A Wnt Oscillator Model for Somitogenesis

Peter B. Jensen, Lykke Pedersen, Sandeep Krishna, and Mogens H. Jensen*
Niels Bohr Institute, Copenhagen, Denmark

ABSTRACT We propose a model for the segmentation clock in vertebrate somitogenesis, based on the Wnt signaling pathway. The core of the model is a negative feedback loop centered around the Axin2 protein. Axin2 is activated by β-catenin, which in turn is degraded by a complex of GSK3β and Axin2. The model produces oscillatory states of the involved constituents with typical time periods of a few hours (ultradian oscillations). The oscillations are robust to changes in parameter values and are often spiky, where low concentration values of β-catenin are interrupted by sharp peaks. Necessary for the oscillations is the saturated degradation of Axin2. Somite formation in chick and mouse embryos is controlled by a spatial Wnt gradient which we introduce in the model through a time-dependent decrease in Wnt3a ligand level. We find that the oscillations disappear as the ligand concentration decreases, in agreement with observations on embryos.

INTRODUCTION

Oscillations are ubiquitous in biological systems. Circadian (24 h) rhythms are well known, but recently ultradian (1–4 h) oscillations have been observed in the expression of genes involved in the immune system, programmed cell death, and embryo development (1,2). We focus on the latter, where the oscillations have an obvious physiological function: the segmentation clock provided by the oscillations is responsible for the periodic spacing of somites, structures that eventually become the vertebrae. We have made a model of the molecular network of genes in the Wnt signaling pathway, based on known experimental data. The model produces oscillations of the observed frequency and clarifies the essential ingredients required for such oscillations. In particular, our model shows how the oscillations can be stopped by a decrease in the Wnt ligand concentration. This is important because experiments have shown that a higher concentration of Wnt ligand demarcates the region where the somites form in the embryo (3).

The embryological process of somitogenesis in vertebrates is the rhythmic formation of vertebrae precursors known as somites in the anterior presomitic mesoderm tissue (PSM). As the embryo elongates in the posterior direction, adding new cells to the posterior PSM, the maturing anterior PSM sequentially buds off pairs of cell clusters—the somites. In mice, a new pair of somites forms approximately every 120 min (4). The strict periodicity of the process suggests the involvement of a cellular clock (the segmentation clock), the elucidation of which has been the focus of many studies. Underlying most models is the idea of locally coupled intracellular clocks controlled by a morphogen gradient in the PSM. This can supply cells with both the temporal information (cycle state) and spatial information (axial position) necessary to form distinct somites at the right time and place. This general idea is known as the clock-and-gradient, or clock-and-wavefront model, originally proposed by Cooke and Zeeman (5).

Although species-dependent, the key to the clock operation seems to lie in the action and interaction of a number of cellular pathways, most notably Notch and Wnt. A number of oscillating Notch target genes has been identified and possible feedback loops have been proposed (reviewed in (6)). In particular, attention has been given to the autoinhibiting *hes*/*her* genes (7,8) and the glycosyltransferase *lfng* believed to alter Notch ligand susceptibility (9,10). In cells in the posterior two-thirds of the PSM, these genes demonstrate oscillation frequencies matching the somite segmentation frequency in different animals (120 min in mice, 90 min in chick embryos, i.e., ultradian periods).

Underlying the oscillations there must necessarily be a negative feedback loop (11). Most of the mathematical models of somite formation so far have focused on delay-driven negative feedback loops in Notch (12–15), or larger Wnt-Notch models operating with Dsh as a direct activator of *axin2* transcription and inhibitor of *hes* transcription (16). This potential role for Dsh is still undocumented experimentally in vertebrates, and circumvents essential parts of canonical Wnt signaling. In 2003, Aulehla et al. (2) discovered that *axin2*, a target gene of the canonical Wnt signaling pathway, displays a 120-min cyclic expression in mice completely out of phase with the Notch target genes. Axin2 is a known inhibitor of its own transcription, thus forming a negative feedback loop, and Aulehla et al. (2) suggests this loop in the Wnt pathway as the driving force behind the segmentation clock.

