Elucidating the interactions between the adhesive and transcriptional functions of β-catenin in normal and cancerous cells

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Abstract

Wnt signalling is involved in a wide range of physiological and pathological processes. The presence of an extracellular Wnt stimulus induces cytoplasmic stabilisation and nuclear translocation of β-catenin, a protein that also plays an essential role in cadherin-mediated adhesion. Two main hypotheses have been proposed concerning the balance between β-catenin's adhesive and transcriptional functions: either β-catenin's fate is determined by competition between its binding partners, or Wnt induces folding of β-catenin into a conformation allocated preferentially to transcription. The experimental data supporting each hypotheses remain inconclusive. In this paper we present a new mathematical model of the Wnt pathway that incorporates β-catenin's dual function. We use this model to carry out a series of *in silico* experiments and compare the behaviour of systems governed by each hypothesis. Our analytical results and model simulations provide further insight into the current understanding of Wnt signalling and, in particular, reveal differences in the response of the two modes of interaction between adhesion and signalling in certain *in silico* settings. We also exploit our model to investigate the impact of the mutations most commonly observed in human colorectal cancer. Simulations show that the amount of functional APC required to maintain a normal phenotype increases with increasing strength of the Wnt signal, a result which illustrates that the environment can substantially influence both tumour initiation and phenotype.

Key words: adherens junction, colorectal cancer, APC mutation, crypt dynamics

1 Introduction

The extensively studied Wingless/Int (Wnt) signalling pathway regulates the expression of target genes involved in fundamental cellular processes, such as cell proliferation, differentiation, migration and death. It plays a central role in, for instance, the coordination of embryonic development (Brennan and Brown, 2004; Kielman et al., 2002), the renewal of the adult intestinal epithelium (Batlle et al., 2002; Es et al., 2005; Gaspar and Fodde, 2004) and the maintenance of stem cell niches (Korinek et al., 1998; Staal, 2005; Van de Wetering et al., 2002). The importance of Wnt signalling is also reflected in the serious consequences of its dysfunction. Pathologies that have been linked to Wnt signalling include Alzheimer's disease (Chevallier et al., 2005), retinal degeneration (Hackman, 2005), osteoporosis (Koay and Brown, 2005; Westendorf et al., 2004) and various human cancers (Giles et al., 2003; Ilyas, 2005). In particular, genetic alterations affecting components of the Wnt pathway are responsible for over 90% of colorectal cancers (Kinzler and Vogelstein, 1996; Radtke and Clevers, 2005).

Wnt signalling regulates gene expression by controlling β-catenin levels. In the ab-

sence of a Wnt-signal ('off' state), cytoplasmic β-catenin is rapidly marked for degradation by a protein complex that includes APC. In contrast, when extracellular Wnt-factors bind to surface receptors ('on' state), the activity of the complex is inhibited and, consequently, unphosphorylated β-catenin accumulates in the cytoplasm. It is then also able to enter to the nucleus, where it binds to transcription factors from the TCF/LEF family and eventually induces the expression of numerous Wnt target genes. Wnt-mediated malignant transformation results from genetic alterations that mimic a continuous Wnt signal and, thereby, cause an aberrant accumulation of β-catenin (Behrens, 2005).

β-catenin is a multifunctional protein that is essential not only for Wnt signalling but also for cell–cell adhesion. At the cell membrane, it binds to the cytoplasmic domain of E-cadherin, which constitutes the primary component of adherens junctions (Jamora and Fuchs, 2002; Perez-Moreno et al., 2003). Although β-catenin's adhesive and transcriptional functions have long been known (Nusslein-Volhard and Wieschaus, 1980; Ozawa, 1989), the mechanisms by which it is allocated to a particular activity remain unclear (Bienz, 2005; Harris and Peifer, 2005; Nelson and Nusse, 2004). It has been proposed that components of the two pathways simply compete for β-catenin binding. However, it has also been hypothesised that β-catenin exists in two molecular forms with different binding affinities (Gottardi and Gumbiner, 2004). Different experimental studies support each hypothesis and an encompassing mechanism remains to be elucidated. In this article, we use mathematical modelling to investigate the various hypotheses concerning the interactions between Wnt signalling and cell–cell adhesion.

Theoretical models constitute an important tool for understanding biochemical pathways. While the classic Michaelis–Menten equation is extensively applied to single–enzyme kinetics, more complex mathematical models are required to analyse larger biological systems, such as the cell–cycle regulatory network (Novák and Tyson, 2004; Swat et al., 2004) and the regulation of ATP kinetics in muscle (Jeneson et al., 2000). Lee and co-workers have recently presented a mathematical model of the Wnt signalling pathway to investigate the importance of axin, a component of the destruction complex, in the transduction of the Wnt signal (Krüger and Heinrich, 2004; Lee et al., 2003). Although this model is based on a detailed reaction scheme, it does not account for the dual role of β-catenin. We have therefore developed a new mathematical model for the Wnt signalling pathway that incorporates cadherin-mediated adhesion and accounts for the proposed mechanisms of interaction between β-catenin's adhesive and transcriptional functions.

It is generally acknowledged that, in developing models of complex biochemical networks, the values of the model parameters constitute a major source of uncertainty. For most biochemical networks, including the Wnt pathway, kinetic coefficients and concentrations of molecular components are largely unknown. Some parameter values can simply not be measured with the experimental techniques available today, whereas others include measurement errors, which might have a significant impact on

the system's behaviour. Consequently, extrapolation of model predictions based on parameter values measured in a particular species or cell line (e.g. Lee et al. (2003)'s model is parameterised with data from *Xenopus* extracts) to another should be regarded with extreme caution (e.g. Cho et al. (2006) draw conclusions about human colorectal cancer from Lee et al. (2003)'s model). Advantages of mathematical modelling are that even when reliable parameter values are unavailable it is possible to determine the generic behaviour of the system, to compare potential mechanisms of action, to generate new hypotheses and to identify those parameters which have the most dramatic effect on the system's dynamics and which should, therefore, be determined most accurately.

This article is organised as follows. In the next section, we describe β-catenin's role in cell–cell adhesion and Wnt signalling, concentrating on the surface epithelium of the gut. We then discuss how the switch between its two functions might be regulated. Section 3 is concerned with the development of our mathematical model. We focus on the assumptions that underlie the model, on the derivation of the model equations and on how the two proposed mechanisms of interaction between β-catenin's adhesive and transcriptional functions correspond to specific model parameter values (further technical details are provided in the Appendix). In Section 4 we explain how our Wnt model performs in the presence and absence of a Wnt signal and use the model to predict how different manipulations, such as mutations in the APC and E-cadherin tumour suppressors, affect the system. This enables us to discriminate between the proposed modes of interaction between cell–cell adhesion and signalling. In section 5, we summarise our model predictions and compare them with existing experimental evidence. In particular, based on our model, we predict that with existing data it is not possible to discriminate between the two hypotheses. However, we suggest new experiments that could be performed to resolve this issue.

2 Adhesion and signalling in the gut

2.1 Adherens junctions

The major component of tight lateral anchoring junctions, also known as adherens junctions or *zonula adherens*, is the transmembrane protein E-cadherin (Perez-Moreno et al., 2003). Disulphide-linked E-cadherin homodimers located on the surface of adjacent cells bind to each other in a Ca^{2+}-dependent manner. Inside the cell, the cytoplasmic tail of E-cadherin binds to catenin p120 and β-catenin (Fig. 1). The latter plays an essential role in maintaining the integrity and cohesion of the protective and selective absorption barrier provided by the surface epithelium of the gut. Firstly, as a structural component of adherens junctions, it bridges E-cadherin to α-catenin, which in turn binds to the actin cytoskeleton. Secondly, it regulates the turnover of the adhesion complexes by controlling the transport of new E-cadherin

molecules to the membrane. For reviews on the assembly and function of adherens junctions, see Gumbiner (2000), Perez-Moreno et al. (2003) and Tepass (2002).

The stability of the cadherin complexes is regulated by phosphorylation (Nelson and Nusse, 2004). Of particular interest is tyrosine phosphorylation of β-catenin, leading to dissociation of the complex. Hence, phosphorylation of Tyr-142 (Brembeck et al., 2004) and Tyr-654 (Roura et al., 1999) impedes association of β-catenin with α-catenin and E-cadherin, respectively. Interestingly, the latter phosphorylation event can be induced by c-Src, a non-receptor tyrosine kinase that is believed to play an oncogenic role in a variety of human cancers (Russello and Shore, 2004). Cadherin-mediated adhesion is further controlled via transcriptional repression of E-cadherin by proteins of the Slug/Snail family (Conacci-Sorrell et al., 2003).

2.2 Canonical Wnt signalling

Wnt signalling plays an essential role in the coordination of the continuous self-renewal of the intestinal surface epithelium, including the maintenance of stem cell niches (Korinek et al., 1998; Van de Wetering et al., 2002) and the control of cell proliferation, migration and differentiation along the crypt axis (Gaspar and Fodde, 2004). Although autocrine production by cancer cells of Wnt factors responsible for the Wnt stimulus has been reported (Bafico et al., 2004), the Wnt source in the normal intestine remains to be identified. A putative source is the mesenchymal cells surrounding the bottom of the crypt (Van de Wetering et al., 2002). In this section we provide an overview of the Wnt signalling pathway (Fig. 2). For more detailed descriptions, we refer the reader to recent reviews (Giles et al., 2003; Ilyas, 2005; Moon et al., 2002) and references therein.

2.2.1 'Off' state

In the absence of a Wnt signal, APC protein, glycogen synthase kinase 3β (GSK3β) and axin assemble into a destruction complex that binds β-catenin. Whereas APC protein and axin act as scaffolds (Kries et al., 2000), GSK3β is responsible for attaching phosphate groups to three specific amino-terminal residues of β-catenin: Thr-41, Ser-37 and Ser-33 (Dajani et al., 2003; Liu et al., 2002). Before this event can take place, a second kinase, casein kinase Iα (CKIα), has to create a priming site by phosphorylating β-catenin at Ser-45 (Liu et al., 2002). The β-catenin mutations detected in cancer lines are often located within the GSK3β-recognition region (residues 32–45) and prevent β-catenin phosphorylation (Ilyas, 1997; Polakis, 1999).

The APC-mediated phosphorylation events mark β-catenin for subsequent ubiquitination: the F-box-containing protein β-TrCP recognises the phosphorylated region and directs the Skp-Cul1-F-box (SCF) system to ubiquitinate β-catenin at specific residues (Wu et al., 2003). The resulting ubiquitinated β-catenin macro-

molecules then undergo rapid degradation by a proteasome system (Aberle et al., 1997; Ciechanover, 1998). In addition, β-catenin is subject to normal proteolytic processes and to phosphorylation-independent ubiquitination/degradation. The latter pathway can be induced by the p53 tumour suppressor (Liu et al., 2001; Matsuzawa and Reed, 2001).

As a result of the activity of the destruction complex, any β-catenin that is not allocated to cadherin-mediated cell–cell adhesion is rapidly degraded. Consequently, no β-catenin enters the nucleus. Under these conditions, the transcriptional repressor Groucho, which binds to the DNA–TCF/LEF complex, ensures that the Wnt target genes are not expressed (Cavalli and Liang, 1998; Daniels and Weis, 2005).

2.2.2 'On' state

Wnt signalling is activated by the presence of extracellular Wnt factors (Miller, 2002), which bind to a receptor (Frizzled) and a coreceptor (LRP5/6) located on the cell surface (Fig. 2). Wnt binding induces, by an unknown mechanism, the activation of CKIϵ (Kishida et al., 2001; Swiatek et al., 2004) and, in addition, Wnt binding to Frizzled results in the hyperphosphorylation of the cytoplasmic protein Dishevelled (Gonzalez-Sancho et al., 2004b; Takada et al., 2005). There is evidence that Frizzled interacts directly with Dishevelled (Wong et al., 2003), but how the activation of the latter takes place remains to be elucidated. Furthermore, in response to the Wnt stimulus, axin translocates to the cell membrane, where binding to LPR5 promotes its degradation (Mao et al., 2001a; Tolwinski and Wieschaus, 2004b). These reactions eventually lead to the inhibition and destabilisation of the destruction complex and, as a result, intracellular levels of β-catenin rise. Genetic alterations in either APC or β-catenin that prevent phosphorylation of the latter similarly cause β-catenin levels to rise (Fodde et al., 2001; Morin et al., 1997).

Once APC-mediated degradation stops, β-catenin can stabilise in the cytoplasm and then travel to the nucleus. It is believed that β-catenin does not shuttle freely from the cytoplasm to the nucleus, but that its nuclear transport requires several other proteins, such as BCL9/Legless (Brembeck et al., 2004; Kramps et al., 2002; Tolwinski and Wieschaus, 2004a) and Pygopus (Thompson et al., 2002; Townsley et al., 2004). Legless is known to bind β-catenin's *Drosophila* homolog Armadillo in the cytoplasm and to transport it into the nucleus. Inside the nucleus, β-catenin can displace Groucho from TCF/LEF (Daniels and Weis, 2005), thereby eliminating the repression exerted on gene expression. Consequently, changes in expression occur in numerous Wnt target genes. Among them are genes coding for proteins involved in cell–cycle control (e.g. cyclin-D1 (Shtutman et al., 1999; Tetsu and McCormick, 1999)), cell migration (e.g. EphB and ephrinB (Batlle et al., 2002)) and differentiation (e.g. c-myc (He et al., 1998)).

Although cadherin-mediated adhesion and Wnt signalling were originally studied independently, recently there has been increasing interest in interactions between the two pathways, stimulated to a large extent by the intriguing observation that the same protein, β-catenin, plays essential roles in adhesion and signalling (Bienz, 2005; Brembeck et al., 2006; Harris and Peifer, 2005). As expressed by Harris and Peifer (2005): "*the challenge ahead is to define how β-catenin interaction decisions are integrated and reflected during cell differentiation and tissue remodelling, and how they go awry during oncogenesis.*"

Fig. 3 maps β-catenin's binding choices onto its primary structure. The 781-residue sequence of human β-catenin is characterised by the presence of 12 highly conserved Armadillo repeats (residues 151–666), which fold into a superhelix of helices. The groove of this superhelix is the binding site for most ligands. Hence, β-catenin can undergo phosphorylation by the destruction complex (Section 2.2.1), which involves binding to APC (repeats 1–12 (Xing et al., 2004)) and axin (repeats 3–5 (Ha et al., 2004; Xing et al., 2003)). Alternatively, at adherens junctions, β-catenin binds to E-cadherin (repeats 1–12 (Huber and Weis, 2001)) and α-catenin (residues 120–146 (Aberle et al., 1994; Pokutta and Weis, 2000)), which prevents APC-mediated degradation and TCF/LEF binding. Finally, during Wnt signal transduction, β-catenin interacts with BCL9 (repeats 1–4 (Kramps et al., 2002)) and TCF/LEF (repeats 3–10 (Graham et al., 2000, 2001)).

Two main hypotheses have been formulated to describe the regulation of the adhesive and transcriptional functions of β-catenin. According to the earlier one ($\mathcal{H}$.I), E-cadherin attenuates Wnt signalling simply by competing with TCF/LEF for β-catenin binding (for review, see Nelson and Nusse (2004)). This mechanism is based on the observation that, as can be seen from Fig. 3, β-catenin's sites for E-cadherin and TCF/LEF binding overlap significantly (Daniels et al., 2001; Graham et al., 2000; Huber and Weis, 2001). Under this purely competitive scenario, stabilisation of the cytoplasmic β-catenin suffices to induce the expression of target genes. Alternatively, a more elaborate hypothesis ($\mathcal{H}$.II) involves a switch that governs β-catenin's fate by controlling its binding affinities via conformational changes. Gottardi and Gumbiner (2004) have recently identified a candidate for such a switch. They propose that Wnt signalling promotes the production of a molecular form of β-catenin that binds preferentially to TCF/LEF (for critical reviews, see Bienz (2005) and Harris and Peifer (2005)). Moreover, they hypothesise that this TCF-selective form is characterised by a 3D-conformation that impedes access of α-catenin to its binding site. As E-cadherin binds preferentially to β-catenin–α-catenin dimers, this *closed-form* of β-catenin is less likely to be recruited to the cell membrane.

According to Brembeck et al. (2004), closed-form β-catenin might result from the phosphorylation of *open-form* β-catenin at Tyr-142, which favours BCL9 and in-

hibits α-catenin binding (see Section 2.1). As explained in Section 2.2.2, BCL9 is required for β-catenin's nuclear transport and, thus, for its transcriptional function. How the Tyr-142 phosphorylation event is regulated is still unknown, however. The primary aim of the theoretical analysis presented in this article is to undertake a series of experiments *in silico* and, in so doing, to discriminate between a system governed by the kinetics associated with $\mathcal{H}$.I and one regulated by $\mathcal{H}$.II.

