Compartmental Modelling of the Wnt Pathway: Elucidating the role of nucleo-cytoplasmic shuttling of β-catenin and its antagonists

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"Sometimes your only available transportation is a leap of faith."
Margaret Shepard

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Abstract

Wnt/β-catenin signalling plays a critical role in development and disease. The key player of the pathway is β-catenin, which is able to shuttle between cytoplasm and nucleus. In the nucleus, complex formation of β-catenin and TCF initiates target gene expression. The activity of β-catenin is mainly regulated by the destruction complex consisting of the scaffolding proteins APC and Axin, and the kinase GSK3. In recent years, it has been shown that these antagonists are also capable of nucleo-cytoplasmic shuttling, although their functional relevance in the nucleus remains to date elusive. This study aims to investigate the impact of nucleo-cytoplasmic shuttling of APC, Axin, and GSK3 on the [β-catenin/TCF] concentration, which is considered as the output of the pathway. To this end, I establish and analyse compartmental models encoded in ordinary differential equations, which are based on experimental findings. These models allow for protein shuttling between the nucleus and the cytoplasm and the resulting regulation of subcellular β-catenin levels through retention and degradation. Using simulations of transient dynamics, as well as steady state and sensitivity analyses, I gain the following key results: Nucleo-cytoplasmic shuttling of β-catenin and its antagonists can lead to an increase of the [β-catenin/TCF] concentration in contrast to a reference model without antagonist shuttling. I demonstrate that the total robustness of the [β-catenin/TCF] output is closely linked to its absolute concentration. Therefore nucleo-cytoplasmic shuttling also leads to an increased robustness of [β-catenin/TCF] signalling against intracellular perturbations. In addition, I show that balanced shuttling of β-catenin antagonists yields maximal relative response of [β-catenin/TCF] to a transient Wnt signal. A sensitivity analysis moreover reveals that nuclear accumulation of the destruction complex renders the pathway robust against fluctuations in the extracellular Wnt signal and against changes in the compartmental distribution of β-catenin. Thus my results strongly suggest that Wnt/β-catenin signalling can benefit from nucleo-cytoplasmic shuttling of APC, Axin and GSK3, although they are in general β-catenin antagonising proteins. These findings provide the basis for continuative experimental investigations of antagonist shuttling in the Wnt pathway.

Zusammenfassung

Der Wnt/β-catenin Signalweg spielt eine entscheidene Rolle in der Entwicklungsbiologie und bei der Entstehung von Krankheiten. Das Protein β-catenin ist der zentrale Protagonist dieses Signalweges. Im Nukleus bildet es gemeinsam mit TCF einen Transkriptionsfaktorkomplex, der die Expression verschiedener Zielgene einleitet. Die Aktivität des β-catenin wird maßgeblich durch einen Abbaukomplex reguliert, der aus den Gerüstproteinen APC und Axin, und der Kinase GSK3 besteht. Diese Proteine sind Antagonisten des β-catenins und wie dieses in der Lage zwischen Zytoplasma und Nukleus hin und her zu shutteln, wobei die funktionelle Relevanz der Antagonisten im Zellkern bis heute nicht geklärt ist. Ziel der vorliegenden Arbeit ist die Untersuchung des nukleo-zytoplasmischen Shuttelns (NZS) der Antagonisten und dessen Auswirkung auf die [β-catenin-TCF] Konzentration, die als Output des Signalweges gilt. Zu diesem Zweck habe ich auf experimenteller Grundlage Kompartimentenmodelle entwickelt und analysiert, die auf Systemen von gekoppelten Differentialgleichungen beruhen. Diese Modelle beschreiben das NZS des β-catenins und seiner Antagonisten und die daraus resultierende Beeinflussung der subzellulären β-catenin Konzentration durch Retention und Abbau. Mittels Simulation der transienten Dynamik, sowie durch Analyse des stationären Zustands und dessen Sensitivität gegenüber Störungen, bin ich zu den folgenden Ergebnissen gekommen: Das NZS der β-catenin Antagonisten kann im Vergleich zu einem Referenzmodel, in dem die Antagonisten nicht shutteln, zu einer Maximierung der [β-catenin/TCF] Konzentration führen. Da ich zeigen konnte, dass die Gesamtrobustheit des [β-catenin/TCF] Output stark an die absoluten Werte der [β-catenin/TCF] Konzentration gekoppelt ist, bewirkt das NZS der Antagonisten zusätzlich eine erhöhte Robustheit der [β-catenin/TCF] Konzentration gegenüber kleinsten intrazellulären Störungen. Die Modellanalyse belegt weiterhin, dass i) das NZS der Antagonisten zwischen den Kompartimenten eine maximale Reaktion der [β-catenin/TCF] Konzentration auf transiente Wnt Signale verursacht und ii) die Akkumulation des Abbaukomplexes im Nukleus in einem robusten Output gegenüber Fluktuationen des extrazellulären Wnt Signals und der zellulären Verteilung des β-catenin resultiert. Diese Erkenntnisse verdeutlichen, dass der effektive Output des Wnt Signalweges von dem NZS dieser Proteine profitieren kann, obgleich sie bisher nur für eine antagonistische Rolle im Zytoplasma bekannt waren. Die Ergebnisse dieser Arbeit bieten die Basis für weiterführende experimentelle Untersuchungen der Rolle der Antagonisten im Wnt Signalweg.

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List of Abbreviations

AAG	APC-Axin-GSK3, i.e. the destruction complex
APC	Adenomatous polyposis coli
CK1	Casein kinase 1
CRM1	Chromosome region maintenance 1
Dkk1	Dickkopf 1
DNA	Deoxyribonucleic acid
Dsh	Dishevelled
Frz	Frizzled
GSK3	Glycogen synthase kinase 3
iFFL	incoherent Feedforward loop
IκB	inhibitor of Nuclear factor-kappa B
Int-1	Integration-1
JAK	Janus kinase
kDa	Kilodalton
LEF	Lymphoid enhancer factor
LRP	LDL-related protein
mRNA	messenger Ribonucleic acid
NES	Nuclear export signal
NF-κB	Nuclear factor-kappa B
NLS	Nuclear localisation signal
NPC	Nuclear pore complex
ODE	Ordinary differential equation
PDE	Partial differential equation
RNA	Ribonucleic acid
sFRP	secreted Frizzled-related protein
STAT	Signal transducer and activator of transcription
TCF	T-cell factor
wg	wingless

1. Motivation and objectives

In order to maintain life, cells must continuously sense their external and internal environment and induce changes on the basis of this information [Alberts et al., 2002]. This processing of information is carried out by signalling pathways and networks [Wolkenhauer and Mesarović, 2005], which enable the cells to adapt to their environment, continue or stop their development, and form more complex structures through intercellular communication [Wolkenhauer et al., 2005]. During evolution, living cells have developed means to control the temporal dynamics of signalling pathways. However, recent findings emphasise the pivotal role that space plays in intra- and intercellular dynamics [Kholodenko, 2006, Klipp et al., 2009]. Crucial cell decisions, including whether to undergo proliferation, apoptosis (programmed cell death) and differentiation, are governed by the temporal dynamics and spatial distribution of key signalling proteins [Kholodenko et al., 2010]. Therefore, cells and organisms show complex spatial structures that are vital for the processes of life. Compartmentalisation is a major characteristic of eukaryotic cells, as demonstrated, for example, for the Toll-like receptor function [Barton and Kagan, 2009]. The partitioning of a cell by membranes results in a separation of functional units and in the formation of reaction spaces that might differ significantly in their molecular composition. This is due to a restricted permeability of the membranes and the controlled shuttling of molecules, such as proteins, protein complexes and mRNA (messenger ribonucleic acid), between the compartments, especially between the cytoplasm and the nucleus [Klipp et al., 2009, Schmierer et al., 2008]. The importance of the localisation of molecules in signalling in general and of compartmental shuttling of proteins in particular, becomes apparent considering the Janus kinase - Signal transducer and activator of transcription (JAK-STAT) pathway, which has been studied in great detail [Beirer and Höfer, 2006, Horvath, 2000]. In the JAK-STAT pathway, the rapid signal transduction from the receptor to the nucleus is mediated by STAT5. This central protein is phosphorylated on recruitment to the activated receptor complex, where it is also dimerised. It then migrates to the nucleus, where it stimulates the transcription of target genes. After dephosphorylation, the STATs

are relocated back into the cytoplasm and recruited to the receptor complex, if it is still active. Hence, STAT proteins continuously translocate between the nucleus and the cytoplasm [Reich and Liu, 2006]. Using mathematical modelling, Swameye et al. [2003] have identified nucleo-cytoplasmic shuttling as an essential feature of the JAK-STAT core signalling module. They predicted that steps of nuclear import and export are most sensitive to perturbations and experimentally verified these predictions by inhibiting nuclear export.
Another example emphasising the central role of space is the nuclear factor-κB (NK-κB) signalling module, whose output is regulated by spatio-temporal coordination involving a negative feedback [Hoffmann et al., 2002, Kholodenko et al., 2010, Scott and Pawson, 2009]. NF-κB is inactive in the cytoplasm when associated with the inhibitor of NK-κB (IκB). Cell stimulation leads to phosphorylation and degradation of IκB, resulting in the release and subsequent translocation of free NF-κB into the nucleus, where it activates target genes. Thereby, NF-κB initiates transcription of its own inhibitor IκB, which can bind nuclear NF-κB and export it back to the cytoplasm. This example shows that the spatial-temporal arrangement of signalling proteins is subject to dynamic regulation and, vice versa, that spatial organisation can specify kinetic activity profiles [Kholodenko et al., 2010]. In particular it demonstrates that the nucleo-cytoplasmic shuttling of antagonists can play an essential role in the regulation of the pathway activity.

In recent years, the nucleo-cytoplasmic shuttling of Wnt pathway components and its role in regulating the signalling activity have come into focus of research [Cao et al., 2009, Caspi et al., 2008, Li et al., 2010, 2012]. Wnt/β-catenin signalling is a key regulatory system in development and disease [MacDonald et al., 2009]. The key player of the pathway is β-catenin [Kikuchi, 2000]. Its activity is mainly regulated by the destruction complex consisting of Adenomatous polyposis coli (APC), Axin and Glycogen synthase kinase 3 (GSK3) [Stamos and Weis, 2012]. In the nucleus, complex formation of β-catenin and T-cell factor (TCF) initiates target gene expression [Behrens et al., 1996, Mosimann et al., 2009]. However, not only β-catenin as a transcriptional cofactor is able to translocate into and out of the nucleus. A surprisingly high number of cytoplasmic Wnt regulators are shuttling proteins that have been proven to also reside in the nucleus [Bijur and Jope, 2003, Neufeld et al., 2000, Rosin-Arbesfeld et al., 2000, Wiechens et al., 2004], while their functional relevance in the nucleus is still unclear. The goal of this thesis is to provide a comprehensive analysis of the role of nucleo-cytoplasmic shuttling of β-catenin and its antagonists

APC, Axin, and GSK3 in Wnt/β-catenin signalling and the resulting regulation of subcellular β-catenin levels. The purpose of my theoretical analysis is to inspire biologists to design and perform new experiments. My study aims at answering the following key questions: Does the nucleo-cytoplasmic shuttling of the β-catenin antagonists APC, Axin and GSK3 affect the [β-catenin/TCF] concentration, which is considered as the output of Wnt signalling? Can the [β-catenin/TCF] concentration be maximised by antagonist shuttling, leading to an optimised signal transduction through the cell? How is the output of the pathway influenced by cytoplasmic and nuclear retention of β-catenin, and by phosphorylation and subsequent degradation of β-catenin?

To answer these questions, I investigate the impact of nucleo-cytoplasmic antagonist shuttling on [β-catenin/TCF] signalling using mathematical modelling. I define a mathematical model as an abstract representation of a (in this case, biological) system in which the interactions and dynamics of its components are described using the language of mathematics. In this work, I design and analyse compartmental models encoded in coupled ordinary differential equations (ODEs) that describe spatio-temporal changes of pathway components as functions of molecular interactions and transport processes. These ODE models are on a subcellular, compartmental scale and allow for the retention of β-catenin by APC, and β-catenin phosphorylation and successive degradation in the nucleus and cytoplasm, respectively.

Outline of this thesis

This thesis is structured into the following chapters: **Chapter 2** provides the biological background regarding the signal transduction of the Wnt/β-catenin pathway as well as intracellular transport mechanisms of the involved proteins. In **Chapter 3**, I give an introduction to mathematical modelling of spatio-temporal biochemical processes in cells. In particular, I present the modelling framework and model analysis techniques I decided to use in this thesis. **Chapter 4** offers a survey of mathematical models dealing with the Wnt pathway on a biochemical and subcellular level. I conclude this literature review with summarising open questions regarding Wnt pathway modelling. In **Chapter 5**, I investigate the impact of nucleo-cytoplasmic shuttling of the antagonist APC on the [β-catenin/TCF] concentration. In this model the β-catenin concentration is mainly regulated through compartmental retention by APC (the "Retention Model"). This work has been published in [Schmitz

et al., 2011] and is reproduced with permission from Elsevier. In **Chapter 6**, I investigate the influence of nucleo-cytoplasmic shuttling of the three antagonists APC, Axin and GSK3 on the output of Wnt/β-catenin signalling. This compartmental model is an extention of the Retention Model and allows for β-catenin degradation in both cellular compartments, cytoplasm and nucleus. It is referred to as the "Degradation Model". The work presented in this chapter has been published as [Schmitz et al., 2013], and is reproduced with permission from Elsevier. **Chapter 7** provides a comparison of the Retention Model and the Degradation Model and gives a consolidation of all obtained results. Final remarks and a brief outlook to future research conclude this thesis. An appendix provides supplemental material.

2. Signal transduction in the Wnt pathway

The goal of this thesis is to provide a comprehensive analysis of the role of nucleo-cytoplasmic shuttling of Wnt pathway components on the output of the pathway. To this end, I build a model on the Wnt/β-catenin pathway and investigate the dynamics of the model with the focus on nucleo-cytoplasmic shuttling of β-catenin and its antagonists. In order to achieve this goal it is indispensable to understand the background of signal transduction and intracellular transport in general, and the Wnt/β-catenin pathway, in particular.
In this chapter I therefore present some of the key biological concepts necessary to motivate, develop and comprehend the models presented in this thesis. I start by giving an introduction to cellular signalling in Section 2.1 and then discuss the Wnt signalling pathway in Section 2.2. Thereby, I provide an introduction to the biological and biochemical function of the relevant key proteins. The description is more detailed than necessary for the models that I present, in line with my intention to illustrate the complex nature of these proteins and their roles in signalling. Section 2.3 summarises intracellular transport mechanisms in general. Finally, the nucleo-cytoplasmic shuttling of the relevant Wnt pathway components is presented in Section 2.4.

2.1. Cellular signalling

All cells, whether they live as individuals or in a multicellular organism, are bombarded by signals in many forms in a continual manner. It is the ability to sense and respond to their environment that is crucial to their survival and therefore a fundamental characteristic of life [Hancock, 2010, Sauro and Kholodenko, 2004]. Cellular signalling is equal to information processing. It controls the inner workings of all organisms, allowing them to respond, adapt and survive [Hancock, 2010, Pollard and Earnshaw, 2008]. The main principles, components and mechanisms of cellular sig-

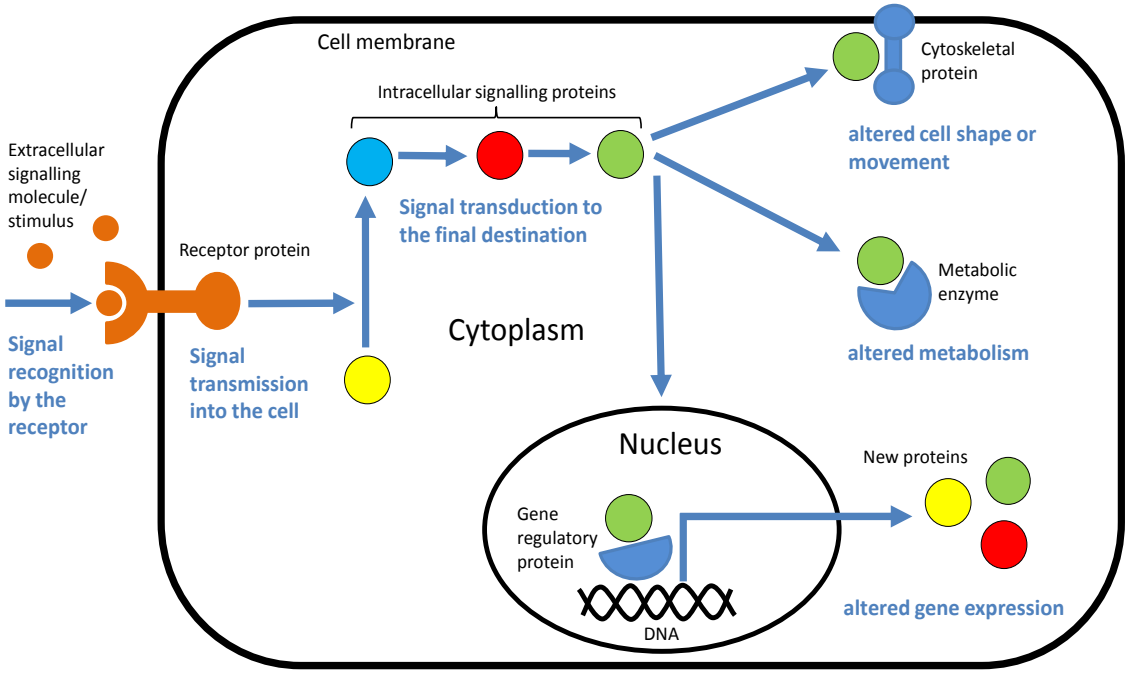

Figure 2.1.: Abstract representation of the main steps of signal transduction. An extracellular signalling molecule binds to a receptor protein, thereby activating an intracellular signalling pathway that is mediated by a series of signalling proteins. Ultimately, the signal reaches its final destination where the signalling protein interacts with a target protein, resulting in a change of behaviour of the cell [Alberts et al., 2002, Figure 15-1, page 832].

nalling are essentially the same across the diverse range of organisms, from bacteria, fungi, plants and animals [Hancock, 2010]. The specificity of cellular responses to external stimulation is encoded by the spatial and temporal dynamics of signalling pathways and networks [Kholodenko, 2006]. In this way, complex biological processes such as development, tissue function, immune response and wound healing are precisely and dynamically regulated [Asthagiri and Lauffenburger, 2000].

Figure 2.1 illustrates the main steps of cellular signalling. The first step is the recognition of the extracellular signalling molecule. Most stimuli from outside the cell, including proteins and peptides, cannot penetrate the cell membrane. These extracellular ligands bind transmembrane receptors on the cell surface that detect physical stimuli and transfer the signal across the lipid bilayer. Most stimuli act through one of about 20 families of receptor proteins, each coupled to distinct signal transduction mechanisms [Pollard and Earnshaw, 2008]. Active receptors generate a chemical signal inside the cell by interacting with one or more cytoplasmic proteins

and thereby transmit the signal into the cell. This transduction step converts one type of signal (stimulus) into another signal (messenger) and commonly amplifies the signal. The signal is then passed on to a series of complex biological interactions and modification of intracellular signalling proteins that lead the signal to its final destination within the cytoplasm or nucleus [Hancock, 2010]. The transfer and processing of a signal can be accomplished through diverse biochemical reactions and posttranslational modifications, such as protein (de)methylation and ubiquitination and the dynamic formation (and dissociation) of protein complexes [Scott and Pawson, 2009]. (De)Phosphorylation is the most common posttranslational modification of proteins and regulates the activity of one or more proteins along most signalling pathways [Pollard and Earnshaw, 2008]. In this case, regulation is achieved by the activity of a protein kinase (addition of a phosphate) or phosphatase (removal of a phosphate). In a last step, the signal arrives at its final destination within the cytoplasm or nucleus, where the signalling molecule may interact with a cytoskeletal protein, a metabolic enzyme or a gene regulatory protein. This interaction leads to the appropriate cellular response and hence results in a change of behaviour of the cell [Alberts et al., 2002]. Depending on the initial signal and the signalling pathway, the cell thus alters its shape or movement, metabolism or gene expression.

Signalling pathways regulate virtually all cellular processes. Understanding signalling pathways, however, is challenging [Pollard and Earnshaw, 2008]. First, cells employ hundreds of distinct signalling pathways, involving hundreds and thousands of different proteins. Second, most pathways include positive or negative feedback loops allowing for complex responses, such as sigmoidal and hysteretic switches, transient responses and oscillators [Tyson et al., 2003]. Third, few signal transduction mechanisms utilize simple linear pathways from a stimulus to a change in behaviour. Rather most pathways branch and converge multiple times and signals propagate through a tangled network of interconnecting proteins and cascades rather than through independent linear routes, making it difficult to predict how information flows through a system [Kholodenko et al., 2010]. Finally, the response of some pathways depends on both, the duration and the amplitude of the stimulus.

Wnt signalling is among the handful of key signalling pathways that play roles in almost all aspects of metazoan biology. It is an ancient system that has been highly conserved during evolution throughout the animal kingdom. It regulates normal embryonic development, tissue homeostasis and regeneration. Aberrations of Wnt signalling cause various diseases, including cancer. Next, I give an introduction to Wnt signalling, its key players and its role in development and disease.

2.2. Wnt signalling in development and disease

In 1982, Varmus and Nusse reported the identification of a tumour virus that induced mammary gland tumours in mice by activating the expression of a hitherto unknown proto-oncogene that they named *Int-1* (Integration-1) [Klaus and Birchmeier, 2008, Nusse and Varmus, 1982]. Independently, a *Drosophila melanogaster* mutant lacking wings was described by Sharma and Chopra [1976] and the responsible fly gene was hence named wingless (*wg*). Subsequently, *Drosophila wg* was also shown to control segment polarity during larval development [Nüsslein-Volhard and Wieschaus, 1980]. Based on protein sequence homology, Rijsewijk et al. [1987] showed that Int-1 and wg were homologs. Accordingly, the name *Wnt* is derived from a combination of both, Wg and Int-1.
Currently, three different Wnt pathways are distinguished: the canonical Wnt/β-catenin cascade, the noncanonical planar cell polarity (PCP) pathway and the noncanonical Wnt/Ca^{2+} pathway. Of these three, the canonical Wnt/β-catenin is best understood and subject of this thesis. For comprehensive overviews to the other Wnt signalling pathways, the reader is referred to [Katoh, 2005] and [Kohn and Moon, 2005].
In the following, I introduce the key players of canonical Wnt/β-catenin signalling, i.e. Wnt proteins, β-catenin and the destruction complex. Subsequently, I discuss the pathway and its role in development and disease.

2.2.1. Wnt proteins

The mammalian Wnts compromise a large highly conserved family of 19 secreted, glycosylated and lipidated protein ligands that have crucial roles in the regulation of diverse processes such as embryonic induction, generation of cell polarity and the specification of cell fate [Anastas and Moon, 2012, Logan and Nusse, 2004]. Wnts are defined by their amino acid sequence rather than by functional properties [Logan and Nusse, 2004]. Wnt proteins are characterised by a high number (23-24) of conserved cysteine residues and have an approximate molecular weight of 40 kDa [Miller, 2002].
After synthesis, Wnts are escorted to the plasma membrane for secretion. The picture of Wnt secretion though is incomplete, with diverse proposed mechanisms that are yet a matter of conjecture (see [Coudreuse and Korswagen, 2007] for a recent review). After secretion, Wnt bind lipoproteins in the extracellular environment. Wnt proteins can signal to the cell that produced them (i.e. autocrine signalling)

and to other cells (i.e. paracrine signalling). They function as morphogens that are capable of both, short- and long-range signalling (reviewed in [Logan and Nusse, 2004]). However, it is unclear how the long-range gradients are generated.
Binding of extracellular Wnt proteins to the membrane receptors Frizzled (Frz) [Bhanot et al., 1996] and LDL-related protein (LRP5/6) [Mao et al., 2001] initiates the Wnt pathway. In Wnt/β-catenin signalling, Wnts strictly control the phosphorylation, degradation and regulation of β-catenin [MacDonald et al., 2009].

2.2.2. The key signalling protein: β-catenin

The protein β-catenin has a molecular weight of ~ 90 kDa and is encoded by a single gene in most animals and humans. The β-catenin protein is a truly dual function protein and a pivot between cell adhesion and Wnt signalling, as it contains binding sites for cadherins, TCF, APC and Axin [Valenta et al., 2012]. Initially, it was discovered for its role in cell adhesion [Kemler, 1993]. As a structural component of adherens junctions, it promotes cell adhesion by binding to the cytoplasmic domains of classical cadherins and linking cadherin to the actin cytoskeleton through the adaptor protein α-catenin [Bienz, 2005, Johnson et al., 2009]. Within this complex, β-catenin is immobile and does not influence Wnt signalling. In contrast, the signalling function of β-catenin is conferred by a soluble cytoplasmic pool that is highly unstable in the absence of a Wnt signal [Bienz, 2005]. It is the key effector in the canonical Wnt signalling pathway [Bienz, 2005, Kikuchi, 2000]. In the nucleus, it forms a complex with transcription factors from the LEF/TCF (Lymphoid enhancer factor 1/T-cell factor) family, which initiates target gene expression [Behrens et al., 1996, Mosimann et al., 2009]. The principal regulatory mechanism that controls the levels of soluble β-catenin is the activity of the so-called destruction complex.

2.2.3. The destruction complex

The destruction complex is a dynamic multiprotein assembly. Minimally, it consists of the scaffolding proteins Axin and APC, and the kinase GSK3 [Doble and Woodgett, 2003, Hart et al., 1998, Kishida et al., 1998, Yost et al., 1996]. As these proteins counteract β-catenin, I refer to them as β-catenin antagonists (see Figure 2.2). Apart from the three mentioned proteins, the destruction complex also includes another kinase, namely casein kinase 1 (CK1) [Liu et al., 2002], as well as the E3-uniquitin ligase β-Trcp. For a very detailed recent review on the destruction complex, see [Stamos and Weis, 2012]. In this thesis however, the focus lies on the

core components Axin, APC and GSK3.

Axin has a molecular weight of $\sim$ 110 kDa [Chia and Costantini, 2005]. In vertebrates, there exist two isoforms, Axin and Axin2/Conductin. Axin is a largely unstructured, flexible protein that contains CK1, GSK3 and β-catenin binding sites [Stamos and Weis, 2012]. Axin is the central phosphorylation scaffold of the Wnt/β-catenin pathway, meaning that phosphorylation of β-catenin is greatly enhanced by the presence of Axin [Stamos and Weis, 2012]. It contains binding sites for all the complex components, and also a binding site for β-catenin.

APC is short for Adenomatous polyposis coli. It is a large $\sim$ 310 kDa multifunctional protein [Brocardo and Henderson, 2008] with several structural domains [Kikuchi, 2000]. It is an important tumour suppressor in the colon. APC mutations occur in 80% of all colon cancers [Roberts et al., 2012]. It is highly conserved and essential for cell survival. APC is a highly mobile protein with multiple destinations in the cell, with multiple roles that include the regulation of directed cell migration, apoptosis and DNA repair [Brocardo and Henderson, 2008]. Apart from the destruction complex, APC can associate with β-catenin itself [Bienz, 2002].

The ability of APC to interact with β-catenin has been demonstrated to be enhanced by phosphorylation of APC in vitro [Rubinfeld et al., 1996]. Seo and Jho [2007] proposed that accumulation of β-catenin induces phosphorylation of APC and that phosphorylated APC in turn retains β-catenin. Moreover, Sierra et al. [2006] concluded from their experiments that β-catenin cannot bind unphosphorylated APC efficiently and that CK1 phosphorylation of APC might induce high-affinity binding to β-catenin and even trigger its dissociation from LEF/TCF. Phosphorylated APC and Axin bind the same surface of β-catenin and therefore directly compete for β-catenin binding.

GSK3 is short for Glycogen synthase kinase 3. It was originally identified in the context of regulation of the glycogen metabolism [Embi et al., 1980], though it is now known to regulate many other cellular processes, including protein synthesis, gene expression and protein degradation [Caspi et al., 2008]. It is highly conserved from yeast to mammals [Wu and Pan, 2010]. Mammals express two isoforms, GSK3α ($\sim$51 kDa) and GSK3β ($\sim$47 kDa). The activity of GSK3 itself is regulated by phosphorylation-dephosphorylation, with phosphorylation being inhibitory [Sugden et al., 2008]. It is emerging as an important therapeutic target in a variety of pathologies, including diabetes, neurogenerative disorders and tumorigenesis [Cohen and Goedert, 2004, Doble and Woodgett, 2003, Wu and Pan, 2010]. It is predominantly located in the cytosol, but is also present in nuclei and mitochondria [Bijur

and Jope, 2003]. GSK3 has been reported to phosphorylate a large number of substrates (at least 40 [Wu and Pan, 2010]). It preferentially phosphorylates substrates like β-catenin residues in a "relay" fashion. For this, a priming phosphorylation on a Serine/Threonin (Ser/Thr) residue is catalysed by a protein kinase distinct from GSK3 [Doble and Woodgett, 2003, Sugden et al., 2008]. In case of β-catenin, the priming phosphorylation is conducted by the kinase CK1 on Ser45. Subsequently, GSK3 sequentially phosphorylates β-catenin at the residues Thr41, Ser37 and Ser33 [Wu and Pan, 2010].

2.2.4. The Wnt/β-catenin signalling pathway

The canonical Wnt/β-catenin pathway has been studied extensively, since it has been experimentally proven to play a crucial role in several developmental processes, including synaptic differentiation in neurogenesis [Cadigan and Nusse, 1997], neuronal connectivity [Ciani and Salinas, 2005] and stem cell control [Nusse, 2008]. It is known to regulate cell fate determination, cell proliferation and tissue homeostasis [Baron and Kneissel, 2013, Cadigan and Peifer, 2009, Clevers and Nusse, 2012, Huang and He, 2008, Logan and Nusse, 2004, Nusse, 2008, van Amerongen and Nusse, 2009]. Mutations and deregulated expression of components of the pathway underlie a wide range of pathologies, including various types of cancer [Anastas and Moon, 2012, Carethers, 2009, Klaus and Birchmeier, 2008, Moon et al., 2004, Reya and Clevers, 2005], bone defects, arthritis, schizophrenia [Clevers, 2006] as well as neurodegenerative diseases such as Alzheimer's and Parkinson's disease [Inestrosa, 2000, Inestrosa and Arenas, 2010]. Wnts are also likely to contribute in the future to improve stem/precursor cell replacement therapy approaches to Parkinson's disease [Castelo-Branco and Arenas, 2006]. The Wnt/β-catenin pathway is subject to a multiplicity of reviews (see [MacDonald et al., 2009] for a well-written, detailed example). For a complete list of reviews and for up-to-date information on the pathway, the reader is also referred to the Wnt Homepage (http://www.stanford.edu/group/nusselab/cgi-bin/wnt/).

