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Nucleo-cytoplasmic shuttling of APC can maximize β -catenin/TCF concentration

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Abstract

β -catenin is the key player of the canonical Wnt pathway. Its activity is mainly regulated via protein degradation. In the nucleus, its interaction with TCF initiates target gene expression. Although the functional relevance is unclear, it has been shown that β -catenin antagonists are also capable of nucleo-cytoplasmic shuttling. The focus of our systems biology analysis lies on the β -catenin subcellular distribution regulated by the antagonist and scaffolding protein APC. We address the following questions: Can the concentration of the transcription factor complex [β -catenin/TCF], which is considered as the output of the pathway, be maximized by APC nucleo-cytoplasmic shuttling and how is retention of β -catenin by APC influencing this output?

We established a mathematical model based on experimental findings to examine the influence of nucleo-cytoplasmic shuttling of APC and retention of β -catenin by APC on the output of the pathway. The model is based on ordinary differential equations and includes protein shuttling between the two compartments nucleus and cytoplasm as well as protein complex formation in each compartment. We discuss how the steady state concentration of [β -catenin/TCF] is influenced by APC shuttling and retention. The analysis of the model shows that the breakdown of β -catenin cytoplasmic retention induced by APC shuttling can enhance nuclear accumulation of β -catenin and hence maximize the output of the pathway.

Using mathematical modelling, we demonstrate that in certain parameter ranges, the steady state concentration of [β -catenin/TCF] benefits from APC shuttling. The inhibitory effect of APC is alleviated due to shuttling of APC. Surprisingly, our study therefore indicates that the nucleo-cytoplasmic shuttling of APC can have a beneficial effect on the output of the pathway in steady state, although APC is in general a β -catenin antagonizing protein. Furthermore, we show 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.

Keywords:

mathematical model, intracellular transport, steady state analysis, Wnt pathway, retention

1 Introduction

1.1 The Wnt Signalling Pathway

The canonical Wnt signalling pathway has been studied extensively, since it has been experimentally proven to have a major impact on cell proliferation, migration and fate decisions as apoptosis and differentiation [1–4]. Dysfunctions within the pathway can lead to serious consequences, such as osteoporosis, cancer, and neurodegenerative diseases (reviewed in [5–9]).

The protein β -catenin is the central signalling molecule of the canonical Wnt signalling pathway [10]. Its complex formation in the nucleus with transcription factors from the LEF/TCF family activates target genes [11, 12]. The principal regulatory mechanism that controls the nuclear accumulation of β -catenin is the activity of the so-called destruction complex, consisting of Axin, Adenomatous polyposis coli (APC) [13] and the kinases glycogen synthase kinase 3 (GSK3) [14, 15] as well as casein kinase 1 (CK1) [16]. As these proteins counteract β -catenin, we refer to them as β -catenin antagonists. In the absence of a Wnt signal (the ‘off’ state; see Figure 1, left), the destruction complex phosphorylates β -catenin. Thereby, β -catenin is targeted for ubiquitylation and subsequent degradation by the proteasome [17]. Thus, in the ‘off’ state β -catenin levels are kept low. Upon binding of Wnt ligands to their receptors Frizzled [18] and LRP5/6 [19], the pathway is activated (Figure 1, right). The presence of an extracellular Wnt stimulus (‘on’ state) inhibits the activity of the destruction complex. Thereby it induces cytoplasmic β -catenin accumulation and β -catenin’s entry into the nucleus, where it acts as a transcriptional co-factor [20]. However, not only β -catenin is able to translocate into and out of the nucleus. A surprisingly high number of cytoplasmic Wnt regulators are shuttling proteins that distribute both, in the cytoplasm and the nucleus, where they may, directly or indirectly, interact with β -catenin and modulate its transcriptional activity [21]. This holds especially for the β -catenin antagonists APC [22, 23], Axin [24, 25] and the kinases GSK3 [26–28] and CK1 [21].

Next we give a short introduction to nucleo-cytoplasmic shuttling mechanisms and we summarize experimentally derived hypotheses on β -catenin and APC shuttling.

1.2 Nucleo-cytoplasmic shuttling

The contents of the eucaryotic nucleus are separated from the cytoplasm by the nuclear envelope. All molecules enter or exit the nucleus through nuclear pore complexes (NPCs) [29]. Small molecules ($\lesssim 50$ kDa [30, 31]) can diffuse freely across the NPC, while large macromolecules in general are transported if they exhibit a particular transport signal sequence, such as the nuclear localization signal (NLS) or

nuclear export signal (NES) [29]. These sequences are required for nucleo-cytoplasmic transport mediated by importin/exportin receptors, such as importin- β and CRM1, respectively [32]. The process of actual translocation across the NPC generally occurs either by diffusion or facilitated transport [32].

