Biophysical Journal, Volume *115*

Supplemental Information

Unraveling the Control of Cell Cycle Periods during Intestinal Stem Cell Differentiation

Richard Ballweg, Suengwon Lee, Xiaonan Han, Philip K. Maini, Helen Byrne, Christian I. Hong, and Tongli Zhang

The four functional modules of the model. The current model focuses on the control of proliferation of intestinal stem cells (ISCs) and their progeny, which maintain the epithelial lining of the intestine. The cell cycle module is at the core of our investigation. As elaborated below, Wnt, an essential niche signal provided by Paneth cells and the surrounding stroma, is required for sustained self-renewal of intestinal stem cells; Notch signaling provided by Paneth cells promotes cell proliferation; the STAT pathway is important for transducing inflammatory signals to the cell cycle machinery during diseases such as inflammatory bowel disease. All four modules are integrated in our model.

The STAT signaling module. In response to inflammatory signals, innate immune cells in the intestine are activated to secrete IL22 which, in turn, activates the STAT signaling pathway within intestinal epithelial cells (1). Activation of STAT enhances the production of Cyclin D and, thereby, the proliferation of ISCs. When the STAT pathway is inactivated, ISC proliferation is impaired, resulting in a weaker intestinal barrier and impaired damage repair (2). While in practice the STAT family is comprises 7 different family members (3), in our model we represent them via a single, generic protein, STAT, which is activated by IL-22.

The WNT **and MAPK crosstalk module.** In their niches, ISCs are exposed to high levels of Wnt ligands produced by adjacent Paneth cells and the surrounding stromal cells (4-6). The canonical Wnt pathway is activated by the binding of these Wnt ligands to cell surface receptors, which in turn stabilizes cytoplasmic β-catenin (7, 8). Stabilized β-catenin then translocates into the nucleus whereupon it activates the expression of down-stream genes (e.g. Cyclin D, c-Myc, Lgr5, and Ascl2). Of these target genes, both Cyclin D and c-Myc promote cell proliferation; in our model they are lumped together as Cell Cycle Starters (CCS). Upon ablation of β-catenin, ISC proliferation is blocked and the stem cell pool is lost (6).

Cyclin D (bound to Cdk4/6) actively sequesters Cdk inhibitors such as p27 and p57. In addition, the Cyclin D:Cdk complex phosphorylates and inactivates Rb, which then promotes the activation of the transcription factor E2F and the transcription of Cyclin E and Cyclin A. After E2F is activated, it also induces the transcription of the APC:Cdh1 inhibitor Emi1.

The Mitogen Activated Protein Kinase (MAPK) pathway also promotes cell cycle progression. Activated MAPK phosphorylates and activates the transcription factor Activator protein 1 (AP1), and promotes the production of Cyclin D (9).

In contrast to Wnt signaling, MAPK signaling is weakest at the bottom of the intestinal crypt and increases with distance from the crypt base (10). The MAPK pathway is known to repress the transcriptional activity of the Wnt signaling pathway by decreasing the active form of Tcf4 (an essential transcriptional partner of nuclear β-catenin) (10). When MAPK signaling is inhibited, levels of active Tcf4 isoforms increase, and hence, the levels of nuclear β-catenin bound to active Tcf4 isoforms increase (10). Consistent with these observations, it has been hypothesized that the transcription programs downstream of the MAPK and Wnt- β-catenin pathways may repress each other (11). On the basis of these reports, the current model incorporates a mutual repression between the transcriptional activities of the MAPK and Wnt signaling pathways, this repression behavior is modelled by assuming that AP-1 and β-catenin inhibit the rate at which the other protein is produced (see **Table 1**).

The Notch signaling module. Four types of Notch receptors and several ligands Delta-like (Dll), Serrate, Lag-2 (DSLs) are functional in mammalian cells (12). Among these, the ligands Dll1 and Dll4 (13), and the receptors Notch1 and Notch2 (14), play significant roles in the intestine. In our model, we denote by Notch the combined activity of Notch1 and Notch2, and by DSL the net effect of Dll1 and Dll4. We assume further that DSL activates Notch.