The protein β-catenin is the central signalling molecule of the canonical Wnt/β-catenin signalling pathway [Kikuchi, 2000]. In the nucleus, it forms a complex with transcription factors from the LEF/TCF family (see Section 2.2.2), which initiates target gene expression [Behrens et al., 1996, Mosimann et al., 2009]. The principal regulatory mechanism that controls the nuclear accumulation of β-catenin is the

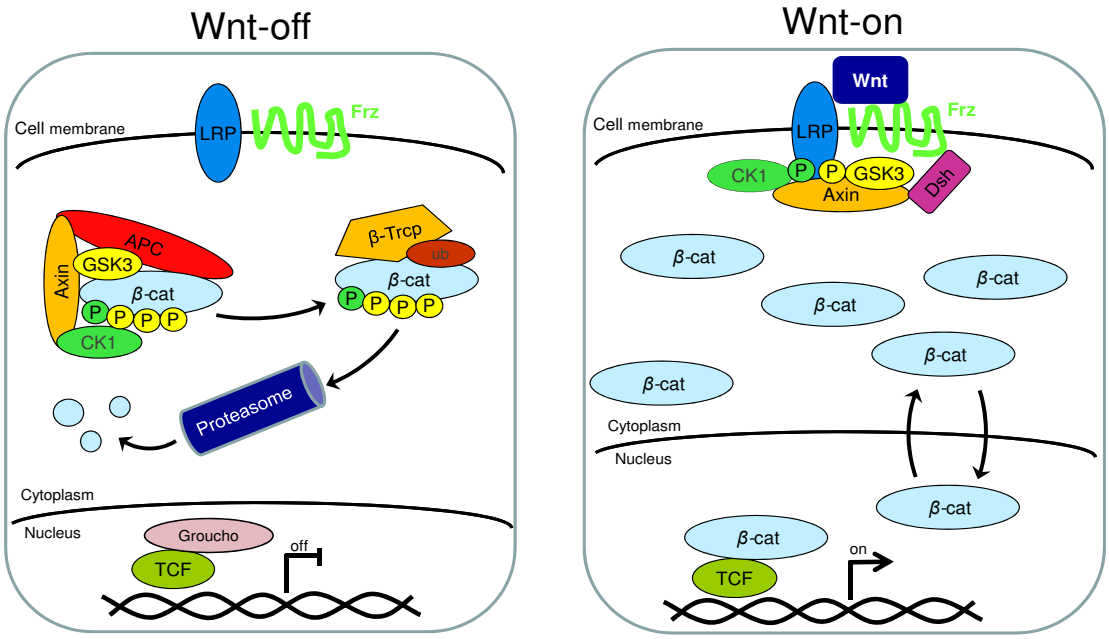

Figure 2.2.: The Wnt/β-catenin signalling pathway. Left: The Wnt-off state. Cytoplasmic degradation keeps β-catenin levels low. Right: The Wnt-on state. The destruction complex is inhibited in the cytoplasm. In the nucleus, the interaction of β-catenin with TCF induces target gene expression. See text for details.

activity of the destruction complex. Basically, Wnt signalling functions in either the on- or off-mode, depending on whether extracellular Wnt ligands (see Section 2.2.1) are present or not.

In the absence of a Wnt stimulus (the off-state; see Figure 2.2, left), β-catenin forms a complex with Axin, APC, GSK3 and CK1 (i.e. the destruction complex, see Section 2.2.3), and is phosphorylated by CK1 and subsequently by GSK3. Afterwards, phosphorylated β-catenin is recognised by the ubiquitin ligase β-Trcp, which targets β-catenin for proteasomal degradation. It is then released from the destruction complex and rapidly degraded by a large protein complex, called the proteasome [Aberle et al., 1997]. Thus, in the off-state β-catenin levels are kept low. In the nucleus, LEF/TCF is maintained in a repressed state by association with inhibitors such as Groucho.

Upon binding of Wnt ligands to their receptors Frz [Bhanot et al., 1996] and LRP5/6 [Mao et al., 2001], the pathway is activated (the on-state; see Figure 2.2, right). In the presence of an extracellular Wnt stimulus, a receptor complex forms between Frz and LRP, resulting in signal transmission into the cell. The first intracellular signalling protein to be activated is Dishevelled (Dsh), which is recruited to the

receptor complex, where it binds Frz. This then leads to LRP phosphorylation and Axin recruitment to the receptor complex (see [Niehrs, 2012] for a review on Wnt receptor signalling). Therefore, in the Wnt-on state, the activity of the destruction complex in the cytoplasm is disrupted, leading to destruction complex dissociation. This induces accumulation of non-phosphorylated β-catenin in the cytoplasm. It then translocates into the nucleus and subsequently accumulates there. In the nucleus, β-catenin displaces Groucho and then serves as a coactivator for TCF to activate Wnt target gene expression [Tolwinski and Wieschaus, 2004].

Currently, 123 genes are known to be target genes of Wnt/β-catenin signalling (for details and updates, see the Wnt homepage provided and regularly updated by the Nusse Lab (http://www.stanford.edu/group/nusselab/cgi-bin/wnt/). Fourteen of these target genes – including Frz, LRP, TCF and Axin2 – are components of the Wnt pathway and therefore provide positive or negative feedback loops.

Since the discovery of the Wnt gene in 1982 [Nusse and Varmus, 1982], Wnt/β-catenin signalling has cemented its role as a key regulatory system in biology. However, even after 30 years of intense study, there are still various mechanisms of the Wnt signalling cascade that remain unclear (see [Clevers and Nusse, 2012], who highlighted ten of those questions, including the subcellular location of Wnt signalling events and the nuclear translocation of stabilised β-catenin), particularly with regard to its role in human disease [Chien et al., 2009]. An important aspect that has recently come into focus of research is the fact that not only β-catenin as a transcriptional cofactor is able to translocate into and out of the nucleus. A surprisingly high number of cytoplasmic Wnt regulators are shuttling proteins that have been proven to reside in both, cytoplasm and nucleus, where they may, directly or indirectly, interact with β-catenin and therefore influence its transcriptional activity [Willert and Jones, 2006]. This holds true especially for the β-catenin antagonists we consider here: APC [Neufeld et al., 2000, Rosin-Arbesfeld et al., 2000], Axin [Cong and Varmus, 2004, Wiechens et al., 2004] and the kinase GSK3 [Bijur and Jope, 2003, Caspi et al., 2008, Franca-Koh et al., 2002]. The question why they do so is still discussed controversially in the community and forms the focus of this thesis. In the next sections, I give a short introduction to intracellular transport and nucleo-cytoplasmic shuttling mechanisms. Afterwards, I summarise experimentally derived hypotheses on the shuttling and nuclear functions of β-catenin and its antagonists APC, Axin and GSK3.

2.3. Intracellular transport

A typical eukaryotic cell contains many thousands of different proteins, almost all of which are synthesised in the cytoplasm [Friedman, 2008]. These proteins include molecules intended to function in one of the many organelles of the cell, or the plasma membrane, or intended for secretion into extracellular space (such as the Wnt proteins, discussed in Section 2.2.1.) There are several mechanisms by which specific proteins are directed to their final destination [Friedman, 2008]. Intracellular transport is most commonly described as either diffusive or directed by other cellular molecules. The transmission of information over intracellular distances of more than a few micrometres requires facilitated transport mechanisms, including movement of phosphorylated kinases on scaffolding proteins and endosomes driven by molecular motors [Kholodenko, 2006].

The contents of the eukaryotic nucleus are separated from the cytoplasm by the nuclear envelope. Transport into or out of the nucleus occurs through bidirectional aqueous pores in the nuclear membrane [Strambio-De-Castilla et al., 2010, Zilman et al., 2007]. There are several thousand such nuclear pore complexes (NPCs) in the nuclear membrane of a typical cell [Friedman, 2008]. The process of actual translocation across the NPC generally occurs either by diffusion or facilitated transport [Xu and Massagué, 2004]. The radius of the pores is found to be 4-5 nm [Friedman, 2008], therefore small molecules of this size and smaller ($\lesssim$ 50 kDa [Kopito and Elbaum, 2007, Macara, 2001]) can exchange freely between the nuclear lumen and the cytoplasm. Large macromolecules are transported through the NPC if they contain a particular amino acid sequence, called the nuclear localisation signal (NLS) (for import) or nuclear export signal (NES) (for export)[Friedman, 2008, Zilman et al., 2007]. These sequences are required for nucleo-cytoplasmic transport mediated by importin/exportin receptors, such as importin-β and Chromosome region maintenance 1 (CRM1), respectively [Xu and Massagué, 2004].

2.4. Nucleo-cytoplasmic shuttling of Wnt pathway components

Unlike NLS-mediated import and despite a molecular weight of $\sim$ 90 kDa, β-catenin enters the nucleus independently of the importin receptor. Instead, it can interact directly with the NPC and can translocate on its own [Fagotto et al., 1998, Yokoya et al., 1999]. This is possible because, like importin-β, β-catenin contains several

HEAT-like repeats, which are protein domains that are both, necessary and sufficient for nuclear import [Xu and Massagué, 2004]. Its nuclear export is "rather nebulous and somewhat controversial" [Städeli et al., 2006]. On the one hand, there is experimental evidence that β-catenin is exported out of the nucleus on its own, using interactions with the nucleoporins to pass through the NPC independently of the CRM1 exportin pathway [Eleftheriou et al., 2001, Wiechens and Fagotto, 2001]. On the other hand, it has been proposed to bind to APC or Axin to exit the nucleus [Cong and Varmus, 2004, Henderson, 2000, Neufeld et al., 2000, Rosin-Arbesfeld et al., 2000, 2003, Wiechens et al., 2004].

Axin can interact directly with the CRM1 receptor, although via non-classical NES sequences [Wiechens et al., 2004]. Although cytoplasmic at steady state, Axin shuttles in fact into and out of the nucleus, where it may also have yet unknown functions [Wiechens et al., 2004].

APC exhibits at least two NES interacting with the CRM1 nuclear export factor and has been proven to shuttle between cytoplasm and nucleus [Neufeld et al., 2000, Rosin-Arbesfeld et al., 2000], but the functional relevance of this is still controversial [Bienz, 2002, Brocardo and Henderson, 2008, Henderson and Fagotto, 2002]. It was proposed that the nuclear export of APC controls the level and hence the transcriptional activity of nuclear β-catenin [Henderson, 2000, Rosin-Arbesfeld et al., 2003]. Most recent findings, however, indicate that APC and Axin (and also Axin2) enrich β-catenin in the cytoplasm, but do not accelerate the nucleo-cytoplasmic shuttling of β-catenin, i.e. increases the rate of β-catenin nuclear import or export [Krieghoff et al., 2006]. From their results, Krieghoff et al. [2006] concluded that β-catenin antagonists, such as APC, mainly regulate β-catenin subcellular localisation by retaining it in the compartment in which they are localised, rather than by active transport into or out of the nucleus.

The kinase GSK3 exhibits a bipartite NLS, which was found to be both necessary and sufficient for nuclear localisation. GSK3 is highly active in the nucleus [Bijur and Jope, 2003], where it can bind the protein FRAT and be exported in a CRM1-dependent fashion [Franca-Koh et al., 2002]. It was proven that GSK3 has a nuclear function in downregulating the activity of β-catenin [Caspi et al., 2008].

The fact that GSK3 can enter the nucleus and might regulate the levels of Wnt signalling prompts to re-evaluate the current view of the pathway [Caspi et al., 2008]. Moreover, as GSK3, Axin and APC all appear in the nucleus and might have a nuclear function in regulating the Wnt signal, it is conceivable that the β-catenin degradation complex is assembled in the nucleus [Caspi et al., 2008].

To sum up, several approaches have been followed in order to understand the functional role of nucleo-cytoplasmic shuttling of Wnt pathway components, in particular APC, Axin and GSK3 as β-catenin antagonists. Different experiments resulted in different hypotheses. However, the functional relevance of antagonist shuttling in the Wnt/β-catenin pathway is yet to be determined.

3. Mathematical modelling of cell signalling

Kinetic modelling of biochemical reactions has a long history. The Michaelis-Menten model for the rate of irreversible reactions in enzyme kinetics, for example, is a milestone in biochemistry and was developed in the early 20th century. Other early milestones include the work of Hodgkin and Huxley [1952], with their groundbreaking mathematical reconstruction of the nerve impulse, and the work of Turing [1952] on the chemical basis of morphogenesis, who reproduced spatial patterns which are ubiquitous in nature, e.g. on sea shells or animal coats, with a simple reaction-diffusion model. The term 'Systems biology' was coined by Mesarović [1968] and is an interdisciplinary approach combining mathematical modelling and quantitative cell biology [Klipp, 2009]. Systems biology models are often based on well-established physical laws, e.g. the thermodynamics of chemical reactions [Klipp et al., 2009]. Since the 1990s, modelling has emerged as a tool to decipher the dynamics of biochemical reaction networks and to provide insights into the complex relationships between cellular stimuli and the corresponding responses [Kholodenko, 2006].

In this chapter, I give an introduction to mathematical modelling of biochemical networks to provide the theoretical background for the models I derive and investigate in the following chapters. I start with defining a model in Section 3.1, where I also explain why modelling is useful. I then present different approaches to mathematical modelling in Section 3.2. In particular, I focus on spatial aspects and on the central question of how to choose an appropriate modelling approach for my research questions. Subsequently, I present the modelling framework I decided to use in this thesis. To this end, I show how to model biochemical networks with ordinary differential equations (ODEs) in Section 3.3. Finally, in Section 3.4, I present relevant techniques for the analysis of models, such as stability analysis and sensitivity analysis. For an extensive discussion of mathematical modelling applied to biological systems, the reader is referred to the textbook by Klipp et al. [2009].

3.1. The purpose of modelling

In a broad sense, a model is an abstract representation of objects or processes that explains features of these objects or processes [Klipp et al., 2009]. A biochemical reaction network is often represented by a graphical sketch showing proteins as nodes and reactions as arrows. The interactions and dynamics of its components can be described using the language of mathematics. As the interactions between the objects and processes of such a dynamical system are often highly nonlinear, the system can exhibit complex – often counter-intuitive – behaviour. As such, a mathematical model is a tool that helps to overcome the linearity of Human thinking [Dörner, 1980].

The quantities of a mathematical model can be classified as variables, parameters and constants. A constant is a quantity with a fixed value, such as Avogadro's number which is the numbers of molecules per mole. Parameters include reaction rate constants or total protein concentrations. Their values are time independent, but can depend on experimental conditions and may change, for example, due to different cellular compositions or different cell types. Variables (in signalling often species or substances) are quantities which values change over time and/or space. A subset of all variables, the state variables, describe the system's behaviour completely. They are linearly independent of each other and form a basis of the state space. Therefore each of them is necessary to define the current state of the system. Models represent only specific aspects of the reality. The intention of modelling is to answer a particular question. Modelling is thus a subjective and selective procedure. It needs to make specific statements about the system of interest. In case of systems biology, these statements are justified by either experiments and biochemical knowledge, or by mere extrapolation from other systems [Klipp et al., 2009]. Typically, systems biology projects on cellular signalling are characterised by close integration of experiments with mathematical models realised through an iterative cycle of quantitative cell biology, computational modelling, parameter estimation, model predictions and experimental validation [Becker et al., 2010, Blüthgen et al., 2009, Chen et al., 2009, Lai et al., 2012, Lange et al., 2012, Rateitschak et al., 2010, Schilling et al., 2009, Schmidt et al., 2012]. Simulation is a simple but powerful tool for studying a model's behaviour and guiding experiments [Aldridge et al., 2006a]. It allows to compare time-dependent concentrations of key species over a range of concentrations, network topologies and rate parameters. Model simulations are cheap compared to wet-lab experiments. They can be repeated often and for many

different conditions. Moreover, modelling also offers benefits other than prediction [Epstein, 2008]. It is even a powerful tool without the availability of experimental data. It highlights gaps of knowledge or understanding and can provide insights into principal mechanisms. Modelling drives conceptual clarification. Examples include the discovery of recurring network motifs or fundamental design principles of signalling pathways, such as feedback and feedforward loops [Mangan and Alon, 2003, Markevich et al., 2004, Tyson et al., 2003], thresholds in transient dynamics [Rateitschak and Wolkenhauer, 2010], as well as defined measures to quantify signalling time, signal duration and signal amplitude [Heinrich et al., 2002].

3.2. Discussion of selected spatio-temporal modelling approaches

Cells and organisms show complex spatial structures, which are vital for the processes of life [Klipp et al., 2009]. Various modelling approaches have been successfully used to address specific questions about biochemical systems. The range of problems that can be tackled is growing steadily and the modelling approaches differ in their modularity and mathematical implementation [Tomlin and Axelrod, 2007]. The 'correct', or rather appropriate mathematical form of a dynamical model depends on many different practical and experimental considerations, such as the goal or purpose of the modelling effort, the available data, the computational power or personal preferences [Aldridge et al., 2006a, Voit et al., 2008].
The central question I focus on in this section is how to describe the spatio-temporal distribution of species or substances in a model, which affects the interactions between these species to a large extent. There exist a large variety of different types of spatio-temporal models, which exhibit different advantages and disadvantages. Ultimately, to choose a suitable modelling approach, one needs to balance computational cost against the biological realism. In the following, I present selected approaches for spatio-temporal modelling, in order to find a suitable approach for my research questions. I then discuss which approach I have chosen, and why.
The interactions and temporal evolution of molecular species in a model can either be represented individually (microscopic scale) or as a population (macroscopic scale). Signalling pathways mainly describe processes that contain a large number of molecules. In this case, a population-based representation of species has the advantage of low computational costs. Models for signalling pathways can be loosely

grouped as follows [Klipp and Liebermeister, 2006]: i) deterministic (with defined states in the future) or probabilistic (stochastic processes); ii) discrete or continuous (with respect to time or component abundances, e.g. molecule numbers or concentrations); and iii) they may or may not account for space. Currently, in most models, biochemical reactions are described in a deterministic, continuous manner by rate equations for concentrations of substances and complexes using a system of ODEs [Aldridge et al., 2006a, Kholodenko et al., 2010]. An ODE denotes an equation in which differentiation occurs with respect to only a single independent variable, mostly time [Kholodenko, 2006]. ODE modelling is population-based and assumes a spatial homogeneity, i.e. a well-stirred medium without any barriers [Klipp et al., 2009]. It furthermore assumes that all molecules move freely and independently and that diffusion is much faster than chemical reactions. Inhomogeneities will thus rapidly disappear and the substances can hence be described by the concentrations averaged over a cell.

If molecules, however, are not homogeneously distributed, e.g. because membranes hamper free movement, then spatial location and structure have to be taken into account. In the deterministic framework, the spatio-temporal distribution of species can be described indirectly by distinguishing different compartments or directly, by describing dynamics in continuous or discrete space [Bittig and Uhrmacher, 2010].

In compartmental models, each species is allowed to be located in one or more compartments and to move between them through elementary reactions [Aldridge et al., 2006a, Bittig and Uhrmacher, 2010]. There are two fundamental assumptions for the compartmentalised ODE formalism [Aldridge et al., 2006a, Takahashi et al., 2005]: First, within a homogeneous compartment, the concentration of each species is high and transport is essentially instantaneous. Second, the transport between compartments is slower and modelled by transport reactions.

If these assumptions are not satisfied, then it is necessary to model changes in species concentrations explicitly with respect to space. In a population-based approach, partial differential equations (PDEs) are typically used to account for space. PDEs contain partial derivatives with respect to two or more independent (in this case, spatial) variables [Kholodenko, 2006]. PDE models assume that concentrations are smooth functions in space, which only holds on a spatial scale much larger than the average distance between molecules and if inhomogeneities of the spatial structure are neglected. In practice, solving PDEs is usually computational demanding. By discretising space, however, spatial structures can be taken into account in a population-based approach without increasing computational costs (e.g. by using

Cellular Automata) [Kossow et al., 2013].
If a substance is present in small amounts, an individual-based representation of molecules and reaction events is often more suitable to yield low computational costs. In systems with small amounts of individuals, stochastic effects due to thermal movement and chemical reactions become relevant [Takahashi et al., 2005]. In this case, the behaviour of individual molecules have to be simulated stochastically. There are several approaches available, most of which are based on Brownian dynamics [Takahashi et al., 2005] or the Gillespie algorithm [Gillespie, 1977, Klipp and Liebermeister, 2006]. To date, a remaining challenge in the field of stochastic spatio-temporal modelling of cellular systems is the lack of standard analysis methods and therefore very limited availability of analytical tools, as most results are obtained using simulations.

Within this thesis, I model the Wnt/β-catenin pathway as a biochemical reaction network including two cellular compartments, cytoplasm and nucleus. These models allow for nucleo-cytoplasmic shuttling of β-catenin and its antagonists, and for protein complex formation of β-catenin with APC and with the destruction complex in the cytoplasm and nucleus, respectively. Within each compartment, I do not consider spatial dimension and assume a well-mixed, homogeneous reaction medium. Transport between the compartments is assumed to be slower than the movement within the compartments and modelled as either diffusion-like motion or active transport by transporter proteins. Therefore, the compartmentalised ODE formalism as a deterministic population-based modelling approach offers the most convenient way to represent the dynamics of the variables within my models. In addition, the ODE modelling approach has the advantage that there exists a large variety of available methods, tools and software from dynamical systems theory to analyse the behaviour of the system. In the next section, I give an introduction to the mathematical structure of a system of coupled ODEs and the methods I use to investigate such a system.

3.3. The mathematical structure of an ODE model

The temporal evolution of a biochemical network in a deterministic approach can be described by a set of (coupled) ODEs [Klipp et al., 2009]. A general ODE system can be written in the form

$$\frac{dX_i}{dt} \equiv \dot{X}_i = f_i(X_1, ..., X_N, k_1, ..., k_M) \qquad i = 1, ..., N, \tag{3.1}$$

where $X_1, ..., X_N$ is a set of variables and t is time. The temporal evolution of the system is determined by the functions $f_1, ..., f_N$ depending on the parameters $k_1, ..., k_M$ and the variables $X_1, ..., X_N$. In this notation, N is the dimension of the state space, whereas M is the dimension of the parameter space. The left hand side of the ODE is the time derivative of a species concentration. If the right hand side of the ODEs is not explicitly dependent on time, the system is called autonomous, which is generally the case in biochemical modelling.
In most ODE models, the right hand side of Equation (3.1) is the algebraic sum of reaction rates for the production and consumption of the involved species, i.e. proteins and complexes:

$$\dot{X}_i = \sum_{j=1}^{r} m_{ij}\nu_j(X_1, ..., X_N, k_1, ..., k_M) \qquad i = 1, ..., N, \tag{3.2}$$

where N is the number of biochemical species with the concentrations X_i and r is the number of reactions with the rates ν_j and the stoichiometric coefficient m_{ij}. Depending on experimental information, the individual reaction rates can be described by sophisticated kinetic laws. However, most commonly mass action kinetics are used [Klipp and Liebermeister, 2006], which is an empirical law stating that the rate of a reaction is proportional to the concentration of the reacting species [Aldridge et al., 2006a]. A biochemical transformation of the form $X_1 + X_2 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} X_3$ is thereby represented by the reaction rate

$$\nu = k_1 X_1 X_2 - k_2 X_3, \tag{3.3}$$

described with forward and reverse reaction rate constants k_1 and k_2, respectively. The rate constant k_2 of monomolecular reactions has the dimension s^{-1}, whereas the rate constant k_1 for bimolecular reaction has the dimension $M^{-1}s^{-1}$ (where M equals mol per litre).
Differential equations can be substituted by time-independent algebraic relations,

resulting in a smaller model with more complex rate terms [Aldridge et al., 2006a]. A common example of an algebraic substitution is replacing mass action kinetics with the Michaelis-Menten equation, which approximates the reaction rate for enzyme-catalysed reactions of a substrate S into a product P ($S \rightarrow P$) as follows:

$$\nu = \frac{v_{max} S}{k_M + S}. \tag{3.4}$$

The quantity v_{max} is the maximal rate. k_M is the half-saturation constant and denotes the substrate concentration ensuring a half-maximal rate [Klipp and Liebermeister, 2006]. Note that this approach can only be applied if the concentration of the substrate is in excess of the enzyme and the quasi-steady state assumption is fulfilled [Millat et al., 2007]. This is only the case if elementary reactions produce short-lived intermediates (like the enzyme-substrate complex) and the rate of formation for an intermediate complex and its dissociation back into the reactants is much faster than its conversion into products [Atkins and De Paula, 2002].

3.4. Methods and concepts chosen for model analysis

When the structure, the mathematical implementation and the parametrisation of the model have been determined or estimated, mathematical exploration and analysis begins. Simulations are a widely-used tool to study the model's behaviour, as they depict the temporal evolution of system variables. In the context of ODEs, model simulation is performed by numerical integration. Other analysis tools relevant for this thesis include stability and sensitivity analysis. In the following sections, I present these tools and discuss how they are applicable to the study of steady states and transient dynamics, respectively.

3.4.1. Steady states and their stability

The concept of steady states is important for the modelling of all dynamical systems. In dynamical systems theory, a system is characterised by its state, which equals a snapshot of the system at a given time [Klipp et al., 2009]. The state of a system is described by a set of variables (state variables). The set of all possible states is the state space. Steady states (or stationary states or fixed points), in contrast to transient dynamics (see Section 3.4.2), are determined by the fact that the values of all state variables remain constant in time. Therefore, to study the steady states,

those states in which the time derivatives vanish are considered:

$$\frac{dX_i}{dt} = 0. \tag{3.5}$$

If a system is in steady state, it stays there, until an external perturbation occurs. The asymptotic behaviour of dynamic systems, i.e. the behaviour after a sufficiently long time, is often stationary. Other types of asymptotic behaviour are oscillatory or chaotic regimes.
To investigate whether a steady state $\mathbf{X}^*$ of an ODE system is (asymptotically) stable, we have to consider small perturbations of the state variables. A linearisation of the evolution equations (3.1) close to the steady state yields the Jacobian matrix. The Jacobian is a real $N \times N$ matrix with its coefficients being

$$J_{ij} = \left.\frac{\partial f_i}{\partial X_j}\right|_{\mathbf{X}=\mathbf{X}^*} \qquad i,j = 1,...,N, \tag{3.6}$$

where N is the dimension of the state space. These coefficients calculated at steady state $\mathbf{X}^*$. If all eigenvalues of the Jacobian have non-vanishing real parts the steady state is called hyperbolic. A hyperbolic steady state is asymptotically stable if (and only if) all eigenvalues of the Jacobian are negative. Transitions in the stability of steady states occur if eigenvalues of the Jacobian cross the imaginary axis (leading to at least one positive eigenvalue) in response to parameter variation. In parameter space, these transition points are called bifurcation points. Crossing a bifurcation point leads to a qualitative transition in state space, which corresponds to a change in the asymptotic long term behaviour of the system. Such a transition is called a bifurcation.
Stability and bifurcation analysis are of interest because they help to explain how a network can switch between different states [Aldridge et al., 2006a]. These methods are however restricted to the analysis of steady states.

3.4.2. Transient dynamics

Transient dynamics describe the transition from a state far away from a steady state (e.g. some initial state of the system) into a steady state and the transition from one steady state to another, respectively. In case of transient processes, other techniques from dynamical systems theory than stability or bifurcation analysis are required to determine how the output of a model will change over time (or time and space) when initial conditions or parameter values change. These methods include the

singular-perturbation theory and finite-time Lyapunov exponents [Aldridge et al., 2006a,b], as well as defined measures to quantify key features of transient stimuli, such as the time, rate and duration of signalling and the amplitude of the signal output [Heinrich et al., 2002, Millat et al., 2008].

3.4.3. Sensitivity analysis

In order to study the influence of small perturbations on a system's behaviour and thereby account for the robustness of the system, one widely used tool is sensitivity analysis. It is a powerful method for systematically determining which concentrations and rate constants in a model have the biggest influence on the overall behaviour [Aldridge et al., 2006a]. In addition to revealing key parameters in a network, sensitivity analysis is valuable in ascertaining which parameters should be in the focus of direct measurements or experimental perturbations. Insensitivity of a model to parameter variation or perturbations is often equated with robustness.
Depending on the strategies used for perturbing the model parameters, sensitivity analysis can be classified into two types: local and global sensitivity analysis. Local sensitivity analysis investigates sensitivities of the model variables with respect to particular points in parameter space, whereas global sensitivity uses the full ranges of parameter space and addresses the global behaviour of model parameters using statistical methods [Satelli et al., 2000]. In the following, I focus on local sensitivity analysis.
For quantifying the effects of the perturbations, so-called control coefficients (also known as sensitivities or response coefficients) are calculated. Originally, they were proposed for quantifying control in metabolic networks and are nowadays a standard quantitative measure in sensitivity analysis [Heinrich and Schuster, 1996], not only in systems biology. Control coefficients describe the relative response of the concentration of a given compound (or state variable) X_i with respect to a relative perturbation of the parameter k_j and are defined by

$$C(X_i(t), k_j) = \left(\frac{k_j}{X_i} \frac{\Delta X_i}{\Delta k_j} \right)_{\Delta k_j \to 0} = \frac{k_j}{X_i} \frac{\partial X_i}{\partial k_j}. \tag{3.7}$$

Note that the analysis is restricted to small perturbations of the reference value only, as the relations between steady state variables and kinetic parameters are usually nonlinear. For $C(X_i(t), k_j) = 0$, a perturbation of the parameter k_j has no influence on the model variable X_i at time point t at all, which is thus most robust. The larger

the value of $C(X_i(t), k_j)$, the higher the influence of the parameter perturbation. In steady state, the concentration control coefficients fulfil the summation theorem

$$\sum_{j=1}^{r} C_{\nu_j}^{X_i^*} = 0, \tag{3.8}$$

for any steady state concentration X_i^*. Again, r is the number of reactions rates ν. Control coefficients can also be calculated for finite times to cover time-dependent response coefficients as described by Ingalls and Sauro [2003] and Hornberg et al. [2005]. Aside from control coefficients for signalling, characteristics such as maximum amplitude or mean signal time of output signals have been defined [Reijenga et al., 2002], and spatial processes such as diffusion can be incorporated [Peletier et al., 2003], as summarised by Klipp and Liebermeister [2006].

4. Modelling the Wnt/β-catenin pathway

In the last decade, several quantitative and qualitative mathematical models have been developed to describe the Wnt/β-catenin pathway in order to gain deeper insight into its functioning in time and space. Kofahl and Wolf [2010] have published a first review on mathematical modelling of Wnt/β-catenin signalling, providing an overview (including a timeline) of the models published from 2003 to 2010. The models focus on different aspects of the Wnt pathway and describe it on different scales, ranging from subcellular and biochemical to cellular and tissue scale. At the biochemical level, there are detailed models of the core canonical pathway and its crosstalk with other pathways [Cho et al., 2006, Kim et al., 2007, Kogan et al., 2012, Krüger and Heinrich, 2004, Lee et al., 2003, Wawra et al., 2007]. Cellular models consider cell-cell interactions [Basan et al., 2010, Shin et al., 2010, van Leeuwen et al., 2007] and tissue level models describe multicellular systems, investigating how Wnt signalling regulates cellular decision-making and the development and maintenance within multicellular systems. The latter also allow for multiscale models that couple cellular and tissue behaviour with subcellular processes [Ramis-Conde et al., 2008, Van Leeuwen et al., 2009].

In this chapter, I review previous modelling approaches of the Wnt pathway on biochemical and subcellular level, as this work also takes biochemical reactions and intracellular localization of Wnt pathway components into account. For an overview on cellular and tissue scale models, the reader is referred to [Lloyd-Lewis et al., 2013], who have recently published a comprehensive review on the understanding the Wnt/β-catenin pathway through simulation and experiment, in which they have classified the models according to the scale in which they are built. I start with the "standard model" of Wnt signalling developed by Lee and colleagues [Lee et al., 2003] and then present the models that have further analysed, extended and validated the Lee Model. I conclude this chapter with summarising open questions of Wnt pathway modelling on the biochemical subcellular level.

