Mathematical modelling of Wnt/β-catenin signalling
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Abstract
The Wnt/β-catenin pathway plays an important role in development and disease. Theoretical approaches have been used to describe this pathway and have provided intriguing insights into its signalling characteristics. In the present paper, we review mathematical models of the pathway. We focus on a quantitative kinetic model for canonical Wnt/β-catenin signalling describing the reactions of the pathway's core compounds [Lee, Salic, Krüger, Heinrich and Kirschner (2003) PLoS Biol. 1, 116–132]. Numerous modifications and further analyses with respect to signalling characteristics, transcriptional feedback and cross-talk were performed. In addition, the role of β-catenin in gene expression and cell–cell adhesion as well as spatial aspects of signalling are investigated in various theoretical models.

Introduction
The Wnt/β-catenin pathway regulates the concentration of β-catenin and is critically involved in processes of development, organogenesis, proliferation, regeneration and cell–cell adhesion. Components of this signalling pathway have been found to be mutated in various diseases [1–10].

In the last few years, quantitative and qualitative mathematical modelling approaches have been used to describe this signalling pathway and have provided deeper insights into its functioning in time and space. The starting point was a quantitative model for canonical Wnt/β-catenin signalling based on measurements in Xenopus oocyte extracts [11]. This kinetic model describes the molecular reactions of the pathway’s core compounds. Numerous modifications and further analyses with respect to signalling characteristics, the interplay of its compounds and feedback loops were performed [12–16]. β-Catenin not only may act as a transcriptional regulator, but also is involved in cell–cell adhesion. This bifunctionality of β-catenin and/or spatial effects of signalling are included in various models [17–20]. The cross-talk of the canonical Wnt pathway with other signalling pathways was analysed in the context of development and tumorigenesis [21–23]. A timeline of the models reviewed is shown in Figure 1.

Biological background
β-Catenin is a bifunctional protein that is involved in cell–cell adhesion by binding to E-cadherin and α-catenin as well as in transcriptional regulation [24]. The protein is produced constantly and its degradation is highly regulated. A prerequisite for the degradation of β-catenin is its sequential phosphorylation. Following a priming phosphorylation at Ser45 mediated by CK1α (casein kinase 1α), β-catenin becomes phosphorylated at three further N-terminal serine and threonine residues (Ser33, Ser37 and Thr41 in humans) by GSK3 (glycogen synthase kinase 3). These modifications occur within the so-called destruction complex that contains additional proteins such as phosphatases and the two scaffold proteins APC (adenomatous polyposis coli) and axin-1. Phosphorylated β-catenin is subsequently recognized by β-TrCP (β-transducin-repeat-containing protein), a subunit of the ubiquitin ligase complex, and marked with ubiquitin. This leads to its proteasome-dependent degradation.
A Wnt stimulus results in the decomposition of the destruction complex. As a consequence, less β-catenin is degraded and the protein may enter the nucleus. How β-catenin is translocated into and out of the nucleus is not completely understood and is still under investigation. Nuclear β-catenin interacts with HMG (high-mobility group) box transcription factors of the TCF (T-cell factor)/LEF (lymphocyte enhancer factor) protein family and further co-factors regulating the transcription of various target genes, for example myc, cyclin D1, siamois and FGF4 (fibroblast growth factor 4). Target genes also include components and regulators of the Wnt/β-catenin pathway itself, e.g. Dkk (Dickkopf), β-TrCP, LEF and axin-2. Axin-2 is a negative regulator of this signalling pathway as it is functionally equivalent to axin-1 and may fulfil the same roles. Axin-1 and axin-2 are denoted as axin in the modelling context. Dickkopf acts as an inhibitor of the pathway by interacting with the co-receptor LRP5/6 (low-density-lipoprotein receptor-related protein-5/6).

How the stimulus induces the decomposition of the destruction complex is still the object of intensive research. A Wnt stimulus brings the receptor Frizzled and the co-receptor LRP5/6 into close proximity. Subsequently, several events may take place, including the phosphorylation of LRP5/6 by CK1 and GSK3, recruitment of proteins participating in the destruction complex, e.g. axin and GSK3, and the phosphorylation of the protein Dsh (Dishevelled). Dsh is involved in the recruitment of proteins as well as in the regulation of the receptors within the pathway. This leads to an inhibition of the destruction complex and hence to a reduced degradation of β-catenin. A schematic description of the pathway is given in Figure 2, and more details can be found on the Wnt homepage (http://www.stanford.edu/~rmusse/wntwindow.html).