1.3 β -catenin and APC shuttle using different mechanisms

Unlike NLS-mediated import and despite β -catenin's ~ 90 kDa, it enters the nucleus independently of the importin receptor. Instead, β -catenin can interact directly with the NPC and can translocate on its own [33,34]. This is possible because, like importin- β , β -catenin contains several HEAT-like repeats, which are both, necessary and sufficient for nuclear import [32]. Its nuclear export is "rather nebulous and somewhat controversial" [35]. 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 [36,37]. On the other hand, it has been proposed to bind to APC or Axin to exit the nucleus [22–25,38,39]. APC is a large ~ 310 kDa protein. It exhibits at least two NES interacting with the CRM1 nuclear export factor and has been proven to shuttle between cytoplasm and nucleus [22,23], but the functional relevance of this is still controversial [40–42]. It was proposed that the nuclear export of APC controls the level and hence the transcriptional activity of nuclear β -catenin [38,39]. Most recent findings, however, indicate that APC (and also Axin and Axin2) enriches β -catenin in the cytoplasm, but does not accelerate the nucleo-cytoplasmic shuttling of β -catenin, i.e. increases the rate of β -catenin nuclear import or export [43]. From their results, Kriehoff et al. [43] concluded that β -catenin antagonists, such as APC, mainly regulate β -catenin subcellular localization by retaining it in the compartment in which they are localized, rather than by active transport into or out of the nucleus.

1.4 Scope of the paper

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 as a β -catenin antagonist. Different experiments resulted in different hypotheses. The functional relevance of antagonist shuttling is yet to be determined.

Contrary to the so far mentioned experimentally focussed studies, we choose a theoretical approach. In the literature one can find several mathematical models describing the canonical Wnt signalling pathway. The models are based on ordinary differential equations and focus on different aspects and influences of the

pathway (e.g. [44–48]). However, none of them accounts for nucleo-cytoplasmic shuttling of Wnt pathway components.

In our work we investigate the impact of nucleo-cytoplasmic APC shuttling on the β -catenin/TCF concentration using mathematical modelling. Thus, our model mimics an activated Wnt pathway in steady state, as β -catenin levels are kept very low in the Wnt-off state (see Figure 1). We neglect β -catenin degradation and membrane associated events, as we focus on the influence of APC shuttling on the β -catenin/TCF concentration. The experimental findings obtained by Kriehoff et al. [43] provide the structural basis of our model: β -catenin and APC shuttle independent of each other; the complexes are however unable to cross the nuclear envelope. Our key questions of interest are: Does the nucleo-cytoplasmic shuttling of APC influence the [β -catenin/TCF] concentration, which we consider as the output of Wnt signalling? Can the [β -catenin/TCF] concentration be maximized by APC shuttling? How is the output of the pathway influenced by cytoplasmic and nuclear retention of β -catenin?

2 Results and Discussion

2.1 Nucleo-cytoplasmic shuttling of β -catenin and APC

We established a simple biochemical reaction network describing nucleo-cytoplasmic shuttling of β -catenin (β -cat), the main protagonist of the canonical Wnt signalling pathway, and its antagonists APC. The biochemical reaction network is depicted in Figure 2. Both key players are able to shuttle between the nucleus and the cytoplasm (ν_1 and ν_2 , respectively) [22, 36, 39]. 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) [43]. These complexes, however, are not able to cross the nuclear envelope [36, 37, 43].

Additionally, nuclear β -catenin can interact with TCF (ν_5) to activate transcription [24]. We hence consider the complex [β -cat/TCF] as the output of both, the Wnt pathway [44] and our shuttling model.

Next, we translated the reaction network into a system of ordinary differential equations (ODEs) describing temporal changes of protein concentrations as functions of interactions and transport processes. The simplifying assumptions are presented in the Methods section. The network depicted in Figure 2 includes neither a time dependent input nor output. Thus, we can study it in steady state. The calculation of the steady state concentration for the network components is presented in the Methods section.

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 [32,36]. We 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 [22,23]. In our approach, we investigate the influence of nucleo-cytoplasmic shuttling of APC on the output of the Wnt signalling by studying different shuttling mechanisms:

In a first step, we 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. We then study nucleo-cytoplasmic shuttling of APC and its effect on the output of the pathway. For the sake of simplicity, we first assume diffusion of APC across the nuclear envelope as proposed for nucleo-cytoplasmic shuttling of β -catenin. We 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 [44], see Table 1. These numbers serve as the default parametrization and hence form the starting point for the investigation of the effect of nucleo-cytoplasmic antagonist shuttling. Next, we analyze the influence of cytoplasmic and nuclear retention of β -catenin by APC, by varying the respective dissociation constants. Then, we 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.

