assigned an integer-valued identifier; all points of a given identifier constitute one biological cell (as shown in Figure 4). The system evolves via a modified Metropolis algorithm, as implemented in Graner and Glazier [85]. A series of lattice point samplings is performed and energy-dependent quantities are calculated via a Hamiltonian, the system being assumed to evolve to one of minimum energy. Typically the Hamiltonian takes the form

$$H = \sum_{(i,j),(i',j')} (1 - \delta_{\sigma_{ij}\sigma_{i'j'}}) J_{\tau,\tau'} + \alpha \sum_{\text{cells } k} (A_k - a_k)^2 \,,$$

where $\sigma_{i,j}$, $\sigma_{i',j'}$ are the cell identifiers of neighbouring lattice points, $J_{\tau,\tau'}$ indicates the cell adhesion strength between neighbouring cell types τ, τ', A_k and a_k are the respective target and actual volumes of cell k and α is a user-defined weighting term. The first summation calculates adhesion contributions from the interactions of adjacent lattice points and the second summation assesses the difference between each cell and its target volume. The Hamiltonian can be extended to include other constraints if desired, for example a target perimeter.

The CPM permits more accurate representation of cell morphology by generalising the pointwise approach of CA methods. Accurately quantified adhesion parameters are rarely available in the experimental literature and so parametrisation and time scaling may prove problematic. In the absence of continuous time measurement, CPM output must first be calibrated against experimental velocity results, and the stochastic nature of CPM evolution can lead to simulations becoming trapped in local energy minima.

Wong *et al.* adopt the 'generational' paradigm and examine the impact of differential adhesion upon cellular migration within the crypt [86]. Adhesion parameters are inferred from knowledge of extracellular gradients of EphB and ephrinB, associated with cell adhesion in intestinal tissue. Division axes are determined by the cell geometry, with the cell dividing along its shortest axis at mitosis. Results from the model provide a good match against experimentally-measured cell migration data [36] and suggest an increase in cell velocity towards the upper regions of the crypt. Unfortunately the model appears highly sensitive to its starting configuration; nonetheless, it would be interesting to remove the pedigree assumption and examine the behaviour of a spatially-typed CPM model, particularly with experimentally-determined adhesion parameters.

Cellular automata and the cellular Potts model both involve discrete timestepping and feature cells which are lattice-bound in space. An in-depth study of realistic cell movement or of the continuous cycling processes employed by biological cells demands continuous representations in space and time. This yields the off-lattice models which we shall now describe.

3.3.2 Off-Lattice Models

Biological cells are not confined to move on a lattice. If this constraint is relaxed, a raft of new models is created. The simplest of these is a cell-centre (CC) model, in which cells are defined by the spatial location of their centrepoints. Cells can be represented as spheres, or by a Voronoi tessellation; connections between the cells can be defined either by nearest neighbours or by constructing a Delaunay triangulation. Springs connecting the centres of neighbouring cells model intercellular adhesion, permitting explicit mechanical control of cell motion and allowing cell motion to be described by a finite number of forces.

Successor to the CA approaches of [16], the cell-centre modelling framework was initially established in Meineke *et al.* [87] and has since yielded several crypt-specific, computational studies of note, as detailed in Figure 3. These address several limitations of their lattice-based predecessors. Firstly, *cell morphology*: a polygonal cell representation accurately captures cell tessellation patterns within the intestinal epithelium, avoiding the global displacement artefacts of the Loeffler CA model [16] and acknowledging that cells need not retain a fixed number of neighbours over time. Secondly, *temporal realisation*: a continuous time representation models growth processes by means of an explicitly defined cell cycle model (CCM), in contrast to the discretised motion employed by the

lattice-based models. This CCM may take the form of a discretised 'clock' or a more sophisticated series of continuous differential equations.

'Overscoring' of cell position is a known artefact of experimental crypt sectioning, resulting from non-longitudinal cuts to a crypt structure and subsequent overassignment of cell position during vertical counting. Such discrepancies motivate the model proposed in [87], based on the traditional 'pedigree concept' of succession, its fixed cell generations inhabiting a cylindrical domain and reliant upon a passive mitotic pressure as the principal stimulus for migration, along with strong, frictional overdamping. Force balances determine the equations of motion of the individual cells; the force F_i exerted on cell i by its neighbours j is as follows:

$$F_i(t) = \mu \sum_{j \in N_i} \hat{\mathbf{r}}_{i,j}(t)(s_{i,j}(t) - |\mathbf{r}_{i,j}(t)|), \qquad \mathbf{r}_i(t + \Delta t) = \mathbf{r}_i(t) + \frac{1}{\eta} F_i(t)\Delta t\,,$$

where $\mathbf{r}_{i,j}$ is the vector from i to j and $\hat{\mathbf{r}}$ its unit equivalent; N_i is the set of cells neighbouring cell i; $s_{ij}(t)$ is the natural spring length between the cells; μ is a spring constant; and t is time. Significantly, the Meineke framework pins stem cells in place and constrains them to divide asymmetrically, thus negating the possibility of NDD studies.

Refinement of this cell-centre model to incorporate subcellular and/or tissue level phenomena has proved fruitful. The cell-centre crypt model of [88] extends the Meineke framework [87] by embedding into each cell ODEs for cell-cycle progression [89], coupled to a separate model for Wnt stimulation [90]. The cell-cycle response varies according to whether or not the prescribed local Wnt stimulus exceeds a threshold value. This permits spatially-derived cell typing and also influences cell-cell adhesion. Plasticity of the cellular response at the crypt base seems sufficient to maintain a proliferative hierarchy that is consistent with experimental observations. The Meineke model [87] is further adapted by unpinning the stem cells, which then rely upon stromal adhesion to remain at the crypt base. This modification permits the study of niche succession; model simulations reveal that competing cell lines can displace one another over time and establish a monoclonal crypt. A similar approach has been used to show that the spatial 'stem cell niche' hypothesis is more likely than a stochastic generational model of stem cell division [91]. Application of a spatial model significantly reduces the time taken to attain a monoclonal state and extension of the model's geometry to incorporate a rounded crypt base brings this time closer to experimentally observed values [91]. Simulations based upon the van Leeuwen approach [88] have examined how cell location and adhesion properties can influence the process of monoclonal conversion [92]. van Leeuwen *et al.* [88] also compare their synthetic labelling simulations with *in vitro* data and identify the occurrence of experimental overscoring.