Upon binding to DSL ligands, the Notch receptor is elevated by the ϒ-secretase enzyme complex, causing the release of the Notch intracellular domain (NICD), which then translocates into the nucleus. Although NICD does not have transcriptional activity, it can recruit transcriptional activators to the DNA binding complex CSL (CBF1, Suppressor of Hairless, Lag-1). Through CSL, NICD promotes the expression of Notch target genes, including Hes1. In our model, we assume that Notch actives Hes1 production. Hes1 is a basic helix-loop-helix transcriptional repressor, which represses the transcription factor Atoh1 (15). When active, Atoh1 drives stem cells towards a the secretory cell fate in the intestine by activating several downstream genes (16). Atoh1 is known to be necessary for expression of the CDK inhibitors p27 and p57 during Notch inhibition (17). Atoh1 has also been shown to induce expression of p21, p27 and p57 in colorectal cancer cell lines (18). While Hes1 has been shown to directly inhibit p27 and p57, in keeping with the minimal nature of the model, and due to Atoh1's role in differentiation, we choose to omit this reaction from our model.

The cell proliferation module. Many mathematical models of varying detail have been constructed for the control of the mammalian cell cycle (19-24). Because the current work focuses on how differentiation interacts with the cell cycle control mechanism, rather than the detailed molecular interactions within the cell cycle control network, we employ an idealized model of cell cycle control.

Cell proliferation is driven by the periodic activation and inactivation of Cyclin-dependent kinases (CDK) (25). In non-proliferating cells, CDK is kept inactive by several layers of regulation. CDK inhibitors are abundant in resting cells, binding to CDKs and preventing them from reaching their physiological targets. Also, the transcriptional repressor RB is active and represses the transcription of Cyclins A and E, which are the activating partners of CDK.

Meanwhile, the degradation machinery of APC/C:Cdh1 causes the degradation of CDK activating cyclins (25). These repressors of CDK are lumped together as CKR in the current model.

CDK actively inhibits its repressors. It phosphorylates inhibitors such as p27, which degrade more rapidly when they are phosphorylated. Activated CDK also phosphorylates Rb and inactivates Rb (25). Additionally, CDK phosphorylates the APC/C protein complex preventing it from binding to Cdh1, thus blocking activation of the APC/C:Cdh1 degradation machinery (25). In this way, CDK and its repressor, CKR, form a mutually antagonistic positive feedback loop. This positive feedback is incorporated in the current model.

The current cell cycle module also incorporated a negative feedback loop, in which CDK activates the degradation machinery APC/C:Cdc20. By degrading cyclins, which are essential activating partners of CDK, APC/C:Cdc20 causes inactivation of CDK (25).