Besides these canonical processes, cross-talk with non-canonical Wnt pathways, such as Wnt/Ca²⁺ and PCP (planar cell polarity) [25], are described in [26] as well as interactions with other signalling pathways, e.g. EGF (epidermal growth factor), NF-κB (nuclear factor κB), BMP (bone morphogenetic protein), Hedgehog, Notch or PI3K (phosphoinositide 3-kinase) [27–35].

**Insights from a quantitative kinetic model of the pathway**

In a combined experimental and theoretical approach, the groups of Marc Kirschner and Reinhart Heinrich developed a mathematical model of the canonical Wnt/β-catenin signalling pathway [11]. The model is based on comprehensive data measured in *Xenopus* oocyte extracts including concentrations, fluxes and characteristic times. In the model, the interactions of the core compounds β-catenin, axin, APC, GSK3, TCF and Dsh are taken into account. The system is described by a set of 15 coupled ODEs (ordinary differential equations). With the knowledge about concentrations and assumptions about timescales, several differential equations were replaced by algebraic equations [11,15]. The reduced model contains seven ODEs and eight algebraic equations. Additional experiments have been used to validate this model. The subsequent analysis led to numerous new insights into the functioning of the pathway (see also [36]). A crucial observation was the low abundance of axin. The fast axin turnover has a strong effect on the steady state, amplitude and duration of the β-catenin signal, leading to the conclusion that the regulation of the axin turnover could be an effective intervention point for drugs. This was emphasized by a sensitivity analysis of the system indicating whether processes or compounds have oncogenic or tumour-suppressor effects. The prediction was confirmed recently by the finding of new inhibitors of axin degradation as potent drugs to reduce the concentration of β-catenin [37,38]. It was discussed that the low axin concentration not only limits the β-catenin degradation, but also might have another implication. As its concentration is very
Without a Wnt stimulus, $\beta$-catenin is constantly degraded by the destruction complex. This complex contains APC, axin and GSK3. A Wnt stimulus leads to its disruption. Hence, less $\beta$-catenin is degraded. The protein may translocate into the nucleus regulating the expression of various target genes, including components or regulators of the Wnt/$\beta$-catenin pathway itself. These regulations act on different levels, indicated by broken lines. $\beta$-Catenin is also able to bind E-cadherin participating in the cell-cell adhesion processes.

Further analysis of the core model led to the experimentally confirmed prediction that the scaffold proteins APC and axin contribute differently to the formation of the degradation complex. Whereas APC binds its interaction partners in an ordered manner, axin binds them randomly [11]. Moreover, it was discussed that variations in the APC level can be compensated for by APC-dependent axin degradation. This opens the possibility of stabilizing the signalling pathway under conditions of decreased APC levels. The theoretical investigation also indicated the potential importance of non-axin-dependent $\beta$-catenin degradation under conditions of low APC concentration.

The dynamics of $\beta$-catenin is determined by the binding affinities of the pathway compounds. Those affinities can be modified. For example, the binding of axin and GSK3 can be enhanced by the phosphorylation of axin by CK1, resulting in a lower TCF-mediated transcription activity. It was demonstrated experimentally that PP1 (protein phosphatase 1) has an opposite effect according to model results [39].

A recently published experimental and theoretical analysis focused on the question of what the cellular reporter of the Wnt/$\beta$-catenin pathway is [14]. On the basis of a dimensional analysis of the core model, the reactions were divided into three subgroups: input, degradation and synthesis. In a detailed theoretical investigation, it was shown that the fold change of $\beta$-catenin, defined as the ratio of the stimulated and the unstimulated steady-state concentration, is quite robust against small perturbations in the degradation module. In contrast, other signalling characteristics, such as the absolute level of $\beta$-catenin, the difference between stimulated and unstimulated steady state, response time, integrated level, integrated difference and integrated fold change, are sensitive to perturbations in all modules. Therefore the $\beta$-catenin fold change was considered to be the relevant readout of the signalling pathway. In that way, variations in concentrations or kinetic parameters can be buffered to a certain extent. It was experimentally proven that the fold change of $\beta$-catenin is relevant for the phenotype. Experiments for two target genes confirm that their expression is insensitive to moderate perturbations in the degradation module. This shows that biological target genes can behave differently from artificial $\beta$-catenin reporters such as TOPflash. It was demonstrated that, on the gene regulation level, detection of the fold change may be provided by the motif of an incoherent feedforward loop under appropriate conditions [40]. Such a motif was found for the regulation of c-myc and E2F1 [41] and has been identified as one of the most common network motifs. Other motifs could also provide fold change detection.
Analyses of transcriptional feedback loops and cross-talk to other signalling pathways

Several gene products of the Wnt/β-catenin pathway participate in the pathway itself, opening the possibility for positive and negative autoregulation (see Figure 2). The feedback loop via axin-2 was included in several modelling approaches [12,13,21]. Its effect with respect to the β-catenin steady state was studied in various mutants [12]. The increase in the β-catenin steady state arising solely from mutations was discussed as a balanced outcome between pathway activation and increased pathway inhibition by axin-2 feedback.