Crypt buckling and fission applications reiterate the versatility of the cell centre approach in modelling physical deformation. Dunn *et al.* address the adhesive interaction between epithelial cells and the underlying stroma [93]. This is achieved by adding a curvature term to the force calculation as a restoring influence on the crypt shape, equivalent to the bending stiffness of [70]. In agreement with the continuum models, the formative stages of carcinogenesis are heavily influenced by the strength of epithelial attachment to the underlying stroma; hyperproliferation of the epithelium heightens the risk of crypt buckling. [93] has since been extended to a two-dimensional model of the colorectal crypt and used to study the relationship between crypt deformation and cell proliferation events [94].

Folding of the crypt epithelium has also been studied by Drasdo *et al.* [95], who model the crypt epithelium as a chain in two-dimensional space, where each cell is a deformable circle whose movement is regulated by the elastic forces it experiences from its neighbours. Changes to the 'bending rigidity' of the chain can induce buckling and crypt fission. Following [95], Buske *et al.* [20] use deformable spheres to create a three-dimensional crypt model. The spheres experience intercellular adhesion and also adhere to a basement membrane. Separate thresholds for proliferation and cell fate decision determine the phenotype of each crypt cell: no predetermined 'pedigree' is assumed. Cell fate is determined locally and arises from averaging over the subcellular variables of neighbouring cells. Spontaneous deletion of a cell during a simulation of healthy tissue appears to

be quickly compensated for by the cells in the immediate vicinity, for example by neighbouring cells reverting from a specialised fate to stem type to replace a deleted stem cell.

Vertex dynamic frameworks provide an alternative to the CC modelling approach. A cell is defined by a series of moving points at its boundary and free energy at these vertices is quantified through terms for deformation, surface tension and intercellular adhesion [96]. Cell vertices move so as to minimise this free energy function. The polygonal tessellations generated by the vertex approach seem to replicate the packing arrangement of the intestinal crypt. A direct comparison between the cell-centre, vertex dynamic and continuum approaches for the crypt is presented in [71]: owing to its multiscale nature and treatment of tumour growth, we defer its discussion to Section 5.

3.4 Remarks

Mathematical and computational models for the intestinal crypt epithelium have allowed us to explore the mechanisms of crypt homeostasis, facets beyond the scope of statistical analyses. Many models in this vast array can apparently capture the basic behaviour of the crypt, such as transit times or monoclonal conversion, if properly parametrised. It is in coupling tissue-level behaviour with subcellular biochemistry that the interesting discussion lies: we therefore defer a fuller comparison until Section 5. Nonetheless, so-called 'model artefacts' must be borne in mind when analysing emergent behaviour: to what extent is the observed output contingent on the choice of modelling framework? If we are to draw conclusions from the output of a model, we must understand its limitations, and the comparative studies already discussed here provide a valuable contribution to the literature.

Many of the Hallmarks of Cancer can be studied using some form of tissue or cell-level model, particularly those relating to cell birth or death; for example, the mathematical models of Sections 3.1 and 3.2, and the mathematical/computational approaches of Section 3.3 frequently examine how *proliferative potential* and *apoptotic resistance* may affect crypt homeostasis. However, such behaviours as *genome instability and mutation*, *reprogramming intracellular circuitry* and *reprogramming energy metabolism* require subcellular detail in the form of biochemical or genetic networks, and we shall explore this in Section 4.

The continued success of mathematical and computational modelling will also require active involvement of experimentalists: for testing a model's predictions, providing accurately quantified parameters and ultimately for translating the model's results into further experimental enquiry. Numerous papers encountered here underline the need for readily available experimental data. Closer association between the biological and mathematical communities, as outlined in Figure 3, would undoubtedly assuage these concerns and produce the experimentally validated models so vital in guiding further enquiry, whether in the laboratory, on paper or on the computer.

4 Subcellular Influences in Colorectal Cancer

Biochemical dysregulation is the first consequence of genetic mutation, and the means by which these aberrations propagate to the cellular scale. The Hallmarks reflect this, describing the '*programming of Hallmark capabilities via intercellular circuitry*' [6] as well as the enabling characteristic, '*genome instability and mutation*' [7]. Genetic studies in the early 1990s postulated sequential mutation as a causal factor in the development of CRC, the so-called *linear* model of carcinogenesis [44]. Nowadays the idea of a universal sequence of consecutive mutations is less popular, although a distinction has been drawn between active *driver* mutations and the coexisting, passive *passenger* mutations, identified through extensive sequencing of cancer genomes [97] and inspiring various mathematical interpretations [98]. We now focus on the key biochemical pathways involved, their crosstalk and corresponding mathematical descriptions and explain how their dysregulation can give rise to some of the other Hallmarks of Cancer. Simplified depictions of all the pathways and their interactions are displayed in Figure 5.

4.1 Mutations and Mechanisms Leading to Cancer

The Wnt pathway, or at least its canonical form, governs proliferation and cellular adhesion [99]. Sufficiently high concentrations of extracellular Wnt inhibit the intracellular β-catenin destruction complex (APC/Axin/GSK3), allowing cytoplasmic β-catenin to translocate to the nucleus and upregulate Wnt target genes. These include: Lgr5, the stem cell marker [21]; cyclin D, required in the cell cycle [100] and c-myc, a cell growth regulator [101]; downregulation of Hath1, involved in Notch signalling [102], is also observed. Wnt signalling contributes to several key Hallmarks, namely *sustaining proliferative signalling* and *enabling replicative immortality* through its disruption of mitosis, and *activating invasion and metastasis* by its role in cell adhesion. The pathway has also been implicated in *resisting cell death* [103].

Wnt's proliferative control can be artifically upregulated *in vitro* (typically via the Wnt agonist R-Spondin1) to induce crypt hyperproliferation, as in the *de novo* synthesis experiments of Ootani *et al.* [23]. Neoplastic cells frequently display 'addiction' to Wnt signalling [104] and Lgr5 expression, associated with Wnt pathway activity, is upregulated in colorectal tumour tissue [105]. An intricate pattern of cellular control is emerging as understanding increases of the interactions of the Wnt pathway with other networks, such as Notch [106] and EGF [107].