Supplementary Table 1. Equations of the model

The Notch Signalling Module	
HES1	NOTCH
Activity of the transcription repressor Hes1	Activity of the Notch pathway
$\frac{dHES}{dt} = \tau_{HES} \cdot (F_{HES} - HES)$	$\frac{dNOC}{dt} = \tau_{NOC} \cdot (F_{NOC} - NOC)$
$F_{HES} = \frac{1}{1 + e^{-\sigma \cdot W_{HES}}}$	$F_{NOC} = \frac{1}{1 + e^{-\sigma \cdot W_{NOC}}}$
$W_{HES} = R_0^{HES} + R_{NOC}^{HES} \cdot NOC$	$W_{NOC} = R_0^{NOC} + R_{DSL}^{NOC} * DSL$
ATOH1	
Activity of Atoh1	
$\frac{dAtoh1}{dt} = \tau_{Atoh1} \cdot (F_{Atoh1} - Atoh1)$	
$F_{Atoh1} = \frac{1}{1 + e^{-\sigma \cdot W_{Atoh1}}}$	
$W_{Atoh1} = R_0^{Atoh1} + R_{Hes}^{Atoh1} \cdot Hes$	
The Stat Signalling Module	
Activity of STAT transcription factors	
$\frac{dStat}{dt} = \tau_{Stat} \cdot (F_{Stat} - Stat)$	
$F_{Stat} = \frac{1}{1 + e^{-\sigma \cdot W_{Stat}}}$	
$W_{Stat} = R_0^{Stat} + R_{Il22}^{Stat} \cdot Il22$	
The Wnt-MAPK Module	
BET	AP1
Activity of the transcription activator β-catenin of the Wnt pathway	Activation of the transcription factor AP1 of the MAPK pathway
$\frac{dBET}{dt} = \tau_{BET} \cdot (F_{BET} - BET)$	$\frac{dAP1}{dt} = \tau_{Ap1} \cdot (F_{AP1} - AP1)$
$F_{BET} = \frac{1}{1 + e^{-\sigma \cdot W_{BET}}}$	$F_{AP1} = \frac{1}{1 + e^{-\sigma \cdot W_{AP1}}}$
$W_{BET} = R_0^{BET} + R_{Wnt}^{BET} \cdot Wnt + R_{AP1}^{BET} \cdot AP1$	$W_{AP1} = R_0^{AP1} + R_{MAPK}^{AP1} \cdot MAPK + R_{BET}^{AP1} \cdot BET$
The Cell Cycle Module	
CKR	CDK
CDK repressors	Cyclin dependent kinases
$\frac{dCKR}{dt} = \tau_{CKR} \cdot (F_{CKR} - CKR)$	$\frac{dCDK}{dt} = \tau_{CDK} \cdot (F_{CDK} - CDK)$
$F_{CKR} = \frac{1}{1 + e^{-\sigma \cdot W_{CKR}}}$	$F_{CDK} = \frac{1}{1 + e^{-\sigma \cdot W_{CDK}}}$
$W_{CKR} = R_0^{CKR} + R_{CDK}^{CKR} \cdot CDK + R_{CCS}^{CKR} \cdot CCS + R_{Atoh1}^{CKR} \cdot Atoh1$	$W_{CDK} = R_0^{CDK} + R_{CKR}^{CDK} \cdot CKR + R_{C20}^{CDK} \cdot C20$
C20	CCS
APC/C:Cdc20 degradation machinery	Cell cycle starters
$\frac{dC20}{dt} = \tau_{C20} \cdot (F_{C20} - C20)$	$\frac{dCCS}{dt} = k_{sBET} \cdot BET + k_{sAP1} \cdot AP1 + k_{sStat} \cdot Stat - k_{dCCS} \cdot CCS$
$F_{C20} = \frac{1}{1 + e^{-\varepsilon \cdot W_{C20}}}$	
$W_{NOC} = R_0^{C20} + R_{CDK}^{C20} \cdot CDK$	

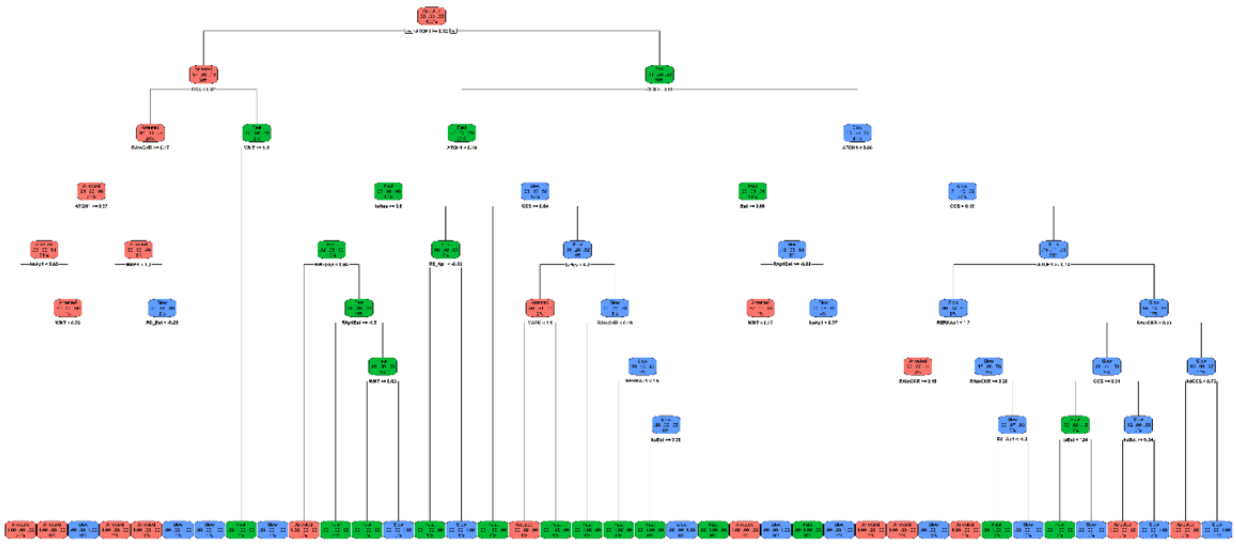
* All initial levels are zero.