2.2 Absence of APC shuttling

Absence of APC shuttling is described by setting the antagonist shuttling constants to zero (see Eqs.(16)). The steady state analysis of the ODE model is performed in the Methods section and leads to the dependency of the steady state concentration $[\beta\text{-cat}/\text{TCF}]^*$ (see Table 2) on the ratio of rate constants for β -catenin nucleo-cytoplasmic shuttling ($K_1 = \frac{k_{-1}}{k_1}$) depicted in Figure 3a. 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 (Eqs.(17)). 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 maximized 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 $[\beta\text{-cat}/\text{TCF}]^* \sim 19$ 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 of the plateau.

In Figure 3a, the blue curve is obtained using the default parametrization (Table 1). 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 parametrization, β -catenin's binding to TCF and to APC occur equally likely. We see that 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. Our results therefore show that cytoplasmic retention of β -catenin by its antagonist APC influences the output of the model without antagonist shuttling.

2.3 Diffusive nucleo-cytoplasmic shuttling of APC

In the following, we investigate the effects of nucleo-cytoplasmic antagonist shuttling on the steady state concentration $[\beta\text{-cat/TCF}]^*$. First, we 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, see Eqs.(19).

The standard (default) parametrization

We start by setting all dissociation constants and total protein concentrations to their default values (see Table 1). As a result of the steady state analysis (see Methods), we obtain that $[\beta\text{-cat/TCF}]^*$ depends on the biochemical binding and shuttling parameters. The steady state concentration $[\beta\text{-cat/TCF}]^*$ as a function of the nucleo-cytoplasmic shuttling rate constant ratios (K_1 and K_2) is presented in Figure 3b.

In this parametrization, 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 3a. 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 parametrization, the steady state concentration $[\beta\text{-cat/TCF}]^*$ 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 1), 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 $[\beta\text{-cat/APC}]^*$ 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, we study the question, whether lower dissociation constants can account for a greater impact of APC shuttling on the steady state concentration $[\beta\text{-cat/TCF}]^*$ in comparison to the values of the reference model.

The impact of the dissociation constants

For the default parametrization, the affinity of β -catenin to bind to APC is low: $K_{3,4} = 1200$ nM (experimentally based estimation by the Kirschner group [44]). However, it has been demonstrated that phosphorylation enhances the ability of APC to interact with β -catenin in vitro [49]. Moreover, Sierra et al. [50] 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 proposed in [51] that accumulation of cytoplasmic β -catenin induces phosphorylation of APC and that phosphorylated APC retains β -catenin. In accordance with these findings, we 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. We choose these specific values to account for a “fair” competition between TCF and APC for β -catenin binding. It is still selected arbitrarily. However, our 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, we change the two different parameter values independently: we first decrease K_3 to consider an enhanced cytoplasmic retention and second decrease K_4 to account for an enhanced nuclear retention of β -catenin by APC. An additional justification for different dissociation constants in nucleus and cytoplasm could also be different biochemical compositions of the two compartments. In a last step we 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 presented in the Methods section leads to the dependency of $[\beta\text{-cat/TCF}]^*$ on the nucleo-cytoplasmic shuttling rate constant ratios presented in Figure 3c. 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 parametrization. In contrast to the default case presented in Figure 3b 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 $[\beta\text{-cat}/\text{TCF}]^*$ 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 $[\beta\text{-cat}/\text{TCF}]^*$ 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 $[\beta\text{-cat}/\text{TCF}]^*$ is larger than the reference concentration of $[\beta\text{-cat}/\text{TCF}]^*$ (Figure 3c). Thus, nucleo-cytoplasmic shuttling of APC can increase the output of Wnt signalling in comparison to the reference model. We can explain our results as follows (see Figure 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 of β -catenin can bind to APC (see Figure 4f). Thus, most of the APC proteins remain free and unbound in the nucleus (Figure 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 4b), hence the concentration of $[\beta\text{-cat}/\text{APC}]$ in the cytoplasm is small (Figure 4c). As a result, more β -catenin remains unbound to APC (Figure 4a and 4d) and can hence bind to TCF (Figure 3c). Cytoplasmic retention of β -catenin is down-regulated due to APC nucleo-cytoplasmic shuttling. Therefore, in this parametrization Wnt signalling benefits from antagonist shuttling.

In order to investigate the impact of nuclear retention, we 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 3d. The steady state concentration of $[\beta\text{-cat}/\text{TCF}]^*$ 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 3a). The curves show that the output of our diffusive shuttling model can only be reduced with these parameter values, in contrast to the results presented in Figure 3c. This is due to an enhanced competition of APC and TCF for β -catenin binding in the nucleus.