Here, we present a mathematical model based on this idea, where the Axin2 feedback loop is closed by its forming a complex with GSK3β and β-catenin (the destruction complex), which results in β-catenin degradation. We have determined most parameter values of the model from experimental data found in the literature, and have explored a range of values for those parameters we could not fix. In the

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*Correspondence: mhjensen@nbi.dk

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particular case where the complex of Axin2 and LPR5/6 coreceptor at the membrane is in quasiequilibrium, our model reduces to a form very similar to that described in Goldbeter and Pourquié (17). The association and dissociation rates of this complex have not been measured, so it is difficult to say whether the quasiequilibrium assumption is a good one in vivo. Our model allows us to investigate the robustness of the oscillations as one moves away from quasiequilibrium to slower association/dissociation of the complex. In addition, it clarifies how the nonlinear degradation terms used in the simpler model of Goldbeter and Pourquié (17) arise from the interaction of Axin2 with the LPR5/6 coreceptor. Lastly, as our model is less coarse-grained, the parameters represent specific physical processes and therefore several of them can be directly ascertained from experimental data. The overall goal of our model is to capture the oscillatory dynamics of key Wnt target genes in individual cells in the posterior two-thirds of the PSM.

THE MODEL

A Wnt oscillator

The key to canonical Wnt signaling (reviewed in Logan and Nusse (18)) is β-catenin. Accumulated β-catenin acts as a part of a transcription complex in the nucleus leading to transcription of Wnt target genes. The regulation of β-catenin levels in the cell is under the control of the destruction complex, which is composed of a large number of proteins. Two key players, whose role is well understood, are the Glycogen Synthase Kinase 3β (GSK3β) and the scaffolding protein Axin. Phosphorylation of β-catenin by GSK3β leads to the degradation of β-catenin. Axin is known to boost this phosphorylation by several thousand-fold (19). Interestingly, whereas Wnt/β-catenin does not regulate transcription of Axin, it does regulate transcription of Axin2 (a homolog of Axin). Along with the oscillatory properties of Axin2 in the PSM tissue, this suggests that Axin2 is a likely component of the negative feedback loop driving oscillations.

Fig. 1 shows the selected key components of our mathematical model of the Wnt feedback network. Wnt signaling is initiated by the binding of a Wnt ligand to the Frizzled/LRP receptor-coreceptor complex on the cell membrane (20,21). How the signal is then mediated to the destruction complex and β-catenin regulation is still not clear, but it is known to involve the protein Disheveled (Dsh) (22). Dsh also binds to the Frizzled receptor, and Wnt activation recruits Axin to the LRP5/6 coreceptor (23). Additionally, Axin and Dsh can bind together and have been demonstrated to colocalize at the membrane (24). This sequestering and possible degradation of Axin at the membrane could lead to a decrease in the concentration of the destruction complex, leading to β-catenin accumulation. We have used this scenario in the model. Axin and Axin2 are functionally equivalent (25), thus Axin2 should be able to fill the role as scaffolding protein in the destruction complex and as binding partner to Dsh and LRP5/6.

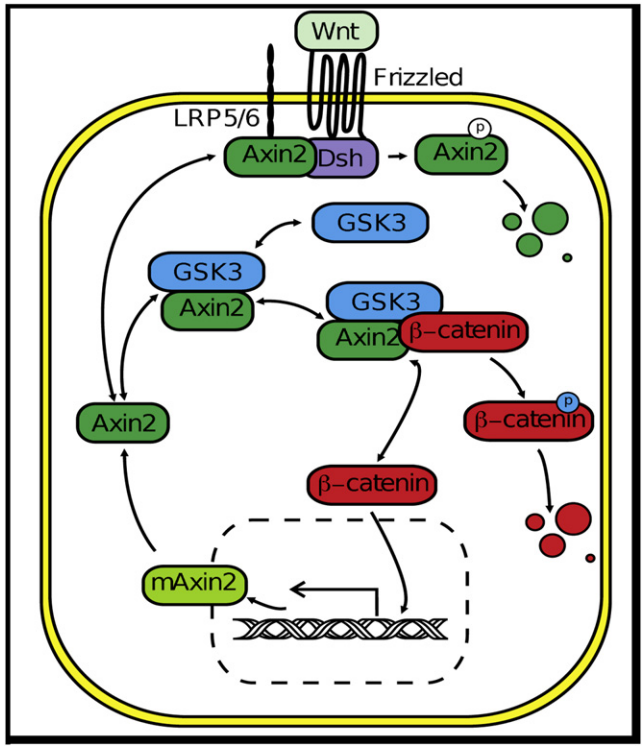

FIGURE 1 Illustration of the processes included in our model of the Wnt system. The core is a negative feedback loop: β-catenin (*red*) activates production of Axin2 (*green*) which, via a complex involving GSK3β (*cyan*), results in degradation of β-catenin. The pathway is controlled by Wnt ligands which, when bound to a receptor-coreceptor complex, catalyze the degradation of Axin2.