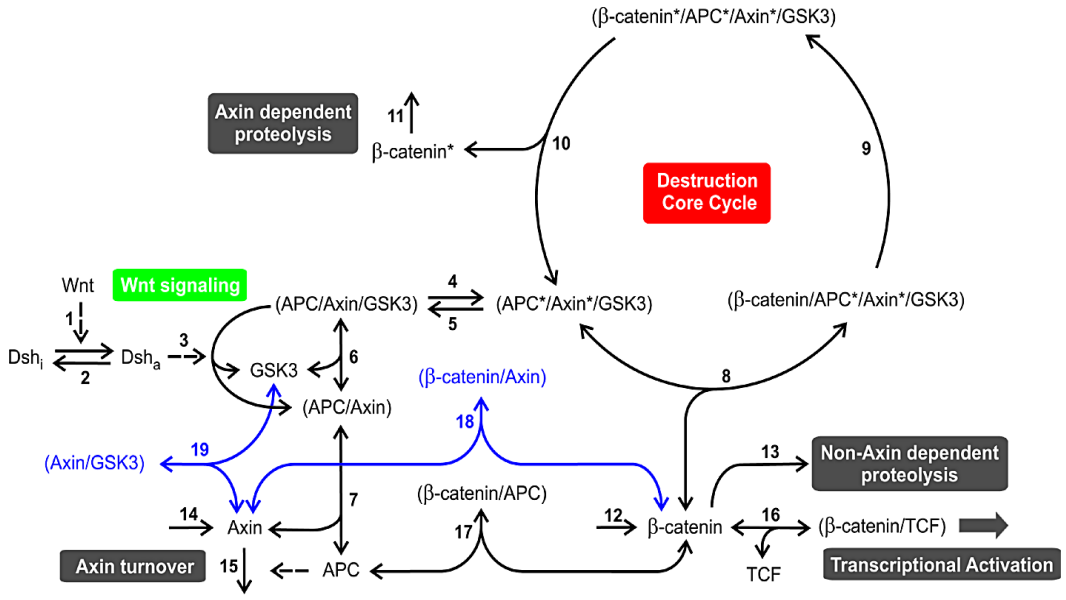

Figure 4.1.: Reaction scheme of the Wnt/β-catenin pathway in the Lee Model (reproduced from [Lee et al., 2003, Figure 1]). Protein complexes are denoted by the names of their components. Phosphorylated components are marked by an asterisk. Single-headed arrows characterise reactions taking place only in the indicated direction. Double-headed arrows denote binding equilibria. The broken arrows indicated that the components mediate, but do not participate stoichiometrically in the reaction scheme. The irreversible reactions 2, 4, 5, 9-11 and 13 are unimolecular and the reactions 6, 7, 8, 16 and 17 are reversible binding steps.

4.1. A quantitative kinetic model of the pathway

The first quantitative model of the Wnt/β-catenin model was proposed by Lee et al. [2003] ("Lee Model" in the following). It describes the interactions among the core components of the pathway (namely Wnt, Dsh, β-catenin, APC, Axin, GSK3β and TCF) excluding receptor dynamics. Figure 4.1 shows the reaction scheme the model is based on. It incorporates the kinetics of protein-protein interactions, protein synthesis/degradation and β-catenin phosphorylation/dephosphorylation (solid arrows). Besides, the model includes reactions that activate a certain process (broken arrows), e.g. the activation of Dsh by Wnt (step 1). The initial reference state was defined on data from experiments using *Xenopus* egg extract, which is basically equivalent to well-stirred cytoplasm. Therefore the model proposed by Lee et al. [2003] is purely temporal and does not take any spatial aspects into account. Parameter values are chosen according to measurements and experimentally based estimations combined with modelling assumptions. All species concentration (except phosphorylated β-catenin) were determined experimentally using Western blot

(or protein immunoblot), which is a widely accepted analytical technique used to detect specific proteins in the given sample of tissue homogenate or extract. Additionally, fluxes and characteristic times have been measured to determine the kinetic parameters describing the turnover of β-catenin and Axin (steps 11, 12, 13 and 14, 15, respectively). In contrast, the total concentrations of Dsh, GSK3β, APC and TCF are assumed to be conserved, meaning that they stay constant over time, as the corresponding biochemical experiments in *Xenopus* indicate a very slow turnover.
The mathematical model is expressed as a system of 15 coupled ODEs. The reactions are described as follows: protein synthesis (arrows 12 and 14 in Figure 4.1) are presented as a constant rate. Binding and dissociation are described using mass-action kinetics, where unimolecular reactions (single-headed arrows) are assumed to be irreversible and described by linear rate equations. Reversible binding (double-headed arrows) is simplified by assuming that the binding step is very fast compared to the dissociation step, such that the corresponding protein complexes are in rapid equilibrium. Therefore, only the dissociation constant is considered in their kinetic description. Using conservation equations and rapid equilibrium approximations, the authors were able to reduce the model to 7 ODEs and 8 algebraic equations [Krüger and Heinrich, 2004, Lee et al., 2003].
The validated Lee Model predicted several unusual features of the Wnt pathway, some of which were tested experimentally. A crucial observation was the low abundance of Axin. The model explains the importance of Axin degradation in amplifying and sharpening the transient Wnt signal. Lee and colleagues thus concluded that Axin is the limiting factor in controlling the β-catenin degradation process. Another key result of the model analysis regards the differential regulation of β-catenin levels by the scaffolding proteins Axin and APC. APC binds its interaction partners in an ordered manner, whereas Axin binds them randomly. The theoretical investigation also indicated that Axin plays an essential role in preventing the accumulation of β-catenin at decreased APC concentrations.
The Lee Model is to date the most cited quantitative model of the Wnt pathway and widely used as a reference regarding further Wnt modelling. It also forms the basis of my modelling approaches.

4.1.1. Including transcriptional feedback and crosstalk

The first extension of the Lee Model was done by Cho et al. [2006] (the "Cho Model"), who added a negative feedback loop provided by β-catenin/TCF-induced

synthesis of Axin2. The added reaction follows mass action kinetics. In their model, Axin1 and Axin2 are combined into one species, i.e. "Axin", since their function is believed to be largely equivalent. The authors also combined the species β-catenin and [β-catenin/TCF] of the Lee Model and termed them "available β-catenin", representing the pool of β-catenin available for nuclear translocation and transcriptional activity. Please note that, although they refer to the nucleus, there are no compartments included in their model. Furthermore, Cho et al. [2006] restricted their analysis to the steady state behaviour of the system. Simulations of the Cho Model evaluate the impact of various APC mutations on Wnt/β-catenin signalling, as these are often observed to be the earliest initiating event for most colorectal tumours. The mutations are modelled via variation of the corresponding parameter values. Their results predicted that APC mutations are selected not based on the maximal level of β-catenin but rather based on a distinct state of activity that appears to be optimal for the tissue-specific tumourigenesis. They concluded that the optimal level of β-catenin is determined by balancing β-catenin increase due to pathway activation and its decrease due to induction of the negative feedback through Axin2.

Similar to the Cho Model, Wawra et al. [2007] extended the Lee Model by introducing negative feedback loops involving Axin or Dickkopf (Dkk1; inhibiting the action of Dsh). These loops have previously been shown to induce oscillating expression patterns in many genes including Wnt pathway components, for example during vertebrate somitogenesis. The additional reactions are modelled using delay differential equations, allowing to consider time delays evoked by transcription, translation and splicing. Gene expression is modelled based on cooperative activation and described by a sigmoidal Hill function. The intermediate products of the transcriptional activity include Axin RNA (Ribonucleic acid) and Dkk1 RNA.
Perturbing the system up to 50% (single and multiple parameter perturbations as well as initial condition variation), the authors performed an extended robustness analysis of the Lee Model. Default parameter values were chosen according to the Lee Model. They demonstrated the very robust behaviour of the pathway. By introducing the feedback loop, the authors were able to induce oscillatory behaviour. However, a remarkably increased β-catenin and Axin throughput and hence unrealistically high axin and β-catenin concentrations are necessary to reach an oscillation threshold. Nevertheless, Wawra et al. [2007] showed that the Wnt/β-catenin pathway is capable of generating oscillatory behaviour independent of external factors.

Based on the Cho Model , Kim et al. [2007] investigated the crosstalk between the Wnt and ERK (extracellular-signal-regulated kinase) pathways, which is known as a major cellular proliferation signalling pathway and also involved in the pathogenesis of various kinds of cancers. The crosstalk was found to create a positive feedback loop. Kim et al. [2007] performed a qualitative comparison of model predictions to experimental results obtained from HEK 293 (Human Embryonic Kidney) cells and proposed that a positive feedback loop between the two pathways stimulates ERK activity and hence increases the levels of β-catenin/TCF in a switch-like manner. The model correctly predicts the consequences of Wnt/ERK crosstalk. However, it incorporates parameters that remain yet to be experimentally validated.

4.1.2. Time scale analysis

Krüger and Heinrich [2004] performed a systematic analysis of the Lee Model. Parts of the analysis have already been published in Lee et al. [2003]. Their results have been used to reduce the number of ODEs in the Lee Model from 15 to 7. The authors also determined how the steady state concentrations of the involved proteins changed when system parameters were varied. Their analysis revealed that (with the exception of β-catenin/TCF) the steady state values of all variables were robust to changes in the parameter values describing Wnt pathway activation.
A detailed asymptotic analysis was performed by Mirams et al. [2010] in order to identify which pathway components are dominant on the different timescales associated with Wnt signalling. Their analysis aimed at systematically simplifying the model to understand how the components of the pathway interact. The authors found that the pathway operates on three different timescales. By highlighting the operation of different pathway components over different time scales associated with the half-life of β-catenin, they derived a simplified model which was shown to retain the essential behaviour of the full pathway. Thus, the original set of ODEs from the Lee Model was reduced to a single ODE that represents the dynamics (accumulation and degradation) of active β-catenin in response to a Wnt signal. The ODE takes three reactions into account: synthesis of β-catenin, non-Axin dependent proteolysis of β-catenin and Axin dependent degradation of β-catenin, where the (mediated) influence of Wnt comes in. As the output of the pathway is usually the main object of interest, the authors also regarded the β-catenin/TCF complex to complete the model. This additional algebraic equation incorporates the equilibrium condition derived from fast-binding kinetics for the reaction between TCF and β-catenin, as

proposed by [Lee et al., 2003]. Taken together, the simplified model consisting of β-catenin and [β-catenin/TCF] only contains seven parameters (expressed as groups of original parameters) and still exhibits the same steady states and similar response to a transient Wnt stimulus as the full model. Moreover, the asymptotic analysis has been applied to the Cho Model. An additional production term was introduced to model Axin2 feedback in response to β-catenin/TCF signalling. Again, the essential behaviour of the model was retained.

4.1.3. Fold-change of β-catenin

A common feature of the models described above is that absolute levels of [β-catenin/TCF] determine the pathway output. Goentoro and Kirschner [2009] in contrast considered fold-changes in β-catenin pre and post pathway activation in the Lee Model. On the basis of a dimensional analysis of the core model, the reactions of the Lee Model were divided into three modules: input, degradation and synthesis. In a detailed theoretical investigation, the authors showed that the fold-change of β-catenin, which is defined as the ratio of the stimulated and the unstimulated steady state concentration, were more robust to perturbations of system parameters than changes in absolute levels of β-catenin. The generated data suggests that Wnt-induced fold-changes in β-catenin are robust against small perturbations in the degradation module. In contrast, other signalling characteristics (including the absolute values of β-catenin) are sensitive to perturbations in all modules. Therefore, the β-catenin fold-change was concluded to be the relevant readout of the Wnt pathway, because in this way the system can compensate for natural biological (both environmental and genetic) noise. Indeed, Goentoro and Kirschner [2009] confirmed their results experimentally, as they perturbed components of the pathway in mammalian cells by gene overexpression and pharmacological inhibitors and thereby proved that fold-change of β-catenin is relevant for the phenotype.

4.2. The dual role of β-catenin

The model developed by van Leeuwen et al. [2007] (the "VanLeeuwen Model") addresses the question of how the binding of β-catenin to E-cadherin alters its availability for gene expression due to Wnt signalling. The model thus incorporates β-catenin's dual – adhesive and transcriptional – function, as an increase in the synthesis rate of E-cadherin enhances the cell-cell adhesion and may transiently de-

crease the expression of target genes. The authors proposed two main hypotheses concerning the balance between both 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 model consists of 11 coupled ODEs, 6 of which describe different pools of β-catenin within the three 'compartments', nucleus, cytoplasm and membrane. Still, following the Lee Model, all kinetic reactions take place within a single cellular compartment. The authors used the model to carry out a series of *in silico* experiments and compared the behaviour of the systems governed by each hypothesis, as the experimental data supporting each hypothesis were inconclusive. The model predicted that dynamic measurements of E-cadherin levels following Wnt stimulation could be used to discriminate between the two hypotheses. To date, this counter-intuitive predictions remains to be tested. Furthermore, van Leeuwen et al. [2007] also exploited the model to investigate the impact of the mutations most commonly observed in human colorectal cancer, where both target gene expression and cell-cell adhesion are increased.

The VanLeeuwen Model was embedded in a multiscale approach combining subcellular, cellular and tissue levels of organisation to investigate the dynamics of intestinal tissue renewal. On the cellular level, the authors studied the contribution of Wnt signalling to the interactions of neighbouring cells and to gene expression and subsequent progression through the cell cycle [Van Leeuwen et al., 2009]. This multiscale model was further analysed by Mirams et al. [2012] and Fletcher et al. [2012]. The contents of their analyses are however beyond the scope of this work and are therefore not described here. For an extensive review on multiscale Wnt modelling, the reader is referred to [Lloyd-Lewis et al., 2013].

4.3. Receptor activation and inhibition

Kogan et al. [2012] developed a detailed mechanistic model (the "Kogan Model") focussing on the initial sequence of events in the Wnt pathway, from ligand binding to β-catenin accumulation, and the effects of pathway inhibitors. The model captures the following processes: i) the pathway is activated by binding of Wnt ligands to the Frizzled receptor; (ii) the resulting ligand-receptor complex may recruit an unoccupied LRP receptor and create a ternary complex Wnt/Frz/LRP; and (iii) the latter complex transduces the signal into the cell and interferes with the destruction

cycle of β-catenin. The Kogan Model is based on the Lee Model. However, in order to reduce the complexity of the intracellular part, the authors have assumed that the destruction complex is also in equilibrium with all of its components, meaning that its total concentration is conserved. Furthermore, β-catenin phosphorylation, dissociation and degradation are modelled as a one-step process. Model parameters were retrieved from experimental data reported previously. The experiments were conducted with mouse fibroblasts (L cells), which do not express cadherin. Hence, the cytoplasmic β-catenin is free and not bound to the membrane.
The predictive ability of this general model was validated using quantitative data from independent experiments (also using L cells), testing the effects of Wnt3a and sFRP (secreted Frizzled-related protein) on β-catenin accumulation. Additional experiments measuring inhibition by Dkk1 also validated corresponding model predictions. Using simulations of the combined effects of sFRP and Dkk1, the authors predicted synergism between the two inhibitors, which yet remains to be verified experimentally.

4.4. The impact of stochastic variation

The first stochastic investigation of the core Wnt/β-catenin pathway was presented by Mazemondet et al. [2012], who proposed a computational modelling approach on cell cycle asynchrony and self-induced signalling in the context of the Wnt/β-catenin pathway in ReNcell VM cells, which are derived from the ventral midbrain of a ten week old fetus [Donato et al., 2007]. The model they developed is based on the Lee Model and validated with wet-lab data obtained from Western blot experiments [Mazemondet et al., 2011]. Their model extensions allow to switch between stochastic and deterministic domains and also cover spatial aspects, i.e. the compartmental location of molecules. Using stochastic simulations, Mazemondet et al. [2012] demonstrated that the impact of the cell cycle asynchrony on the average β-catenin dynamics in cell populations is negligible. Furthermore, the authors showed that low Axin amounts lead to significant stochastic effects that contradict experimental observations and that are not observable in deterministic investigations.

4.5. Regulation of target gene expression

Benary et al. [2013] applied a mathematical modelling approach to investigate the impact of different regulatory mechanisms of target gene expression with Wnt or

APC concentration gradients. They developed a minimal model of Wnt/β-catenin signalling and extended it to different regulatory mechanisms of target gene expression. The core signal transduction module of the model consists of five coupled ODEs and two conservation equations following mass action kinetics. The destruction complex is not modelled explicitly, but indirectly, as β-catenin destruction is influenced by Wnt through active Dsh. Kinetic parameter values are set in strong accordance to the Lee Model. The model extensions are i) linear and cooperative activation of mRNA production, described with linear or Hill kinetics; ii) repression of mRNA production; iii) an incoherent feedforward loop (iFFL), as β-catenin/TCF complexes are proposed to induce the expression of a repressor of the mRNA production, which counteracts the simultaneous direct activation of mRNA expression; and finally iv) transcriptional feedback via TCF. The authors investigated these mechanisms in dependence of the Wnt or APC concentration. They claimed that a combination of these mechanisms with Wnt or APC gradients is sufficient to generate spatially distinct target gene expression patterns as have been observed experimentally in liver. Benary et al. [2013] found that cooperative gene activation in combination with a feedback can establish sharp boarders of target gene expression in dependence of the Wnt or APC concentration, whereas the iFFL renders the gene expression independent of Wnt and APC. Under mutant conditions, their analysis revealed that the impact on gene expression is determined by the gene regulatory mechanisms and the APC concentration in the cell.

4.6. Validation based on mammalian systems

The computational models for the Wnt signalling pathway have so far been largely based on the Lee Model (in which the authors derived their quantitative data underlying the model parametrisation from *Xenopus* egg extracts), due to the lack of corresponding mammalian data for the concentrations of key Wnt pathway components. Therefore, the interpretation of predictions for mammalian systems is limited. In order to close this gap, Tan et al. [2012] reported initial estimates of the concentrations of β-catenin, APC, Axin, GSK3β and E-cadherin in five mammalian cell lines in the basal (non-stimulated) state. To this end, a confocal microscopy technique was developed to measure the average cell volume for each of the cell lines, allowing calculations of the concentrations of the key proteins within cells. Their results demonstrated significant differences in the concentrations of the Wnt signalling proteins between the *Xenopus* egg extracts and mammalian whole cell

lysates, in particular with respect to the Axin concentration. Tan et al. [2012] reported that in mammalian cells, the concentrations of Axin are considerably higher and comparable to other protein concentrations of the pathway. Besides, the authors observed that mammalian cells have higher β-catenin concentrations than measured in *Xenopus* extracts, despite having higher Axin concentrations. The newly acquired quantitative data was integrated in the complete Lee Model. Using computational simulations, the authors concluded that there is a need for recalibration of the model for mammalian cells. To date, the kinetic parameters of the Wnt pathway, such as reaction rate constants or dissociation constants, have not been measured in mammalian systems.

4.7. Open questions

Over the last decade, mathematical models have been used to increase our understanding of the Wnt/β-catenin pathway. The models I have presented in this chapter mainly focus on the biochemical level and thus on intracellular interactions. They are largely based on a small set of experimental observations. Model simulations and analyses led to important insights and generated testable and counter-intuitive predictions. However, several aspects of the pathway have not yet been addressed by modelling. An example for a process that is under extensive experimental investigation and has not yet been modelled is Wnt secretion. Moreover, the idealised biochemical models which have been developed so far are able to provide insights into the cellular dynamics, although they only consider the 'core' Wnt pathway. This raises questions about the role of the large number (i.e. more than 200) of potential Wnt regulators. Incorporating some of these additional regulating components may allow for context-dependent cross-talk of Wnt/β-catenin signalling to non-canonical [Katoh, 2005, Kohn and Moon, 2005] and non-Wnt pathways such as Notch signalling [Lloyd-Lewis et al., 2013].

Another aspect that has not been addressed by modelling is the intercellular compartmentalisation. This issue has also been noticed and mentioned by Kofahl and Wolf [2010]. With the exception of the stochastic model by Mazemondet et al. [2012], all models discussed above treat the interior of the cell as a well-mixed environment. In practice, however, cells comprise distinct regions with proteins migrating between them: β-catenin and other key pathway regulators are present in the cytoplasm and the nucleus, and bind to fixed cellular structures, such as the plasma membrane. This raises questions about the subcellular distribution of concentrations and activ-

ities in the compartments, and the shuttling between them [Kofahl and Wolf, 2010]. As described in Sections 2.2 and 2.4, not only β-catenin as a transcriptional cofactor shuttles between cytoplasm and nucleus. Interestingly, a surprisingly high number of cytoplasmic Wnt regulators, including the β-catenin antagonists APC, Axin and GSK3, have also been localised in the nucleus.

One conceivable hypothesis, attempting to explain why these proteins translocate into the nucleus, is that the antagonists mainly regulate the subcellular localisation of β-catenin by retaining it in the cytoplasm, as well as in the nucleus [Krieghoff et al., 2006]. Another possible hypothesis is that the destruction complex is assembled and also active in the nucleus [Caspi et al., 2008]. In this case, nuclear β-catenin could also be marked for degradation, allowing antagonist shuttling to more efficiently inhibit Wnt signalling. Alternatively, however, the question arises whether it is possible that, in certain scenarios, Wnt signalling benefits from antagonist shuttling.

To date, the functional relevance of antagonist shuttling in the Wnt/β-catenin pathway remains to be determined. This open question is the challenge that inspires the research presented in this thesis. Using mathematical modelling and model analysis, I study the following key questions: Does the nucleo-cytoplasmic shuttling of β-catenin antagonists APC, Axin and GSK3 influence the [β-catenin/TCF] concentration, which is considered as the output of Wnt signalling? Can the [β-catenin/TCF] concentration be maximised by antagonist shuttling, leading to an optimised signal transduction through the cell? How is the output of the pathway influenced by cytoplasmic and nuclear retention of β-catenin, and by phosphorylation and subsequent degradation of β-catenin?

In order to answer these questions, I develop novel compartmental models and analyse them with respect to nucleo-cytoplasmic shuttling of β-catenin and its antagonists. They are currently the only deterministic models of the Wnt/β-catenin pathway on a subcellular, compartmental scale and have been noticed as such in the research community (see [Lloyd-Lewis et al., 2013, Figure 2]). These models account for the compartmental structure of the cell and allow for the retention of β-catenin by APC and the degradation of β-catenin by the destruction complex in the cytoplasm as well as in the nucleus.

The compartmental models and their analyses are presented in the following Chapters 5 and 6.

5. The Retention Model

In this chapter I investigate the impact of nucleo-cytoplasmic shuttling of **one** of the key β-catenin antagonists – namely **APC** – on the [β-catenin/TCF] concentration. To this end, I establish and analyse a mathematical model using the compartmentalised ODE formalism (see Sections 3.2 and 3.3). It is the first deterministic model of the Wnt/β-catenin pathway on a subcellular, compartmental scale [Lloyd-Lewis et al., 2013]. It consists of two compartments, cytoplasm and nucleus, and allows for protein complex formation in each compartment as well as β-catenin and APC shuttling between nucleus and cytoplasm. As in this novel model the β-catenin concentration is mainly regulated through compartmental retention by APC, I refer to it as the **"Retention Model"**. For the model analysis, I focus on the influence of nucleo-cytoplasmic shuttling of APC on the steady state concentration of [β-catenin/TCF] and hence neglect β-catenin degradation as well as membrane associated events. The key questions I address in this chapter are: Does the nucleo-cytoplasmic shuttling of APC influence the [β-catenin/TCF] concentration, which is considered as the output of Wnt signalling? Can the [β-catenin/TCF] concentration be maximised by APC shuttling? How is the output of the pathway influenced by cytoplasmic and nuclear retention of β-catenin?

The experimental results summarised in Chapter 2 provide the structural basis of the Retention model. The main protagonist β-catenin and its antagonist APC shuttle independent of each other between the two compartments; the complexes are, however, unable to cross the nuclear envelope [Krieghoff et al., 2006]. The construction of the Retention Model is presented in Section 5.1. Next, I investigate the influence of nucleo-cytoplasmic shuttling of APC on the output of the Wnt signalling. For this reason I systematically examine the influence of different shuttling mechanisms: In a first step, I neglect the nucleo-cytoplasmic shuttling of APC. I then study diffusive nucleo-cytoplasmic shuttling of APC and analyse the influence of cytoplasmic and nuclear retention of β-catenin by APC. Finally, I choose facilitated nuclear export of APC, which is based on a Michaelis-Menten type rate law. In Section 5.2, the results and the discussion of the model analysis are presented. A conclusion is given

State variable	Original variable	Interpretation
X_1	β-cat$_c$	free cytoplasmic β-catenin
X_2	APC$_c$	free cytoplasmic APC
X_3	β-cat$_n$	free nuclear β-catenin
X_4	APC$_n$	free nuclear APC
X_5	[β-cat/APC]$_c$	cytoplasmic retention complex [β-cat/APC]
X_6	[β-cat/APC]$_n$	nuclear retention complex [β-cat/APC]
X_7	TCF	free nuclear transcription molecules
X_8	[β-cat/TCF]	transcription complex [β-cat/TCF]

Table 5.1.: Definition of the eight state variables of the system. Squared brackets denote protein complexes. The indices n and c denote nuclear and cytoplasmic protein concentrations, respectively. In the text, variables in steady state are annotated by an asterisk, i.e. *.

in Section 5.3. The contents of this chapter is adapted from [Schmitz et al., 2011] and reproduced with permission from Elsevier.

5.1. Construction of the Retention Model

The Retention model is a simple biochemical reaction network model consisting of two compartments, cytoplasm and nucleus. The model describes nucleo-cytoplasmic shuttling of β-catenin (β-cat), the main protagonist of the canonical Wnt signalling pathway, and its antagonists APC. It is depicted in Figure 5.1. This model structure is based on experimental findings (see Chapter 2 for details): Both key players are able to shuttle between the nucleus and the cytoplasm (ν_1 and ν_2, respectively) [Neufeld et al., 2000, Rosin-Arbesfeld et al., 2003, Wiechens and Fagotto, 2001]. Furthermore, they can associate to and dissociate from a common complex [β-cat/APC] within the cytoplasm (ν_3) as well as within the nucleus (ν_4) [Krieghoff et al., 2006]. These complexes, however, are not able to cross the nuclear envelope [Eleftheriou et al., 2001, Krieghoff et al., 2006, Wiechens and Fagotto, 2001].
Additionally, nuclear β-catenin can interact with TCF (ν_5) to activate transcription [Cong and Varmus, 2004]. Hence, the complex [β-cat/TCF] is considered as the output of both, the Wnt pathway [Lee et al., 2003] and the Retention model.
The reaction network is translated into a system of ODEs describing temporal changes of protein concentrations as functions of interactions and transport processes (see Section 3.3 for details). Each protein and protein complex concentration is represented by a variable in the mathematical model (see Table 5.1). Taking the

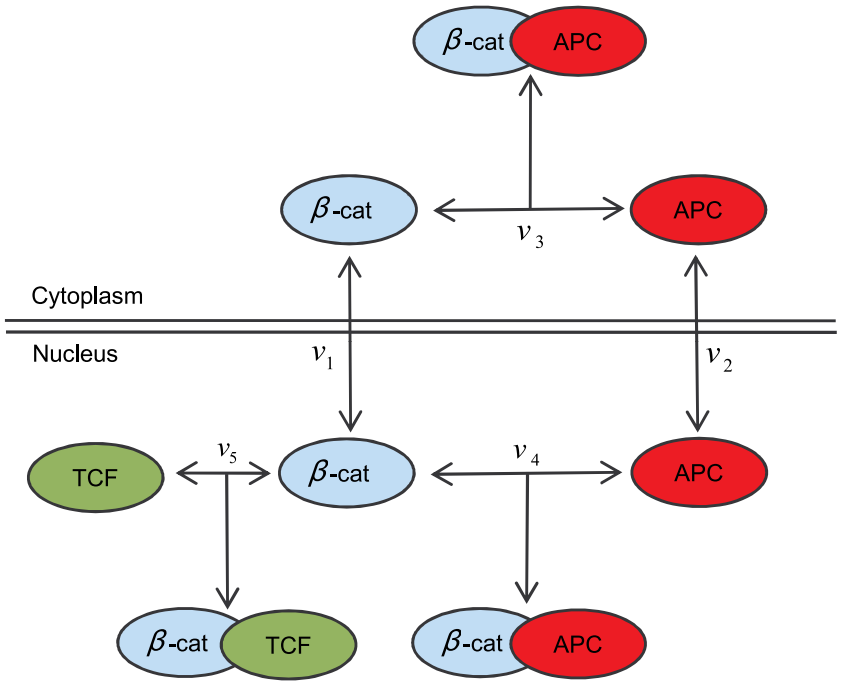

Figure 5.1.: Biochemical reaction network of the Retention Model describing nucleo-cytoplasmic shuttling of β-catenin and APC, and protein complex formation of β-catenin with APC and TCF, respectively. The reactions are numbered 1 to 5. In Table 5.1 the protein names are translated into state variables (X_1 to X_8).

five interactions into account, the dynamics of the biological system are determined by the following eight coupled ODEs:

$$\begin{aligned}
\dot{X}_1 &= -\nu_1 - \nu_3 \\
\dot{X}_2 &= -\nu_2 - \nu_3 \\
\dot{X}_3 &= +\nu_1 - \nu_4 - \nu_5 \\
\dot{X}_4 &= +\nu_2 - \nu_4 \\
\dot{X}_5 &= +\nu_3 \\
\dot{X}_6 &= +\nu_4 \\
\dot{X}_7 &= -\nu_5 \\
\dot{X}_8 &= +\nu_5
\end{aligned} \tag{5.1}$$

where the reaction rates v_i on the right-hand side are functions of the protein or protein complex concentrations, each one of them describing biochemical reactions

or transport processes:

$$\begin{aligned}
\nu_1 &= F_1(X_1, X_3) \\
\nu_2 &= F_2(X_2, X_4) \\
\nu_3 &= k_3\, X_1 X_2 - k_{-3}\, X_5 \\
\nu_4 &= k_4\, X_3 X_4 - k_{-4}\, X_6 \\
\nu_5 &= k_5\, X_3 X_7 - k_{-5}\, X_8.
\end{aligned} \tag{5.2}$$

Binding and dissociation processes (ν_3, ν_4 and ν_5) are described with mass-action kinetics, see Equation (3.3). The functions $F_1(X_1, X_3)$ and $F_2(X_2, X_4)$ account for nucleo-cytoplasmic transport processes of β-catenin and APC, respectively. To investigate the influence of the nucleo-cytoplasmic shuttling on the output of the system, I consider different translocation mechanisms which are presented in the Section 5.2.