Next, we choose all three dissociation constants to have the same value ($K_3 = K_4 = K_5 = 30$ nM). The

steady state concentration $[\beta\text{-cat}/\text{TCF}]^*$ is shown in Figure 3e. 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 $[\beta\text{-cat}/\text{TCF}]^*$ 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 $[\beta\text{-cat}/\text{TCF}]$ signalling can even benefit from APC shuttling if β -catenin is retained by APC also in the nucleus. The intersection point in Figure 3e 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 $[\beta\text{-cat}/\text{TCF}]^*$. Our results demonstrate that the shuttling of the antagonist can yield an enhanced output of the system. We have shown 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 [49]. Phosphorylation of APC can be induced by a high cytoplasmic β -catenin concentration [51]. 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 $[\beta\text{-cat}/\text{APC}]$ concentration is high due to enhanced binding affinity between the two proteins in the nucleus. Therefore the question arises, if $[\beta\text{-cat}/\text{TCF}]^*$ can further be maximized by saturated nuclear export and low binding affinity of β -catenin and APC in the nucleus, which is studied in the following section.

2.4 Facilitated export of APC

Facilitated export of APC is modelled by a Michaelis-Menten type function (see Eqs.(24)). This is reasonable, since APC exhibits a NES [22,23], 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 [52]. 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 maximization of the output of the system. Therefore, and for the sake of simplicity, we restrict our analysis to the saturation of nuclear export. The nuclear import of APC remains diffusive. The steady state analysis of the ODE model is presented in the Methods section. For our analysis, we set $K_3 = K_5 = 30$ nM, $K_4 = 1200$ nM and $K_1 = 1$, as chosen in the previous sections.

For facilitated export, two extreme cases can be considered: First, for $k_m \gg X_4$, the nuclear export of APC changes linearly with the concentration of nuclear APC (X_4), which corresponds to diffusive shuttling. In this case, we obtain

$$K_{lin} = \frac{v_{max}}{k_m k_2} = K_2 \quad (1)$$

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

$$K_{sat} = \frac{v_{max}}{k_2}. \quad (2)$$

Note that K_{sat} is independent of k_m . In Figure 3f, the dependency of $[\beta\text{-cat/TCF}]^*$ on the parameters describing the shuttling of APC is shown.

In the linear extreme case $k_m \gg X_4$, our results depending on K_{lin} are equal to the results obtained assuming diffusive APC export (left border of the scatter-plot), which meets our expectations (see Eq.(1)). 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 $[\beta\text{-cat/TCF}]^*$ is completely determined by K_{sat} (not shown), as expected from Eq.(2). In this case, export is saturated, hence more free APC is located in the nucleus and the steady state solution we obtain is located on the high plateau, which is in agreement with our results for the diffusive model. However, the maximum value of $[\beta\text{-cat/TCF}]^* \approx 14$ nM cannot be exceeded due to saturated export. We therefore conclude that the concentration of $[\beta\text{-cat/TCF}]^*$ cannot be further maximized in response to facilitated and hence saturated export of APC.

We have also considered facilitated import. This results in a more complicated system which is numerically more challenging, but did not gain new insights.

Our 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.

3 Conclusions

Using mathematical modelling, we 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 $[\beta\text{-cat/TCF}]$ concentration, which we consider as the output of the pathway? Can the concentration of $[\beta\text{-cat/TCF}]$ benefit from APC shuttling? How is the output influenced by cytoplasmic and nuclear retention of β -catenin by APC?

To answer these questions, we 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 model based on ordinary differential equations (ODEs). We 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, we studied different shuttling mechanisms.

The analysis of the ODE model leads to the following results: The steady state concentration of $[\beta\text{-cat/TCF}]$ 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 maximization of the transcription factor complex $[\beta\text{-cat/TCF}]$ in contrast to a reference model without APC shuttling. This effect is also visible if the nuclear concentration of $[\beta\text{-cat/APC}]$ is high due to enhanced binding affinity between the two proteins in the nucleus. Thus our findings show that Wnt signalling can benefit from nucleo-cytoplasmic shuttling of APC.

We propose the following mechanism to explain our results: 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 [51]. From their results, Seo and Jho [51] concluded that this is to regulate excessive $[\beta\text{-cat/TCF}]$ signalling. Our results, however, allow for an alternative interpretation as we 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 $[\beta\text{-cat/TCF}]$ signalling. The breakdown of cytoplasmic retention by APC can even lead to an enhanced $[\beta\text{-cat/TCF}]$ concentration, if phosphorylated APC retains β -catenin also 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, our study indicates that the nucleo-cytoplasmic shuttling of APC has a beneficial effect on the steady state output of pathway, although APC is an antagonizing 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, we investigated facilitated nuclear export of APC, which is based on a Michaelis Menten type rate law. We showed that maximization of the output, which we have obtained by considering diffusive shuttling of β -catenin and APC, cannot be further increased by facilitated antagonist export. Our 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 can be simplified.