3 Formulation of the mathematical model

In this Section we introduce a kinetic model for the Wnt pathway that comprises the $\mathcal{H}$.I ('one molecular form of β-catenin') and $\mathcal{H}$.II ('two molecular forms of β-catenin') mechanisms of interaction between adhesion and transcription. The model consists of a system of ordinary differential equations (ODEs) that describes the changes in concentration of eleven compounds involved in Wnt signalling (Table 1): the (active) destruction complex, D, 'free' axin, X, 'free' adhesion molecules (e.g. α-catenin and/or E-cadherin), A, 'free' transcription molecules (e.g. BCL9/Legless and/or TCF), T, a Wnt target protein, Y, and six molecular forms of β-catenin ($C_i,\ i = A$, c, c_T, o, o_T, u). It is the binding of β-catenin to the destruction complex, to an adhesion molecule or to a transcription molecule that determines β-catenin's fate. To facilitate a step-by-step description of the model, we have decomposed the pathway into 24 reactions (Fig. 4). Following Lee et al. (2003), we assume that all kinetic reactions take place within a single cellular compartment (i.e. no distinction between cytoplasmic, nuclear and membrane-bound) and that the size of this compartment remains constant during the simulation period considered, although the model could easily be extended to account for the dilution of these compounds in a growing volume.

3.1 Qualitative description of the model

The central player in our model is β-catenin. In line with $\mathcal{H}$.II (cf. Gottardi and Gumbiner (2004)), we distinguish two basic forms of β-catenin that differ in their binding properties: an open form, C_o, and a closed form, C_c. Whereas the cell can allocate C_o molecules to either adhesion or transcription, it can use C_c molecules only for transcription. Based on Section 2.3 (Fig. 3), we consider three types of protein–protein complexes containing β-catenin, namely adhesion complexes and transcription complexes containing C_o or C_c. As their assembly (reactions 9, 11 and 18) involves reversible binding reactions, the corresponding complexes C_A, C_{o_T} and C_{c_T} undergo certain rates of dissociation (reactions 10, 12 and 19). In our model, the folding of C_o into C_c (reaction 15) is promoted by Wnt signalling and is caused by tyrosine phosphorylation (cf. Brembeck et al. (2004)). In contrast to protein-complex formation, we assume that phosphorylation events, such as this one, are irreversible. The change in conformation affects neither the binding site

for APC protein nor that for axin (Fig. 3) and, therefore, both C_o and C_c are subject to the same rate of irreversible phosphorylation by the destruction complex (reactions 7 and 16). The resulting Ser/Thr-phosphorylated β-catenin molecules, C_u, enter the ubiquitination/degradation pathway (reaction 8; cf. Ciechanover (1998)). In addition to APC-mediated degradation, C_o and C_c can be eliminated by normal protein turnover processes (reactions 4 and 17).

Our model not only specifies the dynamics of six β-catenin pools, but also deals explicitly with changes in the concentrations of 'free' adhesion molecules, A, 'free' axin molecules, X, active destruction complexes, D, 'free' transcription molecules, T, and target protein molecules, Y (Table 1). As can be seen from Fig. 3, β-catenin's TCF-interaction region overlaps with the binding site for APC (cf. Kries et al. (2000)). In our model, therefore, the destruction complex and the transcription molecules compete for β-catenin binding and, when bound to the latter, β-catenin is protected from APC-mediated degradation. The concentration of 'free' adhesion molecules depends on its rate of gene expression (reaction 5), its rate of degradation by proteolysis (reaction 6) and the association/dissociation of adhesion complexes C_A (reactions 9 and 10). When complexed with β-catenin, adhesion molecules are protected against attack by proteases (cf. Huber et al. (2001)), in contrast to their unbound form.

Unlike Lee et al. (2003), for simplicity we do not model the step-by-step assembly of the destruction complex nor its binding to β-catenin. Instead, we characterise the variation in the total concentration of active destruction complex by defining generic production and elimination rates (reactions 1, 2 and 24). According to both hypotheses $\mathcal{H}$.I and $\mathcal{H}$.II, the presence of Wnt factors in the extracellular matrix might affect the availability of active destruction complex in three ways: by increasing its rate of dissociation (reaction 2), by inducing its elimination or inhibition without the release of its components (reaction 24) or indirectly by reducing the level of axin (reaction 23). These lead to a reduction in the cytoplasmic level of active destruction complexes and, consequently, the rate of APC-mediated phosphorylation of β-catenin falls. In addition, under $\mathcal{H}$.II, the Wnt signal also promotes the folding of β-catenin into its closed-form (reaction 15). Finally, the level of the target protein is determined by the corresponding gene expression and protein degradation rates (reactions 13 and 14), the former being strongly enhanced by the transcription complexes, C_{c_T} and C_{o_T}. That is, transcription molecules activate expression of the target gene only when associated with β-catenin (cf. Korinek et al. (1998)).

According to the reaction scheme shown in Fig. 4, the change in concentration of each molecular component is given by:

$$\begin{aligned}
\mathrm{d}[D]/\mathrm{d}t &= r_1 - r_2 - r_{24}, && (1)\\
\mathrm{d}[X]/\mathrm{d}t &= -r_1 + r_2 + r_{22} - r_{23}, && (2)\\
\mathrm{d}[C_u]/\mathrm{d}t &= r_7 - r_8 + r_{16}, && (3)\\
\mathrm{d}[C_o]/\mathrm{d}t &= r_3 - r_4 - r_7 - r_9 + r_{10} - r_{11} + r_{12} - r_{15}, && (4)\\
\mathrm{d}[C_c]/\mathrm{d}t &= r_{15} - r_{16} - r_{17} - r_{18} + r_{19}, && (5)\\
\mathrm{d}[A]/\mathrm{d}t &= r_5 - r_6 - r_9 + r_{10}, && (6)\\
\mathrm{d}[C_A]/\mathrm{d}t &= r_9 - r_{10}, && (7)\\
\mathrm{d}[T]/\mathrm{d}t &= -r_{11} + r_{12} - r_{18} + r_{19} + r_{20} - r_{21}, && (8)\\
\mathrm{d}[C_{o_T}]/\mathrm{d}t &= r_{11} - r_{12}, && (9)\\
\mathrm{d}[C_{c_T}]/\mathrm{d}t &= r_{18} - r_{19}, && (10)\\
\mathrm{d}[Y]/\mathrm{d}t &= r_{13} - r_{14}, && (11)
\end{aligned}$$

where r_i represents the rate of reaction i, for $i = 1, \ldots, 24$ (Table 2). We specify these rates as follows. Reactions 3, 5, 13 20 and 22 represent *de novo* synthesis via gene expression. For β-catenin, adhesion molecules, transcription molecules and axin, we assume that these reactions occur at constant rates, so that: $r_3(t) = s_C$, $r_5(t) = s_A$, $r_{20}(t) = s_T$ and $r_{22}(t) = s_X$. This implies that s_C does not depend on Wnt and, thus, that the Wnt-mediated regulation of the β-catenin level is exclusively post-transcriptional (cf. Bradley et al. (1993)). With regard to reaction 13, as explained above, the rate of production of Y depends on the concentrations of C_{c_T} and C_{o_T}. As we expect this expression rate to reach a maximum value, s_Y, when there is negligible delay between the binding of two successive transcription complexes to the promotor of the target gene, we assume that r_{13} is a saturating function:

$$r_{13}(t) = \frac{s_Y[C_T(t)]}{[C_T(t)] + K_T},$$

with $[C_T] = [C_{c_T}] + [C_{o_T}]$ and K_T a constant saturation coefficient (i.e. if $[C_T] = K_T$, then $r_{13} = s_Y/2$). In using this Michaelis–Menten function we are implicitly assuming that, once in the nucleus, the two forms of β-catenin possess the same ability to induce gene expression.

The phosphorylation rates of open- and closed-form β-catenin (reactions 7 and 16) by the destruction complex depend on the concentration of active destruction complex as follows:

$$r_7(t) = \frac{p_u[D(t)][C_o(t)]}{[C_F(t)]+K_D} \qquad \text{and} \qquad r_{16}(t) = \frac{p_u[D(t)][C_c(t)]}{[C_F(t)]+K_D},$$

where $[C_F]$ denotes the total concentration of 'free' open- and closed-form β-catenin (i.e. $[C_F] = [C_c] + [C_o]$) and K_D is a Michaelis–Menten saturation coefficient (i.e. if $[C_F] = K_D$, then $r_7 + r_{16} = p_u[D]/2$, which is half of the maximum rate of production

of Ser/Thr-phosphorylated β-catenin for a given concentration of active destruction complex). The expressions for r_7 and r_{16} imply that C_c and C_o do not differ in their binding affinities for the destruction complex. Saturation of the destruction complex becomes relevant when, for instance, the cytoplasmic level of β-catenin rises for reasons other than the inhibition/destabilisation of the destruction complex, such as β-catenin overexpression.

Reactions 9, 11 and 18 are typical protein–protein binding processes. The rates of these reactions are assumed to be proportional to the products of the substrate concentrations. The rate of assembly of the multi-protein destruction complex, however, is described differently. Guided by experimental measurements showing that the concentration of axin is much lower than that of any other component of the destruction complex, it has been suggested that the assembly rate (reaction 1) is limited by the availability of axin (cf. Lee et al. (2003)). We therefore express the generic assembly rate as $r_1(t) = s_D[X]$, which implies that the levels of, for instance, GSK3β and APC-protein remain approximately constant during the simulation procedures.

Reactions 4, 6, 8, 10, 12, 14, 17, 19 and 21 correspond to protein degradation and complex dissociation processes. For each of them, we take the reaction rate to be proportional to the substrate concentration. In our model, the two β-catenin conformations, C_c and C_o, differ neither in their degradation rates nor in their binding affinities for the transcription molecules. The quantification of the elimination of active destruction complex (reactions 2 and 24) and axin (reaction 23) is slightly more sophisticated, as the rates are increasing functions of the Wnt signal. For simplicity, throughout this paper we consider a constant Wnt *signal* S_∞, with $0 \leq S_\infty \leq 1$. Although it is generally known that the Wnt signal causes a reduction in the level of active destruction complex, there is no consensus about how this takes place. To cover all likely mechanisms of inactivation/elimination, we set $r_2(t) = \widehat{d}_{D_x}(S_\infty)[D(t)]$ and $r_{24}(t) = \widehat{d}_D(S_\infty)[D(t)]$, with $\widehat{d}_D$ and $\widehat{d}_{D_x}$ arbitrary increasing functions of S_∞. While reaction 2 corresponds to the dissociation of destruction complexes, reaction 24 accounts for the elimination of active destruction complexes by mechanisms other than dissociation, such as inhibition of GSK3β without release of the complex's components. The elimination/destabilisation rate of free axin (reaction 23) is expressed similarly, as an increasing function of the Wnt signal. We note that the total axin elimination rate, $r_{23} + r_{24}$, depends on $[D]$ and, thus, on the level of APC protein (cf. Lee et al. (2003)).

According to hypothesis $\mathcal{H}$.II, Wnt enhances the production of closed-form β-catenin (reaction 15). Although it has been proposed that this might occur via the activation of a Tyr-kinase that phosphorylates C_o (Harris and Peifer, 2005), this mechanism has yet to be confirmed and the Wnt-inducible Tyr-kinase involved remains to be identified. In our model, reaction 15 is characterised as a general enzymatic reaction occurring at rate

$$r_{15}(t) = \frac{\widehat{p}_c(S_\infty)[C_o(t)]}{[C_o(t)] + K_c},$$

with K_c a saturation coefficient. Under hypothesis $\mathcal{H}$.I ('one molecular form of β-catenin'), the rate of Tyr-phosphorylation $\widehat{p}_c$ is independent of Wnt and thus $\widehat{p}_c(S_\infty) \equiv p_c$, with $p_c \equiv \widehat{p}_c(S_\infty = 0)$ a constant parameter. In contrast, under hypothesis $\mathcal{H}$.II ('two molecular forms of β-catenin'), $\widehat{p}_c$ is an increasing function of the Wnt signal and $\widehat{p}_c(S_\infty) > p_c$ for every $S_\infty > 0$. Expressions for the reaction rates r_i $(i = 1 \ldots 24)$ used in the simulations are summarised in Table 2; the resulting model equations are provided in the Appendix.

3.3 Non-dimensionalisation

The state variables in Fig. 4 can be scaled as shown in Table 1. In terms of the dimensionless variables, our model comprises a system of coupled differential equations. For clarity, and in line with Fig. 4, we group the variables in four functional blocks.

- APC-mediated degradation of β-catenin

$$\mathrm{d}D/\mathrm{d}\tau = \alpha_d X - (\widehat{\gamma}_d + \widehat{\gamma}_{dx})D, \tag{12}$$
$$\mathrm{d}X/\mathrm{d}\tau = \alpha_x - (\alpha_d + \widehat{\gamma}_x)X + \widehat{\gamma}_{dx}D, \tag{13}$$
$$\mathrm{d}C_u/\mathrm{d}\tau = \frac{C_F D}{C_F + k_d} - \gamma_u C_u. \tag{14}$$

- Cytoplasmic β-catenin

$$\mathrm{d}C_o/\mathrm{d}\tau = 1 + C_{o_T} + \gamma_{ca}C_A - \alpha_{ca}C_o A - \alpha_{ct}C_o T - \gamma_c C_o - \frac{\widehat{\mu}_c C_o}{C_o + k_c} - \frac{C_o D}{C_F + k_d}, \tag{15}$$
$$\mathrm{d}C_c/\mathrm{d}\tau = \frac{\widehat{\mu}_c C_o}{C_o + k_c} + C_{c_T} - \alpha_{ct}C_c T - \gamma_c C_c - \frac{C_c D}{C_F + k_d}. \tag{16}$$

- E-cadherin-mediated cell–cell adhesion

$$\mathrm{d}A/\mathrm{d}\tau = \alpha_a + \gamma_{ca}C_A - \alpha_{ca}C_o A - \gamma_a A, \tag{17}$$
$$\mathrm{d}C_A/\mathrm{d}\tau = \alpha_{ca}C_o A - \gamma_{ca}C_A. \tag{18}$$

- Transcription of Wnt-target genes

$$\mathrm{d}T/\mathrm{d}\tau = \alpha_t + C_T - \alpha_{ct}C_F T - \gamma_t T, \tag{19}$$
$$\mathrm{d}C_{o_T}/\mathrm{d}\tau = \alpha_{ct}C_o T - C_{o_T}, \tag{20}$$
$$\mathrm{d}C_{c_T}/\mathrm{d}\tau = \alpha_{ct}C_c T - C_{c_T}, \tag{21}$$
$$\mathrm{d}Y/\mathrm{d}\tau = \frac{C_T}{C_T + k_t} - \gamma_y Y. \tag{22}$$

In equations (12)–(22), the parameters α_i are synthesis/assembly coefficients, γ_i and $\widehat{\gamma}_j$ are degradation/dissociation/inhibition coefficients and $\widehat{\mu}_c$ is the Tyr-phosphorylation coefficient. The symbols carrying hats ($\widehat{\gamma}_d$, $\widehat{\gamma}_{dx}$ $\widehat{\gamma}_x$ and $\widehat{\mu}_c$) are those which may increase in response to a Wnt signal; the same symbols without a hat correspond to

values in the absence of Wnt (e.g. $\mu_c \equiv \widehat{\mu}_c(S_\infty = 0)$, with S_∞ the Wnt signal). All parameters and variables are non-negative. We remark that equations (12) and (13), for active destruction complex and axin, decouple from the other equations and can be solved analytically. Moreover, as equations (15)–(21) do not depend on the levels of Ser/Thr-phosphorylated β-catenin (C_u) or Wnt target protein (Y), they can be solved independently of equations (14) and (22). The dimensionless parameters are defined as follows.

$$
\begin{array}{llll}
\alpha_a = s_A/s_C, & k_c = d_{CT}K_c/s_C, & \gamma_d = d_D/d_{CT}, & \gamma_t = d_T/d_{CT}, \\
\alpha_{ca} = s_C s_{CA}/d_{CT}^2, & k_d = d_{CT}K_D/s_C, & \widehat{\gamma}_{dx} = \widehat{d}_{Dx}/d_{CT}, & \gamma_u = d_u/d_{CT}, \\
\alpha_{ct} = s_C s_{CT}/d_{CT}^2, & k_t = d_{CT}K_T/s_C, & \gamma_{dx} = d_{Dx}/d_{CT}, & \widehat{\gamma}_x = \widehat{d}_X/d_{CT}, \\
\alpha_d = s_D/d_{CT}, & \widehat{\mu}_c = \widehat{p}_c/s_C, & \gamma_a = d_A/d_{CT}, & \gamma_x = d_X/d_{CT}, \\
\alpha_t = s_T/s_C, & \mu_c = p_c/s_C & \gamma_c = d_C/d_{CT}, & \gamma_y = d_Y/d_{CT}. \\
\alpha_x = s_X p_u/(s_C d_{CT}), & \widehat{\gamma}_d = \widehat{d}_D/d_{CT}, & \gamma_{ca} = d_{CA}/d_{CT}, &
\end{array}
$$

The parameter values used in the model simulations shown in Section 4 are provided in the Appendix (Table A.1).