5.1.1. Rapid equilibrium approximations and conservation equations

Independent of the translocation mechanism, one can apply the following approximations and conservation equations in order to simplify the model: I assume that the binding and dissociation processes considered in the network (i.e. β-catenin to APC and β-catenin to TCF) will approach the quasi-equilibrium rapidly, leading to:

$$K_3 = \frac{X_1 X_2}{X_5} = \frac{k_{-3}}{k_3} \tag{5.3}$$

$$K_4 = \frac{X_3 X_4}{X_6} = \frac{k_{-4}}{k_4} \tag{5.4}$$

$$K_5 = \frac{X_3 X_7}{X_8} = \frac{k_{-5}}{k_5}. \tag{5.5}$$

The biochemical reaction network (see Figure 5.1) implies the existence of three conservation equations. This means that total amounts of molecules are conserved. In this case, the conserved quantities correspond to the total number of molecules of β-catenin, APC, and TCF. If more than one compartment is considered, conservation sums can only be applied to molecule numbers, not to concentrations. To calculate the corresponding protein concentrations, the compartmental volumes

must be taken into account. For the sake of simplicity, I assume that the volumes and capacities of nucleus and cytoplasm are of the same size. This assumption leads to the following expressions exhibiting the double total concentrations B, A and T, respectively:

$$\text{total } \beta\text{-catenin:} \qquad 2B = X_1 + X_3 + X_5 + X_6 + X_8 \tag{5.6}$$

$$\text{total APC:} \qquad 2A = X_2 + X_4 + X_5 + X_6 \tag{5.7}$$

$$\text{total TCF:} \qquad 2T = X_7 + X_8. \tag{5.8}$$

The set of differential equations can be simplified by considering the conservation equations and the rapid equilibrium approximations to the binding reactions. This results in a subdivision of dependent variables. The dependent variables are algebraic functions of other variables, which are determined as solutions of the remaining equations.

Elimination of X_7 and X_8:

These dependent variables can be expressed as a function of the variable X_3 using the conservation condition for TCF (Equation (5.8)) and the equilibrium condition for β-catenin to TCF binding (Equation (5.5)). The combination of both yields

$$X_7 = \frac{2K_5T}{K_5 + X_3} \quad \text{and} \quad X_8 = \frac{2TX_3}{K_5 + X_3}. \tag{5.9}$$

Elimination of X_5 and X_6:

Both dependent variables are eliminated by the equilibrium conditions for the binding of β-catenin to APC in the cytoplasm and nucleus, respectively (Equations (5.3) and (5.4)).

$$X_5 = \frac{X_1X_2}{K_3} \quad \text{and} \quad X_6 = \frac{X_3X_4}{K_4}. \tag{5.10}$$

5.1.2. Differential equations for the remaining variables

Substituting the dependent variables of Equations (5.9) and (5.10), into the ODE system (5.1), the dynamics of the remaining variables are determined by the nucleo-

cytoplasmic reaction rates:

$$\begin{aligned}\dot{X_1} &= -F_1(X_1, X_3) = -\dot{X_3} \\ \dot{X_2} &= -F_2(X_2, X_4) = -\dot{X_4}.\end{aligned} \tag{5.11}$$

The connection between the variables is given by the conservation relations of β-catenin and APC, described by Equations (5.6) and (5.7), where the dependent variables of Equations (5.9) and (5.10) have been substituted:

total β-catenin:

$$2B = X_1 + X_3 + \frac{X_1 X_2}{K_3} + \frac{X_3 X_4}{K_4} + \frac{2T X_3}{K_5 + X_3} \tag{5.12}$$

total APC:

$$2A = X_2 + X_4 + \frac{X_1 X_2}{K_3} + \frac{X_3 X_4}{K_4} \tag{5.13}$$

Equations (5.11) – (5.13) depend on four variables $(X_1, ..., X_4)$ and can thus be solved in steady state for different nucleo-cytoplasmic transport mechanisms. The other four dependent variables can afterwards be calculated with Equations (5.9) and (5.10).

5.1.3. How to determine the steady state solution

The network depicted in Figure 5.1 includes neither a time dependent input nor output. I investigate a closed system without a stimulus, transcription or degradation, therefore all transport processes are in steady state. To determine the steady states, i.e. the stationary solutions of the model, I calculate the states in which the time derivatives vanish (see Section 3.4.1). The steady state concentrations are annotated by an asterisk. Considering the conservation equations and substituting X_1^* and X_2^* (Equations (5.11)) yields two algebraic equations that depend on X_3^* and X_4^*. Independent of the chosen transport mechanism, substitution always leads to an algebraic equation in which X_3^* (i.e. nuclear β-catenin) is the only remaining variable. It has the form of a polynomial with respect to X_3^*, and depends on dissociation and shuttling constants and total protein concentrations. The solution of the polynomial can be determined in general terms using computer algebra based on symbolic maths (e.g. MAPLE or MATLAB). However, the particular expressions

are far too complex to tell us much about the system's behaviour. The stationary solution has hence determined numerically using the "roots" (for cubic polynomials) or "fzero" (for polynomials of higher order) function in MATLAB. The root finding algorithm used in the "fzero" function depends on the initial value of search. Therefore one has to make sure to find every biological sensible basin of attraction. I investigated parameter space by generating randomised parameter values for those parameters describing nucleo-cytoplasmic shuttling. In the following I consider and analyse different transport mechanisms.

5.2. Results and discussion

In this section, I use the model, which was developed in the previous section, to investigate the impact of nucleo-cytoplasmic shuttling of APC and compartmental retention of β-catenin by APC on the [β-catenin/TCF] concentration.
The protein β-catenin not only exhibits the intrinsic ability to enter the nucleus, but also to move bidirectionally across the nuclear envelope, proposing a free, nondirectional nuclear translocation model for β-catenin [Wiechens and Fagotto, 2001, Xu and Massagué, 2004]. I will therefore consider pure diffusion for nuclear translocation of β-catenin.
The nucleo-cytoplasmic shuttling of APC, however, depends on indirect interaction with the NPCs and occurs in both directions by facilitated transport [Neufeld et al., 2000, Rosin-Arbesfeld et al., 2000]. In my approach, the influence of nucleo-cytoplasmic shuttling of APC on the output of the Wnt signalling is investigated by studying different shuttling mechanisms:
In a first step, I neglect the nucleo-cytoplasmic shuttling of APC, which means that APC is only located in the cytoplasm. This system serves as a reference system in order to examine the influence of APC shuttling. I then study nucleo-cytoplasmic shuttling of APC and its effect on the output of the pathway. For the sake of simplicity, I first assume diffusion of APC across the nuclear envelope as proposed for nucleo-cytoplasmic shuttling of β-catenin. I start by setting all dissociation constants and total concentrations according to measurements and estimations conducted by the Kirschner group in the Wnt off-state in *Xenopus* oocytes extracts to [Lee et al., 2003], see Table 5.2. These numbers serve as the default parametrisation and hence form the starting point for the investigation of the effect of nucleo-cytoplasmic antagonist shuttling. Next, I analyse the influence of cytoplasmic and nuclear retention of β-catenin by APC, by varying the respective dissociation con-

stants. Then, I choose a more complicated but more realistic approach for a APC's shuttling mechanism and assume facilitated nuclear export, which is based on a Michaelis-Menten type rate law.

5.2.1. Absence of APC shuttling

Absence of APC shuttling is described by setting the antagonist shuttling constants to zero (see Equations (5.14)). This system serves as a control for investigating the influence of antagonist shuttling on $X_8^* = [\beta\text{-cat/TCF}]$. In this case, the antagonist APC resides in the cytoplasm and is not able to cross the nuclear envelope.

$$\begin{aligned} F_1(X_1, X_3) &= k_1 X_1 - k_{-1} X_3 \\ F_2(X_2, X_4) &= 0 \\ X_4 &= X_6 = 0 \end{aligned} \tag{5.14}$$

Hence, the system depicted in Figure 5.1 simplifies significantly. The system reduces from eight to six variables. In steady state we find:

$$\begin{aligned} X_1^* &= \frac{k_{-1}}{k_1} X_3^* = K_1 X_3^* \\ X_2^* &= \frac{2K_3 A}{K_3 + X_1^*} \\ X_5^* &= \frac{2AX_1^*}{K_3 + X_1^*}. \end{aligned} \tag{5.15}$$

Substituting these solutions into Equation (5.12), the steady state solutions is obtained as the roots of a cubic polynomial with respect to X_3^*:

$$\begin{aligned} & X_3^{*3}\Big[(K_1+1)K_1\Big] \\ +& X_3^{*2}\Big[2K_1(A-B+T) + (K_1+1)(K_3+K_1K_5)\Big] \\ +& X_3^{*}\Big[2K_1K_5(A-B) + 2K_3(T-B) + K_3K_5(K_1+1)\Big] \\ & - 2BK_3K_5 = 0. \end{aligned} \tag{5.16}$$

Therefore, the steady state concentration of X_8^* is also only, but explicitly, dependent on the dissociation and shuttling constants as well as the conserved total protein

Parameter name	Default value	Description
K_3	1200 nM	binding of β-catenin to APC (cytoplasm)
K_4	1200 nM	binding of β-catenin to APC (nucleus)
K_5	30 nM	binding of β-catenin to TCF (nucleus)
A	100 nM	Total concentration of APC
B	35 nM	Total concentration of β-catenin
T	15 nM	Total concentration of TCF

Table 5.2.: The default parametrisation following Lee et al. [2003]. Numeric values and description of the parameters of the model in its default state. The K_i-parameters are dissociation constants $K_i = k_{-i}/k_i$, the others (A, B, T) illustrate total protein concentrations. These parameters are crucial for simplifying the system using rapid equilibrium approximations and conservation equations.

concentrations.

A cubic polynomial has one to three solutions. We find that there exist three solutions, which raises the question of their ranges and stability. If two biological meaningful and stable steady states coexist, the system exhibits bistability. Hence hysteresis-like behaviour can be expected when crossing bifurcation points. However, in every parametrisation two of the three steady states solutions exhibit at least one negative protein concentration. I investigated parameter space by generating parameter values for the shuttling parameters, i.e. $K_1 \in [10^{-4}, 10^4]$. In a biological sensible range, the system remains monostable. This is therefore a numerically obtained finding, which originates from the strict condition that all variable and parameter values must be positive in order to be biologically meaningful.
The steady state analysis of the ODE model leads to the dependency of the steady state concentration $[\beta\text{-cat/TCF}]^*$ on the ratio of rate constants for nucleo-cytoplasmic shuttling of β-catenin ($K_1 = \frac{k_{-1}}{k_1}$), which depicted in Figure 5.2. The parameter K_1 is defined as the ratio of the shuttling rate constants of β-catenin, which is equal to the ratio of free protein concentrations in the cytoplasm and nucleus calculated in steady state (Equations (5.15), top line). The smaller the value of K_1, the higher the concentration of free β-catenin in the nucleus. If more β-catenin is located in the nucleus, the output of the pathway is maximised in both presented cases. This meets our expectations, as only nuclear β-catenin is able to interact with TCF and therewith initiate target gene expression. However, a certain plateau of $[\beta\text{-cat/TCF}]^* \sim 19$ nM cannot be exceeded. This is due to the fact that the binding of β-catenin to TCF is limited by the value of its dissociation constant K_5. This constant regulates the level of the plateau height: decreasing K_5 increases the height

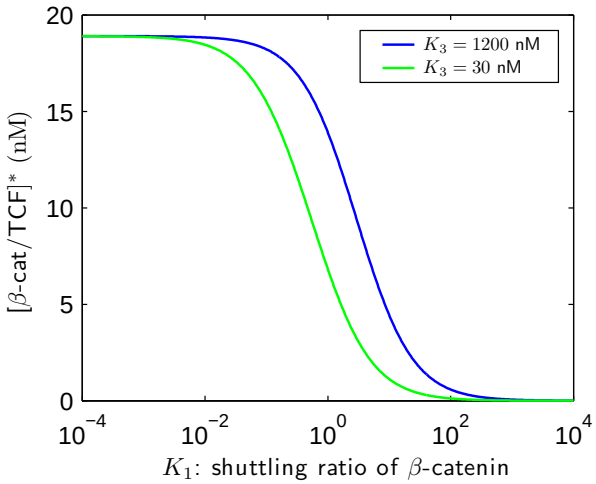

Figure 5.2.: The reference model without APC shuttling. The steady state concentration $[\beta\text{-cat/TCF}]^*$ is depicted in dependence on the ratio of shuttling rate constants of β-catenin (K_1). The blue curve is obtained using the default parametrisation (Table 5.2); for the green curve, $K_3 = K_5 = 30$ nM are chosen to account for increased cytoplasmic retention.

of the plateau.

In Figure 5.2, the blue curve is obtained using the default parametrisation (Table 5.2). The green curve in comparison is obtained if K_3 is reduced to 30 nM. This lower value corresponds to a higher affinity of cytoplasmic β-catenin to bind APC and therefore describes a higher cytoplasmic retention of β-catenin by APC. In this parametrisation, β-catenin's binding to TCF and to APC occurs equally likely. The variation in values for K_3 does not influence the height of the minimal or maximal plateau but shifts the curve to smaller values of K_1. For $K_1 = 1$, the steady state concentration $[\beta\text{-cat/TCF}]^*$ is decreased by half, because of the increased competition of the antagonist with the transcriptional factor for binding to β-catenin. The results therefore show that cytoplasmic retention of β-catenin by its antagonist APC influences the output of the model without antagonist shuttling.

5.2.2. Diffusive nucleo-cytoplasmic shuttling of APC

In the following, I investigate the effects of nucleo-cytoplasmic antagonist shuttling on the steady state concentration $[\beta\text{-cat/TCF}]^*$. First, I assume diffusive shuttling for β-catenin and APC. All translocation processes across the nuclear envelope are modelled as simple as possible, meaning that transport is based on diffusion. Thus,

nucleo-cytoplasmic shuttling is described by linear rate equations:

$$\begin{aligned} F_1(X_1, X_3) &= k_1 X_1 - k_{-1} X_3 \\ F_2(X_2, X_4) &= k_2 X_2 - k_{-2} X_4 \end{aligned} \tag{5.17}$$

The index of the shuttling constants $(k_1, k_{-1}, k_2, k_{-2})$ is positive for transport into the nucleus and negative for the transport back to the cytoplasm. In steady state the following relations are obtained:

$$\begin{aligned} X_1^* &= \frac{k_{-1}}{k_1} X_3^* = K_1 X_3^* \\ X_2^* &= \frac{k_{-2}}{k_2} X_4^* = K_2 X_4^* \end{aligned} \tag{5.18}$$

By considering the conservation equations (5.12) and (5.13) and substituting X_1^* and X_2^* as presented in Equations (5.18), two algebraic equations are obtained, which depend on X_3^* and X_4^*. Solving Equation (5.13) for X_4^* and substituting into Equation (5.12) leads to the following equation in which X_3^* (i.e. nuclear β-catenin) is the only remaining variable. It also has the form of a cubic polynomial with respect to X_3^*:

$$\begin{aligned} & X_3^{*3}\Big[(k_1+1)C_1\Big] \\ +& X_3^{*2}\Big[2C_1(A-B+T) + C_2 + (K_1+1)K_5C_1\Big] \\ +& X_3^{*}\Big[2K_5C_1(A-B) + 2(T-B)(K_2+1) + C_2K_5\Big] \\ & - 2K_5B(K_2+1) = 0. \end{aligned} \tag{5.19}$$

where

$$C_1 = \Big(\frac{K_1K_2}{K_3} + \frac{1}{K_4}\Big) \tag{5.20}$$

and

$$C_2 = (K_1+1)(K_2+1). \tag{5.21}$$

I investigated parameter space by generating parameter values for the shuttling parameters, i.e. $K_{1,2} \in [10^{-4}, 10^4]$. As discussed in the previous case without APC

shuttling, again only one positive, and therefore biological meaningful solution exists.

The standard (default) parameterisation

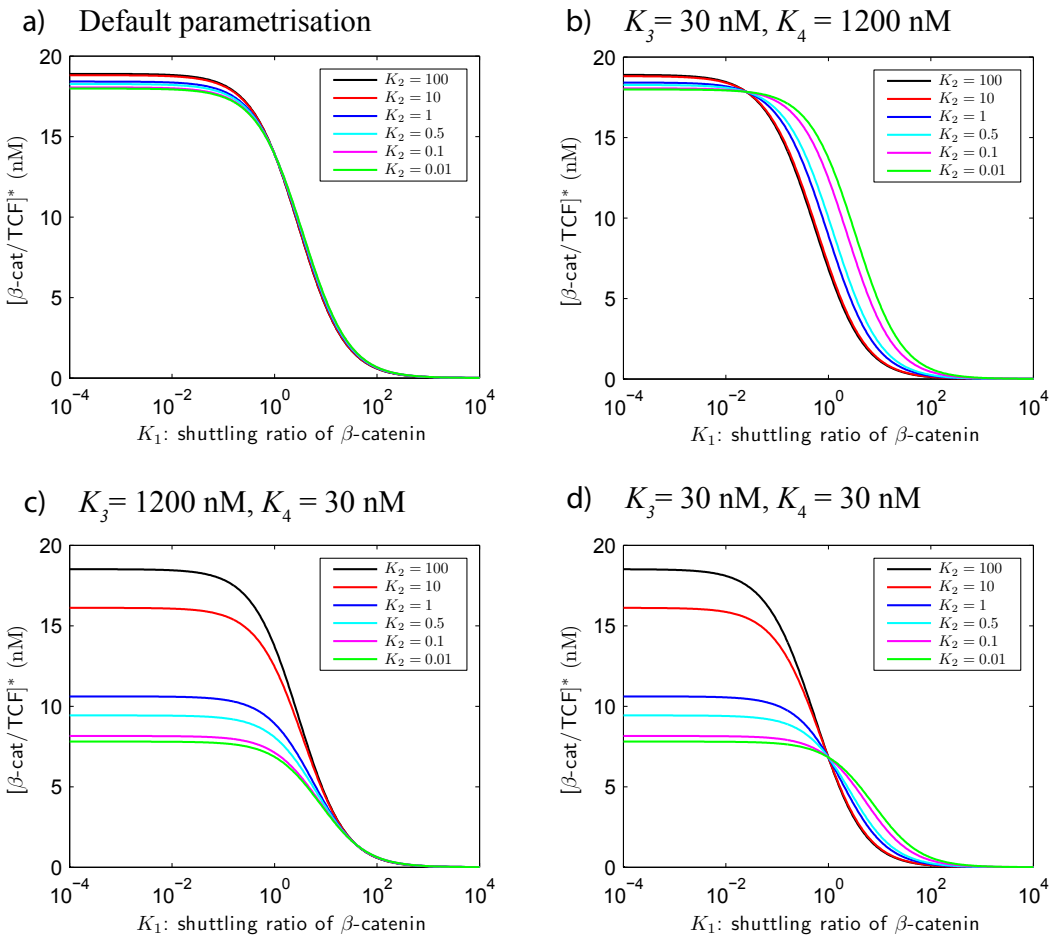

Figure 5.3.: Influence of diffusive nucleo-cytoplasmic shuttling of APC on [β-cat/TCF]*. The steady state concentration [β-cat/TCF]* is depicted in dependence on the ratio of shuttling rate constants of β-catenin (K_1) and APC (K_2). **a)** The default parametrisation: The black curve is equal to the blue curve in Figure 5.2. For this parametrisation, the steady state analysis indicates that the shuttling rate constant ratio of β-catenin has a much higher impact on [β-cat/TCF]* than the ratio of shuttling rate constants of APC. **b)** $K_3 = 30$ nM. The black curve is equal to the green curve in Figure 5.2: The steady state concentration of [β-cat/TCF]* crucially depends not only on β-catenin, but also on APC shuttling. **c)** $K_4 = 30$ nM: The black curve is equal to the green curve in Figure 5.2. The steady state concentration [β-cat/TCF]* only decreases in comparison to the reference curve. **d)** $K_3 = K_4 = 30$ nM: For $K_1 < 1$ the effect of nuclear retention is dominant, for $K_1 > 1$ cytoplasmic retention is mainly determining the steady state concentration of [β-cat/TCF]*.

First, all dissociation constants and total protein concentrations are set to their

default values (see Table 5.2). As a result of the steady state analysis, it is obtained that [β-cat/TCF]* depends on the biochemical binding and shuttling parameters. The steady state concentration [β-cat/TCF]* as a function of the nucleo-cytoplasmic shuttling rate constant ratios (K_1 and K_2) is presented in Figure 5.3a. In this parametrisation, the shuttling ratio of β-catenin (K_1) has a much higher impact on the output than the shuttling ratio of APC (K_2). The shape of the curve is qualitatively very similar to the result obtained with the reference model presented in Figure 5.2. For this model, it is also more favorable for the output of the pathway, if more β-catenin is located in the nucleus. The plateau has the same height as the plateau of the reference model. APC shuttling parameter values between $K_2 = 0.01$ and $K_2 = 100$ exhibit an almost identical output, even if the parameter values of K_2 are further increased or decreased (data not shown). Thus, for the default parametrisation, the steady state concentration [β-cat/TCF]* is only slightly influenced by APC shuttling. This is due to the large dissociation constants describing the binding of APC to β-catenin, i.e. $K_3 = K_4 = 1200$ nM (see Table 5.2), which exceeds the dissociation constant of TCF to β-catenin by a factor of 40. Hence, β-catenin has a much higher affinity to bind to TCF than to APC and the concentration of the complex [β-cat/APC]* can neither reach high numbers in the cytoplasm nor in the nucleus. Subsequently, β-catenin can neither be retained in the nucleus nor cytoplasm by its antagonist APC. Thus, the influence of antagonist shuttling is negligible.
In the next section, I study the question whether lower dissociation constants can account for a greater impact of APC shuttling on the steady state concentration [β-cat/TCF]* in comparison to the values of the reference model.

The impact of the dissociation constants

For the default parametrisation, the affinity of β-catenin to bind to APC is low: $K_{3,4} = 1200$ nM (experimentally based estimation by the Kirschner group [Lee et al., 2003]). However, it has been demonstrated that phosphorylation enhances the ability of APC to interact with β-catenin in vitro [Rubinfeld et al., 1996]. Moreover, Sierra et al. [2006] concluded from their experiments that β-catenin cannot bind unphosphorylated APC efficiently and that CK1 phosphorylation of APC might induce high-affinity binding to β-catenin and even trigger its dissociation from LEF/TCF. Seo and Jho [2007] proposed that accumulation of cytoplasmic β-catenin induces phosphorylation of APC and that phosphorylated APC retains β-catenin. In accordance with these findings, I investigate the influence of small values for dissociation

constants ($K_{3,4} = 30$ nM) between β-catenin and APC. Lower dissociation constants correspond to a higher binding affinity and hence higher possible compartmental retention. I choose these specific values to account for a "fair" competition between TCF and APC for β-catenin binding. It is still selected arbitrarily. However, the results do not change qualitatively if other values (10 to 120 nM) were chosen. Since APC phosphorylation can occur in both compartments, nucleus and cytoplasm, the two different parameter values are changed independently: first, I decrease K_3 to describe an enhanced cytoplasmic retention and second, I decrease K_4 to account for an enhanced nuclear retention of β-catenin by APC. Different dissociation constants in nucleus and cytoplasm can be due to e.g. different biochemical compositions of the two compartments. In a last step I show the combined effect of nuclear and cytoplasmic retention of β-catenin by APC.

For $K_3 = 30$ nM, the binding affinity of β-catenin to APC in the cytoplasm is equal to the binding affinity of β-catenin to TCF in the nucleus ($K_5 = 30$ nM). The steady state analysis leads to the dependency of [β-cat/TCF]* on the nucleo-cytoplasmic shuttling rate constant ratios presented in Figure 5.3b. The dependency on the shuttling ratio of β-catenin (K_1) is plotted for different parameter values of K_2, which captures the shuttling ratio of APC. The shape of all curves is similar to the ones obtained with the default parametrisation. In contrast to the default case presented in Figure 5.3a however, the curves for different K_2 are visibly shifted by different extent along the x-axis. This leads to a qualitatively different dependency of the output of the model on the shuttling of APC. The curves are shifted such that they intersect in one specific point $K_1' \approx 3 \cdot 10^{-2}$. For K_1 values smaller than K_1', the steady state concentration of [β-cat/TCF]* is higher, if the shuttling constant of APC is larger, but it cannot exceed the concentration obtained with the reference model. For small values of K_1, most of the β-catenin proteins are located in the nucleus. In this case, a higher concentration of [β-cat/TCF]* is obtained, if APC remains in the cytoplasm, because cytoplasmic APC and nuclear TCF cannot compete directly for β-catenin binding. However, for $K_1 > K_1'$ we see that the concentration of [β-cat/TCF]* is larger than the reference concentration of [β-cat/TCF]* (Figure 5.3b). Thus, nucleo-cytoplasmic shuttling of APC can increase the output of Wnt signalling in comparison to the reference model. This result can be explained as follows (see Figure 5.4): If APC is highly concentrated in the nucleus (K_2 is small, green curves), and at the same time the dissociation constant of nuclear APC to β-catenin ($K_4 = 1200$ nM) is large, only a small amount

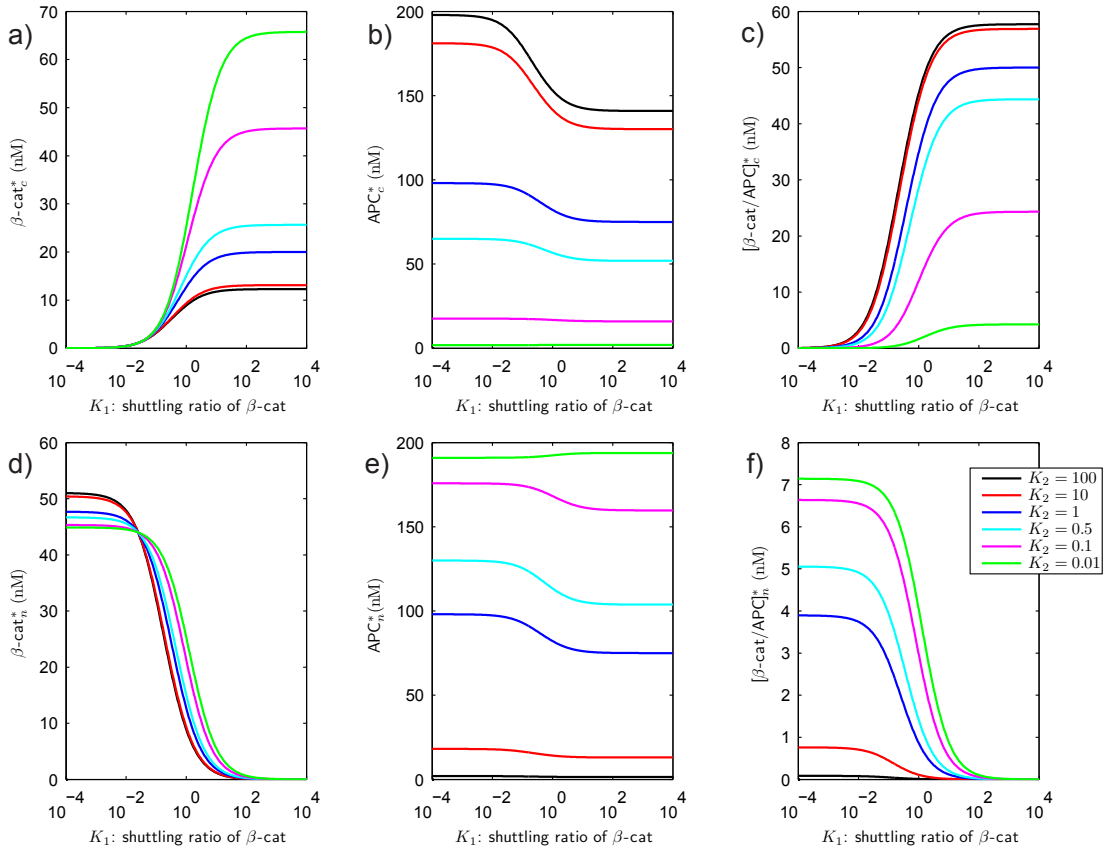

Figure 5.4.: Influence of diffusive shuttling of the protein and complex concentration of β-catenin and APC in the cytoplasm (top row) and nucleus (bottom row) for $K_3 = 30$ nM. The steady state concentrations are plotted in dependence on the ratios of the shuttling rate constants of β-catenin (K_1) and its antagonist APC (K_2), respectively.

of β-catenin can bind to APC (see Figure 5.4f). Thus, most of the APC proteins remain free and unbound in the nucleus (Figure 5.4e). This in turn means that the high binding affinity of cytoplasmic APC and β-catenin cannot be fully utilized due to a low cytoplasmic APC concentration (Figure 5.4b), hence the concentration of [β-cat/APC] in the cytoplasm is small (Figure 5.4c). As a result, more β-catenin remains unbound to APC (Figure 5.4a and 5.4d) and can hence bind to TCF (Figure 5.3b). Cytoplasmic retention of β-catenin is down-regulated due to APC nucleo-cytoplasmic shuttling. Therefore, in this parametrisation Wnt signalling benefits from antagonist shuttling.

In order to investigate the impact of nuclear retention, I now increase the affinity of β-catenin to APC in the nucleus, such that it is equal to the affinity of β-catenin to bind to TCF in the nucleus ($K_4 = K_5 = 30$ nM). The results of the steady state analysis based on these parameter values are shown in Figure 5.3c. The steady state

concentration of [β-cat/TCF]* is plotted with respect to changes in the parameter values K_1 and K_2. The black curve where $K_2 = 100$ also equals the reference case (Figure 5.2). The curves show that the output of the diffusive shuttling model can only be reduced with these parameter values, in contrast to the results presented in Figure 5.3b. This is due to an enhanced competition of APC and TCF for β-catenin binding in the nucleus.

Next, I choose all three dissociation constants to have the same value ($K_3 = K_4 = K_5 = 30$ nM). The steady state concentration [β-cat/TCF]* is shown in Figure 5.3d. We see a combination of the above discussed effects. For $K_1 < 1$ the effect of nuclear retention is dominant, as more β-catenin is located in the nucleus: Higher nuclear concentration of APC hence corresponds to higher competition of nuclear APC and TCF for β-catenin binding and therefore reduces the output. For $K_1 > 1$, the effect of cytoplasmic retention is prevalent; Cytoplasmic retention is down-regulated due to nuclear import of APC. Interestingly, the [β-cat/TCF]* concentration benefits from a decreased competition of cytoplasmic APC and TCF for β-catenin binding, although the binding affinity of β-catenin to APC in the nucleus is equally high. This means that [β-cat/TCF] signalling can even benefit from APC shuttling if β-catenin is retained by APC also in the nucleus. The intersection point in Figure 5.3d corresponds to the situation in which nuclear and cytoplasmic retention are equally powerful.

To sum up, diffusive nucleo-cytoplasmic shuttling of APC has a significant influence on the steady state concentration of [β-cat/TCF]*. My results demonstrate that the shuttling of the antagonist can yield an enhanced output of the system. I showed that this enhancement can be induced by a high binding affinity of APC and β-catenin in the cytoplasm, which can be caused by phosphorylation of APC [Rubinfeld et al., 1996]. Phosphorylation of APC can be induced by a high cytoplasmic β-catenin concentration [Seo and Jho, 2007]. The latter is supported by a high APC shuttling ratio leading to an accumulation of free APC in the nucleus. However, this effect is also visible if the nuclear [β-cat/APC] concentration is high due to enhanced binding affinity between the two proteins in the nucleus. Therefore the question arises, if [β-cat/TCF]* can further be maximised by saturated nuclear export and low binding affinity of β-catenin and APC in the nucleus, which is studied in the following section.