4 Methods

4.1 Mathematical modelling of β -catenin and APC shuttling

The biochemical reaction network is presented in Figure 2. We have translated this network into a system of ordinary differential equations (ODEs). Each protein and protein complex concentration is represented by a variable in the mathematical model (see Table 2). Taking the five interactions into account, the dynamics of the biological system, i.e. the time-dependent changes of the concentrations of the proteins and protein complexes are determined by the following eight coupled ODEs:

$$\begin{aligned}
 \dot{X}_1 &= -\nu_1 - \nu_3 \\
 \dot{X}_2 &= -\nu_2 - \nu_3 \\
 \dot{X}_3 &= +\nu_1 - \nu_4 - \nu_5 \\
 \dot{X}_4 &= +\nu_2 - \nu_4 \\
 \dot{X}_5 &= +\nu_3 \\
 \dot{X}_6 &= +\nu_4 \\
 \dot{X}_7 &= -\nu_5 \\
 \dot{X}_8 &= +\nu_5
 \end{aligned} \tag{3}$$

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:

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

Binding and dissociation processes (ν_3 , ν_4 and ν_5) are described with mass-action kinetics [53]. The functions $F_1(X_1, X_3)$ and $F_2(X_2, X_4)$ describe 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, we consider different translocation mechanisms which are presented in the following.

4.2 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: We assume that the binding and dissociation processes considered in the network (i.e. β -catenin to APC and β -catenin to TCF, see Figure 2) will approach the quasi-equilibrium rapidly, leading to:

$$K_3 = \frac{X_1 X_2}{X_5} = \frac{k_{-3}}{k_3}\tag{5}$$

$$K_4 = \frac{X_3 X_4}{X_6} = \frac{k_{-4}}{k_4}\tag{6}$$

$$K_5 = \frac{X_3 X_7}{X_8} = \frac{k_{-5}}{k_5}.\tag{7}$$

The biochemical reaction network (see Figure 2) implies the existence of three conservation equations. This means, that total amounts of molecules are conserved. In our schema these conserved quantities correspond to the total number of molecules of β -catenin, APC, and TCF. If more than one compartment is considered like in our case, 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, we 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:

$$\text{total } \beta\text{-catenin:} \quad 2B = X_1 + X_3 + X_5 + X_6 + X_8 \quad (8)$$

$$\text{total APC:} \quad 2A = X_2 + X_4 + X_5 + X_6 \quad (9)$$

$$\text{total TCF:} \quad 2T = X_7 + X_8. \quad (10)$$

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 (Eq.(10)) and the equilibrium condition for β -catenin to TCF binding (Eq.(7)). The combination of both yields

$$X_7 = \frac{2K_5T}{K_5 + X_3} \quad \text{and} \quad X_8 = \frac{2TX_3}{K_5 + X_3}. \quad (11)$$

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 (Eqs.(5) and (6)).

$$X_5 = \frac{X_1X_2}{K_3} \quad \text{and} \quad X_6 = \frac{X_3X_4}{K_4}. \quad (12)$$

4.3 Differential equations for the remaining variables

Substituting the dependent variables of Eqs.(11) and (12), into the ODE system (3), the dynamics of the remaining variables are determined by the nucleo-cytoplasmic reaction rates:

$$\begin{aligned} \dot{X}_1 &= -F_1(X_1, X_3) = -\dot{X}_3 \\ \dot{X}_2 &= -F_2(X_2, X_4) = -\dot{X}_4. \end{aligned} \quad (13)$$

The connection between the variables is given by the conservation relations of β -catenin and APC, described by Eqs.(8) and (9), where the dependent variables of Eqs.(11) and (12) have been substituted:

total β -catenin:

$$2B = X_1 + X_3 + \frac{X_1 X_2}{K_3} + \frac{X_3 X_4}{K_4} + \frac{2T X_3}{K_5 + X_3} \quad (14)$$

total APC:

$$2A = X_2 + X_4 + \frac{X_1 X_2}{K_3} + \frac{X_3 X_4}{K_4} \quad (15)$$

Equations (13) – (15) depend on four variables (X_1, \dots, 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 Eqs.(11) and (12).

4.4 Steady states of the ODE model

Since we investigate a closed system without a stimulus, transcription or degradation, all transport processes are in steady state. To determine the steady states, i.e. the stationary solutions of the model, we calculate the states in which the time derivatives vanish: $\dot{X}_i = 0$. We annotate the steady state concentration by an asterisk. By considering the conservation equations and substituting X_1^* and X_2^* (Eqs.(13)), we obtain two algebraic equations, that depend on X_3^* and X_4^* . Independent of the transport mechanism we choose, 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 systems behavior. Therefore we determine the stationary solution 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.). We investigated parameter space by generating randomized parameter values for those parameters describing nucleo-cytoplasmic shuttling. In the following we consider and analyze different transport mechanisms.