Mathematical modelling has aided our understanding of Wnt signalling, the ODE model of [108] shifting experimental focus towards the stability of Axin in the β-catenin destruction complex [109]. Asymptotic analysis has since revealed conditions under which this model may be reduced to a single equation for β-catenin [110], capturing the long-term dynamics of the system. Alternative models have examined Wnt's role in cell-cell adhesion [90] and the consequences of mutation in the APC subunit of the β-catenin destruction complex [111]. A Wnt signalling model [89] has been embedded into a discrete model of the crypt, allowing the consequences for spatial models of crypt homeostasis to be investigated [88].

The Notch pathway regulates cell development through *lineage decisions* at differentiation, *lateral inhibition* amplifying differences between neighbouring cells and *inductive signalling* segregating distinct cell populations. The first event in Notch signalling is the binding of a Delta or Jagged ligand on a signalling cell to a Notch ligand on an adjacent cell (juxtacrine signalling). The Notch ligand splits up (shown by a star in Figure 5), releasing into the signal-receiving cell a fragment which translocates to the nucleus, upregulates Notch and reduces expression of Delta and Jagged. Hes1 is also upregulated, causing crypt epithelial cells to adopt an absorptive phenotype. The Notch pathway influences the distribution of cell types within the crypt epithelium (reviewed in [112, 113]). Notch activation is believed to be a vital step in CRC initiation [106] and its target genes can interact with many other pathways, including Wnt. The protein $\beta-$catenin (shown in Figure 5) acts as a hub linking the Wnt and Notch pathways, serving to upregulate expression of the Notch ligand and transcription of Hes1, which induces cells to adopt an absorptive phenotype [114, 115]. Consequently, the Notch pathway enhances the *intercellular circuitry* required for tumourigenesis.

The multiscale nature of Notch transduction is amenable to mathematical and computational modelling at multiple scales, whether studying cell-cell interactions [116] or the subtleties of gene regulation [117, 118]. The next step in Notch modelling is to link cell fate specification with juxtacrine signalling mechanisms. More recently, simplified models of Notch and Wnt signalling have been incorporated into a cell-based model of the colorectal crypt [20].

The NF-κB pathway is involved in growth, apoptosis and the inflammatory cell response. NF-κB refers to a family of transcription factors, which become activated through molecular binding [119]. Extracellular signals from cytokines and growth factors stimulate phosphorylation of NF-κB subunits by the kinase IKK. The phosphorylated NF-κB translocates to the nucleus to upregulate target genes such as XIAP, an apoptosis inhibitor [120]; IL-6, an inflammatory cytokine [121]; and the proliferative factor Wnt [122]. Negative feedback loops within the healthy NF-κB system are instrumental in regulating the length of its response to stimuli

via time delays and molecular clocks [123]. Overstimulation of the IKK cascade is frequently observed in CRC, often as a result of crosstalk with the Ras-MAPK and PI3K/Akt pathways [124]. Viral-induced activation of the IKK complex is also acknowledged [125]. NF-κB therefore contributes to several Hallmarks, via its role in *sustaining proliferative signalling* [119] and its control of *tumour-promoting inflammation* [126]. It is also associated with apoptosis [127] and therefore aids in *resisting cell death* when dysregulated in CRC.

Ashall *et al.* subject NF-κB to pulsatile stimulation *in vitro* to mimic inflammation signals and measure translocation of NF-κB to the nucleus [128]. This output is used to parametrise a system of ODEs, the resultant model showing that the oscillation frequency of the stimulus can have a significant impact on the magnitude of the transcriptional response. Paszek *et al.* extend this work to explore negative feedback motifs in the NF-κB pathway [129].

The PI3K/Akt pathway relies upon the phosphorylation of PI3Ks, a family of lipid kinases. It effects survival signalling and cell proliferation and is a hub of signal transduction in CRC [130]. Tyrosine kinase receptors at the cell surface (RTKs) induce phosphorylation of PI3K; this recruits the kinase Akt, which translocates to the nucleus, upregulating genes for cell proliferation and survival (reviewed in [131]). The pathway is implicated in several Hallmarks, including *proliferative signalling*, *activating invasion and metastasis* and *resisting cell death*. Artificial inhibition of the pathway in CRC cell lines induces differentiation, characterised by a more flattened cell morphology and loss of CD133+ [132]. Bao *et al.* [133] propose a metastatic role for PI3K signalling, through stimulation of the Akt section of the pathway. Its contribution to *intracellular circuitry* is evident from Figure 5, featuring links to the $\beta-$catenin sequestering complex via GSK3, and to NF-κB via AKT-mediated regulation of IKK and transcriptional downregulation of PTEN. Possible links with mitochondrial function may also provide a route into *reprogramming energy metabolism* [134].

Several authors have developed mathematical models for the pathway, some using ODEs [135] and others using Bayesian network analysis [136]. Bhalla *et al.* parametrise their ODE model against data from mouse fibroblasts [137]. Other mathematical representations are listed in [134].

The TGF-β pathway has two branches, namely Smad-dependent and Smad-independent signalling. Phosphorylation of Smad occurs in the first of these when extracellular TGF-β ligands bind to membrane receptors; Smad translocates to the nucleus, inducing gene targets which can up- or downregulate mitosis and modify the composition of the extracellular matrix [138]. Smad-independent signalling from TGF-β also modulates apoptotic processes through its interaction with the MAPK pathway, as in Figure 5 [139]. Conjectured links with the NF-κB pathway [140] are consistent with observed correlations between TGF-β activity and inflammatory response [141]. The pathway can exhibit either pro- or anti-oncogenic activity [142] and contributes to *resisting cell death* and *sustaining proliferative signalling*. High levels of TGF activity facilitate *tissue invasion and metastasis* [143] and are thought to assist in *inducing angiogenesis* [144]. Disruption to TGF signalling in CRC is often due to mutated membrane receptors [145].

Existing mathematical models have examined the response of Smad to TGF signalling [146, 147] or focused on the receptor dynamics [148], although [149] attempts to unite these perspectives.