5.2.3. Facilitated export of APC

In contrast to the previous cases, the process of the nuclear export of APC across the NPC is now considered as facilitated transport [Xu and Massagué, 2004]. It is reasonable to assume that the concentration of APC is in excess of the NPC. Therefore, the Michaelis-Menten approach based on the quasi-steady state assumption can be applied [Millat et al., 2007] (see Section 3.3). Facilitated export of APC is modelled by a Michaelis-Menten type function (see Equations (3.4)). APC exhibits a NES [Neufeld et al., 2000, Rosin-Arbesfeld et al., 2000], and its export therefore depends on the CRM1 exportin pathway. It is the formation of the transportin-protein complex that restricts export rates and can lead to saturation [Timney et al., 2006]. This holds also true for the nuclear import of APC. However, we have seen in the previous section that especially nuclear accumulation of APC yields a maximisation of the output of the system. Therefore, and for the sake of simplicity, I restrict my analysis to the saturation of nuclear export. The nuclear import of APC remains diffusive.

$$\begin{aligned} F_1(X_1, X_3) &= k_1 X_1 - k_{-1} X_3 \\ F_2(X_2, X_4) &= k_2 X_2 - \frac{v_{max} X_4}{k_m + X_4} \end{aligned} \tag{5.22}$$

In case of facilitated export of APC, the search of the steady states leads to the following dependencies:

$$\begin{aligned} X_1^* &= \frac{k_{-1}}{k_1} X_3^* = K_1 X_3^* \\ X_2^* &= \frac{v_{max}\ X_4^*}{k_2(k_m + X_4^*)} \end{aligned} \tag{5.23}$$

Substituting X_1^* and X_2^* in Equations (5.12) and (5.13), we obtain two algebraic equations, which depend on X_3^* and X_4^*. Considering Equation (5.13) with its substitutes, we see the following equation in which X_3^* (i.e. nuclear β-catenin) is still depending on X_4^*:

$$X_3^* = \frac{2Ak_2k_m + C_3X_4^* - k_2X_4^{*2}}{X_4^*(C_4 + \frac{k_2}{K_4}X_4^*)} \tag{5.24}$$

where

$$C_3 = 2Ak_2 - k_2k_m - v_{max} \tag{5.25}$$

and

$$C_4 = K_1v_{max} + \frac{k_2k_m}{K_4}. \tag{5.26}$$

Substituting these equations into the conservation equation (5.12), we end up with a polynomial of 6th order with respect to X_4^*, which I omit to write down. However, we may obtain up to six different roots and therefore steady state solutions. I investigated parameter space by generating randomized parameter values for the shuttling parameters, i.e. $k_2, v_{max} \in [10^{-3}, 10^3]$ and $k_m \in [10^{-8}, 10^8]$. Again, for this parameter space only one biological sensible and meaningful solution exists.
For steady state analysis, I set $K_3 = K_5 = 30$ nM, $K_4 = 1200$ nM and $K_1 = 1$, as chosen in the previous sections. In case of facilitated export, two extreme cases can be considered: First, for $k_m >> X_4$, the nuclear export of APC changes linearly with the concentration of nuclear APC (X_4), which corresponds to diffusive shuttling. In this case, one obtains

$$K_{lin} = \frac{v_{max}}{k_mk_2} = K_2 \tag{5.27}$$

in steady state. Second, for $k_m << X_4$, the nuclear export is independent of X_4; it is therefore saturated. The nucleo-cytoplasmic shuttling of APC is in this case determined by

$$K_{sat} = \frac{v_{max}}{k_2}. \tag{5.28}$$

Note that K_{sat} is independent of k_m. In Figure 5.5, the dependency of [β-cat/TCF]* on the parameters describing the shuttling of APC is shown.

In the linear extreme case $k_m >> X_4$, the results depending on K_{lin} are equal to the results obtained assuming diffusive APC export (see left border of the scatter-plot presented in Figure 5.5, left panel), which meets our expectations (see Equation (5.27)). For decreasing k_m, more solutions are obtained on the high plateau, but the height of this plateau does not exceed the results we obtain with the diffusive model. For small half-saturation constants ($k_m < 1$ nM), the concentration of [β-cat/TCF]* is completely determined by K_{sat} (see Figure 5.5, right panel), as expected from Equation (5.28). In this case, export is saturated, hence more free APC is located in the nucleus and the corresponding steady state solution is located on the high plateau, which is in agreement with the results obtained for the diffusive model. However, the maximum value of [β-cat/TCF]$^* \approx 14$ nM cannot be exceeded

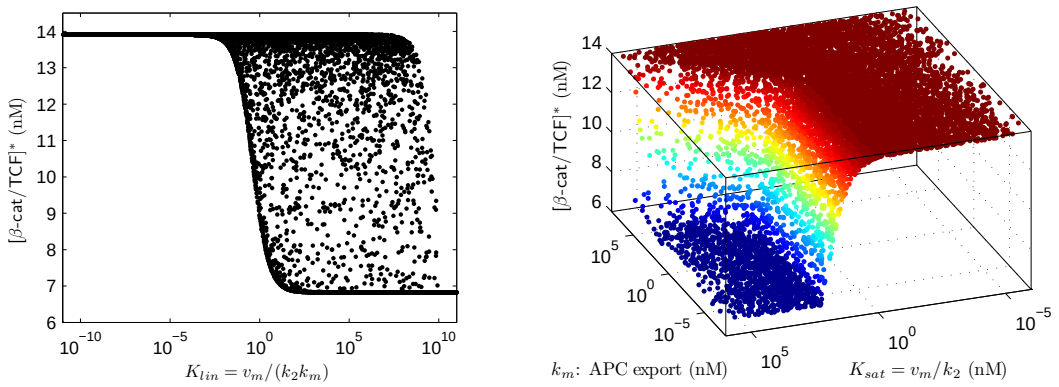

Figure 5.5.: Influence of facilitated nuclear export of APC on [β-cat/TCF]*, K_3 = 30 nM and $K_1 = 1$: The steady state concentration [β-cat/TCF]* is presented in dependence on the ratios of the shuttling rate constant of APC $K_{lin} = v_{max}/k_2 k_m$ (left panel) and $K_{sat} = v_m/k_2$ (nM) and k_m (right panel), respectively. Shuttling parameters are generated randomly. Every dot corresponds to the solution for a specific parametrisation.

due to saturated export. I therefore conclude that the concentration of [β-cat/TCF]* cannot be further maximised in response to facilitated and hence saturated export of APC.

I also considered facilitated import. This results in a more complicated system which is numerically more challenging, but did not yield any new insights.

The presented results thus show that it is possible to simulate the effects of saturation by using pure diffusion, if the shuttling rate constant ratio is chosen appropriately. A difference in the shuttling rate constants of significant orders of magnitude leads to an accumulation of the protein in the respective compartment, which corresponds to saturation in translocation.

5.3. Conclusions

Using compartmental modelling, I investigated the impact of nucleo-cytoplasmic shuttling of APC on the output of the Wnt signalling pathway to answer the following questions: Does nucleo-cytoplasmic shuttling of APC influence the [β-cat/TCF] concentration, which is considered as the output of the pathway? Can the concentration of [β-cat/TCF] benefit from APC shuttling? How is the output influenced by cytoplasmic and nuclear retention of β-catenin by APC?

To answer these questions, I established a simple biochemical reaction network based on experimentally obtained results, which describe the interactions and nucleo-

cytoplasmic shuttling of β-catenin, the main protagonist of the canonical Wnt signalling pathway, and its antagonist APC. The network was translated into a mathematical compartmental model based on ODEs. I assumed diffusive transport for nuclear translocation of β-catenin. To examine the influence of nucleo-cytoplasmic shuttling of APC on the output of Wnt signalling, I studied different shuttling mechanisms.

The analysis of the Retention Model led to the following results: The steady state concentration of [β-cat/TCF] can be enhanced in response to nucleo-cytoplasmic shuttling of APC. If the nuclear import rate constant of APC exceeds its export rate constant, the accumulation of APC in the nucleus can cause a breakdown of β-catenin retention by APC in the cytoplasm. As a consequence, free β-catenin accumulates in the cytoplasm and thus in the nucleus, which leads to a maximisation of the transcription factor complex [β-cat/TCF] in contrast to a reference model without APC shuttling. This effect is also visible if the nuclear concentration of [β-cat/APC] is high due to enhanced binding affinity between the two proteins in the nucleus. These findings thus show that Wnt signalling can benefit from nucleo-cytoplasmic shuttling of APC.

I propose the following mechanism to explain this finding: In the Wnt-on state, where β-catenin levels are high, β-catenin can cause phosphorylation of APC and phosphorylated APC in the cytoplasm may retain β-catenin [Seo and Jho, 2007]. From their results, Seo and Jho [2007] concluded that this is to regulate excessive [β-cat/TCF] signalling. My results, however, allow for an alternative interpretation as I additionally considered nucleo-cytoplasmic shuttling of APC: a high APC shuttling ratio can lead to a breakdown of β-catenin retention in the cytoplasm, therefore enhance nuclear accumulation of β-catenin and hence [β-cat/TCF] signalling. The breakdown of cytoplasmic retention by APC can even lead to an enhanced [β-cat/TCF] concentration, if phosphorylated APC retains β-catenin in the nucleus. This can be explained by a sufficiently high concentration of nuclear β-catenin available to bind TCF. The inhibitory effect of APC is alleviated due to shuttling of APC. In other words, this study indicates that the nucleo-cytoplasmic shuttling of APC has a beneficial effect on the steady state output of the pathway, although APC is an antagonising protein. Nucleo-cytoplasmic shuttling of antagonists may also play a relevant role in other pathways and the advantage it brings may even be a general property in signalling.

Next, I investigated facilitated nuclear export of APC, which is based on a Michaelis-Menten type rate law. I showed that maximal output of the [β-cat/TCF] concen-

tration in steady state, which was obtained by considering diffusive shuttling of β-catenin and APC, cannot be further increased by facilitated antagonist export. My results demonstrate that it is possible to simulate the effects of saturated translocation by pure diffusion. A difference in the shuttling rate constants of significant orders of magnitude leads to an accumulation of the protein in the respective compartment. Based on this result, further modelling approaches of nucleo-cytoplasmic shuttling, including the one presented in the following chapter, can be simplified.

6. The Degradation Model

In this chapter I investigate the influence of nucleo-cytoplasmic shuttling of **three** of the main β-catenin antagonists – the two scaffolding proteins **APC** and **Axin** as well as the kinase **GSK3** – on the output of Wnt/β-catenin signalling. For this purpose, I establish a compartmental model, which is an extension of the Retention model presented in Chapter 5. A detailed comparison of the two models is given in the Chapter 7. One major difference between the two models is that in the Retention Model only one β-catenin antagonist (namely APC) is considered. In this model, the compartmental concentration of β-catenin was mainly regulated through retention by APC. In contrast, I now regard the so-called destruction complex, consisting of three β-catenin antagonists APC, Axin and GSK3. In the following the destruction complex is referred to as AAG. As in this model β-catenin can not only be retained but also degraded in both compartments, cytoplasm and nucleus, I refer to it as the **"Degradation Model"**. Degradation is regarded as the principal regulatory mechanism that controls the activity and hence nuclear accumulation of β-catenin [Aberle et al., 1997]. The second major difference is that with this model we can investigate the impact of different Wnt signals on the output of the system, whereas the previously studied model was a closed system without any external input or signal. In this chapter, I focus on the following questions: Does the nucleo-cytoplasmic shuttling of β-catenin antagonists APC, Axin and GSK3 influence the [β-catenin/TCF] concentration, which we consider as the output of Wnt signalling? Can the [β-catenin/TCF] concentration be maximised by antagonist shuttling, leading to an optimised signal transduction through the cell? How is the output of the pathway influenced by phosphorylation and subsequent degradation of β-catenin?
The construction of the Degradation Model is presented in Section 6.1. Similar to the Retention model, the model structure is based on experimental findings which are presented in Chapter 2. In Section 6.2, I investigate the influence of nucleo-cytoplasmic antagonist shuttling and β-catenin degradation in the nucleus on the [β-catenin/TCF] concentration. To this end, we (i) consider different Wnt on/off scenarios, (ii) investigate the impact of pathway inhibition and (iii) complete this study

with a sensitivity analysis in order to quantify the robustness of the [β-catenin/TCF] concentration against perturbations. A conclusion is given in Section 6.3. The content of this chapter is adapted from [Schmitz et al., 2013] and reproduced with permission from Elsevier.

6.1. Construction of the Degradation Model

Figure 6.1 shows the reaction scheme of the biochemical network we study. The two main components of the model are β-catenin, the main protagonist of the canonical Wnt signalling pathway, and its antagonizing complex AAG. The protein β-catenin and the complex AAG, distribute in both compartments, the cytoplasm and the nucleus.
Reaction rates v_1 and v_2 describe translocation processes across the nuclear envelope. β-catenin can interact directly with the nuclear pore complexes and can also translocate on its own [Fagotto et al., 1998, Henderson and Fagotto, 2002, Wiechens and Fagotto, 2001, Yokoya et al., 1999]. Note that the complex AAG as such is not necessarily able to shuttle between cytoplasm and nucleus. However, its members within their dissociated state are capable of nucleo-cytoplasmic shuttling [Bijur and Jope, 2003, Caspi et al., 2008, Cong and Varmus, 2004, Franca-Koh et al., 2002, Henderson, 2000, Krieghoff et al., 2006, Neufeld et al., 2000, Rosin-Arbesfeld et al., 2000, Wiechens et al., 2004, Willert and Jones, 2006]. Therefore and for the sake of simplicity, reaction rate v_2 is summarising several steps: dissociation of the complex into its parts (Axin, APC and GSK3), the translocation of the proteins Axin, APC and GSK3, association of the proteins back into the destruction complex AAG. The binding and dissociation steps are modelled in detail in the Lee model [Lee et al., 2003]. Here, we neglect the dissociation and binding reactions of the destruction complex and focus on the shuttling. However, in order to account for the multiple reactions modelled by one step within the model, we constrain the reaction rate v_2 in such a way that it is in both directions slow compared to v_1 (we checked differences of the order magnitude of 10 to 1000). The reaction rates v_3, v_4 and v_5 describe binding and dissociation of β-catenin with/from AAG [Hart et al., 1998, Kishida et al., 1998, Yost et al., 1996] in the cytoplasm and nucleus, and TCF, respectively. β-catenin's interaction with TCF can initiate transcription and can therefore activate target gene expression [Behrens et al., 1996, Mosimann et al., 2009]. The complex [β-catenin/TCF] is hence considered as the output of both, the Wnt pathway and the Degradation Model. The arrows v_6 and v_7 describe the rates

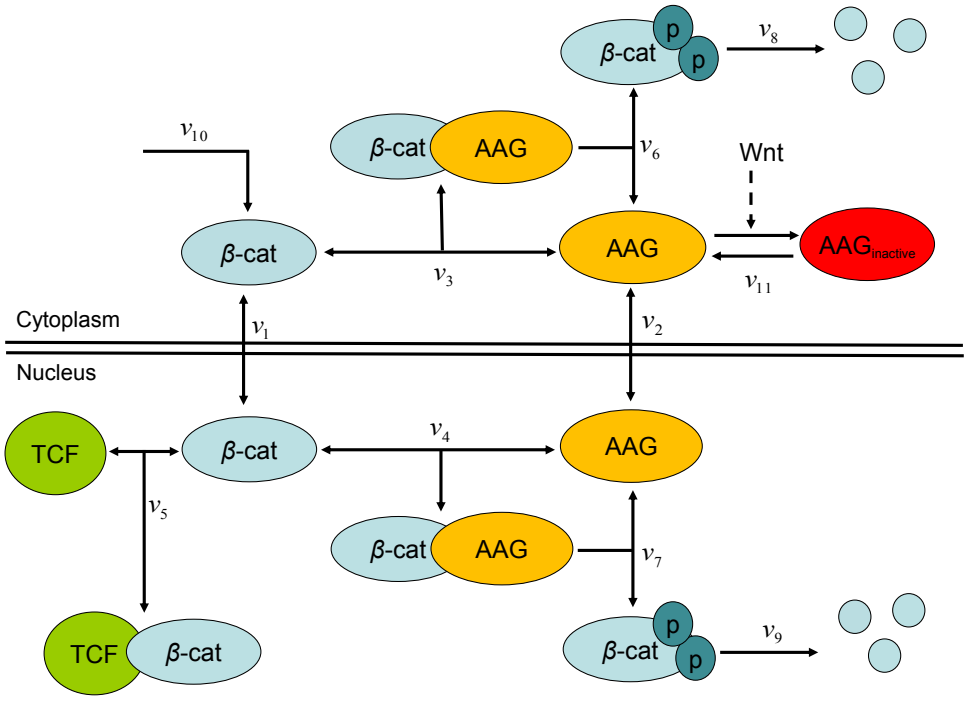

Figure 6.1.: Reaction scheme of the biochemical network. β-catenin and its antagonizing destruction complex AAG are present in nucleus and cytoplasm. The involved proteins are capable of nucleo-cytoplasmic shuttling. β-catenin can associate with AAG in the cytoplasm and the nucleus, can then be phosphorylated by AAG, and subsequently degraded by the proteasome. An incoming Wnt signal inhibits the destruction complex in the cytoplasm. β-catenin is synthesised at constant rate. Complex formation with TCF activates target genes and is considered as the output of the pathway. The reaction rates are numbered $1-11$ and in Table 6.1 the proteins and complexes are translated into state variables (X_1 to X_{11}).

of two combined reactions: phosphorylation of β-catenin by the destruction complex and the irreversible release of phosphorylated β-catenin from the destruction complex (in cytoplasm and nucleus). Reactions v_8 and v_9 account for proteasomal degradation of β-catenin [Aberle et al., 1997]. Synthesis of β-catenin is denoted as v_{10} [Lee et al., 2003]. The arrows denoted with v_{11} represent the influence of Wnt proteins: presence of a Wnt signal inactivates the destruction complex reversibly (see [Lee et al., 2003] for a more detailed model). Therefore, reaction v_{11} combines the inactivation with the counter reaction, i.e. the activation of AAG.

The biochemical reaction network depicted in Figure 6.1 is translated into a system of coupled ordinary differential equations for further analysis. Each protein/protein complex concentration is represented by a variable in the mathematical model (see Table 6.1). Taking all described interactions into account, the dynamics of the

biological system, i.e. the time-dependent changes of the concentrations of these proteins and protein complexes, are determined by 11 coupled ODEs:

$$\begin{aligned}
\dot{X}_1 &= -\nu_1 - \nu_3 + \nu_{10} & \dot{X}_7 &= -\nu_5 \\
\dot{X}_2 &= -\nu_2 - \nu_3 + \nu_6 - \nu_{11} & \dot{X}_8 &= +\nu_5 \\
\dot{X}_3 &= +\nu_1 - \nu_4 - \nu_5 & \dot{X}_9 &= +\nu_6 - \nu_8 \\
\dot{X}_4 &= +\nu_2 - \nu_4 + \nu_7 & \dot{X}_{10} &= +\nu_7 - \nu_9 \\
\dot{X}_5 &= +\nu_3 - \nu_6 & \dot{X}_{11} &= +\nu_{11} \\
\dot{X}_6 &= +\nu_4 - \nu_7 & &
\end{aligned} \tag{6.1}$$

where the reaction rates ν_i on the right-hand side are functions of the protein or complex concentrations, each one of them describing biochemical reactions or transport processes:

$$\begin{aligned}
\nu_1 &= k_1 X_1 - k_{-1} X_3 & \nu_7 &= k_7 X_6 \\
\nu_2 &= k_2 X_2 - k_{-2} X_4 & \nu_8 &= k_8 X_9 \\
\nu_3 &= k_3 X_1 X_2 - k_{-3} X_5 & \nu_9 &= k_9 X_{10} \\
\nu_4 &= k_4 X_3 X_4 - k_{-4} X_6 & \nu_{10} &= k_{10} = const. \\
\nu_5 &= k_5 X_3 X_7 - k_{-5} X_8 & \nu_{11} &= k_{11} W X_2 - k_{-11} X_{11} \\
\nu_6 &= k_6 X_5 & &
\end{aligned} \tag{6.2}$$

We assume all transport processes between the two compartments, nucleus and cytoplasm, to be purely diffusive. In Section 5.2.3, I have shown that it is possible to model facilitated translocation of APC across the nuclear envelope as diffusion-like motion. The effects of saturation can be simulated by using pure diffusion, if the shuttling rate constants are chosen appropriately [Schmitz et al., 2011]. The index of the shuttling constant is positive for transport into the nucleus and negative for the transport back to the cytoplasm.

Binding and dissociation processes are described in the usual manner using mass-action kinetics (see Section 3.3). The index of the respective constant is positive for protein association and negative for protein dissociation. The degradation of phosphorylated β-catenin is modelled linearly and β-catenin is synthesised by a constant rate. The Wnt signal can either be permanent or transient and is discussed in detail in the Section 6.2.

Dissociation constants, reaction rate constants and total concentrations are set fol-

State variable	Original variable	Interpretation
X_1	β-cat$_c$	free cytoplasmic β-catenin
X_2	AAG$_c$	free cytoplasmic destruction complex
X_3	β-cat$_n$	free nuclear β-catenin
X_4	AAG$_n$	free nuclear destruction complex
X_5	[β-cat/AAG]$_c$	cytoplasmic complex [β-catenin/AAG]
X_6	[β-cat/AAG]$_n$	nuclear complex [β-catenin/AAG]
X_7	TCF	free nuclear transcription molecules
X_8	[β-cat/TCF]	transcription complex [β-catenin/TCF]
X_9	β-cat$_c^p$	cytoplasmic phosphorylated β-catenin
X_{10}	β-cat$_n^p$	nuclear phosphorylated β-catenin
X_{11}	AAG$_{inactive}$	inactive destruction complex

Table 6.1.: Definition of the state variables of the Degradation Model. Squared brackets denote protein complexes. The indices n and c denote nuclear and cytoplasmic protein concentrations, respectively, whereas p denotes a phosphorylated protein.

lowing the results of measurements and estimations conducted by the Kirschner group in the Wnt-off state in *Xenopus* oocytes extracts [Lee et al., 2003], see Table 6.2. Recently, concentrations of Wnt pathway components have also been measured for mammalian cells [Tan et al., 2012]. These values have been used to recalibrate the Lee Model, see Section 4.6 for details. However, only initial concentrations have been measured. To incorporate these new findings in this model, one would also need reaction rate constants or dissociation constants. Therefore, I use the parameter values estimated and measured by Lee et al. [2003]. For the shuttling rate constants, I do not use absolute values, but only shuttling rate constant ratios $K_1 = k_{-1}/k_1$ and $K_2 = k_{-2}/k_2$, respectively. The shuttling rate constants are however constrained in such a way that AAG shuttling is in both directions slow compared to β-catenin shuttling, as discussed above. The parameter values for the transient Wnt signal are discussed in Section 6.2.5.

The stoichiometry of the reaction scheme in Figure 6.1 implies the existence of two conservation equations, which correspond to conserved numbers of proteins for the destruction complex AAG and the transcription factor TCF. If more than one compartment is considered, like in this case, conservation sums can only be applied to molecule numbers, not to concentrations (see Section 5.1.1). Assuming that compartment volumes and capacities are of the same size, the following expressions exhibiting the double total concentrations A and T are obtained:

$$\text{total AAG:} \qquad 2A = X_2 + X_4 + X_5 + X_6 + X_{11} \tag{6.3}$$

$$\text{total TCF:} \qquad 2T = X_7 + X_8. \tag{6.4}$$

In case of AAG, the total number of molecules is restricted by the limiting factor, i.e. the amount of available Axin. It therefore equals $A = 0.02$ nM, whereas total TCF equals $T = 15$ nM [Lee et al., 2003].
The total concentration of β-catenin is not conserved. It is denoted by B and composed of the following:

$$\text{total } \beta\text{-catenin:} \qquad 2B = X_1 + X_3 + X_5 + X_6 + X_8. \tag{6.5}$$

According to the Lee model, total β-catenin equals $B = 35$ nM in the Wnt-off state. This number serves as the initial value for the simulation experiments. In the Wnt-on state the concentration of active β-catenin can yield much higher values.
For the reaction rate equations v_3,v_4 and v_5, the quasi equilibrium is approached rapidly, as shown by Lee et al. [2003]. This assumption leads to the dissociation constants K_3, K_4 and K_5, respectively. The model formulated above is investigated in detail in the following section.

6.2. Results and discussion

Using the model developed in the previous section, I next investigate the impact of nucleo-cytoplasmic antagonist shuttling and β-catenin degradation in the nucleus on the [β-catenin/TCF] concentration. In a first step, I assume that the destruction complex is only located in the cytoplasm. This system, which is referred to as the reference model, forms the basis of this analysis. I then allow for nucleo-cytoplasmic shuttling of the destruction complex and study its effect on the output of the pathway. For this, I examine different scenarios in the Wnt-off state in order to provide a comprehensive study of the model. I start by investigating the influence of β-catenin destruction in the nucleus. In a second step, I study the impact of total inhibition of the destruction complex by conducting knock-out experiments of all involved reactions. This is done by setting the according reaction rate constants to zero. In the second part of the analysis, the Wnt pathway is active. I first assume a permanent Wnt signal, and then consider transient Wnt signalling. I complete the results section by performing a sensitivity analysis to systematically investigate the influence of parameter perturbation on the [β-catenin/TCF] concentration.

Parameter name	Default value	Description
K_3	120 nM	Binding of β-catenin to AAG (cytoplasm)
K_4	120 nM	Binding of β-catenin to AAG (nucleus)
K_5	30 nM	Binding of β-catenin to TCF (nucleus)
k_6	200 min^{-1}	Dissociation of [β-cat/AAG] into AAG and phosphorylated β-catenin (cytoplasm)
k_7	200 min^{-1}	Dissociation of [β-cat/AAG] into AAG and phosphorylated β-catenin (nucleus)
k_8	0.4 min^{-1}	Degradation of phosphorylated β-catenin (cytoplasm)
k_9	0.4 min^{-1}	Degradation of phosphorylated β-catenin (nucleus)
k_{10}	0.4 nM min^{-1}	Synthesis (rate) of β-catenin
k_{11}	0.4 min^{-1}	Inactivation of the AAG
k_{12}	0.2 min^{-1}	Activation of AAG
A	0.02 nM	Total concentration of AAG (where Axin is limiting)
T	15 nM	Total concentration of TCF
B	35 nM	Total initial concentration of β-catenin
λ	0.005 min^{-1}	Reciprocal of the characteristic lifetime τ_{Wnt} of receptor stimulation
W_0	10	Maximal stimulation (dimensionless)

Table 6.2.: The default parametrisation of the reference model based on Lee et al. [2003]. Numeric values and description of the parameters of the reference model in its default state. The parameters $K_i = k_{-i}/k_i$ are dissociation constants. A, B, T illustrate total protein concentrations. k_j correspond to rate constants of the remaining involved reactions. λ and W_0 describe the shape of the transient Wnt signal.

6.2.1. The reference model: Wnt-off and the antagonists are only located in the cytoplasm

In Figure 6.2, the steady state concentration of [β-catenin/TCF] is presented in dependence on the ratio of rate constants for β-catenin nucleo-cytoplasmic shuttling ($K_1 = \frac{k_{-1}}{k_1}$). For the reference model the shuttling rate constants of the destruction complex are set to zero. I also set $W = 0$ and fix all other parameters according to Table 6.2. The steady state dynamics of the reference model are illustrated as the red curve in Figure 6.2. The steady states are obtained as follows: initially, the β-catenin and its antagonists are located in the cytoplasm. During the simulation, β-catenin will distribute in both, nucleus and cytoplasm, depending on the shuttling rate constants k_1 and k_{-1}, while the antagonists will remain in the cytoplasm. The system of ODEs (Equations (6.1)) is numerically integrated until the steady state solution is reached for the Wnt-off state. This procedure is repeated for different shuttling rate ratios K_1 of β-catenin. Small values of K_1 yield a higher concentration of [β-catenin/TCF], because more β-catenin is located in the nucleus. Hence, the output of the pathway is maximised, leading to an optimised transcriptional response of the cell. The height of the plateau with a maximal [β-catenin/TCF] concentration of approximately 30nM is essentially due to the value of the corresponding dissociation contant $K_5 = 30$nM.
For $K_1 = 1$, the [β-catenin/TCF] concentration equals approximately 11 nM. In the Wnt-off state of the Lee Model [Lee et al., 2003, Page 121], the value of [β-catenin/TCF] equals 6.83nM. Comparing the two numbers, we see that they differ by a factor of almost two. This is due to the fact that concentrations are regarded, which are naturally related to the size (volume) of the respective compartment. In this model, two compartments of equal size are considered, whereas the Lee Model only consists of one compartment of the same total volume. Therefore, the steady state concentration of [β-catenin/TCF] in the Wnt-off state of the two models should differ by a factor of two and are therefore in good agreement. Next, I consider nucleo-cytoplasmic shuttling of AAG and also include phosporylation and degradation of β-catenin in the nucleus.

6.2.2. Wnt-off and destruction complex formation in the nucleus

In this section, I investigate the effects of nucleo-cytoplasmic antagonist shuttling and destruction of phosphorylated β-catenin in the nucleus on the steady state concentration of [β-catenin/TCF] in the Wnt-off state. For this, I consider different

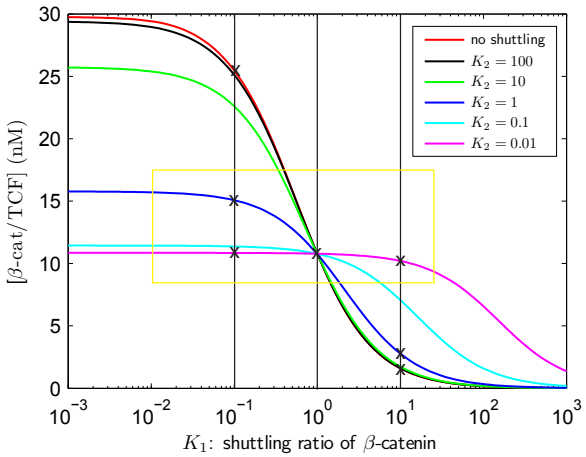

Figure 6.2.: Steady state levels of the [β-catenin/TCF] concentration for different shuttling rate constants ratios of β-catenin (K_1, see x-axis) and the destruction complex (K_2, see legend) in the Wnt-off state. The tags x specify the values for the shuttlings ratios we focus on for the sensitivity analysis. For further information on the tagged positions as well as the yellow box please refer to Section 6.2.6.

ratios of shuttling rate constants of AAG ($K_2 = \frac{k_{-2}}{k_2}$ from 100 to 0.01). Again, the steady state values are obtained using simulation. The parameters are set accordingly (Table 6.2). The results are also depicted in Figure 6.2.

In general, the shapes of the curves are similar. However, the curves are shifted by extent along the x-axis and also compressed along the y-axis such that they intersect in one specific point $K_1' = 1$. For $K_1 < K_1'$, the steady state concentration of [β-catenin/TCF] is smaller, if the shuttling rate constant ratio of AAG is smaller and hence more AAG is located in the nucleus. The maximum value of the reference case 'no shuttling' (red curve) cannot be exceeded. For small values of K_1, most β-catenin is located in the nucleus. In this case, a higher concentration of [β-catenin/TCF] is obtained if the destruction complex remains in the cytoplasm, because cytoplasmic AAG and nuclear TCF cannot compete for β-catenin binding directly.