Supplementary Table 2. Basal parameters of the model

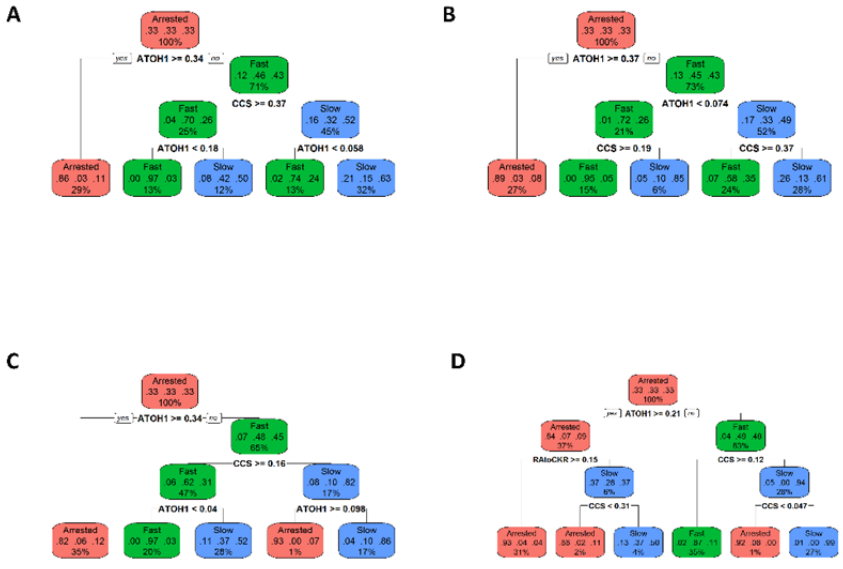
Model Parameters			
Parameters for Notch Signalling module			
Time scale of Hes1	Hes1 background inactivation	Time scale of Notch	Notch background inactivation
$\tau_{HES} = 5$	$R_0^{HES} = -0.6$	$\tau_{NOC} = 5$	$R_0^{NOC} = -0.7$
Nonlinearity adjusting factor	Activation of Hes1 by Notch pathway	Activation of notch pathway by Dsl ligands	
$\sigma = 5$	$R_{NOC}^{HES} = 1$	$R_{DSL}^{NOC} = 1.5$	
Atoh1 background activation	Inactivation of Atoh1 by Hes1	Time scale of Atoh1	
$R_0^{Atoh1} = 0.6$	$R_{Hes}^{Atoh1} = -1.2$	$\tau_{Atoh1} = 5$	
Parameters for Stat Signalling Module			
IL22 Level	Stat background inactivation	Stat activation by IL-22	
$IL22 = 1$	R_0^{Stat} = -0.7	R_{IL22}^{Stat} =1.5	
Parameters for Wnt-MAPK module			
Time scale of β-catenin	β-catenin background inactivation	Time scale of AP1	AP1 background inactivation
$\tau_{Bet} = 100$	$R_0^{BET} = -0.2$	$\tau_{Ap1} = 100$	$R_0^{AP1} = -1$
Activation of β-catenin by Wnt pathway	Inhibition of β-catenin by AP1	Activation of AP1 by MAPK pathway	Inactivation of AP1 by β-catenin
$R_{Wnt}^{BET} = 0.9$	$R_{AP1}^{BET} = -1.2$	$R_{MAPK}^{AP1} = 2$	$R_{BET}^{AP1} = -1.5$
Parameters for cell cycle module			
Time scale of CKR	CKR background activation	Time scale of CDK	CDK background activation
$\tau_{CKR} = 5$	$R_0^{CKR} = 1.03$	$\tau_{CDK} = 5$	$R_0^{CDK} = 0.68$
Activation of CKR by Atoh1	Inhibition of CKR by CDK	Inhibition of CDK by CKR	Inhibition of CDK by C20
$R_{Ato}^{CKR} = 0.23$	$R_{CDK}^{CKR} = -1.5$	$R_{CKR}^{CDK} = -1$	$R_{C20}^{CDK} = -0.5$
Time scale of C20	Inhibition of CKR by CCS	Promotion by β-catenin	CCS degradation
$\tau_{c20} = 0.5$	$R_{CCS}^{CKR} = -0.15$	$k_{sBET} = 0.28$	$k_{dCCS} = 1$
Activation of C20 by CDK	C20 background inactivation	Promotion by AP1	Nonlinearity adjusting factor
$R_{CDK}^{C20} = 1.1$	$R_0^{C20} = -0.7$	$k_{sAP1} = 0.5$	$\varepsilon = 15$
Nonlinearity adjusting factor	Promotion by Stat		
$\sigma = 5$	$k_{sStat} = 0.28$		