3.4 Steady-state solution

The dimensionless steady-state solution of (12)–(22) (denoted by $\star$'s) are:

$$D^\star = \frac{\alpha_d \alpha_x}{\alpha_d \widehat{\gamma}_d + \widehat{\gamma}_x(\widehat{\gamma}_d + \widehat{\gamma}_{dx})}, \tag{23}$$

$$X^\star = \frac{\alpha_x(\widehat{\gamma}_d + \widehat{\gamma}_{dx})}{\alpha_d \widehat{\gamma}_d + \widehat{\gamma}_x(\widehat{\gamma}_d + \widehat{\gamma}_{dx})}, \tag{24}$$

$$C_F^\star = \left((1 - \gamma_c k_d - D^\star) + \sqrt{(1 - \gamma_c k_d - D^\star)^2 + 4\gamma_c k_d}\right)/(2\gamma_c), \tag{25}$$

$$C_u^\star = \frac{D^\star C_F^\star}{\gamma_u(C_F^\star + k_d)}, \tag{26}$$

$$C_o^\star = \left((1 - \widehat{\mu}_c - z^\star k_c) + \sqrt{(1 - \widehat{\mu}_c - z^\star k_c)^2 + 4z^\star k_c}\right)/(2z^\star), \tag{27}$$

$$C_c^\star = C_F^\star - C_o^\star, \tag{28}$$

$$A^\star = \alpha_a/\gamma_a, \tag{29}$$

$$C_A^\star = \alpha_a \alpha_{ca} C_o^\star/(\gamma_a \gamma_{ca}), \tag{30}$$

$$T^\star = \alpha_t/\gamma_t, \tag{31}$$

$$C_{o_T}^\star = \alpha_t \alpha_{ct} C_o^\star/\gamma_t, \tag{32}$$

$$C_{c_T}^\star = \alpha_t \alpha_{ct} C_c^\star/\gamma_t, \tag{33}$$

$$Y^\star = \frac{\alpha_t \alpha_{ct} C_F^\star}{\gamma_y(\alpha_t \alpha_{ct} C_F^\star + \gamma_t k_t)}, \tag{34}$$

with $z^\star = \gamma_c + D^\star/(C_F^\star + k_d)$ and $C_F^\star$ the equilibrium level of total (free) cytoplasmic β-catenin, $C_F^\star = C_c^\star + C_o^\star$. A sensitivity analysis of the equilibrium solution upon the parameter values is performed in the Appendix.

If the value of k_c is small (i.e. if $k_c \ll C_o$ in (15) and (16)), then the production of closed-form β-catenin (C_c) in effect saturates very quickly and the expression for $C_o^\star$ (27) reduces to

$$C_o^\star \approx (1 - \widehat{\mu}_c) \left(\gamma_c + \frac{D^\star}{C_F^\star + k_d} \right)^{-1}. \tag{35}$$

(Note that $\widehat{\mu}_c \leq 1$, as an immediate consequence of its definition; see Appendix). In contrast, if $k_c \gg C_o$, then Tyr-phosphorylation does not saturate and

$$C_o^\star \approx \left(\gamma_c + \frac{\widehat{\mu}_c}{k_c} + \frac{D^\star}{C_F^\star + k_d} \right)^{-1}. \tag{36}$$

We will use this reduced expression to evaluate the impact of β-catenin mutations on the behaviour of a cell (section 4.2).

4 Results

In this section we compare the responses of the $\mathcal{H}$.I ($\widehat{\mu}_c \equiv \mu_c$) and $\mathcal{H}$.II ($\widehat{\mu}_c(S_\infty) > \mu_c$ for $S_\infty > 0$) models to different stimuli under normal and aberrant conditions. First, we investigate the impact of switching on Wnt signalling. We then consider the effect of genetic alterations commonly detected in colorectal cancers (CRC), including the inactivation of the APC tumour suppressor. In addition to these changes, which can occur under normal conditions *in vivo*, we gain further insight by investigating the impact of artificially manipulating the level of E-cadherin. The parameter values used to carry out the simulations are provided in the Appendix. (Readers who are not interested in technical details may omit the next section and move directly to the Discussion without loss of continuity).

4.1 Activation of Wnt signalling

In terms of our model, the Wnt 'off' and 'on' states correspond to absence ($S_\infty = 0$) and presence ($0 < S_\infty \leq 1$) of a Wnt signal, respectively. From (23)–(34) it can be seen that the presence of a Wnt signal results in a decrease in $D^\star$ and an increase in $C_F^\star$, $C_T^\star \equiv C_{c_T}^\star + C_{o_T}^\star$ and $Y^\star$. Thus, as expected, the Wnt stimulus induces a decrease in the level of active destruction complex and an increase in the levels of cytoplasmic β-catenin, transcription complexes and Wnt-target gene expression.

From (25), we note that the equilibrium level of cytoplasmic β-catenin, $C_F^\star$, does not depend on the Tyr-phosphorylation coefficient, $\widehat{\mu}_c$, and, consequently, the equilibrium values of transcription complex ($C_T^\star$) and target protein ($Y^\star$) are identical under $\mathcal{H}$.I and $\mathcal{H}$.II. This can also be seen from Fig. 5, which shows how the levels of transcription complexes, C_T, and target protein, Y, change when a constant Wnt signal is activated at time $\tau = 0$. Although $\mathcal{H}$.II initially responds more quickly to the Wnt stimulus, C_T and Y evolve to the same steady-state values $C_T^\star$ and $Y^\star$ independently of the choice of hypothesis (see also the Sensitivity Analysis in the Appendix).

In contrast, the equilibrium values of open-form β-catenin ($C_o^\star$), closed-form β-catenin ($C_c^\star$) and adhesion complex ($C_A^\star$) do depend on $\widehat{\mu}_c$. That is, our model predicts that, in the presence of Wnt, the levels of open-form β-catenin and adhesion complexes differ depending on whether $\mathcal{H}$.I or $\mathcal{H}$.II is applicable. Fig. 6 shows the effects of a constant Wnt signal initiated at time $\tau = 0$ on cadherin-mediated adhesion. For the non-saturated system it can be shown, from (30) and (36), that the equilibrium concentration of adhesion complexes falls when Wnt is switched on if, and only if,

$$1 > \frac{C_A^\star}{C_A^{\star\star}} = \frac{C_o^\star}{C_o^{\star\star}} = \frac{\left(\gamma_c + \frac{\mu_c}{k_c} + \frac{D^{\star\star}}{C_F^{\star\star}+k_d}\right)}{\left(\gamma_c + \frac{\widehat{\mu}_c}{k_c} + \frac{D^{\star}}{C_F^{\star}+k_d}\right)},$$

that is, $C_A^\star/C_A^{\star\star} < 1$ if, and only if, the condition

$$\frac{\widehat{\mu}_c - \mu_c}{k_c} > \frac{D^{\star\star}}{C_F^{\star\star} + k_d} - \frac{D^\star}{C_F^\star + k_d} \tag{37}$$

holds. The symbols indicated with $\star\star$ correspond to equilibrium values in the 'off' state. An equivalent condition (not shown) can be deduced for the saturated system. Note that the condition above is always false under $\mathcal{H}$.I (i.e. $\widehat{\mu}_c \equiv \mu_c$) and, consequently, Wnt signalling then promotes cadherin-mediated adhesion. Under $\mathcal{H}$.II, however, there exists a threshold value for $\widehat{\mu}_c$ above which Wnt causes a reduction in cell–cell adhesion. As discussed in section 5 below, this Tyr-phosphorylation threshold originates from the competition of the destruction complex and the Tyr-kinase for β-catenin binding.

According to the predictions above, only $\mathcal{H}$.II accounts for a loss of cell–cell adhesion in response to a Wnt signal. However, in most experimental settings this might not help to distinguish between $\mathcal{H}$.I and $\mathcal{H}$.II, since for a wide range of parameter values cell–cell adhesion is enhanced by Wnt in systems governed by either hypothesis (e.g. non-saturated systems for which the condition (37) is false). The magnitude of the Wnt-mediated increase in cell–cell adhesion might provide further information, though. Under $\mathcal{H}$.I, Tyr-phosphorylation is a rare event and, consequently, $C_F^\star \approx C_o^\star$ and $C_F^{\star\star} \approx C_o^{\star\star}$. From (30), this leads to

$$C_A^\star/C_A^{\star\star} \approx C_F^\star/C_F^{\star\star}.$$

That is, a Wnt-mediated x_1-fold increase in 'free' cytoplasmic β-catenin translates eventually into an approximate x_1-fold increase in cell–cell adhesion. In contrast, according to $\mathcal{H}$.II the x_1-fold increase in $C_F^\star$ results in an x_2-fold increase in $C_A^\star$, with $x_2 < x_1$. In summary, a slight difference between x_1 and x_2 provides insufficient information to discriminate between the two hypotheses, whereas a substantial discrepancy between x_1 and x_2 points to $\mathcal{H}$.II.

Complex assembly/dissociation kinetics are normally much faster than protein turnover processes. Consequently, the dynamics of the pool of 'free' adhesion molecules (17) is governed mainly by the change in cell–cell adhesion:

$$\mathrm{d}A/\mathrm{d}\tau = \alpha_a - \gamma_a A - \mathrm{d}C_A/\mathrm{d}\tau.$$

As can be seen from Fig. 6, if C_A increases in response to a Wnt signal then A can initially decrease (and vice-versa). The magnitude and duration of this response depend on the kinetic parameters. After long-term Wnt exposure, however, the 'free' adhesion molecules return to their original level in the absence of Wnt. That is, according to our model, the steady-state value $A^\star$ (Eq.(29)) is not affected by long-term Wnt signalling for either $\mathcal{H}$.I or $\mathcal{H}$.II.

4.2 Malignant transformation

In their original genetic model for colorectal cancer (CRC), Fearon and Vogelstein (1990) identified four candidate cancer genes: *APC*, *DCC*, *K-ras*, and *p53*. Mutations in the *APC* gene are the genetic alterations most commonly observed in both sporadic and hereditary CRC cases. These mutations lead mainly to the production of truncated APC proteins with impaired axin or β-catenin binding. Although APC is a multifunctional protein that can be linked to cancer in several ways (Fodde, 2003), it is currently believed that APC's tumour-suppressor function is related mainly to its ability to downregulate β-catenin (Smits et al., 2000).

Loss of APC function can easily be incorporated into our model, as the drop in the availability of functional APC will lead to a reduction in α_d, the rate at which new destruction complexes are produced. If there is no external Wnt signal, the elimination of axin and active destruction complexes and Tyr-phosphorylation remain unchanged (i.e. $\widehat{\gamma}_x \equiv \gamma_x$, $\widehat{\gamma}_d \equiv \gamma_d$, $\widehat{\gamma}_{dx} \equiv \gamma_{dx}$ and $\widehat{\mu}_c \equiv \mu_c$) and, therefore, systems $\mathcal{H}$.I and $\mathcal{H}$.II behave identically. From the results presented in Section 4.1, we deduce further that this leads to an increase in cytoplasmic β-catenin ($C_F^\star$) and in cell–cell adhesion ($C_A^\star$).

Inactivation of the APC gene is not the only way to induce the expression of Wnt target genes in tumour cells. Genetic alterations in other components of the Wnt-signalling pathway have, indeed, been detected in an important fraction of $\mathrm{APC}^{+/+}$ (wild-type APC) cancers. Mutations in the GSK3β recognition region of β-catenin

(Fig. 3), for instance, prevent β-catenin phosphorylation by the destruction complex without affecting TCF/LEF binding (section 2.2.1). If this kind of mutation is homozygous, it corresponds to the particular case $r_7 \approx 0$ and $r_{16} \approx 0$ of our model (Fig. 4). In contrast, describing the heterozygous state requires an extension of our model to accommodate additional pools of closed- and open-form β-catenin which are resistant to APC-mediated phosphorylation (i.e. $r_7 \approx 0$ and $r_{16} \approx 0$ for these pools only), as well as their associated adhesion and transcription complexes. If Tyr-phosphorylation does not saturate, the equilibrium values for the extended model can be calculated analytically, as shown in the Appendix, which makes it possible to reveal generic properties of the system. As for APC-inactivating mutations, in the absence of a Wnt stimulus β-catenin mutations result in an increase in cytoplasmic β-catenin and cell–cell adhesion under both $\mathcal{H}$.I and $\mathcal{H}$.II.

In Fig. 7 we compare the response of wild-type and mutant cells to a Wnt stimulus. We distinguish four types of genetic alterations: loss of one APC allele (modelled by a 50% reduction in α_d), complete APC inactivation ($\alpha_d \approx 0$), 50% production of APC-resistant β-catenin, and mutations in both β-catenin alleles (i.e. $r_7 \approx 0$ and $r_{16} \approx 0$). According to our model, APC and β-catenin double mutants achieve an identical level of Wnt signalling after being exposed to long-term Wnt, because both genetic alterations result in the complete inhibition of APC-mediated degradation of β-catenin. As can be seen from Fig. 7.A, in wild–type and single–mutant cells the steady-state level of transcription complexes rises with the strength of the Wnt signal. Double mutants, in contrast, maintain the same high level of target-gene expression independently of Wnt, because then $C_F^\star = \gamma_c^{-1}$ does not depend on S_∞. These results are independent of the choice of hypothesis and thus, in line with Fig. 5, gene expression cannot be used to discriminate between $\mathcal{H}$.I and $\mathcal{H}$.II. Cell–cell adhesion provides further insight, though. We predict that under $\mathcal{H}$.I (dashed lines in Fig. 7.B) an increase in Wnt causes a rise in cell–cell adhesion in normal cells and single mutants, but has no effect on adhesion in double mutants. In contrast, under $\mathcal{H}$.II, Wnt affects the level of adhesion complexes in all four cell types: for double mutants, cadherin-mediated adhesion is always a decreasing function of the Wnt signal, whereas for the remaining cell types the response depends on rate of Tyr-phosphorylation.

As depicted in Fig. 7.A, for the chosen parameter values, single β-catenin mutants ($\beta cat1$) achieve a significantly higher level of Wnt-target gene expression than single APC mutants in the absence of Wnt. However, this difference diminishes with increasing strength of the Wnt signal and, eventually, when exposed to high Wnt factor concentrations, $APC^{+/-}$ cells can present the highest transcription complex concentration. These hypotheses are explored further in Fig. 7.C, which shows the threshold APC activity level required to keep the level of transcription complexes lower than in $\beta cat1$ mutants exposed to the same Wnt level. Our simulations suggest that the level of functional APC required to maintain a normal phenotype increases with an increasing Wnt stimulus. When no Wnt stimulus is present, for instance, we predict that only those cells expressing less than 17% functional APC undergo

malignant transformation, a prediction that is in agreement with the estimation by Li et al. (2005) that the threshold level of wild-type APC protein for polyp formation is 15%. The results indicate that the extracellular environment might have a substantial impact on both malignant transformation and tumour phenotype.

4.3 Changes in E-cadherin expression

In the previous sections we have seen how activation of a Wnt signal might significantly affect cadherin-mediated adhesion in normal and aberrant cells (Fig. 6.C and 7.B). The obvious next question is whether changes in cadherin-mediated adhesion might influence Wnt target gene expression. We have sought an answer by analysing the effect of varying the *de novo* synthesis parameter α_a on the level of transcription complexes, C_T. The results are shown in Fig. 8.