The Ras-MAPK pathway is associated with cell differentiation and growth. Extracellular Growth Factors (EGFs) bind to a receptor on the cell surface membrane, which recruits a complex comprising various signalling proteins (including Sos and Grb2) which assist in the phosphorylation of Ras. This induces a series of kinase phosphorylations involving Raf, MEK and ERK/MAPK [124, 150, 151]. The ERK protein kinases then relocate to the nucleus, where they induce chromatin remodelling and transcription of target genes [152]. Oncogenic mutation of Ras genes is frequently implicated in CRC; Jiang *et al.* note the occurrence of K-Ras and

B-Raf mutation in CRC tissue [153]. Current approaches to the problem include inhibiting post-translational modification of Ras to block the initiation of the signalling cascade, and the use of kinase inhibitors to block the MEK/ERK part of the pathway [154]. Ras-MAPK contributes to the Hallmarks of *sustaining proliferative signalling* and *enabling replicative immortality*.

A number of mathematical models for MAPK interaction exist in the literature, many of them ODE based [155, 156, 157]. These approaches usually focus on either the membrane activity or the transcriptional function, given the complexity of the interactions involved; an excellent overview is given in [158].

4.2 Mathematical Approaches to Mutation

Many of the pathway models described in Section 4.1 involve systems of nonlinear coupled ODEs; however, this is not the only possible approach. Bayesian network analysis has been applied to colon cancer data, identifying mutations in Smad (involved in TGF signalling) as a key event in CRC progression [159]; the method has also been used to explore the interactions of the Ras-MAPK pathway [136], for which a signalling logic model has also been proposed [160].

Statistical models provide a means of exploring the probability of various sequences of mutations. For example, Michor *et al.* calculate the probability of a cell acquiring an APC mutation (involved in Wnt signalling) and remaining in the crypt, as a function of the time at which chromosomal instability occurs [161]. Multiple genetic 'hits' have also been explored in stochastic compartment-style models [162, 163]. Multistage statistical knockout models of CRC mutation have been fitted to population data on CRC incidence [164, 165], a useful extension of the investigations of Armitage and Doll [60].

Many models of mutation focus upon one spatial scale, where the consequences of a smaller scale are implicit via a labelling facet, perhaps a simple type tag on cells. This avoids the burden of reconciling disparate scales, at the expense of biochemical detail.

Delitala and Lorenzi [166] extend Tomlinson's compartment framework [62] to account for sequential mutations to crypt cells. Mutations are accounted for in terms of cell-level changes rather than through explicit genetic control. A discrete distribution function, $f_i^r(t)$, characterises the degree of dysplasia in a given compartment, indicating the number of cells in compartment i residing in mutation stage r at time t.

Stability analysis of equilibrium states favours dysregulation of cell birth and death as a key factor in tumourigenesis, more so than acquisition of mutations. In accordance with the results of [62, 64], the onset of mutation within the stem cell compartment may prove a more powerful route to oncogenesis than mutation in the transit amplifying region. However, the stochastic knockout studies of Komarova *et al.* [162, 167, 163] indicate that the actual site of the genetic hits are likely to differ, with one in the stem and one in the transit compartment, rather than both mutations hitting the stem population.

The assumption of well-mixedness implicit in such models limits their usefulness for addressing questions of crypt clonogenesis and local environmental influence, given the strong associations between location and function evident in the tissue.

Computational evolutionary simulations have been used to study the sequential acquisition of genetic mutations underpinning cancer. Attolini *et al.* [168] present the RESIC (Retracing the Evolutionary Steps in Cancer) method for identifying the likely gene trajectory of colorectal cancer, based upon the traditional linear model of [44]. An initially healthy cell population experiences sequential genetic catastrophe as randomly selected cells divide, mutation guided by transition probabilities obtained from statistical analysis of histological samples of human colorectal tumours. A randomised cell death process maintains a constant cell population, and so a mutant lineage may persist and dominate, or die out.

The resulting simulations suggest that colorectal tumourigenesis first involves monozygous APC loss, followed by at least one KRAS mutation and then TP53 inactivation. Criticism has been levelled at the RESIC approach [169], on the grounds that it negates the possibility of polyclonal

tumour formation [170] and presumes that rogue genotypes are retained throughout tumourigenesis. Furthermore, cooperation between distinct mutants in early-stage oncogenesis may cause an initiating aberration to die and therefore not appear in late-stage tissue samples [171]. Nonetheless, the framework could be extended to such cases [172]. One option would be to embed the RESIC approach in a multiscale cell-based model, providing the cellular detail necessary to study polyclonality of colorectal tumours.

4.3 Conclusions

The diverse subcellular underpinnings of colorectal cancer have generated a great deal of informative work in experimental and theoretical disciplines, and provide many routes into examining the origins of the Hallmarks of Cancer. Computational perspectives on cancer biochemistry fall broadly into one of two categories: the stochastic knockout models, and explicit network representations. Protein networks are complex; mathematical modelling requires us to examine key motifs and interactions rather than the entire network. Crosstalk between the pathways of interest, as emphasised in Figure 5, will become increasingly important as our mathematical models become more sophisticated. Bayesian networks can indicate the influence of 'hidden molecules' in a network [136] and direct future biological enquiry. Development of suitable online resources and markup languages will assist in sharing the vast quantity of information from experiment and simulation, facilitating model reuse and validation. Significant progress has already been made through facilities such as the KEGG pathway database [173] and associated format translation software [174].

Although genetic mutation directly affects the biochemical functioning of the cell, it is at cell and tissue level that the now familiar Hallmarks of Cancer become apparent. We have seen how mathematical models for the crypt can be modified to incorporate simple mutated phenotypes [166]; however, explicit inclusion of biochemical networks demands careful consideration of the spatiotemporal scales involved. Processes acting at larger scales typically occur over longer times. Their reconciliation with the relevant biochemical processes requires the development of multiscale models which unite the time and length scales of interest.

5 Multiscale Modelling of Colorectal Cancer

Understanding the interplay between the various Hallmarks of Cancer raises challenges for both experimentalists and theoreticians, because the biochemical foundations for oncogenesis, some of which have been explored in Section 4, ultimately present at cell and tissue scales of interest. How, then, is one to predict the effect a genetic mutation will have upon the tissue as a whole? How are we to characterise multiple mutations? Such questions can be addressed mathematically through *multiscale* modelling.