In the model, we also include the full destruction complex (composed of Axin2, β-catenin and GSK3β) and one of potentially three subcomplexes (Axin2 bound to GSK3β). Other destruction complex components such as the priming kinase CKIα and the other scaffolding protein APC do not play a role in the feedback loop and are therefore absorbed into the parameters. Dsh is similarly absorbed into parameters describing Axin2 binding and degradation at the receptor complex. β-catenin is present in a free form and as part of the destruction complex. Finally, there is GSK3β, which is present in free forms and bound forms in the destruction complex and subcomplex.

The processes shown in Fig. 1 are modeled using standard reaction kinetics, treating all complex formation reactions as reversible processes. This leads to a system of eight coupled ordinary differential equations,

$$\frac{dC}{dt} = c_{\mathrm{fC}}B[\mathrm{GA}] - c_{\mathrm{bC}}C - \alpha C, \tag{1}$$

$$\frac{d[\mathrm{GA}]}{dt} = c_{\mathrm{f[GA]}}GA - c_{\mathrm{b[GA]}}[\mathrm{GA}] - c_{\mathrm{fC}}B[\mathrm{GA}] + c_{\mathrm{bC}}C + \alpha C, \tag{2}$$

$$\frac{dB}{dt} = S - c_{\mathrm{fC}}B[\mathrm{GA}] + c_{\mathrm{bC}}C, \tag{3}$$

$$\frac{dG}{dt} = -c_{\mathrm{f[GA]}}GA + c_{\mathrm{b[GA]}}[\mathrm{GA}], \tag{4}$$

$$\frac{dA}{dt} = -c_{\mathrm{f[GA]}}GA + c_{\mathrm{b[GA]}}[\mathrm{GA}] + c_{\mathrm{tlA}}A_{\mathrm{m}} - c_{\mathrm{f[AL]}}AL + c_{\mathrm{b[AL]}}[\mathrm{AL}], \tag{5}$$

$$\frac{dA_{\mathrm{m}}}{dt} = c_{\mathrm{tsA}}B^2 - \frac{A_{\mathrm{m}}}{\tau_{\mathrm{Am}}}, \tag{6}$$

$$\frac{d[\mathrm{AL}]}{dt} = c_{\mathrm{f[AL]}}AL - c_{\mathrm{b[AL]}}[\mathrm{AL}] - \nu[\mathrm{AL}], \tag{7}$$

$$\frac{dL}{dt} = -c_{\mathrm{f[AL]}}AL + c_{\mathrm{b[AL]}}[\mathrm{AL}] + \nu[\mathrm{AL}], \tag{8}$$

where C, [GA], B, G, A, A_{m}, and [AL] are, respectively, concentrations of the destruction complex, GSK3β-Axin2 complex, β-catenin, GSK3β, Axin2, Axin2 mRNA, and the Axin2-LRP complex.

Subscripts of parameters (c) are named according to the following system: A subscripted f denotes complex formation followed by the name of the complex being formed. Correspondingly, a subscripted b denotes dissociation of a complex. *tlA* and *tsA* denote translation and transcription of Axin2.

Equations 1 and 2 model the breaking and formation of the destruction complex C and the subcomplex [GA]. The α-term models destruction of C due to phosphorylation of β-catenin, whereas the c_{bC}-term is the spontaneous dissociation of the complex into [GA] and B (unphosphorylated β-catenin). Equation 3 deals with the concentration of free β-catenin. S represents constitutive production of β-catenin. Because free β-catenin has been shown to be extremely stable (26), no degradation term is included in Eq. 3. GSK3β is extremely stable and its total concentration is assumed to be fixed on the timescales involved (26); therefore, no source or sink terms are included in Eq. 4.