Firstly, we considered the effect of 50 and 100% repression of E-cadherin expression in the absence of Wnt (panels on the left). As a consequence of (30), these manipulations lead eventually to 50% and nil cell–cell adhesion, respectively (Fig. 8.A). In the absence of Wnt, for the parameter values chosen, most of the cellular β-catenin is located at the cell membrane. Consequently, a reduction in cell-cell adhesion translates into a similar fall in the level of total β-catenin (Fig. 8.C). Nevertheless, we predict only a weak and brief associated increase in C_T, suggesting that E-cadherin repression alone is insufficient to induce Wnt signalling (Fig. 8.E). This result is a consequence of equation (25) for $C_F^\star$, which does not depend on any parameters related to cell–cell adhesion (see also the Sensitivity Analysis in the Appendix). Secondly, we overexpressed E-cadherin in cells exposed to a continuous Wnt stimulus. As can be seen from equation (30) and Fig. 8.B, three- and six-fold increases in α_a translate into proportional increases in cell–cell adhesion under both $\mathcal{H}$.I and $\mathcal{H}$.II. For the parameter values chosen, these changes are accompanied by a rise in the total level of β-catenin (Fig. 8.D); the magnitude of this increase is larger under $\mathcal{H}$.I than under $\mathcal{H}$.II. In contrast to Fig. 8.E, however, these E-cadherin manipulations can cause a significant transient decrease in C_T (Fig. 8.F). That is, E-cadherin overexpression can temporarily inhibit Wnt signalling.

In Fig. 9 we evaluate the impact of E-cadherin manipulations on Wnt-target gene expression in wild-type, $\text{APC}^{+/-}$ (loss of heterozygosity, leading to 50% functional APC protein) and $\text{APC}^{-/-}$ (0% functional APC protein) cells. All three cells types show a transient increase in the level of transcription complexes in response to E-cadherin repression in the absence of an external Wnt stimulus (Fig. 9). For the parameter values chosen, the duration of this transient increase is longer in mutant than in normal cells. Moreover, in all three cell types, E-cadherin overexpression in the presence of Wnt causes a temporary decrease in Wnt signalling. The relative magnitude and duration of this phenomenon in each cell type depend on the model parameter values and, in particular, on whether $\mathcal{H}$.I or $\mathcal{H}$.II is active.

5 Discussion

In this paper we have developed a new model of the Wnt signalling pathway. As such it differs from the model proposed by Lee et al. (2003) and extended by Cho et al. (2006) in several ways. Firstly, we allow for competition between signalling and cell–cell adhesion. We also distinguish two conformations of β-catenin that differ in their binding affinities for E-cadherin. The emphasis of our analysis is also slightly different. Given that not all model parameters have been measured, we aim to reveal generic properties of the system. We now discuss the biological implications of our model predictions. This section is organised as follows. The *in silico* experiments and model predictions described in Section 4 are summarised in boxes without any mathematical notation. Following each box, we discuss the agreement between our model predictions and experimental data from the literature and use our model to provide a novel interpretation of the experimental observations. To conclude, we discuss some alternative model formulations and potential future applications of our work.

5.1 Wnt activation (Summary Box 1)

It is generally accepted that transduction of the Wnt signal involves inhibition of the destruction complex and stabilisation of cytoplasmic β-catenin, leading to increased binding of β-catenin to TCF/LEF and to expression of Wnt target genes (Section 2.2). These basic events are also predicted by our model under both $\mathcal{H}$.I and $\mathcal{H}$.II (see prediction (P.1) in Summary Box 1), but in addition analytical analysis of the model equations provides new insight. In particular, systems governed by $\mathcal{H}$.I and $\mathcal{H}$.II achieve the same level of signalling after long-term Wnt exposure and, consequently, it is not possible to discriminate between them based on target gene expression alone.

The two hypothetical systems can differ in their initial response to Wnt, though. Even if inactivation of the destruction complex occurs rapidly after stimulation of the Wnt receptors, under $\mathcal{H}$.I the accumulation of cytoplasmic β-catenin will take place gradually at a pace defined by its *de novo* synthesis and turnover rates. Consequently, we expect the initial response to Wnt to be quicker under $\mathcal{H}$.II, as less of the newly synthesised β-catenin then goes into cell–cell adhesion and some β-catenin released from adhesion complexes is reallocated to cell signalling. Our model simulations suggest, however, that for these phenomena to have a substantial impact on Wnt signalling, the increase in Tyr-phosphorylation has to be unrealistically large (e.g. 10^3-fold). We can think of at least two ways to enhance the response to a Wnt signal under $\mathcal{H}$.II: (1) the Tyr-kinase phosphorylates 'free' open-form β-catenin and also β-catenin in adhesion complexes and (2) closed-form β-catenin is more stable than open-form β-catenin. (1) is known to cause the release of β-catenin from the

cadherin-bound pool and its subsequent reallocation to signalling (section 2.1). In line with (2), Harris and Peifer (2005) remark about β-catenin's closed-form: "*it is tempting to speculate that APC and/or axin interactions might* (...) *be blocked, thereby saving this form of β-catenin from destruction*". This has an effect only if there are active destruction complexes present in the cytoplasm, though.

Concerning cell–cell adhesion, various experimental studies have quantified the level of adhesion complexes in the presence and absence of Wnt. Bradley et al. (1993), for example, have expressed wild-type Wnt-1 in the rat neural cell line PC12. They concluded that "*Wnt-1 causes increased accumulation of E-cadherin at the borders between adjacent cells and an accompanying change in Ca^{++}-dependent cell adhesion.*" This observation can be explained under either $\mathcal{H}$.I or $\mathcal{H}$.II (see prediction (P.2)). Conacci-Sorrell et al. (2003) have reported a disruption of E-cadherin adhesion in human CRC cells with active Wnt signalling. However, rather than refuting $\mathcal{H}$.I via model prediction (P.3), their study illustrates the transcriptional repression of E-cadherin by Slug (Section 2.1) in very sparse cultures. During normal morphogenesis, loss of cell–cell adhesion due to Wnt-mediated down-regulation of E-cadherin has also been observed by Jamora et al. (2003) and Shimamura et al. (1994). Finally, Hinck et al. (1994) have analysed mammalian AtT20 cells expressing Wnt-1. In line with Bradley et al. (1993), mRNA analysis revealed a similar level of cadherin expression in the presence and absence of Wnt-1. Nevertheless, comparison of steady-state levels by immunoblotting extracts showed a 20–30-fold and 2-fold increase the levels of β-catenin and N-cadherin, respectively. In terms of model (P.3), equivalent observations concerning E-cadherin would point to $\mathcal{H}$.II.

According to our model, under $\mathcal{H}$.II the larger the increase in the rate of Tyr-phosphorylation following a Wnt signal, the lower the associated steady-state level of Ca^{++}-dependent cell–cell adhesion. In particular, we predict that there is a threshold Tyr-phosphorylation rate above which Wnt induces a loss of cadherin complexes from the cell membrane. This threshold results from the competition between the Tyr-kinase and the destruction complex for binding to open-form β-catenin. In the 'off' state, most β-catenin is degraded via the destruction complex, with none or only a residual amount being irreversibly transformed into closed-form β-catenin. According to $\mathcal{H}$.II, in the 'on' state, APC-mediated Ser/Thr-phosphorylation is reduced, whereas Tyr-phosphorylation is enhanced. If the resulting total elimination rate of β-catenin is higher than in the absence of Wnt then the level of open-form β-catenin is reduced and, consequently, the level of cadherin-mediated adhesion also falls.

5.2 Malignant transformation (Summary Box 2)

In addition to analysing the response of normal cells to a Wnt signal, we have used our model to evaluate the effects on signalling and cell–cell adhesion of two types of genetic alterations commonly involved in CRC, namely APC-inactivating mutations

and production of APC-resistant β-catenin. The model predictions are summarised in Summary Box 2.

It has been proposed that, whereas a single β-catenin hit can suffice for malignant transformation to occur, the APC tumour suppressor follows a classical Knudson two-hit model. This does not arise as a natural consequence of the reaction scheme shown in Fig. 4, however. Model simulations suggest that the increase in Wnt target gene expression caused by a certain degree of APC inactivation can vary among different tissue and cell types and that, in particular, it can depend on the presence of an extracellular signal. Hence, the stronger the extracellular Wnt signal, the higher the level of functional APC protein that is required to prevent malignant transformation. Consequently, we predict that partial APC inactivation can suffice for malignant transformation under certain circumstances, which is supported by the experimental observations on *APC* expression levels in CRC cells (Albuquerque et al., 2002) and on dose-dependent effects of APC protein in suppressing intestinal tumourigenesis (Li et al., 2005). This illustrates that the environment might play an essential role in the process of malignant transformation as well as in defining the tumour phenotype.

The results above might explain why genetic alterations of the *APC* gene are responsible for more CRC cases than β-catenin aberrations, despite the fact that the latter requires just one hit. The number of β-catenin point mutations conferring APC-resistance is believed to be very low as they have to modify the GSK3β recognition area specifically (Fig. 3), without affecting β-catenin's transcriptional function. In contrast, a wide range of missense and nonsense mutations in the *APC* sequence can lead to the production of truncated APC proteins with reduced (or non-existent) β-catenin downregulating activity. The accumulation of two such APC hits could thus occur on average before the first *right* β-catenin hit. Our model predictions suggest that these two hits could be able to promote Wnt signalling even if they only partially eliminate APC's tumour suppressor function. The dependence between the distributions of the first and second APC hits described by Albuquerque et al. (2002) might thus be related to the mutational events most likely to overcome a minimum APC inactivation level for tumourigenesis, rather than to a selection for *APC* genotypes retaining a level of APC activity that optimises the balance between increased Wnt target gene expression and cell proliferation (Smits et al., 2000), reduced cell–cell adhesion and enhanced invasiveness (section 4.2) and tumour cell survival (Kim et al., 2000).

5.3 Changes in E-cadherin expression (Summary Box 3)

Various assays have shown that cell–cell adhesion can interfere with Wnt signalling under some circumstances. For instance, a study by Sanson et al. (1996) showed that, in *Drosophila* embryos, overexpression of E-cadherin can antagonise Wnt signalling

by sequestering Armadillo, the *Drosophila* homologue of β-catenin. Analogous results have been obtained in *Xenopus* embryos (Fagotto et al., 1996; Heasman et al., 1994). More recently, Kuphal and Behrens (2006) have shown that reintroduction of E-cadherin in L929 fibroblasts deficient in Wnt signalling and E-cadherin-mediated adhesion has little effect on gene expression. The authors conclude that E-cadherin influences cell signalling in a Wnt-dependent manner. This result is further supported by findings by Gottardi and Wong (2001): they observed a decrease in cell growth after restoring wild-type cadherin expression in a human CRC cell-line lacking cadherin-mediated adhesion. Using a TCF/LEF luciferase reporter plasmid, they revealed that this growth inhibition was due to decreased Wnt signalling, rather than to increased cell–cell adhesion. Our model also predicts (*P*.7; see Summary Box 3) that an increase in the E-cadherin level can prevent the transduction of the Wnt signal. However, as a consequence of normal protein turnover and complex assembly/dissociation kinetics, the predicted 'kidnapping' of β-catenin by E-cadherin is only transient. That is, once the adhesion-complex level reaches a new steady state, β-catenin will slowly return to its original level (the duration and magnitude of these changes depending on the parameter values). A transient drop in Wnt target gene expression is not necessarily in disagreement with the experimental observations, as it could last long enough to, for instance, cause abnormal embryonic development. Moreover, in response to E-cadherin overexpression, our model also predicts a permanent increase in cell–cell adhesion, which could explain the inhibition of both growth and invasiveness observed in cancer cells (Frixen et al., 1991; Vleminckx et al., 1991).

E-cadherin-inactivating mutations have been detected in several human cancers and, in particular, germline mutations have been related to inherited gastric and colorectal cancer syndromes (Christofori and Semb, 1999; Richards et al., 1999), suggesting that E-cadherin acts as a tumour suppressor. The question remains whether the oncogenic effects associated with the loss of E-cadherin function are due to enhanced Wnt signalling or decreased cell–cell adhesion. In the context of our model, (*P*.8), a reduction in E-cadherin expression in the absence of Wnt results in a proportional decrease in the level of adhesion complexes. Nevertheless, according to our simulations, E-cadherin repression has little potential to induce the expression of Wnt target genes in the absence of a Wnt stimulus, because any β-catenin released from the cell membrane is rapidly degraded. In this line, Herzig et al. (2006) have recently reported that, in Rip1Tag2 mice, β-catenin is not present in the nuclei of E-cadherin-negative tumour cells.

The transient nature of the predicted changes (*P*.7 and *P*.8) is a natural consequence of normal protein turnover being slower than complex assembly/dissociation kinetics. The exact duration and magnitude of the changes in Wnt signalling depend on the model parameter values and, in particular, on the total amount of β-catenin present in the cell when E-cadherin inactivation occurs. Consequently, we expect these changes to be, for instance, less pronounced in normal cells than in APC and β-catenin mutants. Furthermore, we conclude that E-cadherin inactivation is likely

to play a role late in tumour progression, mainly through altered cell–cell adhesion. This is supported by the experimental observation that E-cadherin inactivation induces early invasion and metastasis of pancreatic β-cell tumours in mice, whereas E-cadherin expression results in arrest of tumour development at the adenoma stage (Perl et al., 1998). As expressed by the authors, "*the data suggest that E-cadherin counteracts the tumour cell's transition to an invasive phenotype by maintaining intercellular adhesion and cellular organisation.*" Margulis et al. (2005) have recently arrived at an analogous conclusion after analysing the effects of E-cadherin expression on the aggressiveness and invasiveness of human squamous-cell cancers.

5.4 Model properties and limitations

It is important to realise that various phenomena can cloud a clear distinction between $\mathcal{H}$.I and $\mathcal{H}$.II. For example, a decrease in cell–cell adhesion in response to Wnt signalling could be caused by down-regulation of the E-cadherin gene, rather than to a reallocation of β-catenin from the membrane to the nucleus. A study on follicular morphogenesis in mice suggests that the expression of the E-cadherin gene might indeed be regulated by Wnt signalling (Jamora et al., 2003). Moreover, the β-catenin conformation control switch can be overriden. Hence, Gottardi and Gumbiner (2004) have suggested that phosphorylated E-cadherin is able to complex with closed-form β-catenin. Finally, concerning the switch governing β-catenin's fate (Section 2.3), the only assumption incorporated into the model is that the presence of a Wnt stimulus induces the (irreversible) production of a form of β-catenin that is unable to bind to E-cadherin. This generic approach leaves open the possibility that the switch is controlled by mechanisms other than phosphorylation at Tyr-142, such as phosphorylation of β-catenin at Tyr-654 (see Fig. 3).

Several steps within the reaction scheme (Fig. 4) are still poorly understood, such as the role of the Wnt surface receptors (Cadigan and Liu, 2006) and the mechanism of inhibition of the destruction complex (Tolwinski and Wieschaus, 2004b; Wong et al., 2003). To avoid introducing a high level of uncertainty into our equations and predictions, we chose to build a rather generic model implementing generally accepted events as assumptions, rather than to develop a detailed model that would force us into a high degree of speculation about the mechanisms underlying such events. We are also aware that the functioning of the Wnt signalling and cadherin-mediated adhesion pathways may differ between different organisms and cell lines. Fig. 4 should therefore not be interpreted as "the *universal* pathway", but as a generic and flexible framework that can be modified to characterise specific experimental scenarios, cell lines and species. This can be done by extending the model to incorporate additional phenomena or by simply changing the values of the model parameters. The latter includes not only varying the reaction rates and initial concentrations, but also switching particular processes on or off.

While seeking to balance the levels of mathematical and biological complexity, our approach might have oversimplified several aspects of the Wnt pathway. We discuss them only briefly here, as we believe that their detailed analysis is beyond the scope of the present paper. Firstly, in its current form our model does not account for the fact that, like β-catenin, APC is a multifunctional protein (Fodde, 2003). Its interactions with other cellular components, such as the cytoskeleton, might affect its β-catenin-regulating function. Furthermore, we studied the dose-dependence of the response to APC mutations in terms of changes in the assembly rate of the destruction complex, α_d. The relation between the expression level of mutant APC and the parameter α_d is, however, non-trivial. For instance, mutant proteins might dimerise with wild-type proteins, thereby enhancing malignant transformation. Secondly, in the model simulations shown in section 4, we assumed a constant Wnt signal S_∞, with $0 \leq S_\infty \leq 1$. A more general expression for the Wnt signal is given by

$$S_\omega(t) = \frac{[W(t)]}{[W(t)] + K_\omega},$$

with $[W]$ the extracellular concentration of Wnt factors and K_ω a saturation coefficient. If the Wnt-factor/receptor system tends to a steady–state, then we can define $\lim_{t\to\infty} S_\omega(t) = S_\infty$. We expect the strength of the Wnt signal to reach its maximum value of $S_\infty = 1$ when all surface receptors are continuously stimulated. The time-dependency of the Wnt signal becomes specially relevant when considering, for instance, cells exposed to a transient Wnt stimulus or cells migrating in a Wnt-factor gradient. Thirdly, the model does not characterise the nuclear transport and accumulation of β-catenin nor the interactions between β-catenin, Groucho, TCF and the DNA. Fourthly, loss of E-cadherin has been reported to result in N-cadherin upregulation (Cavallaro et al., 2002; Christofori, 2006). This 'cadherin switch' might exacerbate the associated increase in invasiveness. Finally, like the majority of existing subcellular models, Fig. 4 describes protein–protein interactions as chemical reactions taking place in a well-mixed, homogeneous medium. However, a more realistic environment might influence Wnt signalling in several ways, for example: (1) under guidance of protein movement provided by the cytoskeleton, protein interactions might be facilitated, (2) protein transport processes between the cell membrane, cytosol and nucleus might constitute rate-limiting steps in biochemical networks, (3) in cells growing in size, volume/surface relations can influence the relative availability of components of the pathway (the relevant concentration units of, for instance, receptors and adhesion complexes are molecules/(surface area) rather than molecules/volume) and (4) the presence and abundance of specific proteins can vary among cellular compartments, which constrains protein–protein interactions and influences the overall kinetic rates.