5.1 Multiscale Models and the Subcellular Level

Reconcilation of disparate spatiotemporal scales in biological systems represents a serious challenge to the mathematical modeller, but represents a powerful tool in exploring the interplay between Hallmarks. The *sustained proliferative signalling* of CRC cells suggests that cell-cycle control should feature strongly in many multiscale models for cancer. Incorporation of a cell-cycle model therefore represents a natural way to couple a cell-based model to the underlying biochemistry; such models range from the simple fixed-length models used in [87] to the seminal Cdh1/CycB yeast cycle of [175] and the G1/S phase model of [89]. The inclusion of biochemically-governed apoptosis offers another avenue for multiscale development. Section 3 examined mathematical models for the healthy crypt, yielding insights into monoclonal conversion [91], spatial proliferation cues [88] and the influences of Notch and Wnt signalling on crypt organisation [20]; each of these offers a useful perspective on multiscale modelling. In this section, we examine how multiscale approaches have helped us to investigate the effects of mutation upon such models.

The frameworks discussed in Section 3 differ considerably in their representations of cell movement in living tissue. The influence of such nuances upon the observed output have been studied by Osborne *et al.* [71], who compare three different strategies for modelling tumourigenesis in the colorectal epithelium. Cell-centre, cell-vertex and continuum models are applied to a two-dimensional, cylindrical crypt domain, containing a circular region of neoplastic cells. Cells are assumed to proliferate in regions where Wnt levels exceed a given threshold and the extracellular Wnt concentration is presumed to decrease from the crypt base to the lumen. The continuum model relies on a predefined proliferation rate. However, the cell-based nature of the discrete models allows each cell to be given an individual cell-cycle model, in which phase lengths are chosen from a normal distribution. In [71] a mutant phenotype is defined for the discrete models, where cells possess different proliferative and adhesive behaviours. Changes in the position at which the mutant cells are initiated, and the strength of their cell-cell adhesion, affects their ability to dominate the crypt.

Comparison of homeostatic crypts across the three frameworks in [71] yields qualitatively similar velocity profiles, although the continuum model yields higher vertical cell velocities than its discrete counterparts owing to its neglect of cell compression and the lateral displacement of cells found in the cell-based models. Unpinning the stem cells from the crypt base in the discrete models allows a monoclonal crypt to develop, in line with experimental findings. The direction of tumour invasion is also explored by varying the drag (adhesion) of mutant cells. A higher drag coefficient helps mutant cells to resist forces from proliferating cells beneath them, allowing the colony to proliferate and persist for longer in the crypt. In certain cases the colony initially ascends before reversing direction and descending into the crypt. This observation is peculiar to the cell-based models, which allow the colony to attain a critical mass capable of displacing the healthy cells beneath it.

Osborne *et al.* [71] demonstrate that particular representations of tissue impose subtle differences upon the observed output; for example, a continuum approach may not be appropriate if cell numbers are small. Discretised perspectives may prove useful for tracking early tumour development, while continuum approximations are useful in the long term. Much of the experimentally observed crypt behaviour emerges irrespective of the chosen modelling framework, although the influence of mechanical constraints upon cell proliferation and movement varies between models. Experimental quantification of these aspects would prove beneficial if modelling approaches are to be appropriately selected for purpose.

Mirams *et al.* [92] extend the cell-centre based simulations of van Leeuwen *et al.* [88] to study how the adhesion and location of mutant cells affects the capacity of a rogue progeny to dominate the crypt. As before, spatial proliferation cues are assumed via a Wnt gradient up the crypt; cells are endowed with a simple, stochastic cell cycle model; and mutant cells have a reduced adhesion and cell-cycle time, the latter realised through a variable threshold for proliferative cut-off. Results indicate that an ancestor cell need not remain in the crypt for the monoclonal conversion process to occur.

Cell-cycle modelling allows cells to be studied as individual agents, responding to changes in their local environment at varying rates, and opens up possibilities for exploring detailed feedback loops between different spatial scales. However, there exist other ways of including subcellular detail in cell-based models. For instance, the model presented in [20] eschews explicit cell-cycle modelling in favour of a simpler approach, where a cell's Notch activity is either high or low and is influenced by neighbouring cell states and separate differentiated cell types (secretory, absorptive) are included. Wnt activity varies with the local curvature of the basement membrane and cells are undifferentiated if they have high Wnt and high Notch levels; cells differentiate on attaining a low-Wnt state, their cell fate determined by their current Notch status. Functional mutants have the ability to override one or more of these activity thresholds. The use of basic high-low Notch switching allows for whole-crypt simulations involving mutants that are exclusively 'high-Notch' or 'low-Notch'. For example, universal high-Notch conditions produce a crypt devoid of secretory cells; a universal low-Notch crypt loses all undifferentiated and absorptive cells and consists only of Paneth and secretory cells.

We have seen how models such as [20] and [88] differ in their treatment of intercellular forces and how they incorporate proliferation and cell death. In [20] cells only respond to an external cue, while in [88] the subcellular reactions that govern proliferation in each cell are explicitly represented.

Under this latter model, either external cues or cellular maturation could play a part in cell fate selection. Explicit cell-cycle embedding would also permit the study of time delays in biochemical feedback if desired, which a simpler approach would not allow. Regarding apoptosis, in [88] cells die automatically on reaching the top of the crypt [88], whereas in [20] they die on detachment from the basement membrane. It would be interesting to vary the apoptotic site in both these cases and examine how robust the simulation results are in the face of such changes.

Multiscale methods are not confined to discrete spatial models; we now explore a continuum example. Murray *et al.* [19] use a continuum approach to explore how spatially varying morphogen gradients influence cell density in the colorectal crypt. The model comprises a one-dimensional partial differential equation for cell density, incorporating a 1D continuum approximation of the spring based mechanical model from [88] to describe cell movement up the crypt. Here, multiscale methods are used to characterise cell proliferation, by reconciling subcellular scale information with the local tissue environment. An external Wnt gradient is defined throughout the domain, exponentially decaying up the crypt axis, and is coupled to the cellular proliferative response by incorporating the subcellular Wnt model [110] into the PDE. The model can predict the likely β-catenin distribution up the crypt from a given external Wnt gradient, by tracking the rate of change local $\beta-$catenin. Under this model, cells proliferate if their local Wnt concentration exceeds a threshold and if the local cell density is sufficiently low. Apoptosis is presumed to occur at the crypt mouth only. Mutations are simulated through parameter modification in the underlying Wnt model [110], such that mutants exhibit a reduction in β-catenin degradation and can proliferate in lower Wnt conditions than healthy cells.