No antagonist shuttling

This system serves as a control in order to investigate the influence of antagonist shuttling on $X_8^* = [\beta\text{-cat}/\text{TCF}]$. In this case, the antagonist APC resides in the cytoplasm and is not able to cross the nuclear envelope.

$$\begin{aligned} F_1(X_1, X_3) &= k_1 X_1 - k_{-1} X_3 \\ F_2(X_2, X_4) &= 0 \\ X_4 &= X_6 = 0 \end{aligned} \quad (16)$$

Hence, the system depicted in Figure 2 simplifies significantly. The system reduces from eight to six variables.

In steady state we find:

$$\begin{aligned} X_1^* &= \frac{k_{-1}}{k_1} X_3^* = K_1 X_3^* \\ X_2^* &= \frac{2K_3 A}{K_3 + X_1^*} \\ X_5^* &= \frac{2A X_1^*}{K_3 + X_1^*} \end{aligned} \quad (17)$$

Substituting these solutions into Eq.(14), we obtain the steady state solutions as the roots of a cubic polynomial with respect to X_3^* :

$$\begin{aligned} &X_3^{*3} \left[(K_1 + 1)K_1 \right] \\ &+ X_3^{*2} \left[2K_1(A - B + T) + (K_1 + 1)(K_3 + K_1 K_5) \right] \\ &+ X_3^* \left[2K_1 K_5(A - B) + 2K_3(T - B) + K_3 K_5(K_1 + 1) \right] \\ &- 2BK_3 K_5 = 0. \end{aligned} \quad (18)$$

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 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 behavior can be expected when crossing bifurcation points. However, in every parametrization two of the three steady states solutions exhibit at least one negative protein concentration. We 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.

Free diffusive shuttling of APC

The simplest assumption for nucleo-cytoplasmic shuttling is diffusion, which is described by a linear rate equation:

$$\begin{aligned} F_1(X_1, X_3) &= k_1 X_1 - k_{-1} X_3 \\ F_2(X_2, X_4) &= k_2 X_2 - k_{-2} X_4 \end{aligned} \quad (19)$$

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 we obtain the following relations:

$$\begin{aligned} X_1^* &= \frac{k_{-1}}{k_1} X_3^* = K_1 X_3^* \\ X_2^* &= \frac{k_{-2}}{k_2} X_4^* = K_2 X_4^* \end{aligned} \quad (20)$$

By considering the conservation equations (14) and (15) and substituting X_1^* and X_2^* as presented in Eqs.(20), we obtain two algebraic equations, which depend on X_3^* and X_4^* . Solving Eq.(15) for X_4^* and substituting into Eq.(14) 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^* :

$$\begin{aligned} &X_3^{*3} \left[(k_1 + 1)C_1 \right] \\ &+ X_3^{*2} \left[2C_1(A - B + T) + C_2 + (K_1 + 1)K_5C_1 \right] \\ &+ X_3^* \left[2K_5C_1(A - B) + 2(T - B)(K_2 + 1) + C_2K_5 \right] \\ &- 2K_5B(K_2 + 1) = 0. \end{aligned} \quad (21)$$

where

$$C_1 = \left(\frac{K_1 K_2}{K_3} + \frac{1}{K_4} \right) \quad (22)$$

and

$$C_2 = (K_1 + 1)(K_2 + 1). \quad (23)$$

We 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, we again obtain only one positive, and therefore biological meaningful solution.

Facilitated nuclear 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 [32]. 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 [54].

$$\begin{aligned} F_1(X_1, X_3) &= k_1 X_1 - k_{-1} X_3 \\ F_2(X_2, X_4) &= k_2 X_2 - \frac{v_{max} X_4}{k_m + X_4} \end{aligned} \quad (24)$$

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

$$\begin{aligned} X_1^* &= \frac{k_{-1}}{k_1} X_3^* = K_1 X_3^* \\ X_2^* &= \frac{v_{max} X_4^*}{k_2(k_m + X_4^*)} \end{aligned} \quad (25)$$

Substituting X_1^* and X_2^* in Eqs.(14) and (15), we obtain two algebraic equations, which depend on X_3^* and X_4^* . Considering Eq.(15) with its substitutes, we see the following equation in which X_3^* (i.e. nuclear β -catenin) is still depending on X_4^* :

$$X_3^* = \frac{2Ak_2k_m + C_3X_4^* - k_2X_4^{*2}}{X_4^*(C_4 + \frac{k_2}{K_4}X_4^*)} \quad (26)$$

where

$$C_3 = 2Ak_2 - k_2k_m - v_{max} \quad (27)$$

and

$$C_4 = K_1v_{max} + \frac{k_2k_m}{K_4}. \quad (28)$$

Substituting these equations into the conservation equation (14), we end up with a polynomial of 6th order with respect to X_4^* , which we omit to write down. However, we may obtain up to six different roots and therefore steady state solutions. We 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.