For $K_1 > K_1'$, however, more β-catenin is located in the cytoplasm than in the nucleus. In this case we see that the concentration of [β-catenin/TCF] can be increased due to nucleo-cytoplasmic shuttling of β-catenin antagonists in comparison to the previously discussed reference model. For decreasing values of K_2, which corresponds to an increasing nuclear concentration of AAG, the concentration of [β-catenin/TCF] increases significantly, compared to the reference model without AAG shuttling. Therefore, nucleo-cytoplasmic shuttling of β-catenin antagonists

can increase the output of Wnt signalling.
This increase can be explained as follows: For high values of K_1, most β-catenin is located in the cytoplasm, whereas for small values of K_2, most AAG is located in the nucleus. This separation into two different compartments prevents β-catenin from interacting with the destruction complex. Hence, most β-catenin remains free and unbound in the cytoplasm, whereas the cytoplasmic concentration of [β-catenin/AAG] is minimal. Thus, β-catenin cannot be degraded and therefore cytoplasmic β-catenin levels increase. This in turn means that more β-catenin can translocate into the nucleus, which leads to an increase of the [β-catenin/TCF] concentration.
To sum up, I conclude that on the left side ($K_1 < K_1'$) of Figure 6.2 the phosphorylation and subsequent destruction of β-catenin in the nucleus is dominantly influencing the maximal levels of the curves. Thus, the destruction of nuclear β-catenin by AAG is reducing the [β-catenin/TCF] levels. On the right side of Figure 6.2, however, the effects of the processes that occur in the cytoplasm are dominant. The breakdown of degradation of cytoplasmic β-catenin leads to higher levels of total β-catenin and hence to maximised [β-catenin/TCF] concentration. In both cases, the separation of β-catenin and AAG in two different compartments leads to an increase of the steady state concentration of [β-catenin/TCF]. Counterintuitively, these results provide therefore evidence that nucleo-cytoplamic shuttling of the members of the destruction complex can yield a maximisation of [β-catenin/TCF] signalling. In spite of being β-catenin antagonists and thus actively reducing β-catenin levels, they are still capable of positively influencing the output of the pathway. To provide a comprehensive analysis of the model, we next investigate the maximal impact of the activity of the destruction complex in the cytoplasm and the nucleus, by independently inhibiting the involved reactions.

6.2.3. Wnt-off and the impact of total inhibition

In order to investigate the maximal influence of the degradation of β-catenin in the cytoplasm and the nucleus on [β-catenin/TCF] signalling, we independently inhibit, i.e. knock-out, all reactions related to the activity of the destruction complex. From cancer drug development it is known that possible targets for inhibitors include β-catenin protein-protein interactions and the involved kinases [Polakis, 2012]. We here, however, focus on pathway output maximisation, rather than Wnt inhibition,

since this yields an optimised transcriptional activity of the cell. The key question remains whether [β-catenin/TCF] concentration can be maximised by antagonist shuttling. I consider the Wnt-off state, the results are however similar for the Wnt-on state ($W = 1$, data not shown). I focus on the reactions concerning the activity of the destruction complex (see Figure 6.1), namely the binding of β-catenin to the destruction complex in the cytoplasm (ν_3) and in the nucleus (ν_4), as well as the activity of GSK3, which causes the phosphorylation of β-catenin and release of phosphorylated β-catenin from the destruction complex in the cytoplasm (ν_6) and the nucleus (ν_7). All interactions are inhibited independently of each other in order to investigate the maximal influence of intervention. Inhibiting the kinase in both compartments, cytoplasm and nucleus, at once, would lead to a model structure we have analysed before (the Retention Model, see Chapter 5). Inhibiting the binding processes of AAG and β-catenin in both compartments at once, leads to a model structure where β-catenin is not interacting with any molecule, except TCF, and is hence trivial.

The effects of the **inhibition** of β-catenin–AAG binding and of the activity of the kinase GSK3 on the steady state concentration of [β-catenin/TCF] in the **cytoplasm** are depicted in the top row of Figure 6.3. In the left panel, the binding of β-catenin to the destruction complex is inhibited in the cytoplasm. Compared to the reference model discussed in Section 6.2.1, see Figure 6.2, the curves are shifted along the x-axis to a different extent, such that higher values of [β-catenin/TCF] are obtained for shuttling ratios of $K_1 > 1$, where more β-catenin is located in the cytoplasm. Therefore, the inhibition of the destruction complex in the cytoplasm leads to an increase of the [β-catenin/TCF] concentration, because more free β-catenin is available to bind TCF. This increase is particularly significant for higher shuttling ratios of K_2, causing more AAG to be located in the cytoplasm (black and red curves). The more AAG is located in the nucleus, the higher the effect of phosphorylation and hence inactivation of β-catenin by the destruction complex. Accordingly, the levels of [β-catenin/TCF] are decreasing for decreasing AAG shuttling constant ratio K_2, compared to the 'no shuttling' (red) curve with inhibited cytoplasmic binding.

In the right panel of the top row, the kinase activity leading to the degradation of cytoplasmic β-catenin by the destruction complex is inhibited. Hence, AAG is still able to bind and retain β-catenin, but not to further process, i.e. phosphorylate and degrade, it. Therefore, the complex can only release β-catenin in its active state. Interestingly, the output concentration of [β-catenin/TCF] is even more increased

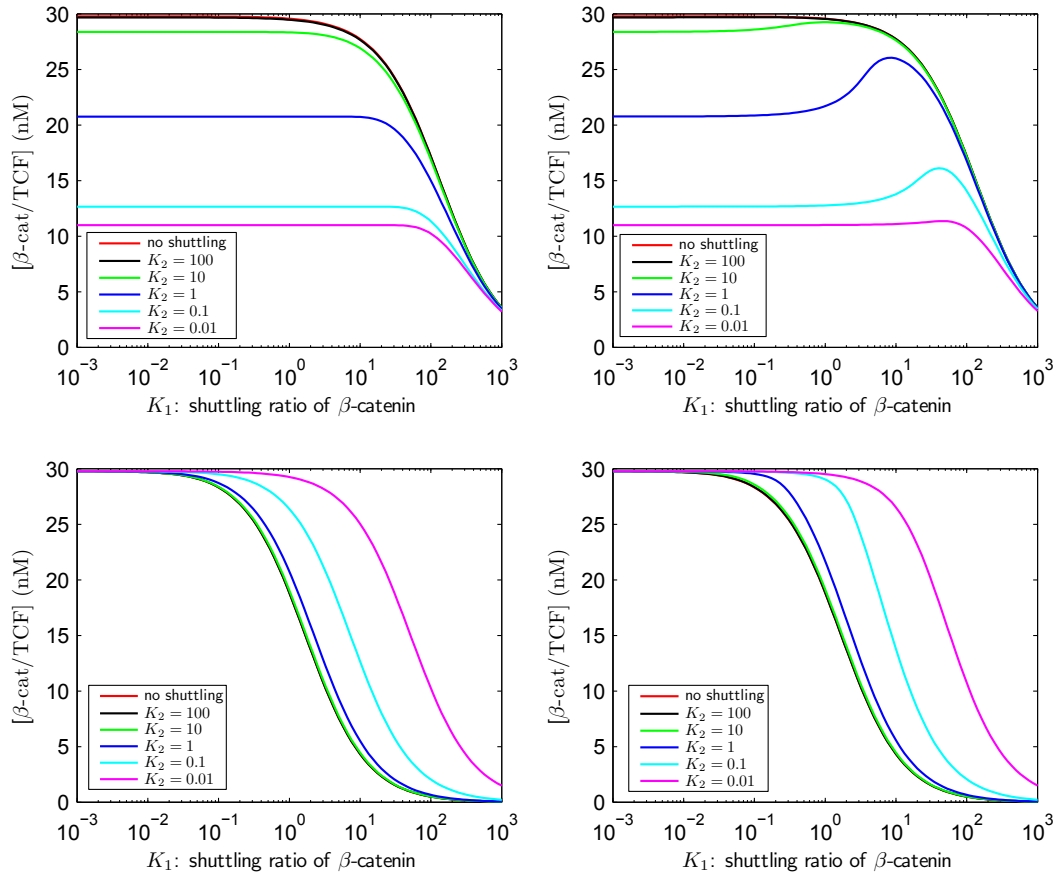

Figure 6.3.: Steady state levels of the [β-catenin/TCF] concentration for different inhibition scenarios and different shuttling rate constants ratios of β-catenin (x-axis) and the destruction complex (see legend). Top row, left panel: Binding of β-catenin to the destruction complex is inhibited in the cytoplasm ($\nu_3 = 0$). Top row, right panel: Phosphorylation and degradation of β-catenin by the destruction complex is inhibited in the cytoplasm ($\nu_6 = 0$). Bottom row, left panel: Binding of β-catenin to the destruction complex is inhibited in the nucleus ($\nu_4 = 0$). Bottom row, right panel: Phosphorylation and degradation of β-catenin by the destruction complex is inhibited in nucleus ($\nu_7 = 0$).

due to nucleo-cytoplasmic shuttling of AAG in comparison to the previously discussed case where complex formation of AAG and β-catenin was inhibited (top row, left panel), if $K_2 = 1$ (blue curve). The effect is also visible for $K_2 = 0.1$ to 10. The mechanism that leads to this increase is based on a combination of β-catenin retention and degradation: if in this parameterisation, AAG is only or mainly located in the cytoplasm (red and black curves), and β-catenin is located in the nucleus, β-catenin levels are high, because of the compartmental separation of β-catenin and AAG. If most AAG is in the nucleus (magenta curve), the competition of AAG and

TCF for β-catenin binding reduces the [β-catenin/TCF] concentration. If, however, AAG is distributed in both, cytoplasm and nucleus (green and blue curves), cytoplasmic AAG is retaining β-catenin (and vice versa) in the cytoplasm and thereby prevents it from being degraded by the destruction complex in the nucleus. Concurrently, the concentration of the destruction complex in the nucleus is reduced and hence the output concentration of [β-catenin/TCF] is enhanced. Therefore, the inhibition of the cytoplasmic kinase activity is leading to β-catenin degradation in the nucleus, and β-catenin/AAG retention in the cytoplasm, and thereby yields an increased [β-catenin/TCF] concentration.

Next, we independently **inhibit** the binding of β-catenin to the destruction complex and the activity of the kinase GSK3 in the **nucleus**, respectively. The effects of these inhibitions on the steady state concentration of [β-catenin/TCF] are presented in the bottom row of Figure 6.3. In the left panel, the binding of β-catenin to the destruction complex is inhibited in the nucleus. In this case, the curves are again shifted, but not only in direction of the x-axis; for small values of K_2 the curves are also visibly shifted along the y-axis, such that a much higher concentration for [β-catenin/TCF] is obtained due to nucleo-cytoplasmic shuttling of AAG in comparison to the reference model (see Figure 6.2). The mechanism that leads to this increase is closely related to the one we have previously discussed in Section 6.2.2: For high values of K_1 and small values of K_2, β-catenin and AAG are separated into two different compartments, which prevents β-catenin from interacting with the destruction complex. Hence, cytoplasmic β-catenin levels increase and more β-catenin translocates into the nucleus. This leads to a [β-catenin/TCF] concentration, which is enhanced in comparison to the model with active destruction complex. In this case, TCF is not competing with AAG for β-catenin binding, due to the inhibited binding ability of the destruction complex.

In the right panel of the bottom row of Figure 6.3, the activity of the kinase GSK3, and hence degradation of β-catenin by the destruction complex, is inhibited in the nucleus. However, AAG is still able to bind and thus retain β-catenin. Surprisingly, both types of inhibitions lead to almost identical steady state concentrations of [β-catenin/TCF]. This is due to the very small concentration of AAG (0.02 nM in total, limited by the Axin concentration [Lee et al., 2003], see Table 6.2) in comparison to the concentration of β-catenin. Therefore, the fraction of retained β-catenin is too small to make a visible difference. In summary, we show that inhibition of the destruction complex is enhancing the [β-catenin/TCF] concentration and hence the output of the pathway. Unexpectedly, given the premise that APC, Axin and

GSK3 are able to translocate into the nucleus, these results strongly suggest that the [β-catenin/TCF] concentration is more effectively enhanced by inhibiting the activity of the kinase GSK3 rather than the binding of β-catenin to the destruction complex. There are many GSK3 inhibitors known and available, with lithium being the best-characterized, although fairly specific for GSK3 [Doble and Woodgett, 2003]. However, several new inhibitors have recently been developed with different mode of actions and therapeutical potential [Cohen and Goedert, 2004, Doble and Woodgett, 2003].
So far, I focussed on the Wnt-off state of the pathway. In the following sections, I consider permanent and transient Wnt signals and investigate their impact on the [β-catenin/TCF] concentration especially with respect to changes in β-catenin and AAG nucleo-cytoplasmic shuttling.

6.2.4. The influence of a permanent Wnt signal

In previous sections 6.2.1 to 6.2.3, I investigated the impact of nucleo-cytoplasmic antagonist shuttling on the [β-catenin/TCF] concentration in the Wnt-off state. In the present section, I examine the influence of a permanent Wnt signal on the [β-catenin/TCF] concentration, in order to provide a comprehensive analysis of the Degradation Model. By applying permanent Wnt signals of different strengths, I ascertain the maximal influence of these Wnt signals on the output of the pathway by determining the according new steady state solutions. Wnt signalling yields an inactivation of the destruction complex and is therefore critical for the concentration of the key protein β-catenin in the cell (see Figure 2.2). As shown in Figure 6.1, I assume that the Wnt signal ($W > 0$) can only inhibit the destruction complex in the cytoplasm, not in the nucleus, as Wnt binds the receptors in the cell membrane. In the presence of Wnt ligands, the destruction complex is recruited to the Wnt-receptor complex and subsequently inactivated (see Section 2.2). I assume that the spatial distance from the membrane to the inner nucleus prevents the destruction complex from being inactivated while it is located in the nucleus. Steady state levels of the [β-catenin/TCF] concentration for permanent Wnt stimuli of increasing strength are presented in Figure 6.4 (top left: $W = 1$, top right: $W = 10$, bottom left: $W = 100$, bottom right: $W = 1000$). We see that the influence of a growing permanent Wnt signal is visible in the shift of the curves along the x- and y-axes and hence the intersection point of the curves: For $W = 1$ (top left panel of Figure 6.4, the intersection point at K_1' is shifted towards a higher shutt-

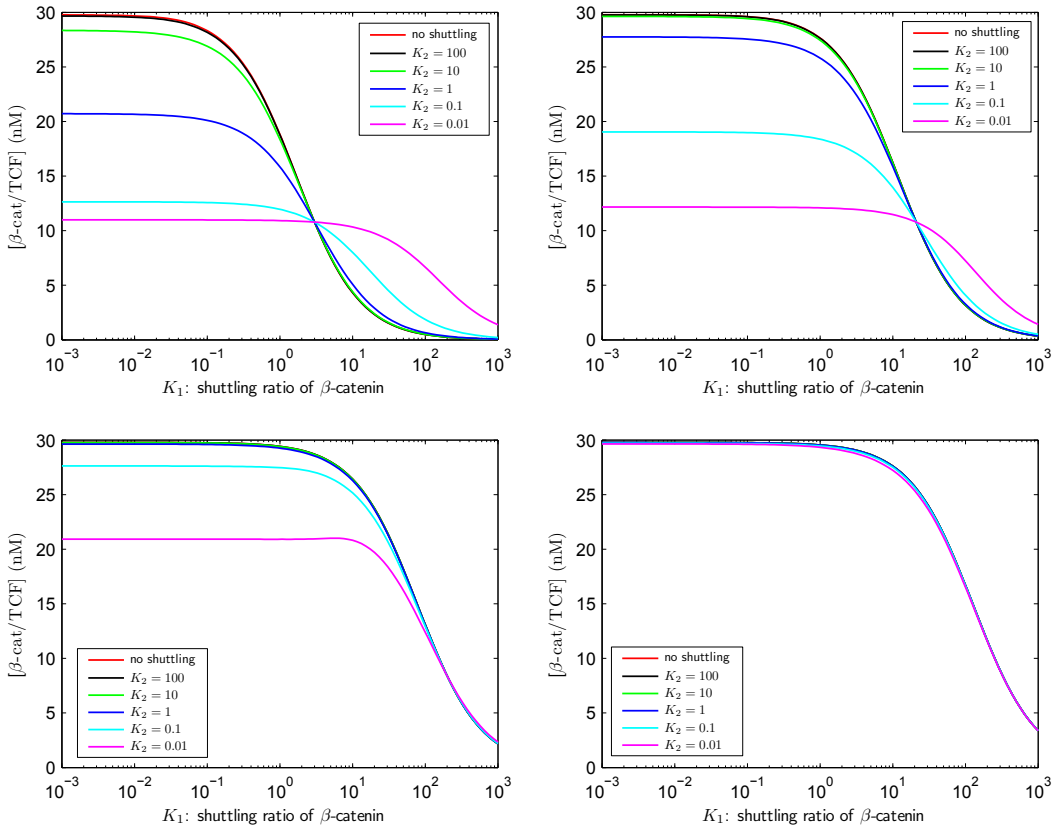

Figure 6.4.: Steady state levels of the [β-catenin/TCF] concentration for different permanent Wnt stimuli levels given in arbitrary dimensionless units (top left: $W = 1$, top right: $W = 10$, bottom left: $W = 100$, bottom right: $W = 1000$). The [β-catenin/TCF] steady state concentrations are plotted in dependence of different shuttling rate constants ratios of β-catenin (x-axis) and the destruction complex (see legend).

ling rate constant ratio of β-catenin compared to the Wnt-off state presented in Figure 6.2. For further increased strength of the Wnt signal (W =10, 100, 1000), the intersection point shifts even further towards higher values of K_1. Thereby, the concentration of [β-catenin/TCF] increases for larger shuttling rate constant ratios of β-catenin and smaller shuttling rate constant ratios of the destruction complex. As expected these results confirm that a weak Wnt signal only reduces the effect of cytoplasmic degradation (right hand side of the intersection point). The impact of nuclear degradation on the output of the pathway (left hand side of the intersection point) is only significantly reduced at much stronger Wnt signals. For very strong Wnt signals almost all the AAG is present in its inactivated/inhibited state. This leads to a higher concentration of free β-catenin and hence a higher concentration of [β-catenin/TCF] for higher values of K_1 and smaller values of K_2. Accordingly,

I conclude that the impact of antagonist shuttling on the [β-catenin/TCF] output becomes weaker with an increasing Wnt signal.

6.2.5. A transient Wnt signal

In the previous section, I examined the impact of a permanent Wnt signal of different strengths on the [β-catenin/TCF] concentration. We have thereby seen that a very strong Wnt signal reduces the influence of AAG shuttling notably. *In vivo* however, Wnt stimulation is transient, likely due to receptor inactivation/internalization and/or other downregulatory processes [Lee et al., 2003]. Transient activation of the Wnt pathway is modelled assuming a Wnt stimulus that decays exponentially:

$$W(t) = \begin{cases} 0 & , \text{for } t < t_0 \\ W_0\, e^{-\lambda(t-t_0)} & , \text{for } t \geq t_0 \end{cases} \tag{6.6}$$

where λ is the reciprocal of the characteristic lifetime of receptor stimulation [Lee et al., 2003], t_0 denotes the onset of signalling and W_0 is the initial stimulation of the pathway. Parameter values are chosen arbitrarily and given in Table 6.2.
I first discuss the trajectories of the involved proteins and protein complexes during a transient Wnt signal for $K_1 = 0.1$ and different AAG shuttling ratios (K_2) and afterwards compare and discuss the trajectories of the [β-catenin/TCF] for varying shuttling ratios for β-catenin during the same transient Wnt signal ($K_1 = 0.1$,1 and 10). The results of the simulations are presented in Figures 6.5 and 6.6, respectively. Before I consider a transient Wnt signal, the system is first simulated in the Wnt-off state, until the steady state has been reached. Then the Wnt signal is switched on according to Equation (6.6). With off-fading Wnt signal, the system again approaches the Wnt-off steady state.

Figure 6.5 shows the trajectories of the β-catenin and AAG concentrations in the cytoplasm (cyto) and the nucleus (nuc), as well as the output concentration [β-catenin/TCF] during a transient Wnt signal in dependence of the shuttling rate constants of the destruction complex. In this parameterisation ($K_1 = 0.1$), more β-catenin is present in the nucleus than in the cytoplasm. We see that during the Wnt signal the concentration of inactive AAG quickly reaches a maximum (Figure 6.5g). The more AAG is available in the cytoplasm, the higher the value of maximal inactive AAG (red, black lines). For $K_2 = 0.01$ (magenta lines), the Wnt signal has hardly any effect on the concentration of AAG, as most AAG is located in the nu-

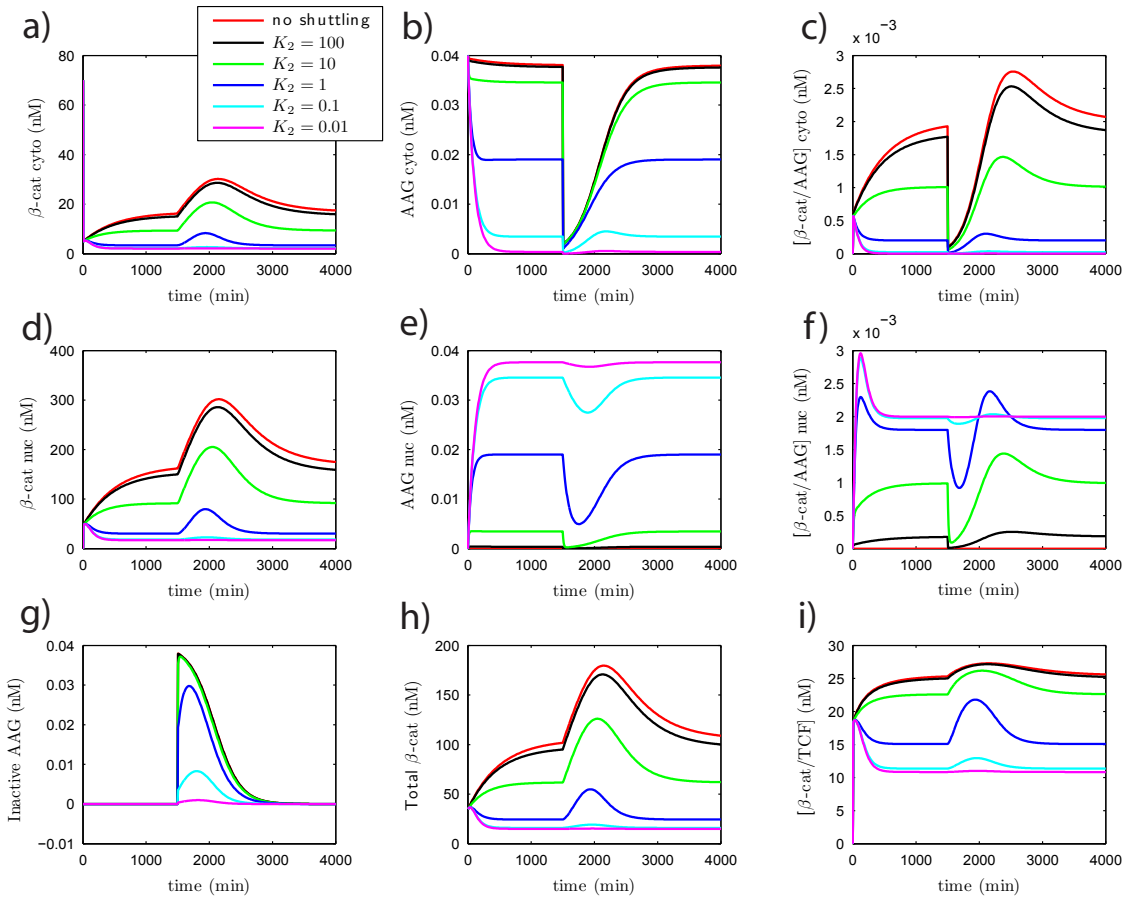

Figure 6.5.: Trajectories of protein and protein complex concentrations during a transient Wnt signal for $K_1 = 0.1$ and different shuttling rate constants ratios for the destruction complex (K_2, see legend). In the simulation, the steady state is first reached in the Wnt-off state, before the transient Wnt signal is applied to the system. The simulation time is given on the x-axis of each subfigure. The state variable/protein concentration in the cytoplasm (cyto) and nucleus (nuc) is given on the y-axis.

cleus and Wnt can only inhibit cytoplasmic AAG. Concurrently, the concentrations of active AAG reach a minimum (in cytoplasm and nucleus, Figure 6.5b+e). The inactivation of AAG is followed by an increase of total β-catenin (Figure 6.5h). This is attended by an increase of free β-catenin, in the cytoplasm (Figure 6.5 a) as well as in the nucleus (Figure 6.5d). Interestingly, the concentration of [β-catenin/AAG] first reaches a minimum, followed by a maximum, before it approaches the Wnt-off steady state again. This behaviour can be observed in the cytoplasm (Figure 6.5c) as well as in the nucleus (Figure 6.5f). This is due to the high amounts of total β-catenin during the Wnt signal (Figure 6.5h), which can bind (re-)activated AAG with decreasing Wnt stimulus. Increased levels of [β-catenin/AAG] are only

of short duration until most of the free β-catenin is phosphorylated by the destruction complex and subsequently degraded by the proteasome. After the Wnt signal, β-catenin levels are kept low in the Wnt-off state due to continuous degradation. The dynamics of the [β-catenin/TCF] concentration (see Figure 6.5i) in principal follow the dynamics of the total β-catenin (Figure 6.5h) for the different shuttling rate constants ratios of AAG. We see the expected increase of the [β-catenin/TCF] concentration with the onset of the Wnt signal, but in contrast to the β-catenin dynamics, we observe a maximal peak for $K_2 = 1$ (blue curve). Thus, these simulation results predict that balanced nucleo-cytoplasmic shuttling of AAG can increase the output of the pathway in relative response to a transient Wnt signal.

I now compare the results for the trajectories of the [β-catenin/TCF] in this parameterisation for β-catenin shuttling ($K_1 = 0.1$, i.e. more β-catenin is located in the nucleus, shown in Figure 6.5 i) with the results depicted in Figure 6.6, where $K_1 = 1$ (left plot) and $K_1 = 10$ (right plot), respectively. Remember that the different basal levels (just before the on-set of the Wnt signal) correspond to the different Wnt-off steady state concentrations for the different K_1 parameter values. I relate the peak of the transient Wnt signal to the basal steady state levels, and hence consider the relative response of the [β-catenin/TCF] concentration to the Wnt signal [Cohen-Saidon et al., 2009].
We see that in the three scenarios, different shuttling rate constant ratios of AAG yield maximal relative response of the [β-catenin/TCF] concentration during transient Wnt signalling, but that this maximal response does not necessarily correspond to maximal absolute levels of [β-catenin/TCF]:
As stated above, for $K_1 = 0.1$, the blue curve ($K_2 = 1$) shows maximal relative response to Wnt signalling. In this case the output increases from 15 nM to 22 nM (see Figure 6.5i). The maximal absolute concentration is, however, obtained for the red curve, where AAG cannot translocate into the nucleus, although in this case the [β-catenin/TCF] concentration is hardly responding to Wnt signalling at all. For $K_1 = 1$ (Figure 6.6, left panel), the 'no shuttling' (red) curve also exhibits maximal relative responses to Wnt signalling and also exhibits a maximal absolute [β-catenin/TCF] concentration of 20 nM. Contrariwise, for $K_1 = 10$ (Figure 6.6, right panel), the 'no shuttling' case reveals maximal relative response to Wnt signalling, but yields minimal output compared to the other AAG shuttling parameter values. Here, maximal output is obtained for $K_2 = 0.01$ (magenta curve), where most AAG is located in the nucleus.

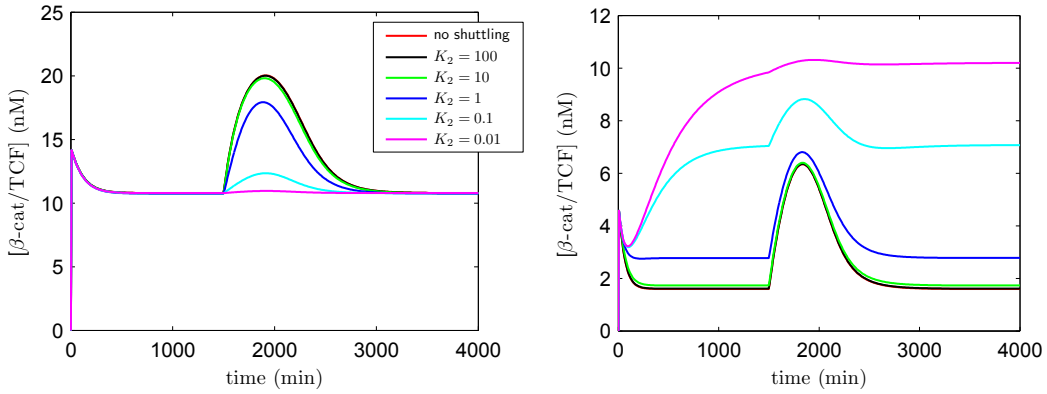

Figure 6.6.: Trajectories of the [β-catenin/TCF] concentration during a transient Wnt signal for different shuttling rate constants ratios for the destruction complex (K_2, see legend) and β-catenin (left panel: $K_1 = 1$, right panel: $K_1 = 10$). In the simulation, the steady state is first reached in the Wnt-off state, before the transient Wnt signal is applied to the system.

To sum up, we see very divers responses to the Wnt signal depending on the cellular distribution of β-catenin and AAG, which is primarily determined by the nucleo-cytoplasmic shuttling ratios. However, I want to highlight two major outcomes of my analysis: first, these results indicate that for a high β-catenin concentration in the nucleus (Figure 6.5i), which is very likely to be the case in the on-state of the pathway, well-balanced AAG shuttling leads to the maximal relative response to Wnt signalling. This finding is unexpected and yet promising, as the underlying relations between the shuttling ratios and concentrations of β-catenin and AAG can be used to design experiments in order to efficiently manipulate the cells such that the [β-catenin/TCF] concentration is maximised.
Second, the results for a transient Wnt signal confirm what we discovered with respect to permanent signalling: the transient Wnt signal has a bigger impact on the [β-catenin/TCF] concentration if more AAG is located in the cytoplasm. In all three scenarios I investigated, the magenta curves, which correspond to an increased nuclear AAG concentration, show minimal response to transient Wnt signalling. From this result I conclude that nucleo-cytoplasmic shuttling of AAG renders the pathway robust against fluctuations in Wnt signalling. Thus, a threshold concentration of Wnt ligands and a minimal duration of the Wnt Signal is needed to overcome the influence of the nuclear destruction complex against background fluctuations of Wnt.

So far, I investigated the impact of the shuttling parameters and of Wnt signalling on the output of the pathway. However, it is interesting to quantify the sensitivity of the system's output towards changes or perturbations of not only the shuttling parameters, but also towards individual perturbations of all involved parameters. This provides us with the opportunity to directly compare the sensitivities, and additionally to determine the total robustness of the [β-catenin/TCF] concentration with respect to perturbations of all parameters. In the next section, I therefore conduct a sensitivity analysis of the [β-catenin/TCF] concentration for a transient Wnt signal.

6.2.6. Time dependent sensitivity analysis for a transient signal

One goal of this chapter is to provide a comprehensive study of the Wnt signalling pathway with respect to β-catenin degradation and nucleo-cytoplasmic shuttling of β-catenin itself as well as its antagonists. Similar to the Lee Model, the Degradation Model contains several parameters that affect the output of the system in different ways and to various extent. In order to account for the robustness of the system against perturbations, one widely used tool is the so-called sensitivity analysis. In this section, I perform a sensitivity analysis to systematically investigate the perturbation of which parameter is influencing the [β-catenin/TCF] concentration most dramatically. For quantifying the effects of the perturbations, I calculate control coefficients, as defined in Section 3.4.3.