Supplementary Figures:

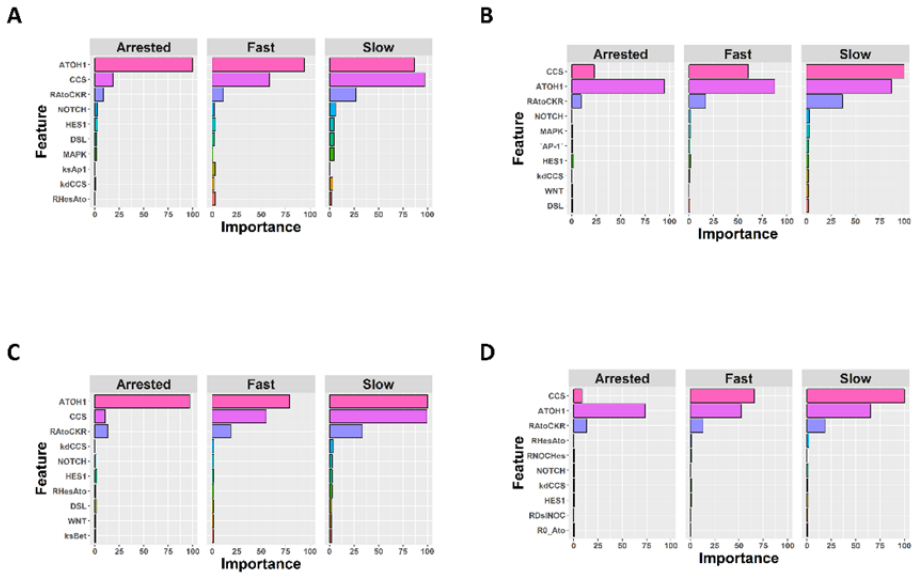
Supplementary Figure 1.