The concentrations of Axin2 and Axin2 mRNA are determined, respectively, by Eqs. 5 and 6. Translation is directly proportional to the amount of Axin2 mRNA transcript. In the activation of transcription by β-catenin, we use a Hill coefficient of 2, that is, we assume some cooperativity. In the absence of cooperativity, i.e., for a Hill coefficient of 1, there are no sustained oscillations, whereas higher values increase the region of parameter space where oscillations are found, but otherwise do not change our results. As there is some evidence for cooperativity in the action of β-catenin (27,28), using a value of 2 for the Hill coefficient seems reasonable.

τ_{Am} is the average lifetime of the Axin2 mRNA. Finally, Eqs. 7 and 8 deal with the binding of Axin2 to the LRP5/6 coreceptor and its subsequent degradation (the ν-term). As with GSK3β, we assume a constant total concentration of LRP5/6. Note that activation of Wnt signaling, when wnt3a ligand binds to the receptor, is exclusively mediated down the pathway through the interaction of the LRP5/6 coreceptor with Axin2, and wnt3a ligand levels are therefore absorbed into the parameter ν. Thus, keeping ν constant corresponds to a constant level of Wnt signaling.

Default parameter values

The literature contains limited quantitative measurements of concentrations and kinetics for the Wnt components in mouse PSM tissue. We therefore use results from general experiments on Wnt signaling. The primary source of parameter values is Lee et al. (26), where a number of component concentrations and dissociation constants are measured and calculated in *Xenopus* (frog) extracts, which has the same canonical Wnt signaling pathway as in mice. These include dissociation constants for the destruction complex and its primary subcomplex:

$$D_{\mathrm{C}} = \frac{c_{\mathrm{bC}}}{c_{\mathrm{fC}}}$$

and

$$D_{\mathrm{[GA]}} = \frac{c_{\mathrm{b[GA]}}}{c_{\mathrm{f[GA]}}}.$$

Lee et al. (26) also supplies the total concentration of GSK3β and the source of β-catenin synthesis and allows for a good estimation of the α-value. The measurements in Lee et al. (26) are done for Axin, but as Axin2 is functionally equivalent to Axin (25), we assume it has the same binding affinities.

Table 1 shows the 14 parameters in the model, together with their estimated values and units. These default parameter values define a reference state.

The first six parameter values are from Lee et al. (26). From the values of $D_{\mathrm{[GA]}}$ and D_{C} given in Lee et al. (26),

TABLE 1 Parameters in the eight-variable model of the Wnt system and their default values

Parameter	Process	Default value
c_{fC}	Binding of B to [GA] to form destruction complex C	0.1 nM^{-1} min^{-1}
c_{bC}	Dissociation of C into B and [GA]	7 min^{-1}
α	Dissociation of C due to destruction of β-catenin	200 min^{-1}
$c_{\mathrm{f[GA]}}$	Binding of G to A to form [GA]	0.2 nM^{-1} min^{-1}
$c_{\mathrm{b[GA]}}$	Dissociation of [GA] into G and A	1.2 min^{-1}
S	Constant source of β-catenin	0.4 nM min^{-1}
$c_{\mathrm{f[AL]}}$	Binding of A to L	10 nM^{-1} min^{-1}
$c_{\mathrm{b[AL]}}$	Dissociation of [AL] into A and L	0.08 min^{-1}
c_{tsA}	Transcription of *axin2* gene	0.7 nM^{-1} min^{-1}
c_{tlA}	Translation of Axin2 mRNA	0.7 min^{-1}
τ_{Am}	Average lifetime of Axin2 mRNA	40 min
ν	Degradation of Axin2 in [AL] complex	0.1 min^{-1}
$GSK3\beta_{\mathrm{tot}}$	Total G level	50 nM
L_{tot}	Total L level	70 nM

the values of c_{fC}, c_{bC}, $c_{f[GA]}$, and $c_{b[GA]}$ are estimated. mRNA half-lives are known to range from a few minutes to several hours, thus the half-life of mRNA Axin2 τ_{Am} is set to 40 min. Looking at Eqs. 5 and 6 it is evident that c_{tlA} and c_{tsA} have a similar effect; the behavior of the system depends only on their product. Therefore, we simply assign them equal values. The constants concerned with the degradation of Axin2 are also unknown. The values in Table 1 were tuned to produce oscillations with an ultradian period of ~120 min. The table also gives the total amounts of GSK3β and LRP5/6 in the reference state, which are needed as a boundary condition for the system, as neither source nor sink are included for these variables in our model.