There are two obvious directions for extending the work presented in this paper. Firstly, there are several experiments that could be performed to validate our modelling assumptions and test our model predictions. Secondly, the model could be extended to include additional features. We expand on each of these themes below.

Given the importance of Wnt dysregulation in colorectal pathogenesis, β-catenin is naturally being considered as a molecular target in drug design for CRC (Clapper et al., 2004; Daniels et al., 2001). Several chemical agents, such as aspirin, indomethacin and sulindac sulfone, are known to interfere with the Wnt signalling pathway and influence β-catenin levels in CRC cells. Their specific mechanisms of action remain unknown, however. As summarised by Clapper et al. (2004), "*possibilities include:* (1) *physical inhibition of the β-catenin/TCF complex formation*, (2) *upregulation of the ubiquitin-mediated proteosomal degradation of β-catenin*, (3) *accelerated nuclear export of β-catenin and* (4) *enhanced sequestration of β-catenin by E-cadherin*." The non-steroidal anti-inflammatory drug (NSAID) indomethacin, for instance, has recently been proposed to act either by enhancing β-catenin phosphorylation (Dihlmann et al., 2003) or by promoting cadherin-mediated adhesion (Kapitanović et al., 2006). Our model provides a useful tool to investigate these mechanisms of action as well as to explore safely alternative therapeutic targets within the Wnt signalling and cadherin-mediated adhesion pathways.

Wnt signalling depends not only on the molecular components in Fig. 4 and on the presence of Wnt factors in the extracellular environment; it is tightly controlled by several layers of regulation. In the nucleus, for example, the protein Chibby can compete with TCF/LEF for β-catenin binding (Takemaru et al., 2002). In the extracellular environment, Wnt inhibitory factor 1 (WIF-1) (Hsieh et al., 1999), Cerberus (Piccolo et al., 1999) and Coco (Bell et al., 2003) can antagonise Wnt signalling by sequestering Wnt factors. Moreover, secreted Frizzled-related proteins (FRPs) can bind Wnt factors, as well as forming inert complexes with Frizzled (Jones and Jomary, 2002; Üren et al., 2000), and secreted Dickkopf proteins can inhibit LRP5/6 and induce its Kremen-mediated removal from the cell membrane (Mao et al., 2001b, 2002). Genetic alterations in several of these modulators of Wnt activity have been detected in human cancers (e.g. Bafico et al. (2004); Holcombe et al. (2002); Suzuki et al. (2004)). Our model can be extended to study these phenomena. Furthermore, feedback loops exist, as the expression of some Wnt components and modulators is regulated by Wnt signalling (e.g. Filali et al. (2002); Gonzalez-Sancho et al. (2004a); Jho et al. (2002); Leung et al. (2002)). Several human cancer cell lines show Wnt-mediated activation of *AXIN2* expression, which gives rise to a negative feedback loop capable of downregulating β-catenin levels. This mechanism can easily be incorporated into our model, as illustrated in Fig. 10. When the feedback loop is active, the level of active APC protein has to fall drastically to achieve a substantial effect on target gene expression. The observation that Wnt signalling and *AXIN2*

expression are not invariably linked (Jho et al., 2002; Leung et al., 2002) suggests that certain cells can switch on the axin2 feedback-loop to reduce the duration and intensity of the Wnt response under certain circumstances. Further research is needed to deepen our understanding of the role of this feedback loop, its possible synergy with other loops, and its importance relative to other feedback mechanisms.

Our simulations and steady state analysis suggest that it is not possible to discriminate between $\mathcal{H}$.I and $\mathcal{H}$.II by measuring gene expression alone. However, by measuring how E-cadherin levels change following a Wnt stimulus, it may be possible to distinguish between $\mathcal{H}$.I and $\mathcal{H}$.II: in particular, in the absence of Wnt-mediated E-cadherin repression, a reduction in (total) E-cadherin levels following a Wnt stimulus is consistent with $\mathcal{H}$.II only. Another detectable difference between the two hypotheses may be revealed by measuring cell–cell adhesion (or total E-cadherin levels) in $\mathrm{APC}^{-/-}$ cells. Under $\mathcal{H}$.I there is no change in cell–cell adhesion, whereas under $\mathcal{H}$.II it decreases as the Wnt stimulus increases.

The work on Wnt signalling and cell–cell adhesion presented in this paper was carried out as part of the *Integrative Biology* (IB) e-science project (`http://www.integrativebiology.ox.ac.uk/`), which is bringing together computer scientists, modellers and experimental researchers to build a virtual heart and a multiscale model of the normal and aberrant intestine. Existing models for colorectal cancer are largely very simple and generally focus on a single time and length scale (for a review, see Van Leeuwen et al. (2006)). The IB approach, instead, aims to integrate descriptions of all levels of organisation from molecule to tissue. Hence, the presence of Wnt gradients along the crypt axis would determine spatially-dependent rates of gene expression, cell proliferation, adhesion, differentiation, migration and death (cf. Gaspar and Fodde (2004)). Moreover, Wnt signalling and cell–cell adhesion are involved directly in colorectal cancer (e.g. Radtke and Clevers (2005)). As illustrated by progress in developing multiscale models (e.g. Alarcón et al. (2004); Noble (2002); Ribba et al. (2006)), integrating the hierarchy of processes occurring at different levels of organisation has the advantage of allowing researchers to investigate possible interactions between such processes, to combine biochemical, histological and clinical data, and to test drugs *in silico* on the system as a whole. We hope that the current model represents a worthwhile contribution to such developments.

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Appendix

Model equations

Adopting the functional forms specified in Table 2 for r_i (i =1, ..., 24), our model can be written as a system of first-order nonlinear ODEs as follows.

- APC-mediated degradation of β-catenin

$$\begin{aligned}
\mathrm{d}[D]/\mathrm{d}t &= s_D[X] - (\widehat{d}_D(S_\infty) + \widehat{d}_{D_x}(S_\infty))[D],\\
\mathrm{d}[X]/\mathrm{d}t &= s_X - s_D[X] - \widehat{d}_X(S_\infty)[X] + \widehat{d}_{D_x}(S_\infty)[D],\\
\mathrm{d}[C_u]/\mathrm{d}t &= \frac{p_u[D][C_F]}{[C_F]+K_D} - d_u[C_u].
\end{aligned}$$

- Cytoplasmic β-catenin

$$\begin{aligned}
\mathrm{d}[C_o]/\mathrm{d}t &= s_C + d_{CA}[C_A] + d_{CT}[C_{o_T}] - (s_{CA}[A] + s_{CT}[T] + d_C)[C_o]\\
&\quad - \frac{\widehat{p}_c(S_\infty)[C_o]}{[C_o]+K_c} - \frac{p_u[D][C_o]}{[C_F]+K_D},\\
\mathrm{d}[C_c]/\mathrm{d}t &= \frac{\widehat{p}_c(S_\infty)[C_o]}{[C_o]+K_c} + d_{CT}[C_{c_T}] - (s_{CT}[T] + d_C)[C_c] - \frac{p_u[D][C_c]}{[C_F]+K_D}.
\end{aligned}$$

- E-cadherin-mediated cell–cell adhesion

$$\begin{aligned}
\mathrm{d}[A]/\mathrm{d}t &= s_A + d_{CA}[C_A] - (s_{CA}[C_o] + d_A)[A],\\
\mathrm{d}[C_A]/\mathrm{d}t &= s_{CA}[C_o][A] - d_{CA}[C_A].
\end{aligned}$$

- Transcription of Wnt-target genes

$$\begin{aligned}
\mathrm{d}[T]/\mathrm{d}t &= s_T + d_{CT}[C_T] - (s_{CT}[C_F] + d_T)[T],\\
\mathrm{d}[C_{o_T}]/\mathrm{d}t &= s_{CT}[C_o][T] - d_{CT}[C_{o_T}],\\
\mathrm{d}[C_{c_T}]/\mathrm{d}t &= s_{CT}[C_c][T] - d_{CT}[C_{c_T}],\\
\mathrm{d}[Y]/\mathrm{d}t &= \frac{s_Y[C_T]}{[C_T]+K_T} - d_Y[Y].
\end{aligned}$$

In the equations above, $[C_F] = [C_c] + [C_o]$ and $[C_T] = [C_{c_T}] + [C_{o_T}]$. The symbols s_i denote synthesis/assembly rates of the substrate/complex indicated as subscript. Similarly, d_i and $\widehat{d}_j$ denote degradation/dissociation/inhibition rates. The symbol $\widehat{p}_c$ represents the Tyr-phosphorylation rate of open-form β-catenin. Finally, p_u is the rate of Ser/Thr-phosphorylation of β-catenin by the destruction complex. The

symbols carrying hats ($\widehat{d}_D$, $\widehat{d}_{D_x}$ $\widehat{d}_X$ and $\widehat{p}_c$) are those which may vary in response to a Wnt signal. Hypothesis $\mathcal{H}$.I corresponds to $\widehat{p}_c(S_\infty) \equiv p_c$ and hypothesis $\mathcal{H}$.II to $\widehat{p}_c(S_\infty) > p_c$ for $S_\infty > 0$. To arrive at the conclusions presented in the main text, we have assumed that $\widehat{d}_D$, $\widehat{d}_{D_x}$ $\widehat{d}_X$ and $\widehat{p}_c$ are increasing functions of S_∞. For simplicity, the Wnt-dependent functions used in the simulations are linear:

$$\widehat{d}_D(S_\infty) = d_D + \xi_D S_\infty, \qquad \widehat{d}_{D_x}(S_\infty) = d_{D_x} + \xi_{D_x} S_\infty,$$
$$\widehat{d}_X(S_\infty) = d_X + \xi_X S_\infty, \qquad \widehat{p}_c(S_\infty) = p_c + \xi_c S_\infty,$$

with ξ_c, ξ_D, ξ_{D_x}, and ξ_X non-negative parameters. All simulations were performed using *Mathematica*'s numerical ODE solver `NDSolve`.

Initial conditions and parameter values

As mentioned previously, the kinetic coefficients and concentrations of the components of the Wnt pathway are largely unknown and are likely to vary under different conditions and among different species and cell lines. In this paper, a set of baseline parameter values (Table A.1) has been chosen in order to illustrate the model's behaviour graphically. It is important to note that the choice of these values does not affect the general conclusions from our mathematical analysis.

The values for the model parameters have been chosen based on the following premises.

- All parameters are non-negative.
- The total concentration of axin is relatively low: $[X^{\star\star}]+[D^{\star\star}] < \min([A^{\star\star}], \sum_i [C_i^{\star\star}], [T^{\star\star}])$ for $i = A, c, c_T, o, o_T, u$.
- β-catenin has a higher affinity for adhesion molecules than for transcription molecules: $s_{CA} > s_{CT}$ and $d_{CA} < d_{CT}$.
- Complex assembly/dissociation kinetics are faster than protein turnover processes: s_{CA}, $d_{CA} > \max(s_A, s_C, d_A, d_C)$ and s_{CT}, $d_{CT} > \max(s_C, s_T, d_C, d_T)$.
- The rate of β-catenin elimination via the destruction complex is higher than the rate of β-catenin protein turnover: $d_u > d_C$.
- In the absence of Wnt, most β-catenin is located in adhesion complexes: $[C_A^{\star\star}] > \sum_i [C_i^{\star\star}]$ for $i = c, c_T, o, o_T, u$.
- In the absence of Wnt, the level of target protein is low: $[Y^{\star\star}] < \min([A^{\star\star}], \sum_i [C_i^{\star\star}], [T^{\star\star}])$ for $i = A, c, c_T, o, o_T, u$.
- The level of Tyr-phosphorylation is negligibly low in the absence of Wnt: $p_c = 0$, $[C_c^{\star\star}] = 0$, $[C_{c_T}^{\star\star}] = 0$.

Given a variable f:$\Re^n \rightarrow \Re$ and an initial point $\bar{p} = (\bar{p}_1, \ldots, \bar{p}_n) \in \Re^n$, the normalised relative sensitivity function

$$\phi_i(f) = \frac{\bar{p}_i}{f(\bar{p})} \frac{\partial f}{\partial p_i}(\bar{p})$$

characterises the local sensitivity of f to deviations of the parameter p_i from its initial value $\bar{p}_i$ $(i = 1, \ldots, n)$. We have used this technique to assess the dependence of the equilibrium solution upon the model parameters. The value of $\phi_i(f)$ can be roughly interpreted as follows. If $\mid \phi_i(f) \mid = 1$, then 1% change in p_i results in approximately 1% change in the value of f. If $\mid \phi_i(f) \mid < 1$, the system is less sensitive to variation in this particular parameter.

The steady state solution (Eqs.(23)–(34)) can be scaled as follows:

$$\begin{aligned}
&\bar{D} = D^\star = p_u[D^\star]/s_C, && \bar{A} = \gamma_a A^\star/\alpha_a = d_A[A^\star]/s_A,\\
&\bar{X} = \widehat{\gamma}_x X^\star/\alpha_x = \widehat{d}_X[X^\star]/s_X, && \bar{C_A} = \tfrac{\gamma_a \gamma_{ca}}{\alpha_a \alpha_{ca} k_d} C_A^\star = \tfrac{d_A d_{CA}}{s_A s_{CA}}[C_A^\star],\\
&\bar{C_F} = C_F^\star/k_d = [C_F^\star]/K_D, && \bar{T} = \gamma_t T^\star/\alpha_t = d_T[T^\star]/d_T,\\
&\bar{C_u} = \gamma_u C_u^\star = d_u[C_u^\star]/s_C, && \bar{C_{o_T}} = \tfrac{\gamma_t}{\alpha_t \alpha_{ct} k_d} C_{o_T}^\star = \tfrac{d_{CT} d_T}{s_{CT} s_T}[C_{o_T}^\star],\\
&\bar{C_o} = C_o^\star/k_d = [C_o^\star]/K_D, && \bar{C_{c_T}} = \tfrac{\gamma_t}{\alpha_t \alpha_{ct} k_d} C_{c_T}^\star = \tfrac{d_{CT} d_T}{s_{CT} s_T}[C_{c_T}^\star],\\
&\bar{C_c} = C_c^\star/k_d = [C_c^\star]/K_D, && \bar{Y} = \gamma_y Y^\star = d_Y[Y^\star]/s_Y.
\end{aligned}$$

If we now define

$$\begin{aligned}
&p_1 = \alpha_x/\widehat{\gamma}_d = \tfrac{s_X p_u}{s_C \widehat{d}_D}, && p_4 = \widehat{\mu}_c = \widehat{p}_c/s_C,\\
&p_2 = \tfrac{\widehat{\gamma}_x}{\alpha_d}(1 + \tfrac{\widehat{\gamma}_{dx}}{\widehat{\gamma}_d}) = \tfrac{\widehat{d}_X}{s_D}(1 + \tfrac{\widehat{d}_{Dx}}{\widehat{d}_D}), && p_5 = k_c/k_d = K_c/K_D,\\
&p_3 = \gamma_c k_d = d_C K_D/s_C, && p_6 = \tfrac{\gamma_t k_t}{\alpha_{ct} \alpha_t k_d} = \tfrac{d_{CT} d_T K_T}{s_{CT} s_T K_D},
\end{aligned}$$

then the scaled steady-state solution above reduces to

$$\bar{D} = p_1/(1+p_2), \qquad \bar{A} = 1,$$
$$\bar{X} = p_2/(1+p_2), \qquad \bar{C_A} = \bar{C}_o,$$
$$\bar{C_F} = \frac{(1-p_3-\bar{D})+\sqrt{(1-p_3-\bar{D})^2+4p_3}}{2p_3}, \qquad \bar{T} = 1,$$
$$\bar{C}_u = \bar{C_F}\bar{D}/(\bar{C_F}+1), \qquad \bar{C_{o_T}} = \bar{C}_o,$$
$$\bar{C}_o = \frac{(1-p_4-\bar{z}p_5)+\sqrt{(1-p_4-\bar{z}p_5)^2+4\bar{z}p_5}}{2\bar{z}}, \qquad \bar{C_{c_T}} = \bar{C}_c,$$
$$\bar{C}_c = \bar{C_F} - \bar{C}_o, \qquad \bar{Y} = \bar{C_F}/(\bar{C_F}+p_6),$$

with $\bar{z} = p_3 + \bar{D}/(\bar{C_F}+1)$. As a result, the number of parameters has been reduced from 20 in Eqs.(23)–(34) to only 6; this simplifies enormously our sensitivity analysis of the system, the results of which are presented in Table A.2.