Authors contributions

K.R. designed the study; Y.S. performed the mathematical modelling and analysis; Y.S. and K.R. wrote the manuscript; Y.S., O.W. and K.R. discussed the results and approved the final article.

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Figures

Figure 1 - The Wnt signalling pathway

Left: The Wnt-off state. Cytoplasmic degradation keeps β -catenin levels low. Right: The Wnt-on state. The destruction complex is inhibited. β -catenin and its antagonists, for example APC can shuttle between cytoplasm and nucleus. In the nucleus, β -catenin forms a transcription factor complex with TCF.

Figure 2 - Biochemical reaction network

Biochemical reaction network describing β -catenin and APC nucleo-cytoplasmic shuttling and protein complex formation of β -catenin and APC as well as β -catenin and TCF. The reactions are numbered 1 to 5. In Table 2 the protein names are translated into state variables (X_1 to X_8) as used in the Methods section.

Figure 3 - Influence of nucleo-cytoplasmic shuttling of APC on $[\beta\text{-cat/TCF}]^*$

The steady state concentration $[\beta\text{-cat/TCF}]^*$ is depicted in dependence on the ratio of shuttling rate constants of β -catenin (K_1) and APC (K_2). **a)** The reference model without APC shuttling. The blue curve is obtained using the default parametrization (Table 1); for the green curve we chose $K_3 = K_5 = 30$ nM to account for increased cytoplasmic retention. **b-e)** Diffusive APC shuttling: **b)** The default parametrization: The black curve is equal to the blue curve in Figure 3a. For this parametrization, the steady state analysis indicates that the shuttling rate constant ratio of β -catenin has a much higher impact on $[\beta\text{-cat/TCF}]^*$ than the ratio of shuttling rate constants of APC. **c)** $K_3 = 30$ nM. The black curve is equal to the green curve in Figure 3a: The steady state concentration of $[\beta\text{-cat/TCF}]^*$ crucially depends not only on β -catenin, but also on APC shuttling. **d)** $K_4 = 30$ nM: The black curve is equal to the green curve in Figure 3a. The steady state concentration $[\beta\text{-cat/TCF}]^*$ only decreases in comparison to the reference curve. **e)** $K_3 = K_4 = 30$ 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 $[\beta\text{-cat/TCF}]^*$. **f)** Facilitated export of APC, $K_3 = 30$ nM and $K_1 = 1$: The steady state concentration $[\beta\text{-cat/TCF}]^*$ is presented in dependence on the ratios of the shuttling rate constant of APC ($K_{lin} = v_{max}/k_2k_m$). Shuttling parameters are generated randomly. Every dot corresponds to the solution for a specific parametrization.

Figure 4 - Influence of diffusive shuttling on 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.

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Tables

Table 1 - The default parametrization following Lee et al., 2003 [44]