Transcriptional feedbacks may give rise to oscillatory behaviour [42]. Interestingly, axin-2 was shown to play a critical role in the segmentation clock that is a molecular oscillator determining the timing of somitogenesis in vertebrates. Besides the Wnt/β-catenin pathway, other signalling pathways such as Notch and FGF are involved in that clock [43,44]. Several models focus on the occurrence and characteristics of oscillations in Wnt signalling. The work of Wawra et al. [13] extended the core model of Lee et al. [11] by feedback loops for axin-2 and Dkk. The two feedback loops reinforce their effect on the β-catenin steady state and the oscillations. It was shown that, for sustained oscillations, the involvement of the two feedback loops is not sufficient, but also the parameters of the core model, especially the β-catenin throughput, have to be adapted.

The possibility of oscillations was also analysed in models combining the transcriptional feedback via axin-2 and cross-talk to Notch and FGF signalling. These approaches describe the Wnt/β-catenin pathway by reduced models, using either ODEs [21] or delay differential equations [22]. Oscillations can arise in the separated as well as linked pathways where the cross-talk determines the phase relationship [21].

Interactions with other signalling pathways are also analysed with respect to tumorigenesis. The cross-talk of Wnt/β-catenin and ERK (extracellular-signal-regulated kinase) signalling results in a positive-feedback loop between both pathways and can generate a switch between two stable steady states. As a consequence, mutations in one pathway have an impact on the behaviour of the other pathway. They may lead to a sustained activation of both pathways even though the stimulus is removed. This may contribute to tumorigenesis and has to be considered for the development of effective drugs [23]. For the analysis of larger signalling networks, a further reduction of the Wnt/β-catenin core model might be advantageous. A first minimal model derived by timescale analyses describes the of β-catenin dynamics very well [16].

Models taking the binding of β-catenin to E-cadherin and spatial aspects into account

A model including E-cadherin was used to address the questions of how the binding of β-catenin to E-cadherin alters its availability for gene expression and how the distribution between the two roles of β-catenin is regulated [17]. An increase in the synthesis rate of E-cadherin enhances the cell–cell adhesion and may transiently decrease the expression of target genes. In cases of mutations in β-catenin or APC, both the target gene expression and the cell–cell adhesion are increased. This model was embedded in a multiscale approach combining the subcellular, cellular and tissue levels of organization to investigate the dynamics of intestinal tissue renewal. On the cellular level, the contribution of Wnt signalling to the interactions of neighbouring cells and to gene expression and subsequent progression through the cell cycle was studied [18].

The interaction of β-catenin with E-cadherin and its impact in tumorigenesis are analysed in a model including the interactions for a cell and a cell layer [19]. It was predicted that more β-catenin is bound to E-cadherin in cells in the centre of a tumour, whereas more β-catenin is available for signalling in cells at its border. The theoretical β-catenin distribution obtained confirmed experimental observations (see [28]). Spatial effects were also investigated within the scope of epidermal appendages [20]. For this analysis, a reaction–diffusion model concentrating on the interplay of Wnt and Dkk was used. Details of the signalling pathway were neglected.

Open questions

The mathematical modelling of the intra- and inter-cellular Wnt/β-catenin signalling led to important insights into the functioning of the pathway. However, several aspects have not been addressed by modelling. One is the intracellular compartmentation. β-Catenin and other proteins of the pathway act in the cytoplasm and nucleus and bind to the plasma membrane. This raises questions about the distribution of the concentrations and activities in the compartments and the shuttle between them. Additional processes such as receptor activation, cross-talk to other signalling pathways or Wnt secretion are objects of intensive experimental research and will influence future modelling approaches.

Modelling was applied to the analysis of disease states, mainly tumorigenesis so far. It led to the prediction of effective intervention points and was recently confirmed by the finding of new drug candidates. The characteristics and regulation of the pathway in other diseases and aging remain to be investigated. A critical point will be the experimental investigation of the pathway in different cell types and tissues. So far, all quantitative modelling attempts are based on the detailed kinetic measurements in *Xenopus* extracts.

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