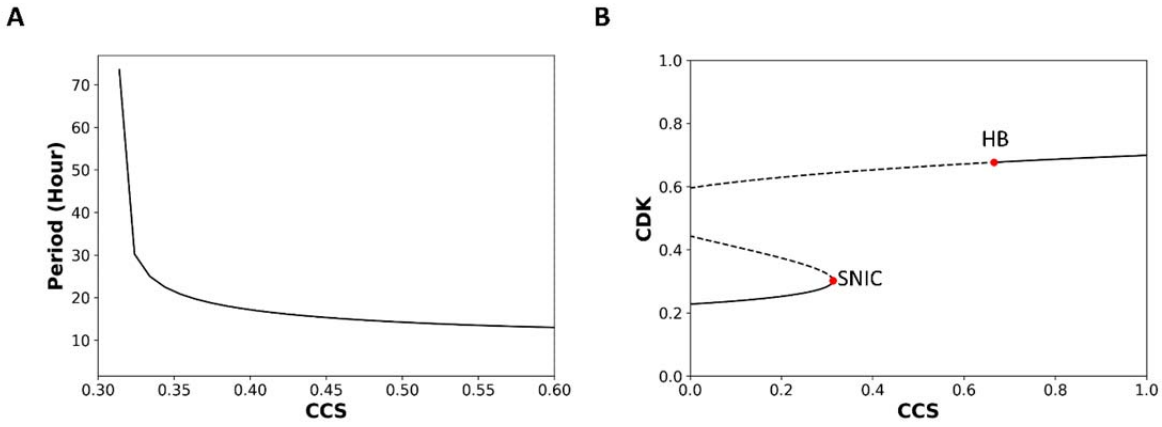
Supplementary Figure 2



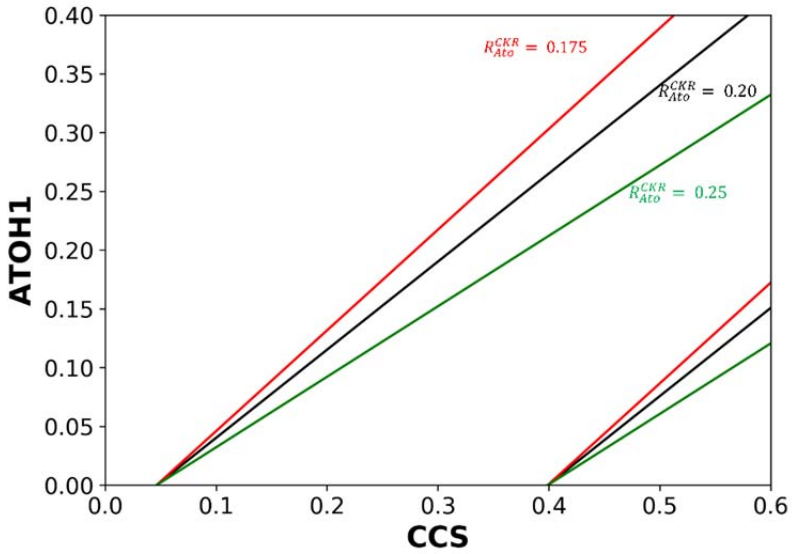
Supplementary Figure 3.



Supplementary Figure 4.



Supplementary Figure 5.



Supplementary Figure Legends:

Supplementary Figure 1.

An example of a fully filled Classification Tree for a total of 300 cells. Nodes are labeled Slow, Fast or Arrested based on the dominant cell type.

Supplementary Figure 2.

Classification Trees generated with different ranges of parameter variability and different numbers of models, this figure accompanies **Figure 2C** of the main text. **A**. Variability: 70% to 130%, 3000 models. **B**. Variability: 70% to 130% 10,000 cells. **C.** Variability: 50% to 200% ~10,000 cells. **D.** Variability: 20% to 500% 10,000 cells. Nodes are labeled Slow, Fast or Arrested based on the dominating cell type.

Supplementary Figure 3.

Variable importance analysis with Random Forest, computed with different ranges of parameter variability and different numbers of models. This accompanies **Figure 2D** of the main text. **A**. Variability: 70% to 130%, 3000 models. **B**. Variability: 70% to 130%, ~10,000 models. **C.** Variability: 50% to 200%, ~10,000 models. **D.** Variability: 20% to 500% , ~10,000 models.

Supplementary Figure 4

A. Period diagram depicting the change in cell cycle period (in hours) as a function of CCS; accompanies **Figure 4A** of the main text. **B**. Full 1 parameter bifurcation diagram with CDK as the representative variable and CCS as the control variable. A SNIC bifurcation and a Hopf Bifurcation are separated by an unstable, oscillatory region; accompanies **Figure 4A** of the main text.

Supplementary Figure 5

2-D plot showing how the locations of the SNIC boundaries change as R_{Ato}^{CKR} varies ($R_{Ato}^{CKR} = 0.175$, Red; $R_{Ato}^{CKR} = 0.20$, Black; $R_{Ato}^{CKR} = 0.25$, Green) ; accompanies **Figures 4C** and **4D** of the main text.