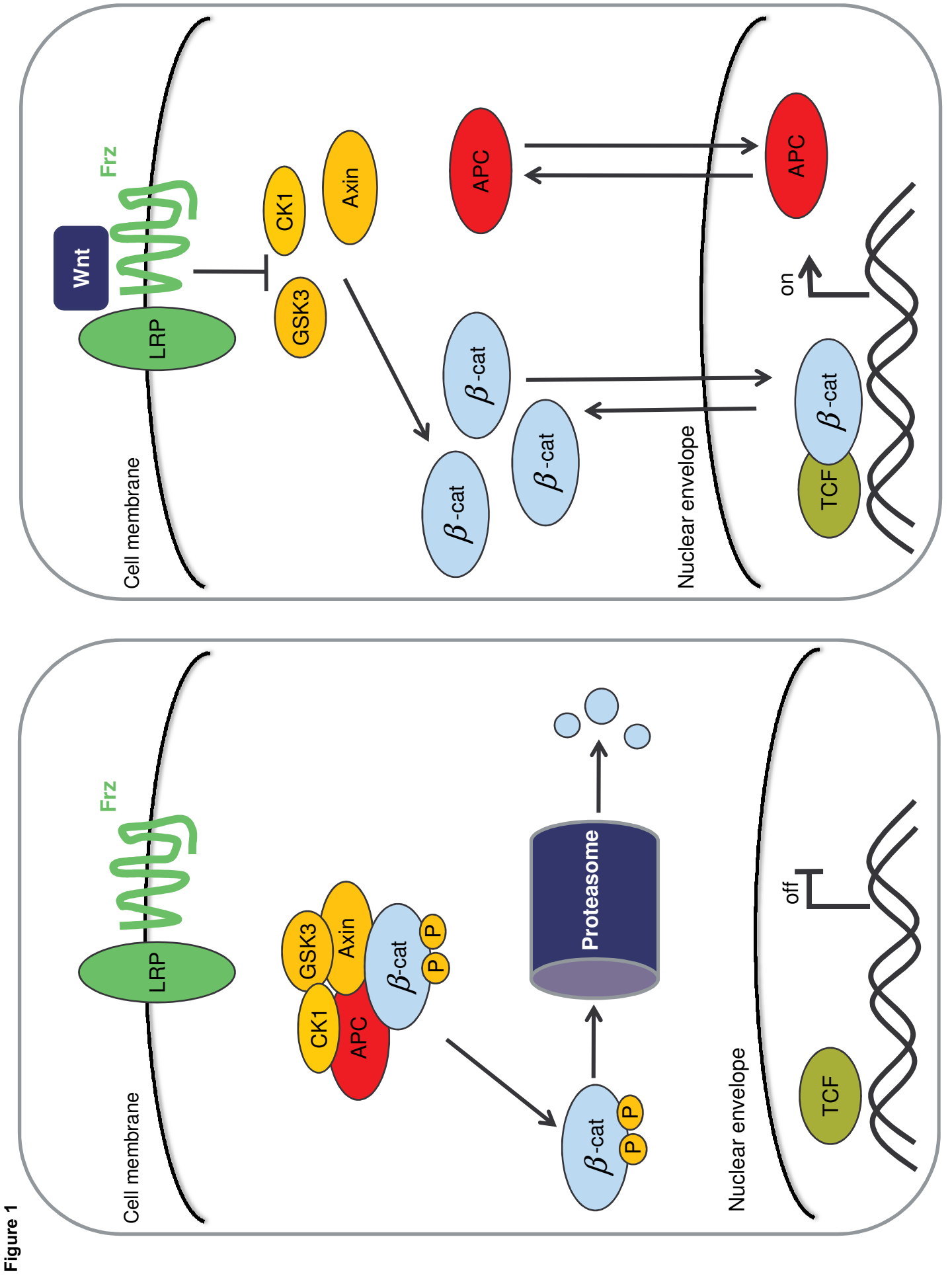
Numeric values and description of the parameters of the model in its default state. The K_i -parameters are dissociation constants $K_i = k_{-i}/k_i$, the others (A, B, T) illustrate total protein concentrations. These parameters are crucial for simplifying the system using rapid equilibrium approximations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

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 2 - 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. *.

State variable as used in Methods	Original variable as used in Results and Figure 2	Interpretation
X_1	$\beta\text{-cat}_c$	free cytoplasmic β -catenin
X_2	APC_c	free cytoplasmic APC
X_3	$\beta\text{-cat}_n$	free nuclear β -catenin
X_4	APC_n	free nuclear APC
X_5	$[\beta\text{-cat/APC}]_c$	cytoplasmic retention complex $[\beta\text{-catenin/APC}]$
X_6	$[\beta\text{-cat/APC}]_n$	nuclear retention complex $[\beta\text{-catenin/APC}]$
X_7	TCF	free nuclear transcription molecules
X_8	$[\beta\text{-cat/TCF}]$	transcription complex $[\beta\text{-catenin/TCF}]$



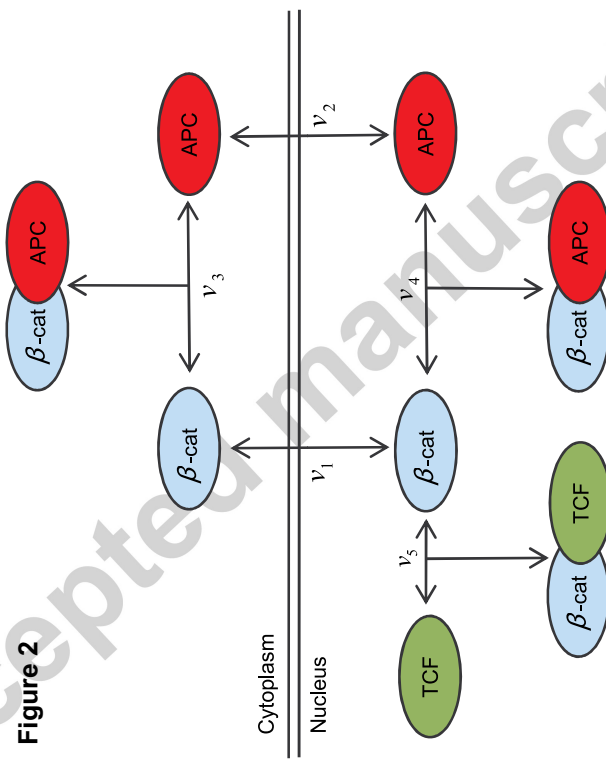


Figure 3