The control coefficients are calculated according to Equation (3.7) at discrete time-points during the course of a transient Wnt signal, which is described with Equation 6.6. For the last time-point, the system has returned to its steady state. I focus on [β-catenin/TCF] and calculate the responses of the concentration of this compound upon changes of about $\pm 1\%$ in the rate constants of all parameters k_j involved in the network.

We perform the analysis at different positions in parameter space for the shuttling rate constants ratios. In particular, we focus on $K_1 = 0.1$, 1 and 10 and $K_2 = 0.01$, 1 and 'no AAG shuttling' (which corresponds to $k_2 = 0$, hence all AAG is present in the cytoplasm). Accordingly, we end up with nine sets of results for 19 perturbed parameters each. All results are presented in Appendix A, Figures A.1 - A.9.

In general, the parameters that effect the system most efficiently are k_5 and k_{-5}, describing the binding and dissociation of β-catenin and TCF, and k_{10}, which accounts for β-catenin synthesis. These parameters have a major impact on the [β-

AAG vs. β-cat shuttling	$K_1 = 0.1$	$K_1 = 1$	$K_1 = 10$
K_2 : no shuttling (red)	4.70	26.65	58.72
$K_2 = 1$ (blue)	21.44	29.50	55.53
$K_2 = 0.01$ (magenta)	26.38	26.24	27.12

Table 6.3.: Quantified total robustness. Sums of the control coefficients. We consider the absolute values of the control coefficients for a parameter perturbation of +1% over all time points and all parameters for different shuttling rate constant ratios. The according positions in parameter space are tagged in Figure 6.2. The colours given with K_2 correspond to the curves presented in Figure 6.2.

catenin/TCF], because the ratio of k_{-5} to k_5 directly describes the binding affinity of β-catenin and TCF and the synthesis rate k_{10} determines the amount of produced and hence the available β-catenin.
The other parameters that have a significant influence on the [β-catenin/TCF] concentration depend on the parametrisation and hence the compartmental distribution of AAG and β-catenin. Therefore, either the control coefficients of k_3, k_{-3} and k_6 (activity of the destruction complex in the cytoplasm) or k_4, k_{-4} and k_7 (activity of the destruction complex in the nucleus) exhibit larger values. In contrast to the Lee model, where the control coefficients of binding and dissociation parameters sum up to zero [Lee et al., 2003], we here observe that the control coefficients of k_{-3} (dissociation rate constant of AAG and β-catenin) and k_6 (constant that describes phosphorylation of β-catenin and irreversible release of phosphorylated β-catenin) sum up to zero. This applies analogously to k_{-4} and k_7 and therefore holds true for these processes in both compartments, cytoplasm and nucleus.

In order to quantify the total sensitivity of the system, I sum up the absolute values of the control coefficients over all time-points for each parameter (for a perturbation of +1%; red dots in Figures A.1 - A.9, Appendix A). The results are shown in Subfigure 20 in each of the supplementary figures. Subsequently, I add up these temporal sums over all parameters. I thereby arrive at nine sums of control coefficients for different shuttling ratio pairs (K_1, K_2), which are presented in Table 6.3. These sums are considered as the quantification of the total robustness, where small numbers correspond to high robustness. The according positions in parameter space are tagged in Figure 6.2. The tagged concentrations of [β-catenin/TCF] correspond to the steady state concentrations just before the transient Wnt signal is applied to the system, which of course equals the steady state the system reaches

after the transient signal. Relating the numbers presented in Table 6.3 to Figure 6.2, we see that the total robustness is linked to the output levels of [β-catenin/TCF]: for high [β-catenin/TCF] levels in steady state ($K_1 = 0.1$ and no AAG shuttling), where all AAG is located in the cytoplasm and most β-catenin is in the nucleus, the system exhibits the most robust output [β-catenin/TCF] ($\sum C = 4.70$). This is due to the fact that if β-catenin and its antagonists reside in different compartments, and hence cannot interfere with each other. Unexpectedly, these results therefore reveal that the compartmental separation of β-catenin and the destruction complex does not only lead to a maximisation of [β-catenin/TCF], but also to an increased robustness of [β-catenin/TCF] signalling. On the other hand, when the [β-catenin/TCF] concentration is small ($K_1 = 10$, no AAG shuttling) and both, AAG and β-catenin, are mostly located in the cytoplasm, the system is the least robust to parameter perturbations ($\sum C = 58.72$). Consequently, in this case the [β-catenin/TCF] concentration reacts most sensitive towards cellular modifications. This, however, could also be of advantage, because already weak signals may trigger the on-set of signalling, helping the system to quickly adapt to changes in its cellular environment.

For intermediate levels of [β-catenin/TCF] (highlighted with a yellow box in Figure 6.2), the system exhibits intermediate levels of robustness. Here, the total robustness sums equal values between 26 and 30. Provided that AAG is not able to translocate into the nucleus (red line in Figure 6.2, and first line in Table 6.3), we furthermore observe the largest and the smallest value for the total robustness of the [β-catenin/TCF] concentration for the studied β-catenin shuttling rate ratios, indicating that the system's robustness is highly dependent on β-catenin's shuttling ratio. If, however, the destruction complex is present and active in the nucleus (magenta line in Figure 6.2, and bottom line in Table 6.3), the total sensitivity of the [β-catenin/TCF] concentration remains almost the same for all β-catenin shuttling scenarios. From this I deduce that if most β-catenin is present in the nucleus, AAG shuttling acts sensibilising on the [β-catenin/TCF] concentration. In contrast, if most β-catenin is located in the cytoplasm, nucleo-cytoplasmic shuttling of the destruction complex does not only yield higher levels [β-catenin/TCF], but the [β-catenin/TCF] output concentration is also more robust against perturbations in general. From this analysis, I therefore conclude that AAG shuttling, which causes a higher nuclear AAG concentration, results in a [β-catenin/TCF] output that is especially robust against changes in the compartmental distribution of β-catenin.

6.3. Conclusions

In this chapter, I established a novel compartmental model of Wnt pathway to investigate the influence of nucleo-cytoplasmic shuttling of β-catenin and its antagonists APC, Axin and GSK3, on the [β-catenin/TCF] concentration. In order to provide a comprehensive analysis, I conducted several case studies: (i) I considered different Wnt on/off scenarios, (ii) I investigated the impact of pathway inhibition and (iii) I completed my study with a sensitivity analysis. The investigation addressed the following concrete questions: Does the nucleo-cytoplasmic shuttling of β-catenin antagonists influence the [β-catenin/TCF] concentration? Can the [β-catenin/TCF] concentration be maximised by antagonist shuttling, leading to an optimised signal transduction through the cell? How is the output of the pathway influenced by phosphorylation and subsequent degradation of nuclear β-catenin?

My analysis revealed the following key findings: Nucleo-cytoplasmic shuttling of the destruction complex AAG can yield a spatial separation of β-catenin and AAG into cytoplasm and nucleus. This separation can prevent β-catenin from interacting with the destruction complex, and thus causes a breakdown of β-catenin degradation. Subsequently, levels of total β-catenin are enhanced and this hence results in an increase of the steady state concentration of the transcription factor complex [β-catenin/TCF]. My results provided therefore unexpected evidence that nucleo-cytoplamic shuttling of the destruction complex can yield a maximisation of [β-catenin/TCF] signalling in comparison to a reference model without AAG shuttling. In spite of being β-catenin antagonists and thus actively reducing β-catenin levels, these findings demonstrate that Wnt signalling can benefit from nucleo-cytoplasmic shuttling of AAG.

Another objective of my analysis was to elucidate the impact of destruction complex inhibition. The results of my analysis support the expectation that inhibition of the destruction complex is enhancing the [β-catenin/TCF] concentration and hence the output of the pathway. Unexpectedly, given the premise that the destruction complex is assembled in the nucleus, I showed that the [β-catenin/TCF] concentration is more effectively enhanced by inhibition of the kinase GSK3 rather than the binding of β-catenin to the destruction complex. There are many GSK3 inhibitors known and available, with lithium being the best-characterized, although fairly specific for GSK3 [Doble and Woodgett, 2003]. Recently, several new inhibitors have been developed [Cohen and Goedert, 2004, Doble and Woodgett, 2003], offering various possibilities for experimental intervention.

Next, I studied the influence of permanent and transient Wnt signals. The results in this regard confirmed the intuitive assumption that a weak permanent Wnt signal only reduces the effect of cytoplasmic β-catenin degradation, as Wnt only causes the dissociation of the destruction complex if it is located in the cytoplasm. For an increased nuclear AAG concentration, however, [β-catenin/TCF] signalling exhibits minimal response to Wnt. From this result, I conclude that nucleo-cytoplasmic shuttling of AAG renders the pathway robust against fluctuations in Wnt signalling. Thus, a threshold concentration of Wnt ligands and a minimal duration of the Wnt Signal is needed to overcome the influence of the nuclear destruction complex against background fluctuations of Wnt. Furthermore, simulation experiments revealed very diverse responses of [β-catenin/TCF] to transient Wnt signalling. The response depends on the cellular distribution of β-catenin and AAG, which is primarily determined by the nucleo-cytoplasmic shuttling ratios. In particular, for a high β-catenin concentration in the nucleus, well-balanced AAG shuttling yields maximal relative response to Wnt signalling. This is very likely to be the case in the on-state of the Wnt pathway. This finding is both, unexpected and yet promising, as the underlying relations between the shuttling ratios and concentrations of β-catenin and AAG can be used to design wet-lab experiments in order to efficiently manipulate the cells such that the [β-catenin/TCF] concentration is maximised.

In complete this study, I conducted a sensitivity analysis to systematically investigate the influence of parameter perturbations on the [β-catenin/TCF] concentration. Surprisingly, my results demonstrated that the total robustness of the [β-catenin/TCF] output is closely linked to its absolute concentration levels. The system exhibits the most robust [β-catenin/TCF] concentration, if β-catenin and its antagonists cannot interfere with each other. This is the case when they reside in different compartments, which is due to nucleo-cytoplasmic shuttling. These findings therefore revealed that the compartmental separation of β-catenin and the destruction complex does not only lead to a maximisation, but additionally to an increased robustness of the [β-catenin/TCF] concentration. Contrariwise, if AAG and β-catenin are mostly located in the cytoplasm, the concentration of [β-catenin/TCF] is very low. In this case, I proved the [β-catenin/TCF] concentration to be very sensitive to parameter perturbations and thus to react most sensitive towards intracellular modifications. This could also be of advantage, since for low [β-catenin/TCF] concentrations weak signals may already trigger the activation of the pathway, helping the system to quickly adapt to changes in its cellular environment.

Finally, my results revealed that the system's robustness is, on the one hand, highly

dependent on β-catenin's shuttling ratio if AAG was unable to translocate into the nucleus. If, on the other hand, the destruction complex is present and active in the nucleus, the [β-catenin/TCF] concentration is very robust towards changes in β-catenin shuttling. From the results of the sensitivity analysis, I therefore conclude that nucleo-cytoplasmic antagonist shuttling yields a [β-catenin/TCF] output that is more robust against perturbations in the cellular environment in general. Moreover, it renders Wnt/β-catenin signalling particularly robust against changes in the compartmental distribution of β-catenin.

7. Synthesis of results and final remarks

Over the last 30 years, Wnt/β-catenin has consolidated its role as a critical signalling pathway in development and disease. The key player of the pathway is β-catenin. Its activity is mainly regulated by the destruction complex consisting of APC, Axin and GSK3. In the nucleus, complex formation of β-catenin and TCF initiates target gene expression. In 2000, it was for the first time shown that APC, a β-catenin antagonist whose activity was believed to be restricted to the cytoplasm, is also capable of nucleo-cytoplasmic shuttling. Since then, many cytoplasmic components of the Wnt/β-catenin pathway have been experimentally proven to also reside in the nucleus. However, the functional relevance of these proteins in regulating the Wnt/β-catenin pathway in the nucleus, remains yet to be determined.

In this thesis, I investigated the role of nucleo-cytoplasmic shuttling of β-catenin and its antagonists APC, Axin, and GSK3 in Wnt/β-catenin signalling and the resulting regulation of subcellular β-catenin levels. My analysis concentrated on the following key questions: Does the nucleo-cytoplasmic shuttling of the β-catenin antagonists APC, Axin and GSK3 affect the [β-catenin/TCF] concentration, which is considered as the output of Wnt signalling? Can the [β-catenin/TCF] concentration be maximised by antagonist shuttling, leading to an optimised signal transduction through the cell? How is the output of the pathway influenced by cytoplasmic and nuclear retention of β-catenin, and by phosphorylation and subsequent degradation of β-catenin?
To answer these questions, I established, analysed and subsequently published the first compartmental models of the Wnt pathway: the Retention Model [Schmitz et al., 2011] and the Degradation Model [Schmitz et al., 2013]. They are currently the only deterministic ODE models of the Wnt/β-catenin pathway on a subcellular, compartmental scale and aim at examining the influence of nucleo-cytoplasmic shuttling of β-catenin antagonists on the output of the pathway. These models allow

Dynamics of the state variable	... in the Retention Model	... in the Degradation Model
$\dot{X}_1$	$-\nu_1 - \nu_3$	$-\nu_1 - \nu_3$ $\mathbf{+\nu_{10}}$
$\dot{X}_2$	$-\nu_2 - \nu_3$	$-\nu_2 - \nu_3$ $\mathbf{+\nu_6 - \nu_{11}}$
$\dot{X}_3$	$+\nu_1 - \nu_4 - \nu_5$	$+\nu_1 - \nu_4 - \nu_5$
$\dot{X}_4$	$+\nu_2 - \nu_4$	$+\nu_2 - \nu_4$ $\mathbf{+\nu_7}$
$\dot{X}_5$	$+\nu_3$	$+\nu_3$ $\mathbf{-\nu_6}$
$\dot{X}_6$	$+\nu_4$	$+\nu_4$ $\mathbf{-\nu_7}$
$\dot{X}_7$	$-\nu_5$	$-\nu_5$
$\dot{X}_8$	$+\nu_5$	$+\nu_5$
$\dot{X}_9$		$\mathbf{+\nu_6 - \nu_8}$
$\dot{X}_{10}$		$\mathbf{+\nu_7 - \nu_9}$
$\dot{X}_{11}$		$\mathbf{+\nu_{11}}$

Table 7.1.: Comparison of the Retention and Degradation Model. The first column describes the time-dependent changes of the concentrations of all proteins and protein complexes included in the models. The second column presents the reaction rates that determine the dynamics of the variables of the Retention Model, the third column presents the reaction rates that determine the dynamics of the variables of the Degradation Model. The definitions of the state variables are given in Tables 5.1 and 6.1. The reaction rates are defined by Equations 6.2. The reaction rates ν_{1-5} are used in both models, whereas the bold characters denote the reaction rates only used in the Degradation Model, and therefore highlight how the Degradation Model is an extension of the Retention Model.

for the retention of β-catenin by APC, and β-catenin phosphorylation and successive degradation in the nucleus and cytoplasm, respectively.
The Retention Model and the Degradation Model share some commonalities, but also exhibit some differences in terms of i) their structure, ii) their parametrisation and iii) the focus of their analysis. Table 7.1 presents a survey of fundamental differences between the two models and thus shows how the Degradation Model is an extension of the Retention Model.

i) In terms of the **model structure**, common features of both models include nucleo-cytoplasmic shuttling of β-catenin and complex formation of β-catenin and TCF, which is regarded as the output of both models. Both models neglect membrane associated events. They also have in common that an antagonist is shuttling between the cytoplasm and the nucleus and interfering with β-catenin in both cellular compartments. Shuttling assumptions are based on experimental findings obtained by Krieghoff et al. [2006] and presume that β-catenin and its antagonists shuttle independently of each other and that the complexes cannot cross the nuclear envelope.

However, the way the antagonists interfere with β-catenin is completely different in the two models. The main structural difference is already implied by their names, i.e. "Retention Model" and "Degradation Model". In the Retention Model, I considered only one β-catenin antagonist – namely APC, which regulates β-catenin levels through compartmental retention. In the Degradation Model, the destruction complex, consisting of the three β-catenin antagonists APC, Axin and GSK3, can bind and phosphorylate β-catenin, and thereby mark it for proteasomal degradation in the cytoplasm and in the nucleus. The second major difference is that the Retention Model is a closed system without any external input or signal, because the presence of an external (transient) Wnt signal does not influence the total levels of APC. The steady state solutions were therefore determined analytically. By contrast, in the Degradation Model the impact of different Wnt signals on the output of the system was investigated. In addition, the total amount of available β-catenin is not conserved, as β-catenin is continuously produced and degraded. The dynamics of the Degradation Model (steady state and transients) were obtained using computer simulations.

ii) The default **parametrisation** of both models is based on the measurements and estimations conducted by Lee et al. [2003], who developed and analysed the "standard model" of the Wnt pathway. Many parameter values describing total protein concentrations, dissociation constants and reaction rate constants are thus the same. For the shuttling rate constants, both models consider ratios rather than absolute values. The most obvious difference between the Retention Model and the Degradation Model in terms of their parametrisation, is the huge difference in available antagonist concentration. In the Retention Model, APC is available in excess (100 nM) compared to β-catenin (35nM). In the Degradation Model, however, the concentration of the destruction complex is limited by the amount of available Axin, and is thus very small (0.02 nM) [Lee et al., 2003]. Therefore, in comparison, the concentrations of the antagonists differ by a factor of 5000.

iii) In terms of the **analysis** of both models, the influence of the nucleo-cytoplasmic shuttling of β-catenin and its antagonists on the steady states of the [β-catenin/TCF] concentration was elucidated. Surprisingly though, despite different model structures, different types of interactions and different parametrisations, the results of the steady state analysis of the Retention Model presented in Figure 5.3 look qualitatively similar to the results obtained for the Degradation Model, which are shown in Figure 6.2. This is a counter-intuitive result, which can be explained as follows: In the Retention Model, APC retains β-catenin through complex formation in the

compartment, in which they are localised. In the Degradation Model, the active destruction complex binds free β-catenin, phosphorylates it and then releases it in its inactive state, marked for subsequent degradation by the proteasome (see Figure 2.2). The destruction complex is therefore able to rapidly bind one β-catenin molecule after another. This rapid process of binding-phosphorylation-dissociation-degradation increases the effectively available amount of destruction complex. In other words, the rapid binding of active β-catenin to the destruction complex and the subsequent release of inactive β-catenin mimics a kind of retention of inactive β-catenin, which afterwards cannot be activated anymore. The steady state analyses of the models yield thus similar results for the steady state concentration of [β-catenin/TCF], although the underlying mechanisms leading to these results are of fundamental difference.
The two models were also investigated in terms of different aspects, as different reaction rates and reaction rate constants were applied to the models (see Table 7.1). For the Retention Model, the focus of the analysis was on the shuttling mechanisms of APC and β-catenin. To this end, I investigated different functions for ν_2, which describes the shuttling of APC, and different parameter values for the shuttling rate constant ratios for β-catenin and APC. Besides, I considered different parametrisations of ν_3 and ν_4, which represent the retention of β-catenin by APC, and hence depict different compartmental retention scenarios.
In the Degradation Model the focus lay on the analysis of the following reaction rates: In order to investigate the impact of destruction complex inhibition, the reaction rates ν_3, ν_4, ν_6 and ν_7 were knocked out independently, as they describe the processes of binding and inactivation of β-catenin by the destruction complex. To account for different Wnt on/off scenarios, I considered different functions for ν_{11}, which describes the inactivation of the destruction complex through Wnt. Finally, the investigation of the Degradation Model concludes with a detailed local sensitivity analysis. The Retention Model was also studied in terms of the sensitivity of its solution towards perturbations. The results are however not presented in this thesis, because they did not provide any new insights.

Summarising the results, the analysis of both models led to the following answers to the initially asked questions:

1. For certain parametrisations, nucleo-cytoplasmic shuttling of β-catenin and its antagonists can yield a spatial separation between the said proteins, which results in a breakdown of β-catenin retention and degradation, respectively. This is fol-

lowed by an accumulation of β-catenin leading to an increase of the [β-catenin/TCF] concentration. Strikingly, both compartmental models I presented in this thesis reveal counter-intuitive behaviour of [β-catenin/TCF] signalling in response to nucleo-cytoplasmic shuttling of β-catenin antagonists. The inhibitory effect of these proteins can be alleviated due to nucleo-cytoplasmic shuttling. In fact, I showed that nucleo-cytoplasmic shuttling of APC, Axin and GSK3 can even maximise the output of Wnt signalling, although they are in general β-catenin antagonising proteins.

2. The investigation of the shuttling mechanisms in the Retention Model showed that saturated protein translocation can under certain conditions be modelled by pure diffusion. A difference in the shuttling rate constants of sufficient orders of magnitude leads to an accumulation in either compartment, which corresponds to saturation in translocation. Based on this result, further modelling approaches on protein shuttling can be simplified significantly, because diffusive translocation is described by a linear rate equation. Such a system is easier to solve than a system containing nonlinear interactions. This result was utilised to describe the shuttling of β-catenin and its antagonists in the Degradation Model. It is however also applicable to other systems dealing with saturated bidirectional translocation processes.

3. The analysis of the Degradation Model also provided other interesting insights. Elucidating the impact of destruction complex inhibition, I demonstrated that the inhibiting the activity of the kinase GSK3 more effectively enhances the [β-catenin/TCF] concentration than the binding of β-catenin to the destruction complex. This is yet another unexpected result, as the destruction complex first binds β-catenin, before GSK3 can phosphorylate and thus inactivate it. Furthermore, I showed that well-balanced antagonist shuttling yields maximal relative response of [β-catenin/TCF] to a transient Wnt signal.

4. The results of the sensitivity analysis indicated that a nuclear accumulation of the destruction complex renders the pathway robust against fluctuations in Wnt signalling and against changes in the compartmental distribution of β-catenin. Additionally, I showed that the total robustness of the [β-catenin/TCF] output is closely linked to its absolute concentration. Finally, this study revealed that the compartmental separation of β-catenin and the destruction complex not only leads to a maximisation, but additionally to an increased robustness of the [β-catenin/TCF] concentration against intracellular perturbations.

These major findings demonstrate that nucleo-cytoplasmic shuttling of β-catenin antagonists accounts for several interesting phenomena, which were discussed in de-

tail in the course of this thesis. I was able to find answers to the questions that biologically motivated this work. This study, however, not only aims at finding answers, but also at inspiring new wet-lab experiments. The next step would be to experimentally verify the theoretically obtained results.
Regarding the results obtains with the Degradation Model, it is necessary to first prove that the destruction complex is active in the nucleus. To this end, I suggest as a possible approach to isolate the nuclei of the cells of interest and subsequently measure if phosphorylated β-catenin has been produced in the isolated nuclei. The detection procedure is not easy, as phosphorylated β-catenin is marked for proteasomal degradation and therefore has a very short half-life time. Thus, one needs experimental (microscopic) devices with high resolution to prove the existence of phosphorylated β-catenin. Alternatively, the activity of the proteasome could be inhibited to increase the half-life of β-catenin. In this case, the chance to detect phosphorylated β-catenin increases.
To conclude, a great deal of the nature of β-catenin and its antagonists, as the key players of the Wnt pathway, is known. A complete understanding of their function has to date remained elusive. More biochemical, microscopic and theoretical studies, and a closer collaboration between experimentalists and modellers will be important to resolve open questions about these proteins in general and their nuclear functioning in particular.

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Appendix

A. Graphical illustrations of the sensitivity analysis

The following Figures A.1 - A.9 show the temporal evolution of the control coefficients for the [β-catenin/TCF] concentration of the Degradation Model. The control coefficients are calculated at discrete time-points during the course of a transient Wnt signal. The figures correspond to different points in parameter space regarding different values for the shuttling rate constant ratios of β-catenin (K_1) and AAG (K_2). Each parameter is independently perturbed by $\pm$ 1% (red: + 1%, blue: - 1 %.). The control coefficient ($C(x_8(t), k_j)$) is given on the corresponding y-axis. The simulation time is given on the x-axis of subfigures 1-19. The panel on the bottom right shows the sum of the absolute values of control coefficients for positive perturbation for each parameter over time. Note that the index j refers to the number of the subfigure in which the control coefficient for the respective perturbed parameter k_j is depicted. To that effect, the parameter λ for example equals k_{18}.

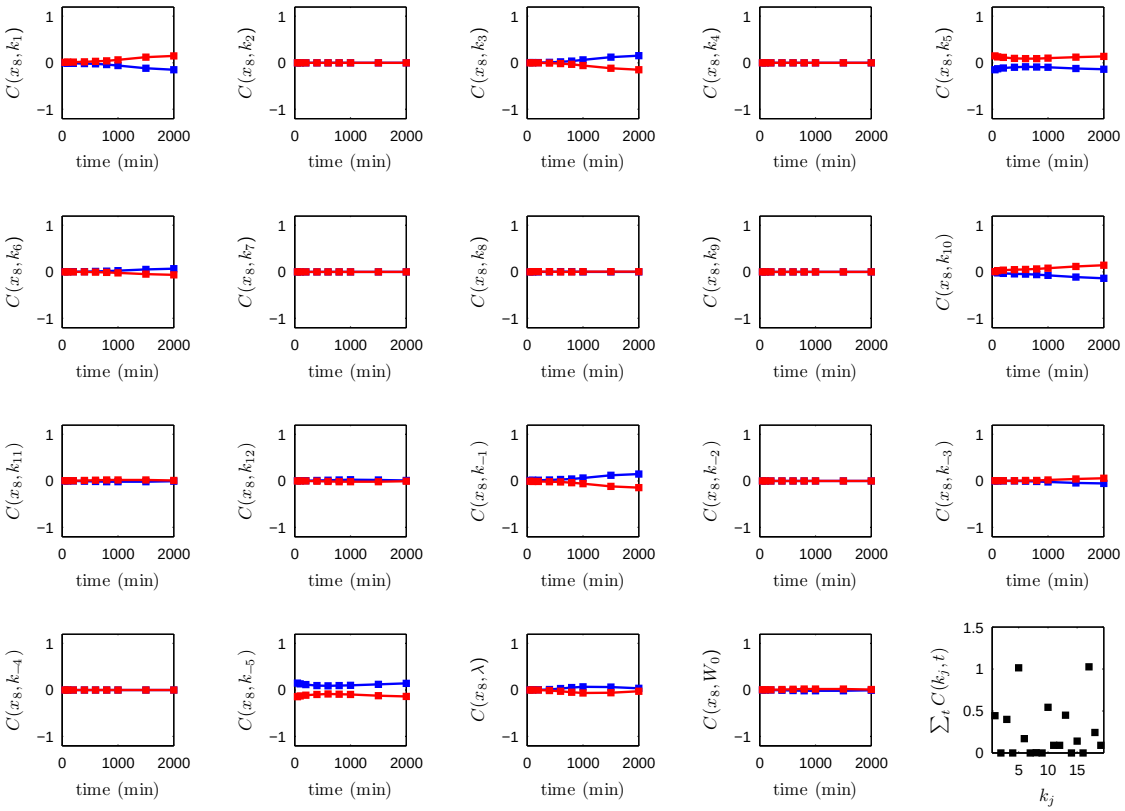

Figure A.1.: Time dependent control coefficients for $K_1 = 0.1$ and no AAG shuttling (i.e. AAG is only located in the cytoplasm; more β-catenin is located in the nucleus than in the cytoplasm). Note that k_2 and k_{-2} are not perturbed in negative direction in order to avoid negative parameter values as the initial values of the shuttling parameters equal zero (no AAG shuttling).

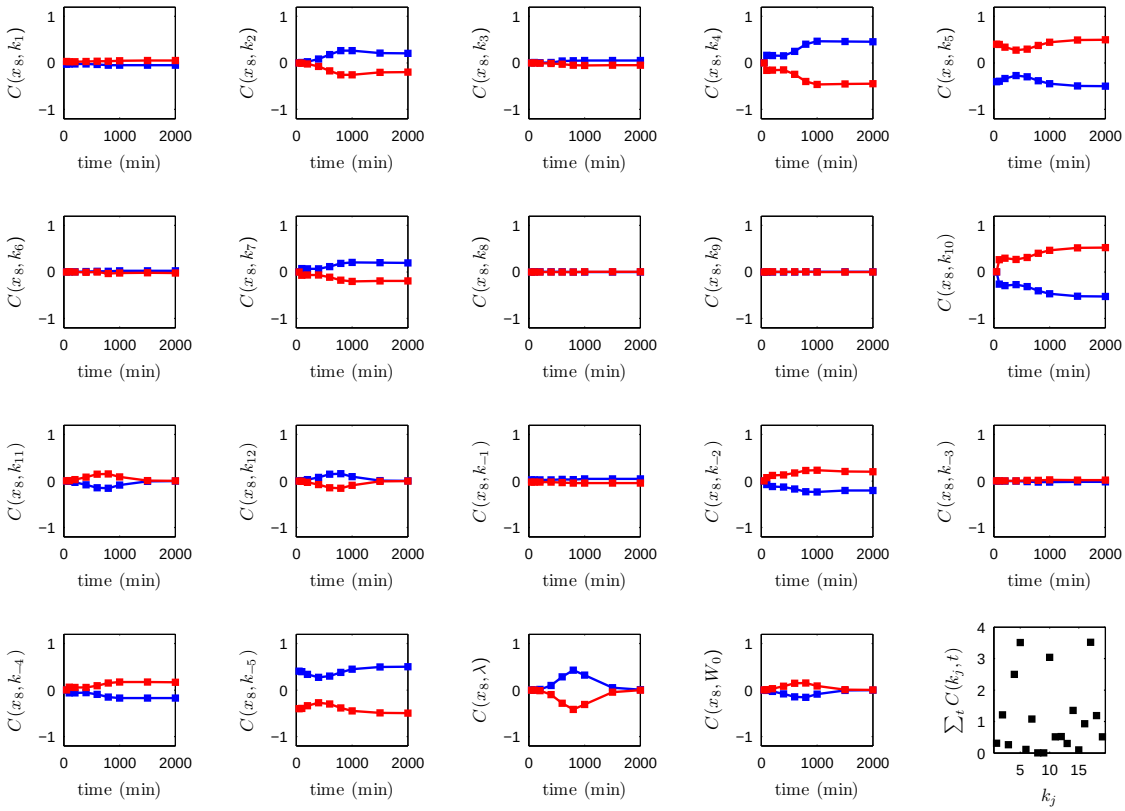

Figure A.2.: Time dependent control coefficients for $K_1 = 0.1$ and $K_2 = 1$ (i.e. in this parameterisation, β-catenin is highly concentrated in the nucleus, and AAG is equally distributed in cytoplasm and nucleus).