REFERENCES

1. Lindemans CA, *et al.* (2015) Interleukin-22 promotes intestinal-stem-cell-mediated epithelial regeneration. *Nature* 528(7583):560-564.
2. Gilbert S, *et al.* (2015) Activated STAT5 confers resistance to intestinal injury by increasing intestinal stem cell proliferation and regeneration. *Stem Cell Reports* 4(2):209-225.
3. Shuai K & Liu B (2003) Regulation of JAK-STAT signalling in the immune system. *Nat Rev Immunol* 3(11):900-911.
4. Clevers H (2013) The intestinal crypt, a prototype stem cell compartment. *Cell* 154(2):274-284.
5. Crosnier C, Stamataki D, & Lewis J (2006) Organizing cell renewal in the intestine: stem cells, signals and combinatorial control. *Nat Rev Genet* 7(5):349-359.
6. Fevr T, Robine S, Louvard D, & Huelsken J (2007) Wnt/beta-catenin is essential for intestinal homeostasis and maintenance of intestinal stem cells. *Mol Cell Biol* 27(21):7551-7559.
7. Clevers H, Loh KM, & Nusse R (2014) Stem cell signaling. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. *Science* 346(6205):1248012.
8. Clevers H & Nusse R (2012) Wnt/beta-catenin signaling and disease. *Cell* 149(6):1192-1205.
9. Wilkinson MG & Millar JB (2000) Control of the eukaryotic cell cycle by MAP kinase signaling pathways. *FASEB J* 14(14):2147-2157.
10. Heuberger J, *et al.* (2014) Shp2/MAPK signaling controls goblet/paneth cell fate decisions in the intestine. *Proc Natl Acad Sci U S A* 111(9):3472-3477.
11. Riemer P, *et al.* (2015) Transgenic expression of oncogenic BRAF induces loss of stem cells in the mouse intestine, which is antagonized by beta-catenin activity. *Oncogene* 34(24):3164-3175.
12. Kopan R (2012) Notch signaling. *Cold Spring Harb Perspect Biol* 4(10).
13. Pellegrinet L, *et al.* (2011) Dll1- and dll4-mediated notch signaling are required for homeostasis of intestinal stem cells. *Gastroenterology* 140(4):1230-1240 e1231-1237.
14. Wu Y, *et al.* (2010) Therapeutic antibody targeting of individual Notch receptors. *Nature* 464(7291):1052-1057.
15. Kazanjian A & Shroyer NF (2011) NOTCH Signaling and ATOH1 in Colorectal Cancers. *Curr Colorectal Cancer Rep* 7(2):121-127.
16. Noah TK & Shroyer NF (2013) Notch in the intestine: regulation of homeostasis and pathogenesis. *Annu Rev Physiol* 75:263-288.
17. Kim TH & Shivdasani RA (2011) Genetic evidence that intestinal Notch functions vary regionally and operate through a common mechanism of Math1 repression. *J Biol Chem* 286(13):11427-11433.
18. Bossuyt W, *et al.* (2009) Atonal homolog 1 is a tumor suppressor gene. *PLoS Biol* 7(2):e39.
19. Novak B & Tyson JJ (2004) A model for restriction point control of the mammalian cell cycle. *J Theor Biol* 230(4):563-579.
20. Csikasz-Nagy A, Battogtokh D, Chen KC, Novak B, & Tyson JJ (2006) Analysis of a generic model of eukaryotic cell-cycle regulation. *Biophys J* 90(12):4361-4379.

21. Gerard C & Goldbeter A (2009) Temporal self-organization of the cyclin/Cdk network driving the mammalian cell cycle. *Proc Natl Acad Sci U S A* 106(51):21643-21648.
22. Toettcher JE*, et al.* (2009) Distinct mechanisms act in concert to mediate cell cycle arrest. *Proc Natl Acad Sci U S A* 106(3):785-790.
23. Singhania R, Sramkoski RM, Jacobberger JW, & Tyson JJ (2011) A hybrid model of mammalian cell cycle regulation. *PLoS Comput Biol* 7(2):e1001077.
24. Weis MC, Avva J, Jacobberger JW, & Sreenath SN (2014) A data-driven, mathematical model of mammalian cell cycle regulation. *PLoS One* 9(5):e97130.
25. Morgan DO (2007) *The cell cycle : principles of control* (Published by New Science Press in association with Oxford University Press ;
Distributed inside North America by Sinauer Associates, Publishers, London
Sunderland, MA) pp xxvii, 297 p.