In Table A.2, the initial values $\bar{p}_i$ correspond to the Wnt 'off' state in Table A.1, except for $p_4 = \widehat{\mu}_c = 100$. The value of $\bar{p}_4$ has been increased significantly for this analysis because $\mu_c = 0$ (Table A.1) would imply $\phi_4(f) = 0, \forall f$. Moreover, small values of μ_c would result in small values of $\bar{C}_c$ and, consequently, $\phi_i(\bar{C}_c)$ would provide relative changes to a value that is close to zero. Among the implications of the $\phi_i(f)$ values in the table above, we would like to highlight the following:

- $\mid \phi_1(C_F^\star) \mid < \mid \phi_2(\bar{C_F}) \mid$, which supports the hypothesis that changes in axin expression have a stronger effect than changes in APC expression;
- $\phi_4(\bar{C}_T^\star) = \phi_4(\bar{Y}) = 0$, in agreement with our conclusion that it is not possible to discriminate between $\mathcal{H}$.I and $\mathcal{H}$.II based on long-term gene expression alone;
- changes in E-cadherin expression (i.e. changes in s_A and α_a) do not affect the value of $\phi_i(\bar{C_F})$, in agreement with our prediction that changes in E-cadherin expression do not affect the steady-state level of cytoplasmic β-catenin;
- as the entries in Table A.2 are all at most O(1) in magnitude, the system does not exhibit extreme sensitivity to any single parameter.

Model extension for mutant β-catenin

The model can easily be extended to include mutant open-form β-catenin, M_o, which behaves identically to wild-type open-form β-catenin, C_o, except for being resistant to APC-mediated degradation. These two pools of open-form β-catenin are not independent, as they compete for binding to adhesion molecules, transcription molecules and Tyr-kinases. Hence, the extended equations for 'free' cytoplasmic β-catenin are

$$
\begin{aligned}
\mathrm{d}[C_o]/\mathrm{d}t &= (1-\sigma)s_C + d_{CA}[C_A] + d_{CT}[C_{o_T}] - (s_{CA}[A] + s_{CT}[T] + d_C)[C_o] \\
&\quad - \frac{\widehat{p}_c[C_o]}{[C_o]+[M_o]+K_c} - \frac{p_u[D][C_o]}{[C_F]+K_D}, \\
\mathrm{d}[C_c]/\mathrm{d}t &= \frac{\widehat{p}_c[C_o]}{[C_o]+[M_o]+K_c} + d_{CT}[C_{c_T}] - (s_{CT}[T] + d_C)[C_c] - \frac{p_u[D][C_c]}{[C_F]+K_D}, \\
\mathrm{d}[M_o]/\mathrm{d}t &= \sigma s_C + d_{CA}[M_A] + d_{CT}[M_{o_T}] - (s_{CA}[A] + s_{CT}[T] + d_C)[M_o] \\
&\quad - \frac{\widehat{p}_c[M_o]}{[C_o]+[M_o]+K_c}, \\
\mathrm{d}[M_c]/\mathrm{d}t &= \frac{\widehat{p}_c[M_o]}{[C_o]+[M_o]+K_c} + d_{CT}[M_{c_T}] - (s_{CT}[T] + d_C)[M_c],
\end{aligned}
$$

where the symbols M_i denote the mutant equivalents of C_i. The parameter σ is taken to adopt the values $\sigma = 0$ in normal cells, $\sigma = 0.5$ in cells with mutations in a single β-catenin allele, and $\sigma = 1$ in double mutants, implying that the three cell types share the same total level of β-catenin expression. If Tyr-phosphorylation does not saturate (i.e. if $[C_o] + [M_o] << K_c$ in the equations above), then the equilibrium values for the extended model can be calculated analytically, as shown below.

- 'Free' wild-type and mutant open-form β-catenin:

$$
\begin{aligned}
C_o^\star &\approx (1-\sigma)\left(\gamma_c + \frac{\widehat{\mu}_c}{k_c} + \frac{D^\star}{C_F^\star + k_d}\right)^{-1}, \\
M_o^\star &\approx \sigma\left(\gamma_c + \widehat{\mu}_c/k_c\right)^{-1}.
\end{aligned}
$$

- Total 'free' wild-type and mutant β-catenin:

$$
\begin{aligned}
C_F^\star &= \left((1-\sigma-\gamma_u k_d - D^\star) + \sqrt{(1-\sigma-\gamma_c k_d - D^\star)^2 + 4\gamma_u(1-\sigma)k_d}\right)/(2\gamma_c), \\
M_F^\star &= \sigma/\gamma_u.
\end{aligned}
$$

- Total transcription and adhesion complexes

$$
\begin{aligned}
(\text{transcription complexes})^\star &= \alpha_t\alpha_{ct}(C_F^\star + M_F^\star)/\gamma_t, \\
(\text{adhesion complexes})^\star &= \alpha_a\alpha_{ca}(C_o^\star + M_o^\star)/(\gamma_a\gamma_{ca}).
\end{aligned}
$$

These equations have been used in the simulations shown in Fig. 7.

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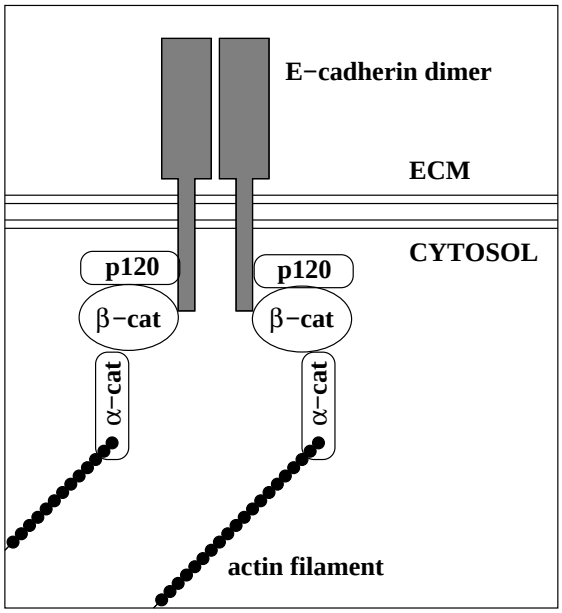

Fig. 1. Schematic of a cadherin complex in an adherens junction. As a link between E-cadherin and α-catenin, β-catenin constitutes an essential component of these tight junctions. In turn, α-catenin is responsible for binding to the actin cytoskeleton. Finally, another catenin, p120, interacts with E-cadherin's cytoplasmic domain and regulates the stability of the adhesion complex (Davis et al., 2003). In intestinal columnar cells, these tight junctions align along each cell's apical circumference, forming sealing strands between adjacent cells. ECM = extracellular matrix.

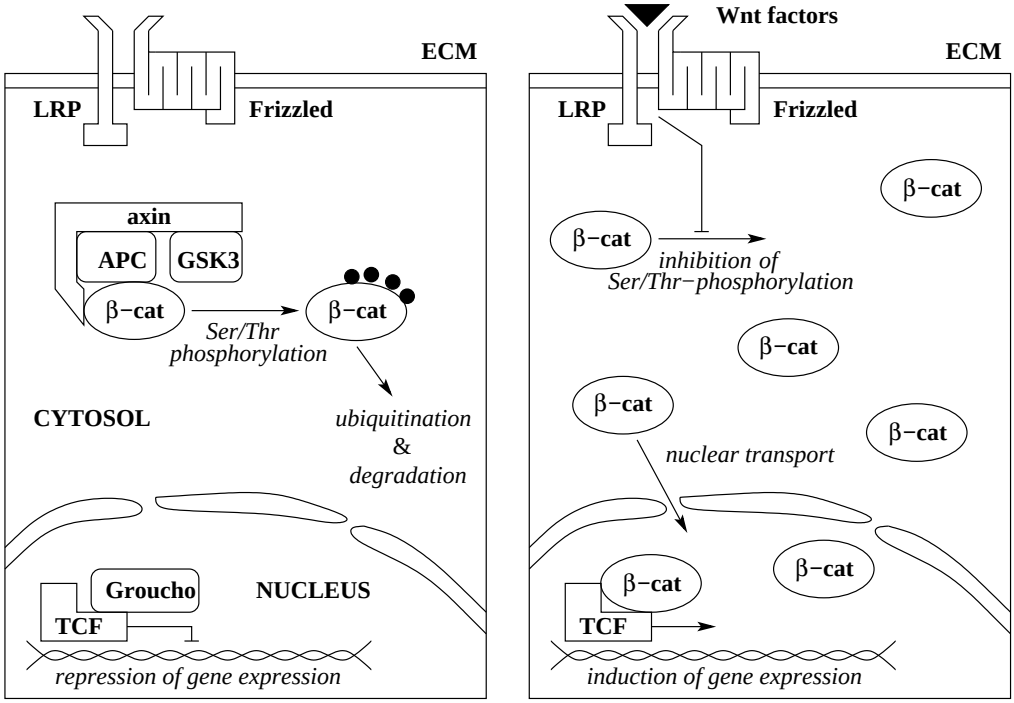

Fig. 2. Schematic of the Wnt signalling pathway. In the absence of Wnt (*left*), β-catenin is phosphorylated at specific serine and threonine residues by the destruction complex and then undergoes ubiquitination-mediated degradation. Under these conditions, most β-catenin is located at the cell membrane as part of adhesion complexes (Fig. 1). As a result, no β-catenin travels to the nucleus and, thus, the co-repressor Groucho is able to bind to DNA–TCF/LEF and prevent the expression of the Wnt target genes. In contrast (*right*), if Wnt factors are present in the extracellular matrix (ECM), the stimulation of the Wnt receptors (Frizzled and LRP5/6) causes loss of activity of the destruction complex. Consequently, unphosphorylated β-catenin accumulates and travels to the nucleus. By binding to TCF/LEF, β-catenin inhibits Groucho's repressing activity and, thereby, induces the expression of the Wnt target genes.

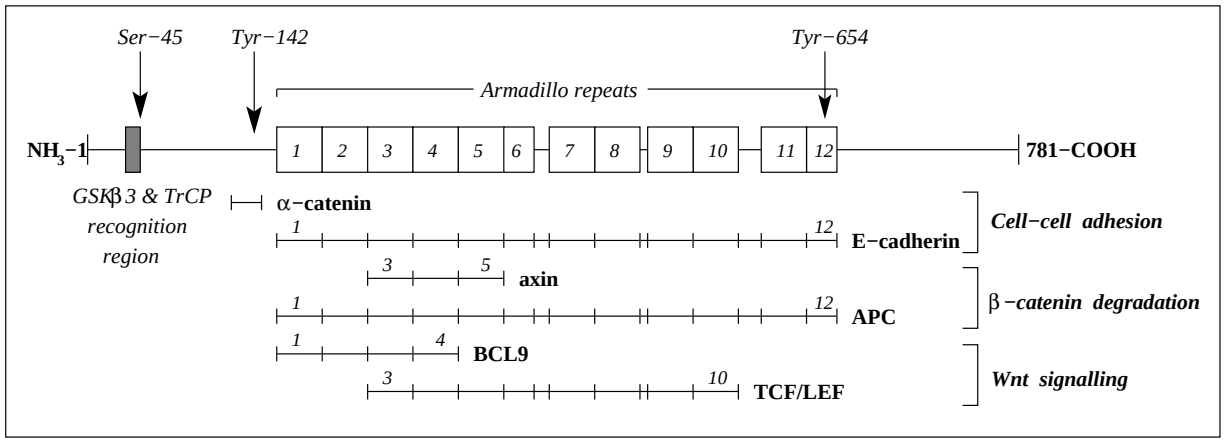

Fig. 3. Overview of β-catenin's binding options. The human β-catenin (P35222; CTNB1_HUMAN) macromolecule is 781-residues long and weighs about 85.5kDa. Binding sites for three major types of interaction are shown. In *cadherin-mediated adhesion* (Section 2.1), β-catenin binds to α-catenin and E-cadherin. The former binding can be inhibited by phosphorylation of β-catenin at Tyr-142 and the latter by Tyr-654 phosphorylation. In *APC-mediated degradation of β-catenin* (Section 2.2.1), β-catenin binds to axin and APC-protein, which act as scaffolds in the destruction-complex. Finally, in *Wnt signalling* (Section 2.2.2), β-catenin interacts with BCL9 and TCF/LEF.

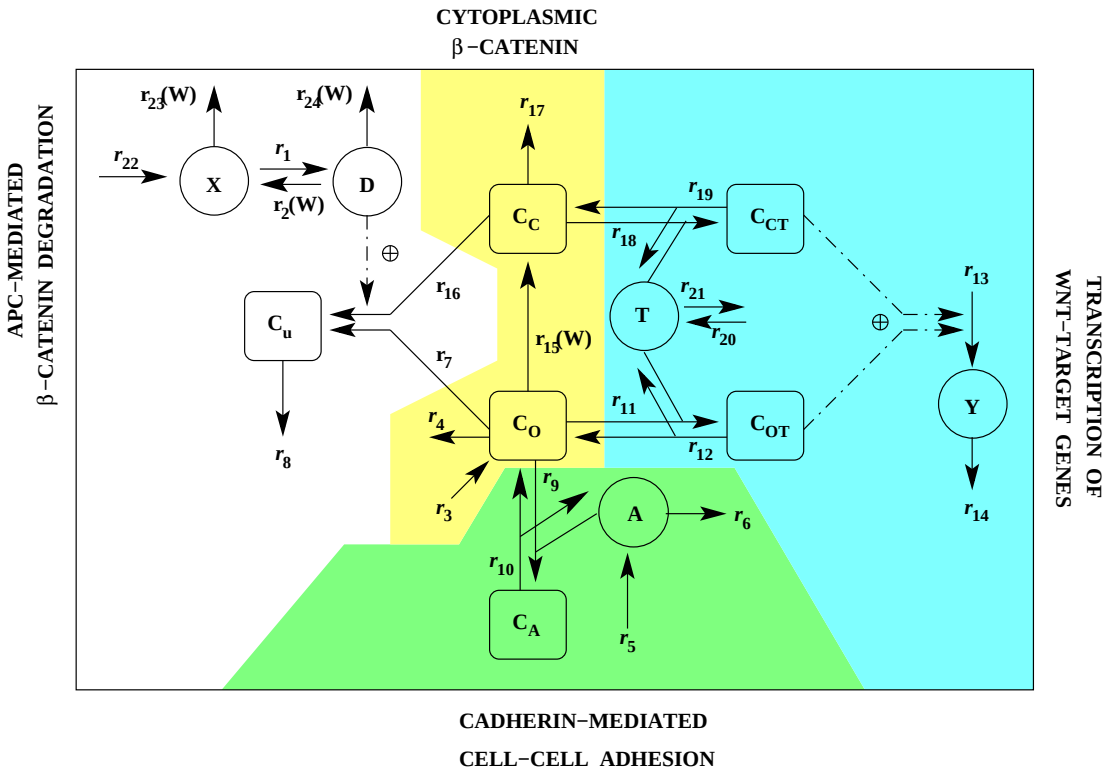

Fig. 4. Schematic representation of the kinetic model, which describes the dynamics of active destruction complexes (D), axin molecules (X), adhesion molecules (A), transcription molecules (T), Wnt target proteins (Y) and six pools of β-catenin (C_i, $i = A, c, c_T, o, o_T, u$). Expression of the β-catenin gene produces an open form C_o (reaction 3), which can undergo APC-mediated phosphorylation (reaction 7), interact with adhesion or transcription molecules (reaction 9 or 11), undergo APC-independent degradation (reaction 4) or fold into a closed conformation C_c (reaction 15). The APC-mediated phosphorylation of C_o and C_c molecules (reactions 7 and 16) produces C_u, which is then recognised for rapid ubiquitination and subsequent degradation (reaction 8). Moreover, both C_o and C_c can bind to a transcription molecule (reactions 11 and 18), giving C_{o_T} and C_{c_T} complexes that promote *de novo* synthesis of the target protein, Y (reaction 13). Like C_o, C_c is subject to APC-independent elimination (reaction 17). Dashed arrows indicate interactions in which the concentration of a compound influences a certain reaction rate in a positive ($\oplus$) way. Moreover, as emphasised by the bold arrows, the presence of extracellular Wnt factors (W) might directly affect several reaction rates. Hence, according to hypothesis $\mathcal{H}$.I, Wnt enhances the elimination of active destruction complexes (D) and axin (X) (reactions 2, 23 and 24). According to hypothesis $\mathcal{H}$.II, Wnt also promotes tyrosine phosphorylation of open-form β-catenin (reaction 15). Reactions 1–2, 9–10, 11–12 and 18–19 are complex assembly/dissociation reactions; reactions 3, 5, 13, 20 and 22 involve *de novo* synthesis from gene expression; reactions 6, 14, 21 and 23 correspond to normal protein elimination via proteolysis. Reaction 24 accounts for irreversible inhibition/elimination of active destruction complexes without release of axin. For $i = 1, \ldots, 24$, the symbol r_i represents the rate of reaction i.