Intercrypt invasion on a periodic domain provides insights into the spread of malignancies, by tracking densities of mutant and healthy cells. Colonisation of neighbouring crypts is predicted when the mutant population has both a proliferative advantage and an ability to evade apoptosis. A proliferative advantage alone is not enough, as mutant cells cannot achieve a sufficiently high velocity to invade neighbouring crypts. It would be interesting to introduce apoptosis in lower regions of the crypt and examine how robust mutant colonisation is to variation in the sites of apoptosis.

5.2 Multiscale Models: what can we learn from them?

The multiscale models discussed in this section illustrate how mutations in the colorectal epithelium can be modelled. They also: highlight the need for consistent parametrisation; demonstrate the potential to study intricate feedback mechanisms and their effect on multiple scales of function; and motivate a future focus on the interplay between, and timing of, genetic aberrations. Pharmaceutical-based research may drive further development of such models, especially given the demand for *in silico* predictive tools to facilitate target selection in drug discovery and thus improve the success of the clinical trial phase [176].

Recent shifts toward multiscale modelling place further onus on model validation. Knowledge of the reaction rates and timescales involved is a prerequisite when attempting to link processes that operate at or between different spatial levels, yet in many cases data for any one species or cell line is incomplete. For example, the multiscale coupling used in [19] relies upon parameters from a Wnt model obtained from experiments on *Xenopus* oocytes [108], while the mutation parameters are supplied from a computational study [111]. Patient-calibrated studies have been performed for agent-based models of ductal carcinoma of the breast [177] and it would be encouraging to see similar work performed for CRC.

Multiscale models for cytoplasmic force transduction are also being developed, such as the subcellular element model of Newman *et al.* [178] and the tensegrity model of Sultan *et al.* [179]. Emphasis is on the mechanics of the cytoskeleton rather than on the biochemical function of the cell, although possible routes for multiscale linking have been identified [180]. While these methods have yet to be applied to colorectal tissue, the potential for exploring crypt mechanics via force transduction at the subcellular level offers new possibilities for multiscale studies of buckling and fission.

Moreover, Hanahan and Weinberg's revised Hallmarks of Cancer encompass the genome, biochemical and cellular scales, emphasising the need for a multiscale, rather than a reductionist, approach to modelling.

6 Discussion

The disruption CRC causes at cell and tissue level, and the attendant complexity of its underlying genetics, offers much potential as a candidate problem for modern biomedical science, within the wider context of understanding oncogenesis and cancer treatment as a whole. In seeking to understand colorectal cancer, we must also address fundamental questions of cell behaviour within healthy tissue. For example, investigation of homeostasis in the crypt has identified neutral drift dynamics; *de novo* crypt synthesis has challenged traditional notions of stemness. The pursuit of understanding CRC has produced a broad canon of work, as is evident from the variety of discoveries discussed in Section 1 of this review.

Mathematical and computational models for CRC hold great potential for building *in silico* predictive tools, whether for tissue engineering applications or for drug target selection. Advances in imaging technology have permitted experimentalists the most detailed view yet of crypt structure, albeit a static one; it would seem expedient to develop computational frameworks to the point where dynamic simulation of the tissue is possible, able to be used in tandem with experimental work to explore hypotheses and guide subsequent *in vitro* studies. Such progress will require close collaboration between the biological community and their mathematical counterparts if the models are to have clinical relevance.

Many of the recent mathematical and computational papers discussed in Sections 3 and 5 present as yet untested predictions relating to homeostasis or oncogenesis. Experimental testing to confirm or refute such predictions would provide a firm basis for further biological enquiry *or* indicate shortcomings in current mathematical models. For example, Buske *et al.* [20] suggest the existence of flexible fate decisions in the crypt and surmise that any single subpopulation of cells can be deleted without long term consequences for crypt organisation. Mirams *et al.* [92] indicate that monoclonal conversion processes can occur without the originating cell remaining in the crypt. Verification of these ideas could give us valuable insights that will guide us to improved tumour treatment.

The *in vitro* genesis of crypt megacolonies as described in Section 2.1 [23, 22] provides a good starting point for the task of parametrisation, and even for *in vitro* testing of the above predictions. *De novo* cryptogenesis is beginning to elucidate the emergence of cell type from initially undifferentiated cells and their self-assembly into a coherent, functional crypt structure. Experiments on these colonies to determine the structure of the major governing pathways would greatly benefit biochemical modelling if these data were fed into mathematical models, for the purpose of validation and parametrisation. Presently most experimental results focus upon qualitative assessment of the megacolonies; parametrisation of mathematical models would help to establish a quantified basis for the observed behaviour. Various colony manipulations could also be performed, such as varying the composition of the gel to explore adhesive constraints, or by introducing specific cell types (e.g. Paneth cells, fibroblasts) at the initial plating and exploring the effect on the emergent cell types.

The diverse behaviour evinced by the Hallmarks of Cancer emphasises the need for a variety of experimental and mathematical approaches, and care in the selection of modelling framework. The interplay of Hallmarks at the genetic, biochemical, cell and tissue levels of interest will become more apparent as we develop our awareness of this complex disease and pursuit of a multiscale picture of CRC may well reveal further Hallmarks.