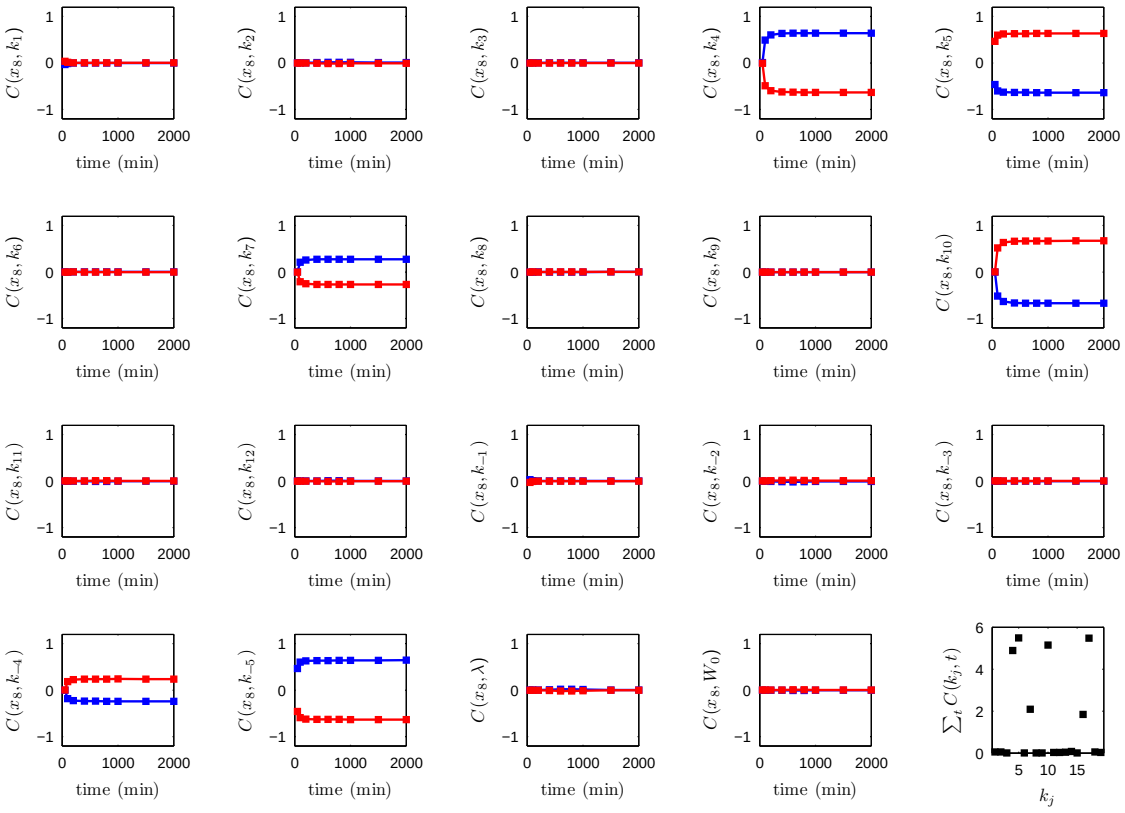

Figure A.3.: Time dependent control coefficients for $K_1 = 0.1$ and $K_2 = 0.01$ (i.e. both, AAG and β-catenin, are highly concentrated in the nucleus).

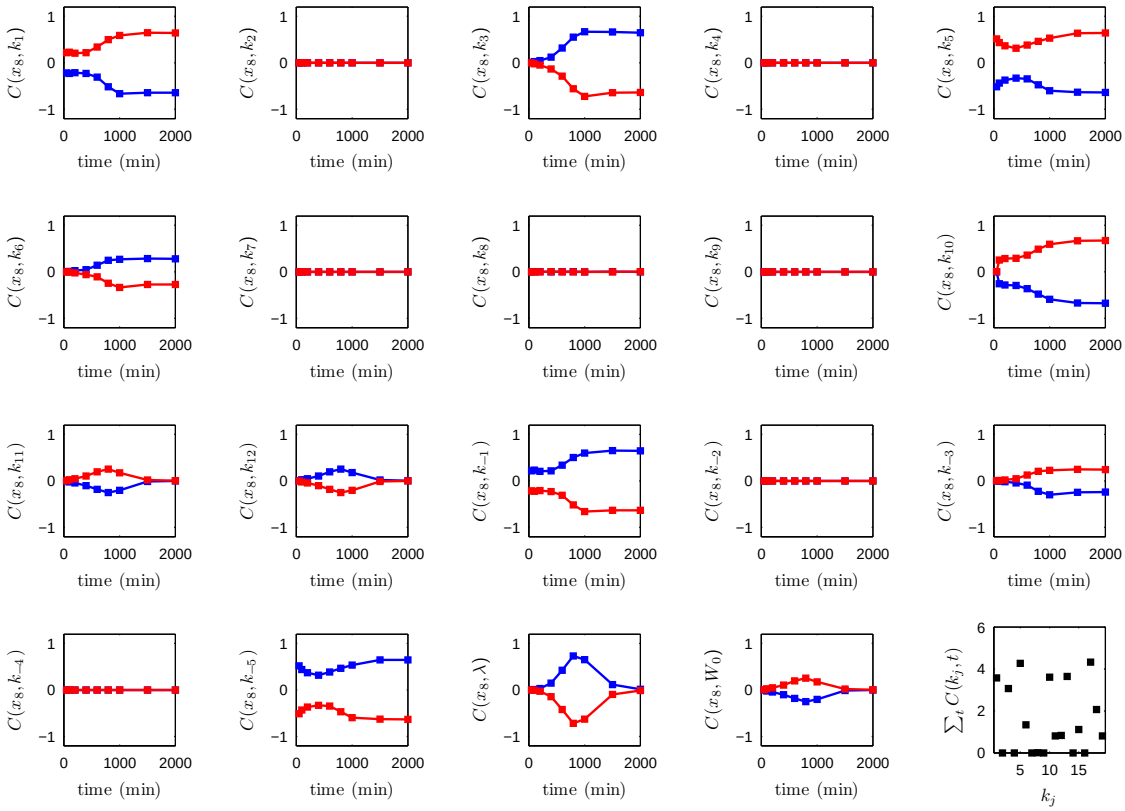

Figure A.4.: Time dependent control coefficients for $K_1 = 1$ and no AAG shuttling (i.e. the destruction complex AAG is only in cytoplasm; β-catenin equally distributes in nucleus and cytoplasm). Note that k_2 and k_{-2} are not perturbed in negative direction in order to avoid negative parameter values as the initial values of the shuttling parameters equal zero (no AAG shuttling).

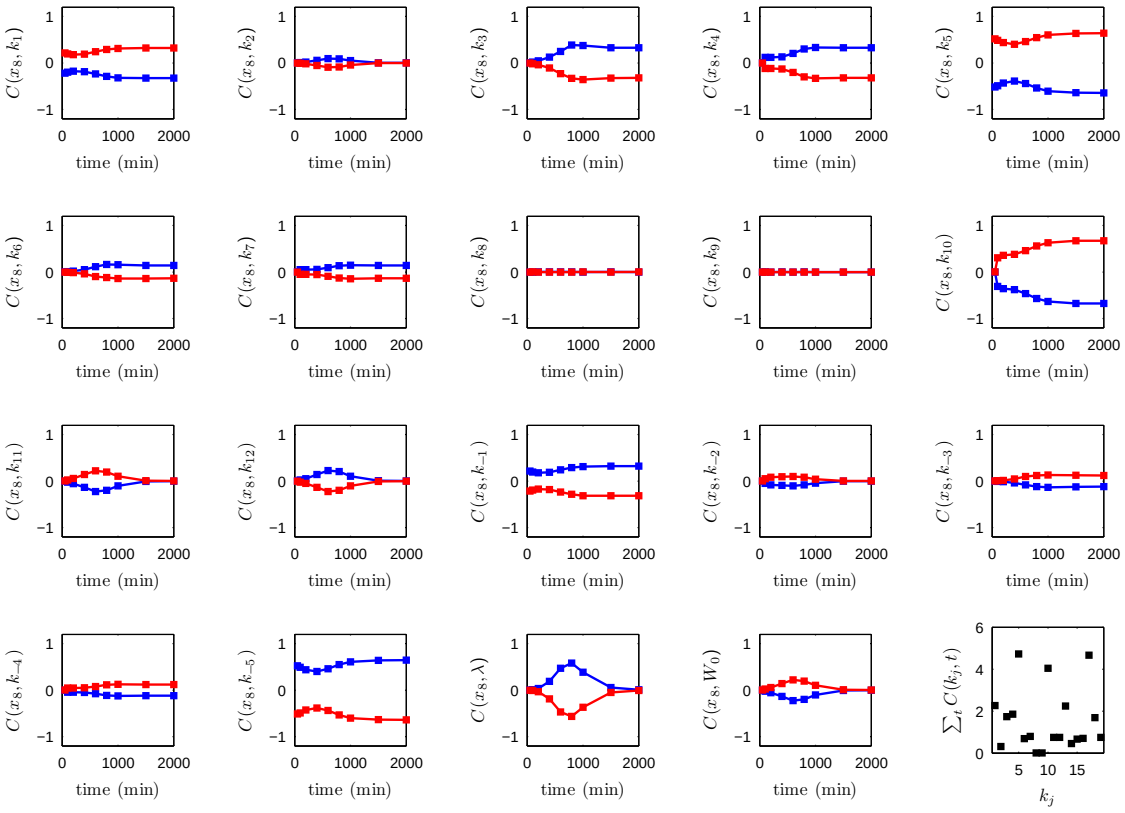

Figure A.5.: Time dependent control coefficients for $K_1 = 1$ and $K_2 = 1$ (i.e. both, β-catenin and AAG, equally distribute in cytoplasm and nucleus).

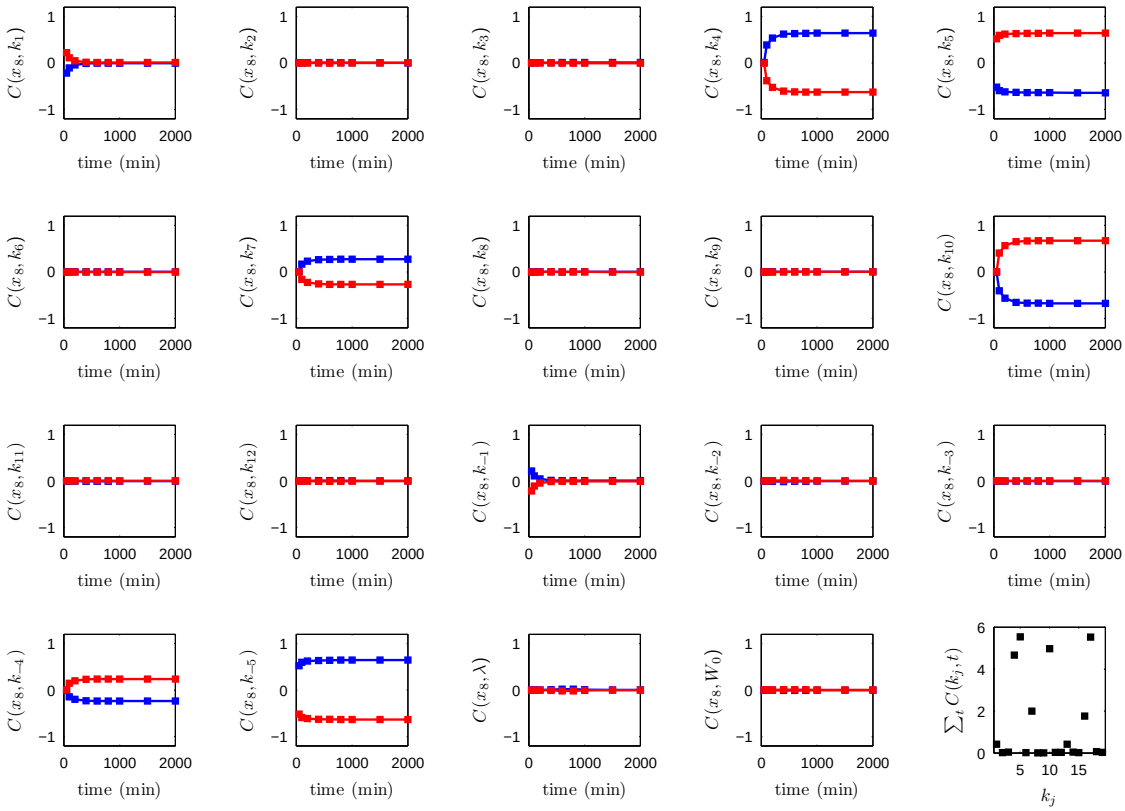

Figure A.6.: Time dependent control coefficients for $K_1 = 1$ and $K_2 = 0.01$ (i.e. β-catenin equally distributes in cytoplasm and nucleus, whereas AAG is highly concentrated in the nucleus).

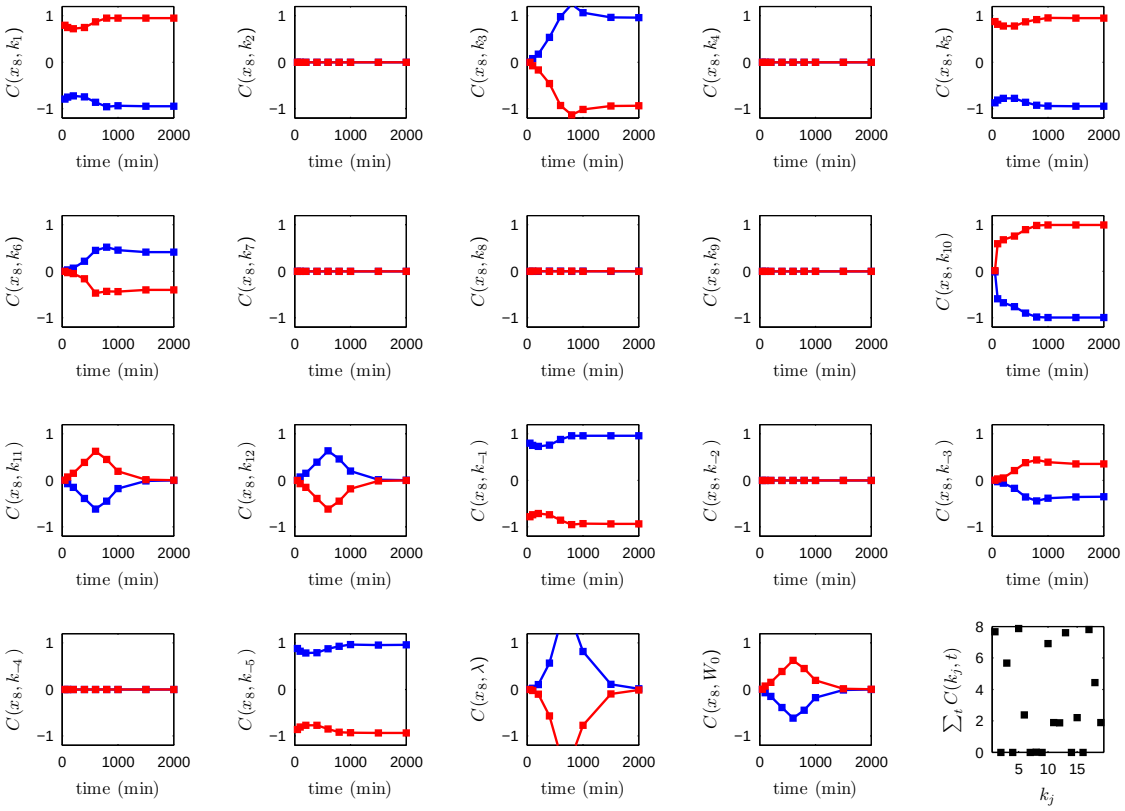

Figure A.7.: Time dependent control coefficients for $K_1 = 10$ and no AAG shuttling (i.e. AAG is only present in the cytoplasm; but more β-catenin is located in the cytoplasm than in the nucleus). Note that k_2 and k_{-2} are not perturbed in negative direction in order to avoid negative parameter values as the initial values of the shuttling parameters equal zero (no AAG shuttling).

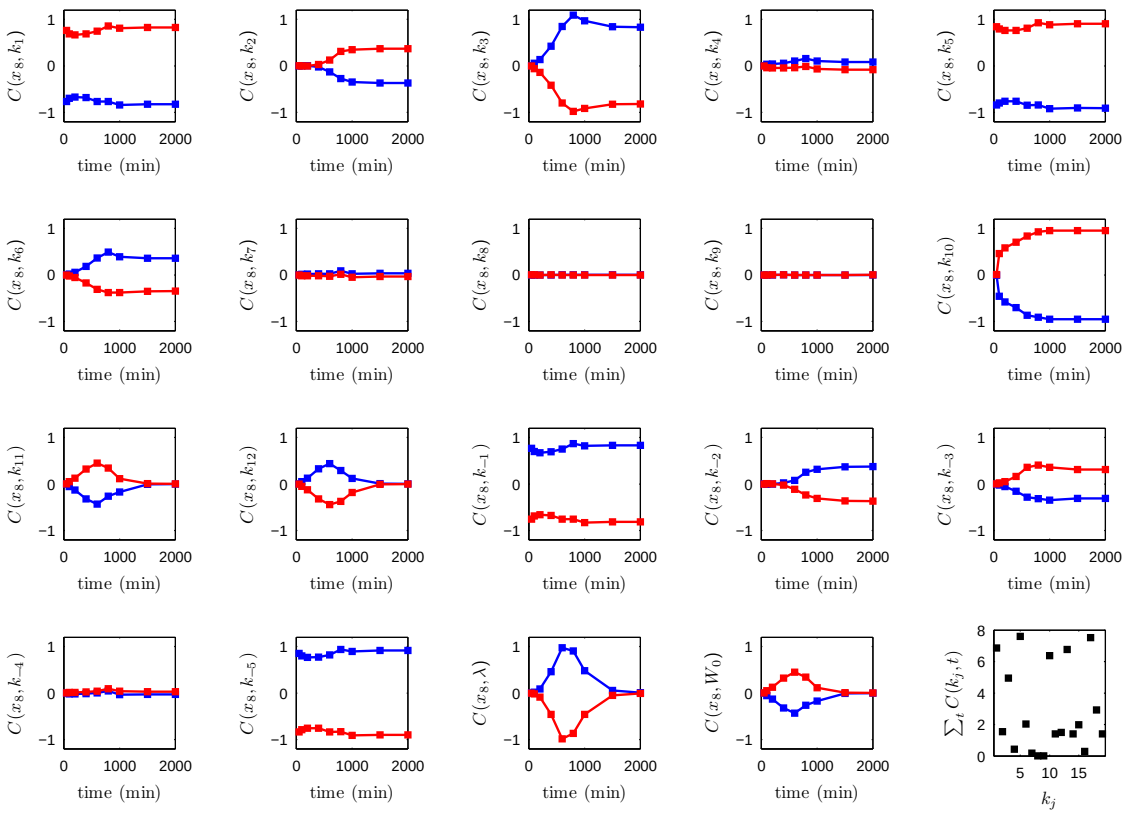

Figure A.8.: Time dependent control coefficients for $K_1 = 10$ and $K_2 = 1$ (i.e. in this parameterisation, β-catenin is highly concentrated in the cytoplasm, whereas AAG equally distributes in cytoplasm and nucleus).

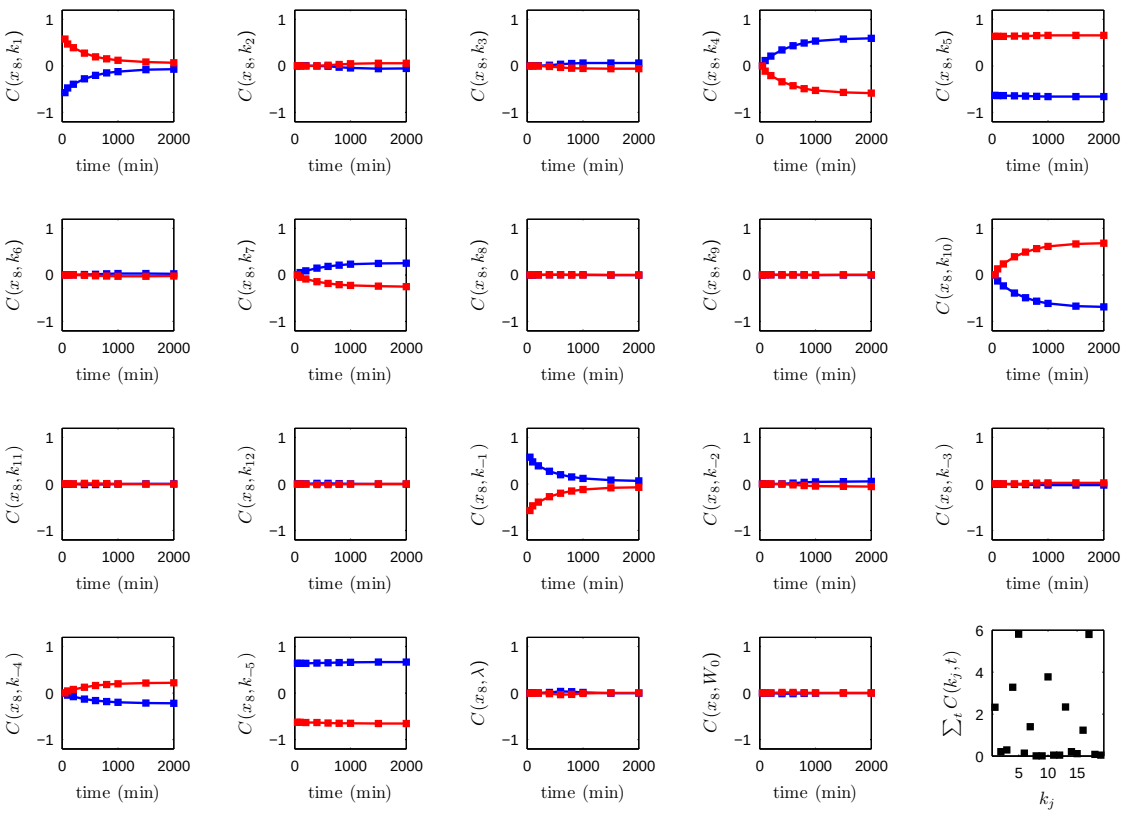

Figure A.9.: Time dependent control coefficients for $K_1 = 10$ and $K_2 = 0.01$ (i.e. more β-catenin is located in the cytoplasm than in the nucleus; AAG is, however, highly concentrated in the nucleus).

Theses

1. The Wnt/β-catenin pathway plays a key role in development and disease. The concentration of its central signalling protein β-catenin is regulated through the activitiy of the destruction complex, which consists of the APC, Axin and GSK3.

2. Depending on the state of signalling, β-catenin and its antagonists APC, Axin and GSK3 are spatio-temporally distributed in the subcellular compartments nucleus and cytoplasm. A Wnt signal leads to nuclear accumulation of β-catenin. Interaction of β-catenin with TCF initiates target gene expression.

3. The compartmental ODE formalism provides a modelling approach allowing to investigate the impact of nucleo-cytoplasmic shuttling of β-catenin antagonists on the [β-catenin/TCF] concentration, which is considered as the output of the Wnt pathway.

4. Nucleo-cytoplasmic shuttling of β-catenin antagonists can lead to a breakdown of retention of cytoplasmic β-catenin and can hence maximise [β-catenin/TCF] signalling.

5. Saturated protein translocation can be modelled by pure diffusion, if the shuttling rate constants are chosen appropriately.

6. Inhibition of the kinase GSK3 is more efficiently enhancing the [β-catenin/TCF] concentration than inhibition of β-catenin's binding to the destruction complex.

7. Balanced nucleo-cytoplasmic shuttling of β-catenin antagonists yields maximal relative response to a transient Wnt signal.

8. Nuclear accumulation of the destruction complex renders the pathway robust against fluctuations in the extracellular Wnt signal and against changes in the compartmental distribution of β-catenin.

9. Sensitivity analysis allows to quantify the total robustness of the [β-catenin/TCF] concentration and demonstrates that the total robustness of [β-catenin/TCF] is linked to its absolute concentration.

10. The described results of the model analyses provide the basis for continuative experimental investigations of antagonist shuttling in the Wnt pathway.

List of publications

Peer-reviewed Journal publications and contributions

The written thesis is mainly based on two publications, which are listed in the following. In both publications, Dr. Katja Rateitschak and I designed the study. I performed the mathematical modelling and analysis, and wrote the manuscript. All authors discussed the results and approved the final article.

- **Y. Schmitz**, O. Wolkenhauer, K. Rateitschak: Nucleo-cytoplasmic shuttling of APC can maximize β-catenin/TCF concentration, *Journal of Theoretical Biology*, 279(1): 132-142, 2011, doi:10.1016/j.jtbi.2011.03.018.

- **Y. Schmitz**, K. Rateitschak, O. Wolkenhauer: Analysing the impact of nucleo-cytoplasmic shuttling of β-catenin and its antagonists APC, Axin and GSK3 on Wnt/β-catenin signalling. *Cellular Signalling*, 25(11): 2210-2221, 2013, doi:10.1016/j.cellsig.2013.07.005.

In addition to the work on Wnt pathway modelling presented here, I participated in and worked on other projects from 2007 to 2012, which led to the following Journal publications.

- **Y. Schmitz**, M. Baurmann, B. Engelen, U. Feudel. Pattern Formation of Competing Microorganisms in Sediments. *Mathematical Modelling of Natural Phenomena*, 2(4) Special Issue: Pattern and waves in ecology and evolution: 74-104, 2007, doi:10.1051/mmnp:2008023.

  The paper is based on my Diploma Thesis, which I wrote under supervision of Dr. Martin Baurmann and Prof. Ulrike Feudel, who together designed the study. I performed the mathematical modelling and analysis and also wrote the manuscript. All authors discussed the results and approved the final manuscript.

- V. Schmidt, K. Baum, A. Lao, K. Rateitschak, **Y. Schmitz**, A. Teichmann, B. Wiesner, C.M. Petersen, A. Nykjaer, J. Wolf, O. Wolkenhauer, T.E. Willnow. Quantitative modeling of amyloidogenic processing and its influence by SORLA in Alzheimer's disease. *The EMBO Journal*, 31(1): 187 - 200, 2012, doi:10.1038/emboj.2011.352.

This publication presents the results generated in an interdisciplinary research project supported by the Helmholtz Society as part of the MDC systems biology network (MSBN: Systems Biology of cardiovascular and neurodegenerative disease processes). I had a major impact on the formulation of the model, the mathematical equations and their analyses. Additionally, I wrote parts of the manuscript dealing with the modelling. All authors discussed the (experimental and theoretical) results and approved the final manuscript.

- A. Lao, V. Schmidt, **Y. Schmitz**, T.E. Willnow, O. Wolkenhauer: Multi-compartmental modeling of SORLA's influence on amyloidogenic processing in Alzheimer's disease. *BMC Systems Biology*, 6(1):74, 2012, doi:10.1186/1752-0509-6-74.

  The model developed in this publication is an (multi-compartment) extension the previous model (Schmidt et al., 2012). The publication is the result of the interdisciplinary research project by the Helmholtz Society as part of the MDC systems biology network (MSBN: Systems Biology of cardiovascular and neurodegenerative disease processes). I supported the work of Angelyn Lao by checking assumptions, programming codes, and results. All authors discussed the results and approved the final manuscript.

- B.M. Bader, **Y. Schmitz**, S.A. Kuznetsov, D.G. Weiss: Spatio-temporal distribution changes and nucleo-cytoplasmic shuttling rates regulate differentiation in human neural progenitor cells. Submitted for publication, 2013.

  This work was performed as part of the DFG-funded research training school 1387 (GRK dIEM oSiRiS). In this project, I provided an automated quantification analysis for subcellular microscopy data to the experimentalists. I had a major contribution in data analyses and interpretation. All authors critically discussed and finally approved the manuscript.

Poster at international conferences

- B.M. Bader, **Y. Schmitz**, B. Redlich, K. Rateitschak, O. Wolkenhauer and D.G. Weiss: Quantitative 3D image analysis to study the β-catenin translocation during differentiation of human neural progenitor cells. - Poster, Dresden, Proceedings of '2nd International Congress on Stem Cells and Tissue Formation', July 2008

- **Y. Schmitz**, B.M. Bader, D.G. Weiss, O. Wolkenhauer and K. Rateitschak: Elucidating the role of nucleo-cytoplasmic shuttling of β-catenin antagonists

by mathematical modelling. - Poster, Summer School on Systems Biology for Medical Applications, Tenerife, Spain, September/October 2008

- **Y. Schmitz**, O. Wolkenhauer, K. Rateitschak: Mathematical modelling of nucleo-cytoplasmic shuttling of β-catenin and its antagonist APC. - Poster, 11th International Conference on Systems Biology (ICSB), Edinburgh (GB), October 2010

- A. Lao, **Y. Schmitz**, V. Schmidt, K. Rateitschak, J. Wolf, T. Willnow, O. Wolkenhauer: Mathematical Modeling of APP Processing influenced by SORLA in Alzheimer's Disease. - Poster, 12th International Conference on Systems Biology (ICSB), Heidelberg/Mannheim (Germany), August/September 2011

- **Y. Schmitz**, K. Rateitschak, O. Wolkenhauer: Mathematical modelling of the Wnt pathway: the influence of nucleo-cytoplasmic shuttling of β-catenin and its antagonists APC, Axin and GSK3. - Poster, 12th International Conference on Systems Biology (ICSB), Heidelberg/Mannheim (Germany), August/September 2011

- A. Lao, V. Schmidt, **Y. Schmitz**, T. Willnow, O. Wolkenhauer: Multi-compartmental modeling of APP processing influenced by SORLA in Alzheimer's disease. - Poster, 13th International Conference on Systems Biology (ICSB), Toronto (Canada), August 2012

- A. Lao, V. Schmidt, **Y. Schmitz**, T. Willnow, O. Wolkenhauer: Regulated Trafficking of APP by SORLA in Alzheimer's Disease. - Poster, Systems Medicine International Conference (SYSMED), Dublin (Ireland), September 2012

Presentations

- Computersimulationen in der Biomedizin-Forschung (with U. Liebal and H. Assmus). - Lange Nacht der Wissenschaft, Rostock, April 2008

- Systems biology of neural differentiation (with B. Bader) - Invited talk, Baltic Winter School on neurodegenerative diseases, Binz, Island of Rügen, December 6, 2008

- The Wnt Signaling pathway – Mathematical modeling and experiments, Paper clinic, Cornell University, Ithaca, NY, USA, May 2009

- Was verbindet Waschmittel, Hautkrebs, Alzheimer, Kraftstoff und Diabetes? (with U. Liebal, U. Schmitz and T. Millat), Lange Nacht der Wissenschaft, Rostock, April 2009 and April 2010

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Academic background

05/2009 - 07/2009	Research Internship at the Cornell University, Ithaca, NY, USA Laboratory of Atomic and Solid State Physics Topic: "Sloppy Parameter Sensitivities in the Wnt Signalling Pathway" Supervisor: Prof. James P. Sethna
since 02/2007	PhD student at the University of Rostock, Rostock, Germany Department of Systems Biology and BioInformatics Thesis title: "Compartmental Modelling of the Wnt Pathway: Elucidating the role of nucleo-cytoplasmic shuttling of β-catenin and its antagonists" Supervisors: Prof. Olaf Wolkenhauer and Dr. Katja Rateitschak
08/2002 - 06/2003	Courses in the masters program of the Department of Physics, as part of an exchange programme University of Liverpool, Liverpool, England
10/1999 - 01/2007	Diplom in Physics (Dipl.Phys.), Carl-von-Ossietzky University of Oldenburg, Oldenburg, Germany Department of Theoretical Physics and Complex Systems Thesis title: "Pattern formation of competing bacteria in sediments: A conceptual model" Supervisors: Prof. Ulrike Feudel and Dr. Martin Baurmann
07/1999	Abitur, Käthe-Kollwitz-Gymnasium, Wilhelmshaven, Germany

Selbstständigkeitserklärung

Ich erkläre hiermit, dass ich die vorliegende Dissertation selbstständig und ohne unerlaubte Hilfe angefertigt habe. Ich versichere, dass ich ausschliesslich die angegebenen Quellen und Hilfsmittel verwendet habe und ich die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Rostock, 06. September 2013 Yvonne Schmitz