Table 1: State variables.

Original variable	**Interpretation**	**Dimensionless variable**
$[A]$	'Free' adhesion molecules	$A = d_{CT}[A]/s_C$
$[C_A]$	Adhesion complex (i.e. C_o bound to A)	$C_A = d_{CT}[C_A]/s_C$
$[C_c]$	Closed-form (Tyr-phosphorylated) β-catenin	$C_c = d_{CT}[C_c]/s_C$
$[C_{c_T}]$	Closed-form β-catenin in transcription complex	$C_{c_T} = d_{CT}[C_{c_T}]/s_C$
$[C_u]$	β-catenin marked for ubiquitination	$C_u = d_{CT}[C_u]/s_C$
$[C_F]$	Total 'free' cytoplasmic β-catenin, $C_F = C_c + C_o$	$C_F = d_{CT}[C_F]/s_C$
$[C_o]$	Open-form (unphosphorylated) β-catenin	$C_o = d_{CT}[C_o]/s_C$
$[C_{o_T}]$	Open-form β-catenin in transcription complex	$C_{o_T} = d_{CT}[C_{o_T}]/s_C$
$[C_T]$	Transcription complex, $C_T = C_{c_T} + C_{o_T}$	$C_T = d_{CT}[C_T]/s_C$
$[D]$	Destruction complex	$D = p_u[D]/s_C$
$[T]$	'Free' transcription molecules	$T = d_{CT}[T]/s_C$
$[X]$	Axin	$X = p_u[X]/s_C$
$[Y]$	Wnt target protein	$Y = d_{CT}[Y]/s_Y$
t	Time	$\tau = d_{CT}t$

Table 2: Reaction rates. $[M(t)]$ = concentration of macromolecule M at time t.

	INTERPRETATION	**EXPRESSION**
r_1	Assembly of active destruction complexes	$r_1(t) = s_D[X(t)]$
r_2	Dissociation of active destruction complexes	$r_2(t) = \widehat{d}_{D_x}(S_\infty)[D(t)]$
r_3	β-catenin *de novo* synthesis	$r_3(t) = s_C$
r_4	APC-independent degradation of open-form β-catenin	$r_4(t) = d_C[C_o(t)]$
r_5	*De novo* synthesis of adhesion molecules	$r_5(t) = s_A$
r_6	Elimination of adhesion molecules	$r_6(t) = d_A[A(t)]$
r_7	Open-form β-catenin Ser/Thr-phosphorylation	$r_7(t) = \frac{p_u[D(t)][C_o(t)]}{[C_F(t)]+K_D}$
r_8	Elimination of Ser/Thr-phosphorylated β-catenin	$r_8(t) = d_u[C_u(t)]$
r_9	Assembly of adhesion–complexes	$r_9(t) = s_{CA}[C_o(t)][A(t)]$
r_{10}	Dissociation of adhesion–complexes	$r_{10}(t) = d_{CA}[C_A(t)]$
r_{11}	Assembly of C_{o_T} complexes	$r_{11}(t) = s_{CT}[C_o(t)][T(t)]$
r_{12}	Dissociation of C_{o_T} complexes	$r_{12}(t) = d_{CT}[C_{o_T}(t)]$
r_{13}	Target protein *de novo* synthesis	$r_{13}(t) = \frac{s_Y[C_T(t)]}{[C_T(t)]+K_Y}$
r_{14}	Target gene elimination	$r_{14}(t) = d_Y[Y(t)]$
r_{15}	β-catenin Tyr-phosphorylation (production of closed-form)	$r_{15}(t) = \frac{\widehat{p}_c(S_\infty)[C_o(t)]}{[C_o(t)]+K_c}$
r_{16}	Closed-form β-catenin Ser/Thr-phosphorylation	$r_{16}(t) = \frac{p_u[D(t)][C_c(t)]}{[C_F(t)]+K_D}$
r_{17}	APC-independent degradation of closed-form β-catenin	$r_{17}(t) = d_C[C_c(t)]$
r_{18}	Assembly of C_{c_T} complexes	$r_{18}(t) = s_{CT}[C_c(t)][T(t)]$
r_{19}	Dissociation of C_{c_T} complexes	$r_{19}(t) = d_{CT}[C_{c_T}(t)]$
r_{20}	*De novo* synthesis of transcription molecules	$r_{20}(t) = s_T$
r_{21}	Elimination of transcription molecules	$r_{21}(t) = d_T[T(t)]$
r_{22}	Axin *de novo* synthesis	$r_{22}(t) = s_X$
r_{23}	Axin elimination	$r_{23}(t) = \widehat{d}_X(S_\infty)[X(t)]$
r_{24}	Inhibition of destruction complex (without axin release)	$r_{24}(t) = \widehat{d}_D(S_\infty)[D(t)]$

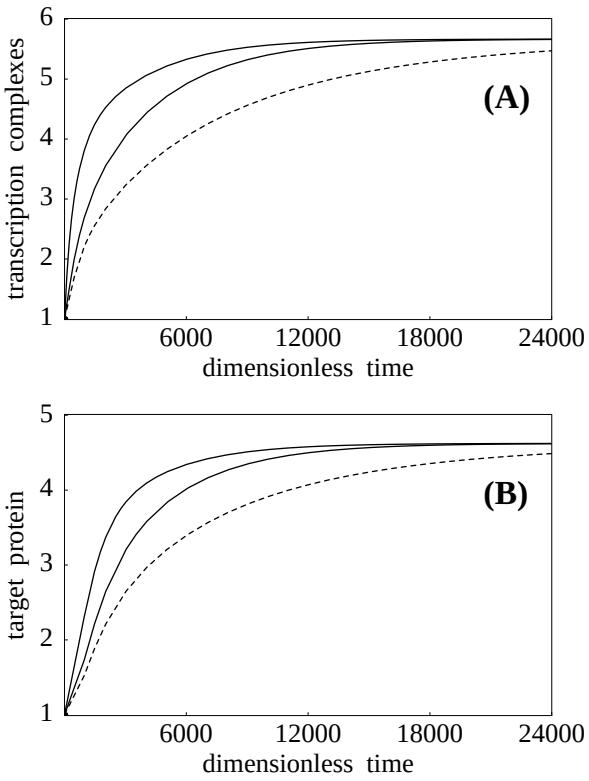

Fig. 5. Effects of continuous Wnt-exposure on gene expression. The Wnt signal $S_\infty \equiv 1$ starts at time $\tau = 0$. The Wnt-induced increase in the rate of Tyr-phosphorylation, $\widehat{\mu}_c(1) - \mu_c$, is given by bottom-up values of 0 ($\mathcal{H}$.I; dashed lines), 25 ($\mathcal{H}$.II) and 175 ($\mathcal{H}$.II), respectively. The higher the rate of Tyr-phosphorylation, the quicker is the initial response to the Wnt stimulus. **(A)** Normalised level of transcription complexes, $C_T(\tau)/C_T^{\star\star}$. **(B)** Normalised level of target protein, $Y(\tau)/Y^{\star\star}$. The $\star\star$'s denote steady-state values in the absence of Wnt. For the parameter values chosen (see Appendix), 1500 dimensionless-time units correspond to 2 hours.

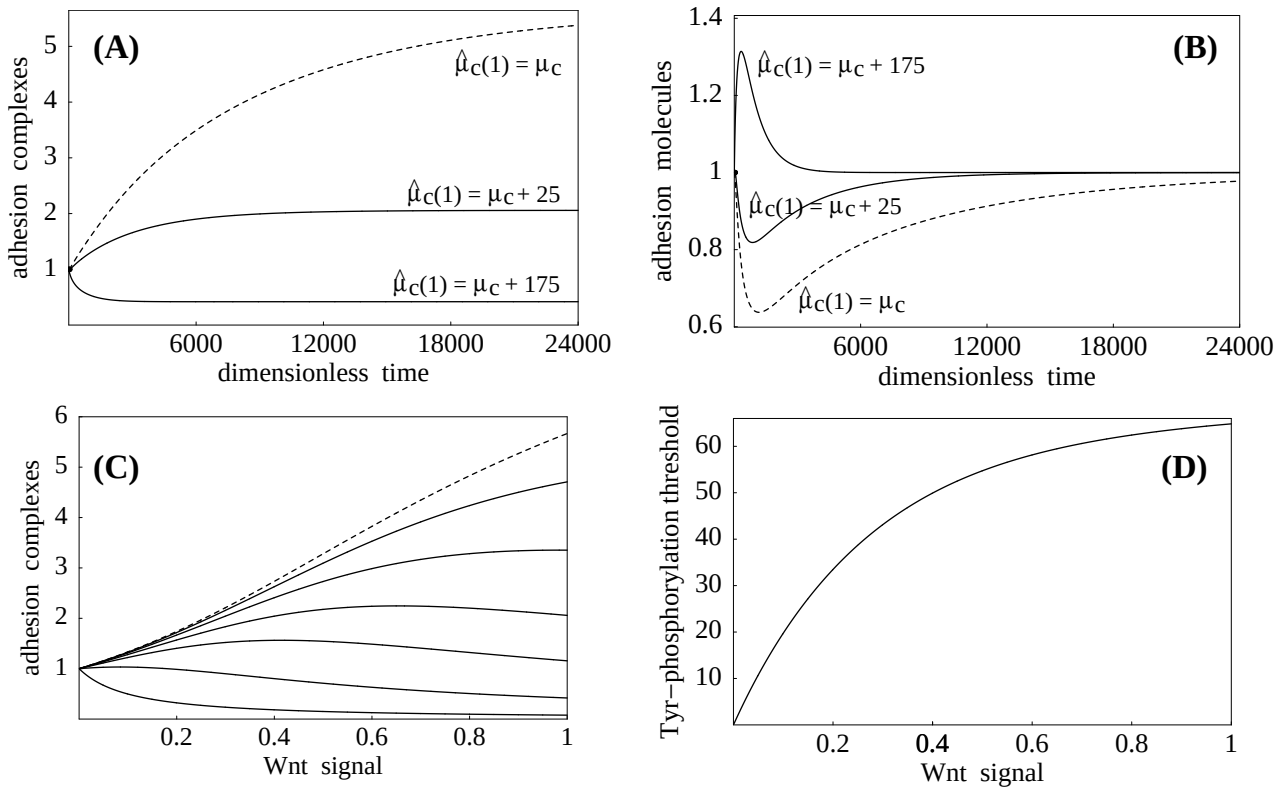

Fig. 6. Effects of long-term Wnt exposure on cell–cell adhesion under $\mathcal{H}$.I (dashed lines) and $\mathcal{H}$.II (solid lines). The Wnt signal starts at time $\tau_\omega = 0$; in the upper panels, $S_\infty = 1$. For the parameter values chosen (see Appendix), 1500 dimensionless-time units correspond to 2 hours. **(A)** Normalised level of adhesion complexes, $C_A(\tau)/C_A^{\star\star}$. Under $\mathcal{H}$.I, cell–cell adhesion always increases in response to the Wnt stimulus. Under $\mathcal{H}$.II, however, a low value of $\widehat{\mu}_c$ can result in an increase in cell–cell adhesion, whereas a higher value can cause a decrease. **(B)** Normalised 'free' adhesion molecules, $A(\tau)/A^{\star\star}$. The transient behaviour differs, but long term behaviour is independent of whethere $\mathcal{H}$.I or $\mathcal{H}$.II is active. **(C)** Normalised equilibrium level of adhesion complexes, $C_A^\star(S_\infty)/C_A^{\star\star}$. The broken line corresponds to $\mathcal{H}$.I and the solid lines to $\mathcal{H}$.II, with top-down $\widehat{\mu}_c(1) - \mu_c$ values of 0, 3, 10, 25, 55, 175 and 1000. Interestingly, two levels of Wnt stimulus can give rise to the same level of cell–cell adhesion for certain values of $\widehat{\mu}_c$. **(D)** Threshold $\widehat{\mu}_c(S_\infty)$ value for which the relation (37) is an equality; that is, in the parameter space above the curve $C_A^\star < C_A^{\star\star}$ holds and Wnt induces a loss of adhesion complexes from the cell membrane.

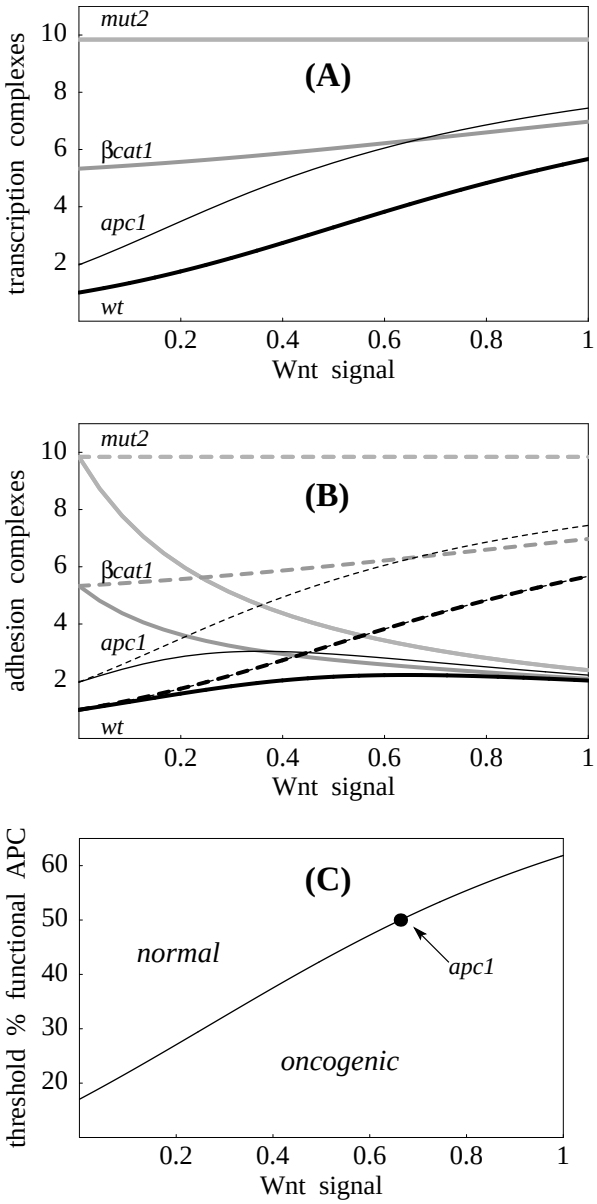

Fig. 7. Response of wild-type (wt) and mutant cells to a Wnt gradient. As before, dashed and solid lines correspond to hypotheses $\mathcal{H}$.I and $\mathcal{H}$.II, respectively. Key: $apc1$ = cells that have lost a single APC-allele (i.e. 50% reduction in α_d); $\beta cat1$ = cells that produce 50% of APC-resistant β-catenin; and $mut2$ = double mutants (i.e. $\alpha_d \approx 0$ or $r_7 \approx 0$ and $r_{16} \approx 0$). To facilitate comparison, all curves have been normalised by the Wnt 'off' steady-state value for normal cells. **(A)** Steady-state value of normalised transcription complexes, which is independent of $\mathcal{H}$.I and $\mathcal{H}$.II. **(B)** Steady-state value of normalised adhesion complexes. **(C)** Percentage of APC activity that gives rise to a level of Wnt signalling equal to that of single β-catenin mutants as a function of the Wnt signal; that is, in the lower region of the parameter space, APC mutants are more oncogenic than single β-catenin mutants. The bullet $(0.663, 50)$ corresponds to the intersection point $(0.663, 6.34)$ in Fig. 7.A. These simulations are based on the non-saturation of Tyr-phosphorylation: the approximate $C_o^\star$ value shown in (36) has been used for the APC mutants (the approximate equilibrium values for the β-catenin mutants are provided in the Appendix).