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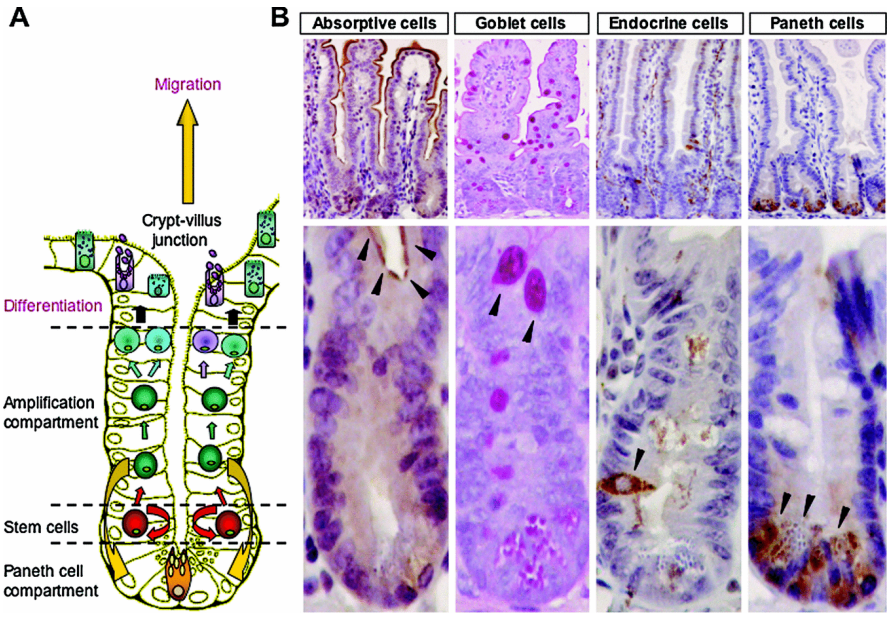

Figure 1: (A) Schematic of a crypt from the small intestine, reproduced with permission from Pinto & Clevers (2005) [181]. (B) L-to-R, the stained sections indicate: absorptive, Goblet, endocrine and Paneth cells respectively. Paneth cells only occur in the small intestine and are therefore not discussed in this review.

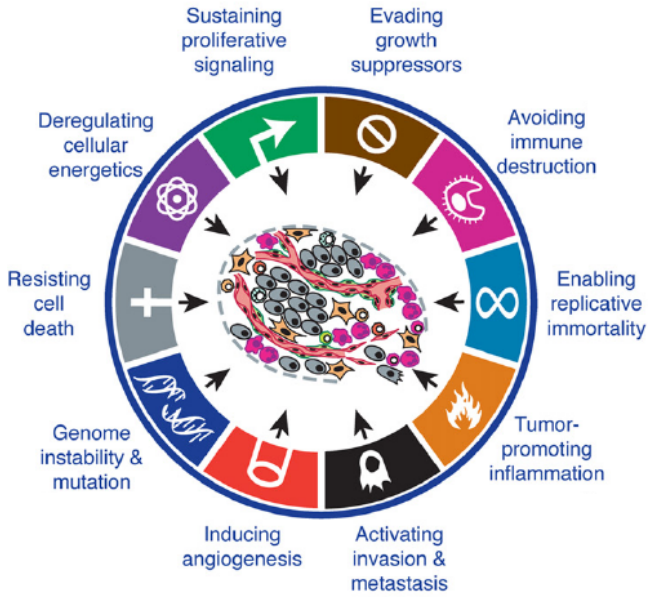

Figure 2: Schematic of Hanahan and Weinberg's *Hallmarks of Cancer*, adapted by permission from Hanahan & Weinberg (2011) [7].

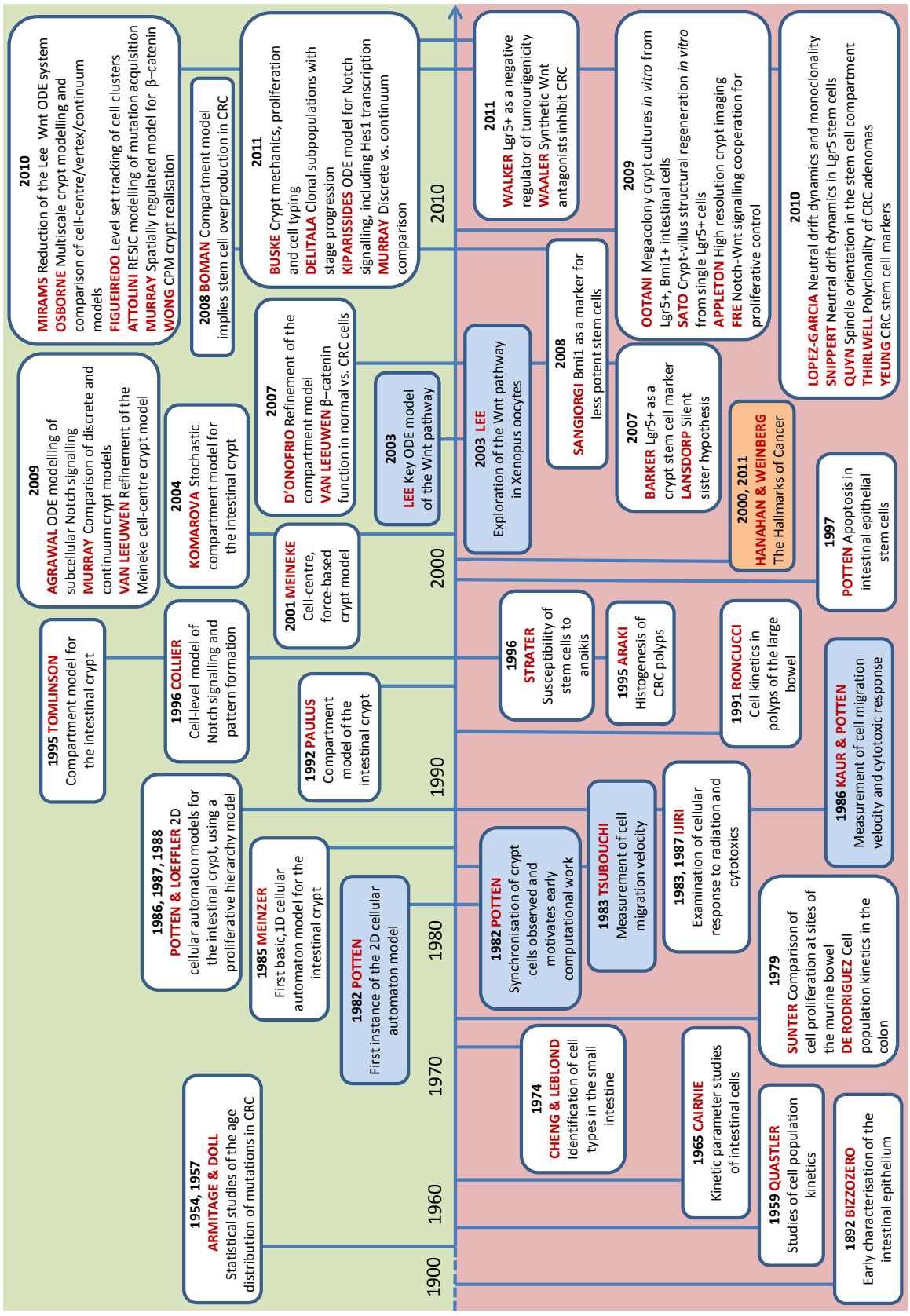

Figure 3: Timeline of experimental development (lower chart) against corresponding computational results (upper). Crossover papers which combine mathematical and theoretical work, or which supply data for parametrisation of mathematical simulations, are shown in blue. For brevity we use only the names of the first author(s) here; the timeline is not drawn to scale.