RESULTS

Oscillatory behavior with default parameters

Fig. 2 shows a timeseries of the behavior of the eight variables of the Wnt system for default parameters (Table 1). The system is clearly oscillatory in all variables with a period close to 120 min. All variables are initially set to zero, except for GSK3β and LRP5/6 which are set to 50 nM and 70 nM, respectively.

The phases of the individual variables appear according to their relative positions in the negative feedback loop (as expected (11)). Starting with, for example, the onset of a β-catenin peak we observe Axin2 mRNA levels and then protein levels rise with a slight delay. The newly produced Axin2 then binds to the free GSK3β to form the [GA] complex. GSK3β and [GA] have mirror image profiles. As soon as [GA] levels go up, destruction complexes C will also form, causing a depletion of β-catenin. The kinetics of the destruction complex is extremely fast ($\alpha = 200\ \text{min}^{-1}$). Hence any formed C breaks up very quickly with a resulting loss of β-catenin in the process.

Robustness of the oscillations

Although the default parameters produce oscillations that are qualitatively similar to those observed in Wnt target genes and have the correct time period, it is worth examining what happens as the parameters are varied. We find in general that the oscillations are quite robust to changes in parameter values. Their shape remains qualitatively the same, with short spikes in β-catenin-separating periods, where its level is close to zero. The period of the oscillations is quite sensitive to changes in some of the parameters, e.g., D_C, $D_{[GA]}$, and τ_{Am}—although being almost independent of other parameters, e.g., α (see the Supporting Material).

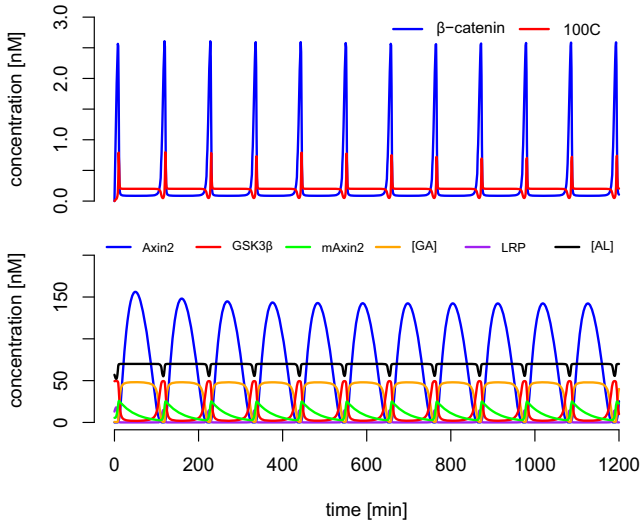

FIGURE 2 Oscillations of the Wnt model for default parameter values (see Table 1): the reference state. (*Upper panel*) β-catenin and C concentrations (the latter is multiplied by 100 for visibility). (*Lower panel*) Axin2, GSK3β, mAxin2, and [GA] concentrations, as a function of time.

Fig. 3 shows a plot of the amplitude and the oscillation period as a function of D_C and $D_{[GA]}$. These two parameters affect the formation and dissociation of the destruction complex and the [GA] complex. Robustness to other parameters is described in the Supporting Material. The reference state is indicated on the plot, as well as four other parameter sets. Timeseries corresponding to the latter are shown in Fig. 4.

A mathematical analysis of the oscillatory properties of this system is presented in the Supporting Material. There we show that the model equations allow only one physically feasible steady state, for any choice of parameter values. Whether the system shows sustained oscillations at given