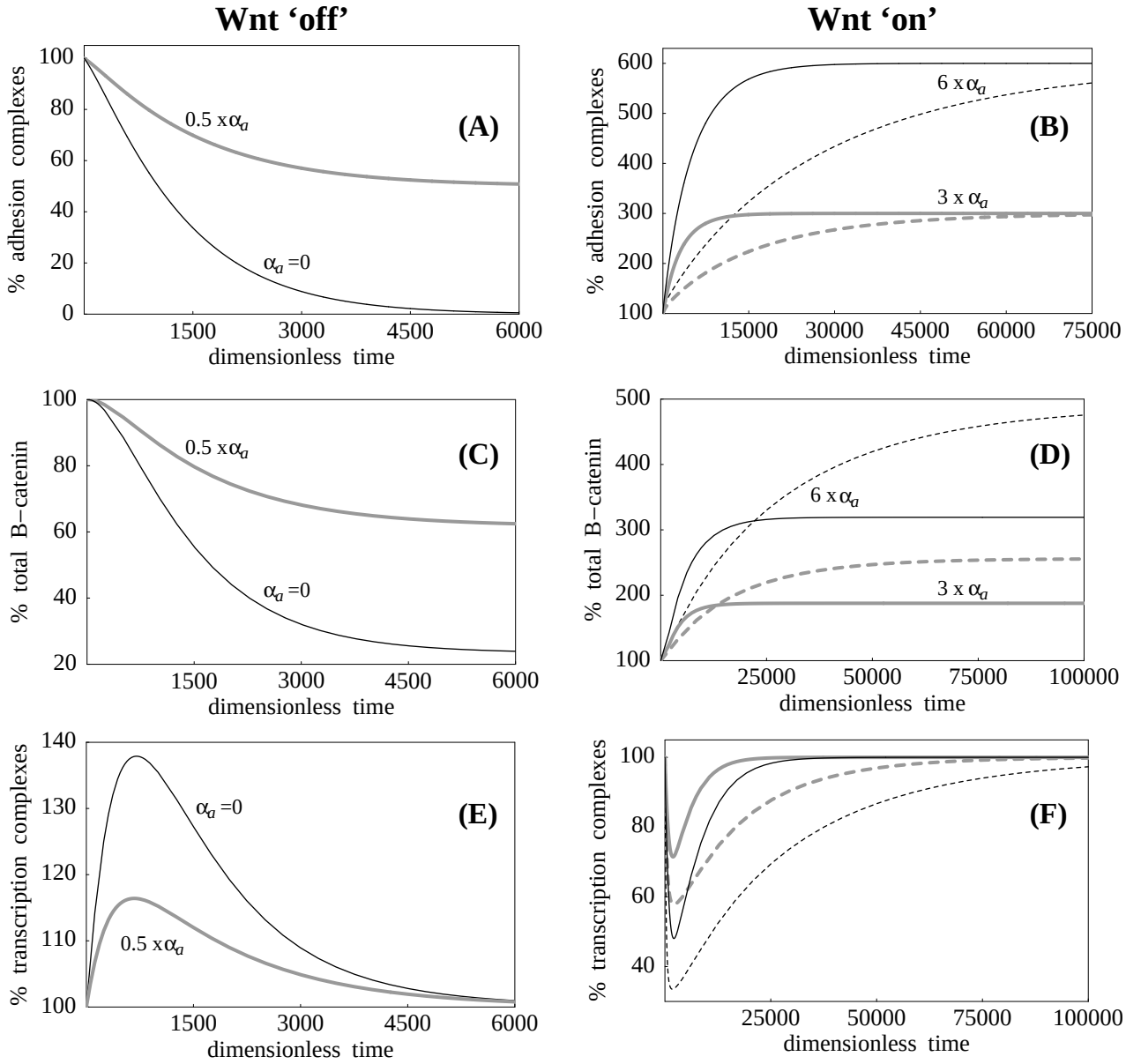

Fig. 8. Impact of changes in E-cadherin expression (occurring at time $\tau = 0$) on cell–cell adhesion and Wnt signalling. For the parameter values chosen (see Appendix), 1500 dimensionless-time units correspond to 2 hours. In the figures on the left, there is no external Wnt signal. In the figures on the right, where Wnt is present, dashed and solid lines correspond to $\mathcal{H}$.I and $\mathcal{H}$.II, respectively. The upper, middle and lower panels show the percentage changes in cell–cell adhesion, $100 \times C_A(\tau)/C_A(0)$; in total cellular β-catenin, $100 \times (C_A(\tau)+C_F(\tau)+C_T(\tau)+C_u(\tau))/(C_A(0)+C_F(0)+C_T(0)+C_u(0))$, and in transcription complexes, $100 \times C_T(\tau)/C_T(0)$, respectively. **(A)** Impact of E-cadherin repression on cadherin-mediated adhesion in the absence of Wnt. **(C)** and **(E)** show the corresponding changes in the percentage of total β-catenin and transcription complexes, respectively. **(B)** Effects of E-cadherin overexpression on cell–cell adhesion in the presence of a Wnt signal. **(D)** and **(F)** show the corresponding changes in the percentage of total β-catenin and transcription complexes, respectively. Note the difference in time scale between the panels (E) and (F).

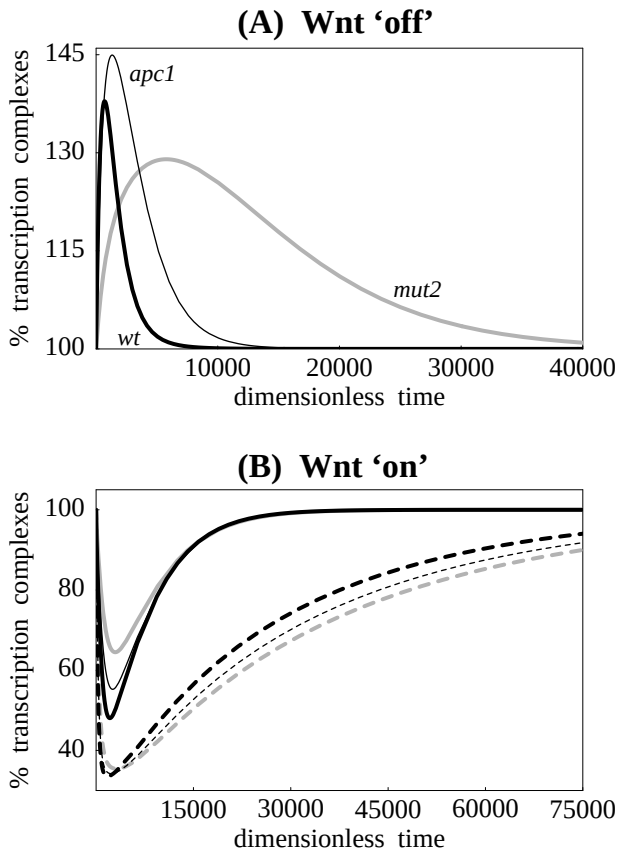

Fig. 9. Impact of E-cadherin expression on Wnt target gene expression in wild-type cells (black bold lines) and APC mutants. The curves show the time course of the percentage of transcription complexes, starting from 100%. Labels as in Fig. 7. Manipulation of E-cadherin expression occurs at time $\tau = 0$. **(A)** Response to complete E-cadherin knock-out ($\alpha_A = 0$) in the absence of an extracellular Wnt signal. **(B)** Response to a six-fold increase in E-cadherin expression ($6 \times \alpha_A$) in the presence of an external Wnt signal, under $\mathcal{H}$.I (dashed lines) and $\mathcal{H}$.II (solid lines).

Summary Box 1: Wnt activation

In silico experiment

Starting from the Wnt 'off' equilibrium concentrations, we expose wild-type cells governed by $\mathcal{H}$.I and $\mathcal{H}$.II to a constant Wnt signal.

Model predictions

(P.1) In response to Wnt, the level of active destruction complexes decreases, whereas the levels of cytoplasmic β-catenin, transcription complexes and target protein increase.

(P.2) Although initially system $\mathcal{H}$.I reacts more quickly to the Wnt signal, the final levels of transcription complexes and target protein are the same for $\mathcal{H}$.I and $\mathcal{H}$.II after long-term Wnt exposure.

(P.3) According to $\mathcal{H}$.I, activation of Wnt signalling always leads to a (similar) increase in both cytoplasmic β-catenin and cadherin-mediated adhesion. In contrast, under $\mathcal{H}$.II, the effect of Wnt on cell–cell adhesion depends on the rate of Tyr-phosphorylation.

Conclusions

It is not possible to discriminate between the two hypotheses on the basis of gene expression following long-term Wnt signalling. In the absence of E-cadherin repression, a Wnt-mediated reduction in cadherin-mediated adhesion can, however, only be explained by $\mathcal{H}$.II.

Summary Box 2: Malignant transformation

In silico experiment

Evaluation of cell–cell adhesion and signalling in wild-type cells (*wt*), cells that have lost a single APC allele (*apc1*), cells producing 50% of APC-resistant β-catenin (β*cat1*) and double (APC or β-catenin) mutants (*mut2*) following a long-term exposure to a constant Wnt signal.

Model predictions

(P.4) In line with (P.2), for any of the cell types above, the concentration of transcription complexes does not depend on the choice of $\mathcal{H}$.I or $\mathcal{H}$.II after long-term Wnt exposure. Moreover, in the absence of Wnt, APC and β-catenin mutations always give rise to an increase in cell–cell adhesion.

(P.5) The relative steady-state levels of transcription complexes in *apc1* and β*cat1* mutants depends on the strength of the Wnt signal. In contrast, *mut2* mutants maintain the same (high) level of Wnt signalling, independently of the Wnt gradient.

(P.6) Under $\mathcal{H}$.I, the steady-state level of cell–cell adhesion in *mut2* mutants does not depend on Wnt, whereas in the other three cell types it increases with Wnt. In contrast, under $\mathcal{H}$.II, cell–cell adhesion decreases with increasing Wnt in *mut2* mutants. The response of the other three cell types depends on the rate of Tyr-phosphorylation.

Conclusions

Autocrine production of Wnt factors can enhance Wnt signalling in *apc1* and β*cat1* mutants, but not in *mut2* mutants. Moreover, oncogenic activation of a Tyr-kinase (e.g. Src) does not promote Wnt signalling but, under $\mathcal{H}$.II and Wnt exposure, may favour tumour invasion by reducing cell–cell adhesion. Finally, the level of APC inactivation required for malignant transformation can vary significantly depending on the values of the kinetic parameters and, in particular, on the presence of extracellular Wnt factors. The extracellular environment can thus have a substantial impact on tumour initiation and progression.

Summary Box 3: Changes in E-cadherin expression

In silico experiment

Changes in the *de novo* synthesis rate of E-cadherin.

Model predictions

(P.7) In the presence of Wnt, an x-fold increase in E-cadherin expressioncauses an x-fold increase in cadherin-mediated adhesion as well as a transient reduction in Wnt signalling in both normal cells and APC mutants, under either $\mathcal{H}$.I or $\mathcal{H}$.II.

(P.8) In the absence of Wnt, an x-fold decrease in E-cadherin expression leads to an x-fold reduction in cadherin-mediated adhesion. This is accompanied by a transient increase in Wnt signalling in both normal cells and APC mutants.

Conclusions

It is not possible to discriminate between $\mathcal{H}$.I and $\mathcal{H}$.II based on manipulation of E-cadherin expression. E-cadherin repression has little oncogenic potential via activating Wnt signalling, but can increase invasiveness by significantly decreasing cell–cell adhesion. In contrast, E-cadherin overexpression can temporarily inhibit Wnt signalling as well as significantly enhancing cell–cell adhesion.

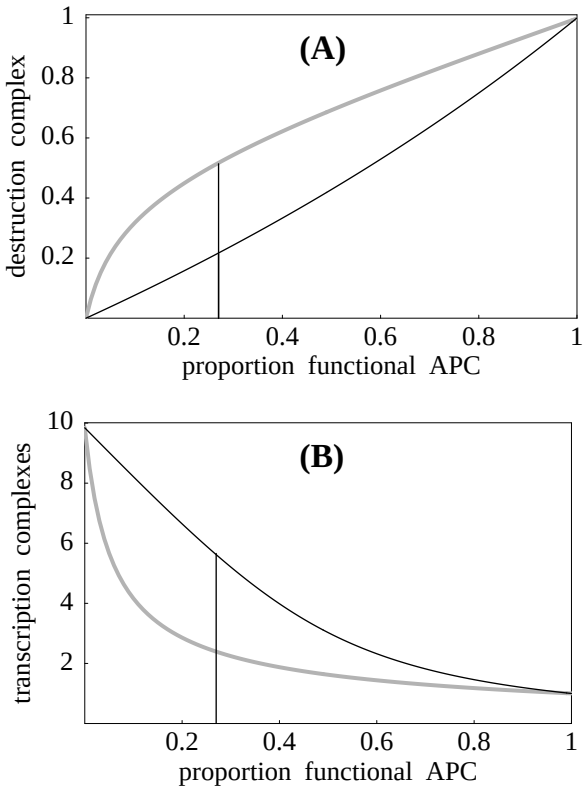

Fig. 10. Impact of the axin2 transcriptional feedback loop. Black lines = feedback-loop inactive ($r_{22} = s_X$); and grey lines = feedback-loop active ($r_{22} = \tilde{s}_X[C_T]/([C_T] + K_X)$). **(A)** and **(B)** show the normalised levels of destruction and transcription complexes as a function of the proportion of active APC protein, respectively. The vertical lines indicate the proportion of active APC protein that has the same effect on Wnt target gene expression as a maximum extracellular Wnt signal. We thus predict that, if the axin feedback loop is active in normal cells, exposure to an extracellular Wnt signal induces negligibly small changes in Wnt-target gene expression. However, we note that these simulations are likely to have overestimated the negative feedback, as in its current form the model does not account for: (1) the presence of axin1 (*AXIN1* is not a Wnt target gene); and (2) the possible dimerisation of mutant and normal APC proteins. We note that the feedback loop has no effect in APC$^{-/-}$ cells nor in APC-resistant β-catenin mutants.

Table A.1: Initial conditions and parameter values.

$[A^{\star\star}] = 10$ nM	$s_A = 20$ nM/h	$d_{CT} = 750$ h^{-1}	$p_c = 0$ h^{-1}
$[C_A^{\star\star}] = 18.14$ nM	$s_{CA} = 250$ (nM h)$^{-1}$	$d_D = 5$ h^{-1}	$p_u = 100$ h^{-1}
$[C_o^{\star\star}] = 2.54$ nM	$s_C = 25$ nM/h	$d_{D_x} = 5$ h^{-1}	$\xi_D = 5$ h^{-1}
$[C_{oT}^{\star\star}] = 2.54$ nM	$s_{CT} = 30$ (nM h)$^{-1}$	$d_T = 0.4$ h^{-1}	$\xi_{D_x} = 5$ h^{-1}
$[D^{\star\star}] = 0.67$ nM	$s_D = 100$ h^{-1}	$d_u = 50$ h^{-1}	$\xi_X = 200$ h^{-1}
$[T^{\star\star}] = 25$ nM	$s_T = 10$ nM/h	$d_X = 100$ h^{-1}	
$[X^{\star\star}] = 0.067$ nM	$s_X = 10$ nM/h	$d_Y = 1$ h^{-1}	
$[Y^{\star\star}] = 0.48$ nM	$s_Y = 10$ h^{-1}	$K_c = 200$ nM	
$[C_c^{\star\star}] = 0$ nM	$d_A = 2$ h^{-1}	$K_D = 5$ nM	
$[C_{cT}^{\star\star}] = 0$ nM	$d_{CA} = 350$ h^{-1}	$K_T = 50$ nM	
$[C_u^{\star\star}] = 0.45$ nM	$d_C = 1$ h^{-1}		

Table A.2: Sensitivity analysis results.

	$\bar{p}_i$	$\phi_i(\bar{D})$	$\phi_i(\bar{X})$	$\phi_i(\bar{C}_F)$, $\phi_i(\bar{C}_T)$	$\phi_i(\bar{C}_u)$	$\phi_i(\bar{C}_o)$, $\phi_i(\bar{C}_A)$	$\phi_i(\bar{C}_c)$	$\phi_i(\bar{Y})$
$i=1$	8	1	0	-1.29	0.14	-0.57	-1.86	-1.23
$i=2$	2	-0.67	0.33	0.86	-0.1	0.38	1.24	0.82
$i=3$	0.2	0	0	-0.14	-0.1	-0.06	-0.21	-0.14
$i=4$	100	0	0	0	0	-0.56	0.44	0
$i=5$	40	0	0	0	0	0.56	-0.44	0
$i=6$	10	0	0	0	0	0	0	-0.95