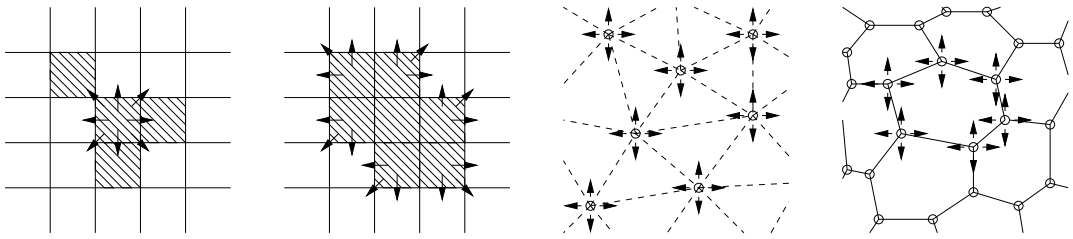

Figure 4: Typical cell constructions for various cell-based frameworks. L-to-R: cellular automaton; cellular Potts; cell-centre; cell vertex models. The latter two are reproduced with permission from Osborne *et al.* [71].

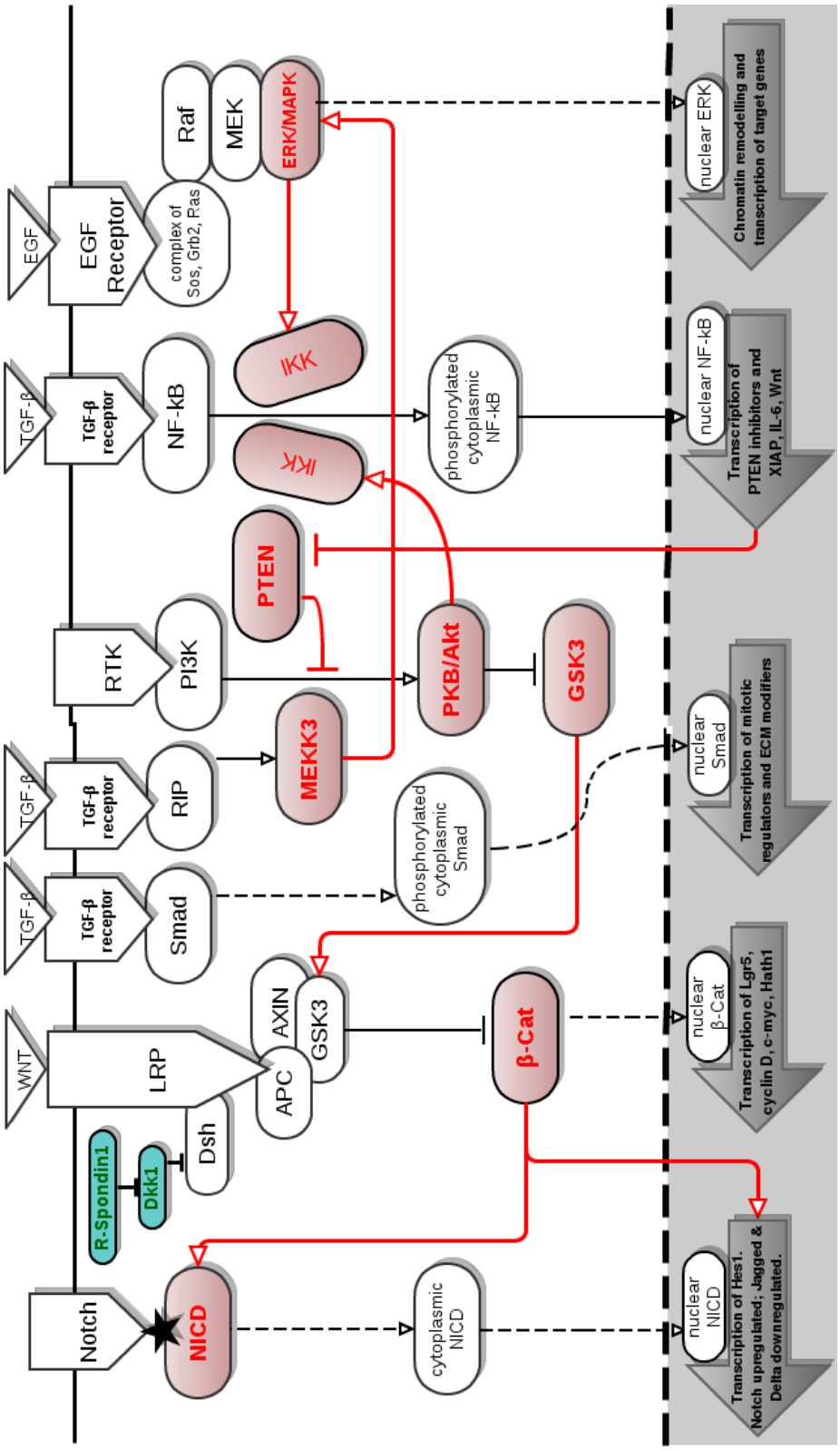

Figure 5: Simplified schematic of the subcellular pathways implicated in CRC, as described in Section 4.1. L-to-R: Notch, Wnt, TGF-β, PI3K, NF-κB, Ras-MAPK. Red proteins indicate principal crosstalk hubs; entities in green are regulators of the Wnt pathway used in [22]. The star indicates a molecular cleavage event which releases the NICD fragment into the cytoplasm.