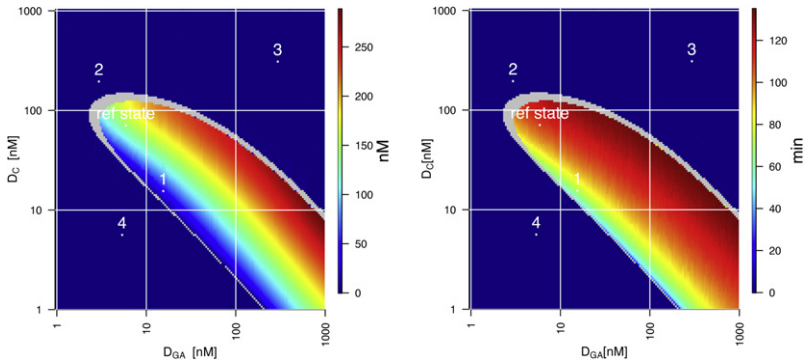

FIGURE 3 The D_C and $D_{[GA]}$ parameter plane. (*Left panel*) The amplitude of the Axin2 oscillations. (*Right panel*) Oscillation period of Axin2. The shaded borderline indicates the boundary of sustained oscillations, derived from a stability analysis of the system. The reference state (see Fig. 2) is indicated by the dot labeled "ref state". Timeseries corresponding to the four numbered dots are shown in Fig. 4.

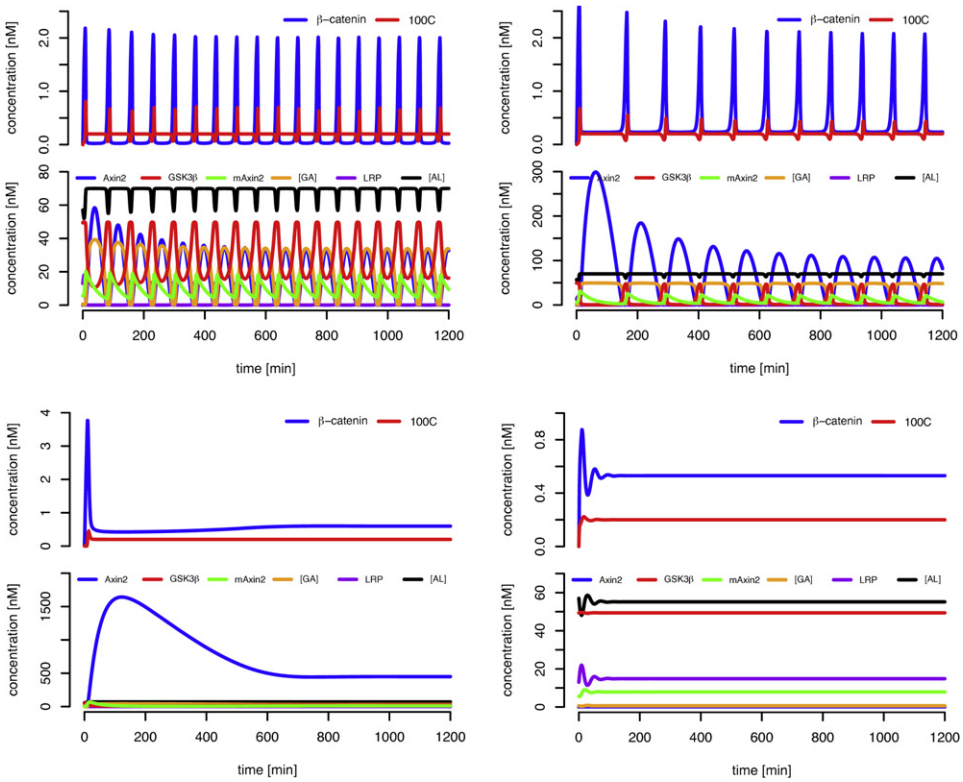

FIGURE 4 Four selected timeseries corresponding to the four numbered dots in Fig. 3. (*Top left*) State 1 shows sustained oscillations with a time period at approximately two-thirds of the reference state. (*Top right*) State 2 has very slowly damped oscillations (in practice, such oscillations would be indistinguishable from sustained oscillations in an embryo). (*Bottom left* and *right*) States 3 and 4 showing clearly damped oscillations.

parameter values can then be determined by examining the linear stability of the steady state via the eigenvalues of the Jacobian. This is explained in more detail in the Supporting Material. The shaded contour in Fig. 3 shows the boundary of the oscillatory region as determined by this stability analysis.

With all other parameters fixed at their default values, we observe that there is a large domain across the diagonal of the $D_C - D_{[GA]}$ plane where the system can oscillate with significant amplitude and a period at ~100 min. The region of oscillation spans almost three orders of magnitude in both D_C and $D_{[GA]}$. We find that the reference state in fact lies close to the upper border of states with sustained oscillations.

Importance of saturated degradation of Axin2

It turns out to be crucial for the oscillatory behavior that the rate of degradation of Axin2 is proportional not to A, the Axin2 concentration, but rather to [AL], the concentration of the Axin2-LRP complex. This is because, in the model, the only route for degradation of Axin2 lies via the formation of this complex. The rates of association and dissociation of the [AL] complex have not been measured experimentally. We therefore examined the effect of varying these rates in our model.

From Fig. 5 it is seen that the oscillatory domain increases as the rate of association and dissociation of [AL] increases (while keeping the binding strength of the complex constant). In the limit of very fast association and dissociation, the complex can be considered to be in quasiequilibrium, and we can reduce the model to a six-variable version, as shown in the Supporting Material. This six-variable model is very similar to the model of Goldbeter and Pourquié (17).

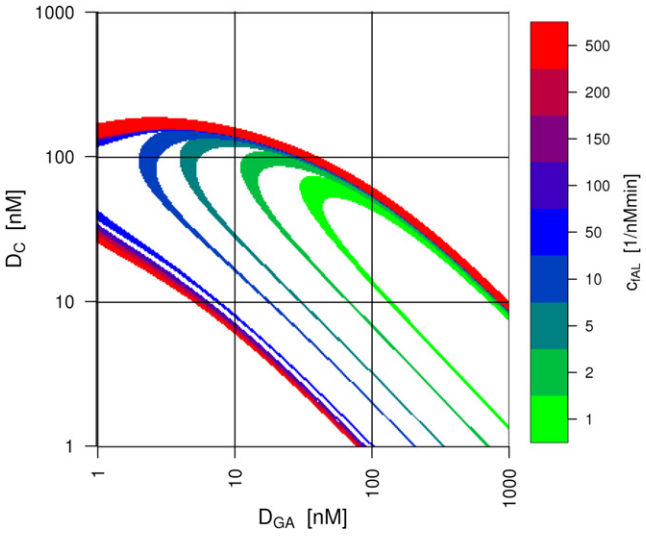

FIGURE 5 The oscillating regime for varying values of $c_{f[AL]}$. The $c_{b[AL]}$ value is also varied so as to keep the ratio $c_{f[AL]}/c_{b[AL]}$ constant, i.e., to keep the binding strength of the [AL] complex unvarying. Oscillations occur within the tongue-shape bounded by the colored lines, where the largest real part of the eigenvalues of the Jacobian is positive. Outside these boundaries all eigenvalues have negative real parts and therefore there can be no sustained oscillations.

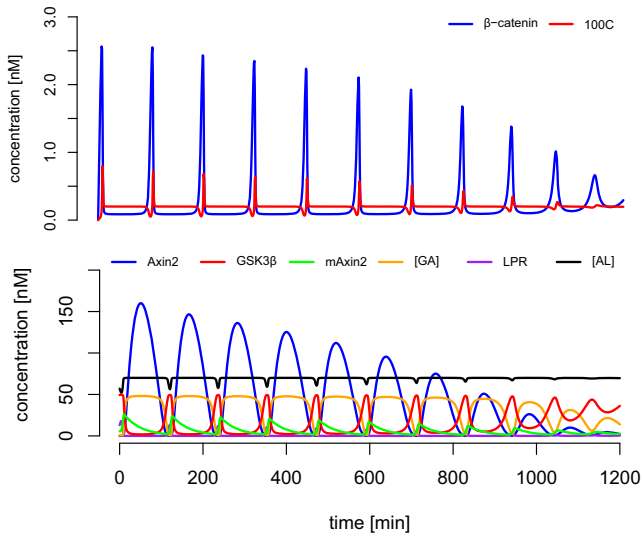

FIGURE 6 Oscillations of the Wnt variables as ν is linearly decreased from 0.1 to 0.03 min^{-1} in 1200 min. Note that the oscillations cease when the Wnt signal falls below a certain threshold level.

In the six-variable equations it is now clear that the degradation rate of Axin2 is not proportional to A. The degradation is instead given by $-c_A A/(k_A + A)$. We call this saturated degradation (29) because when A becomes much larger than the constant k_A, the degradation rate approaches a saturation value, c_A, beyond which it cannot increase further. This saturated degradation is a direct consequence of the complex formation between Axin2 and LRP5/6, especially evident in the case where the complex can be considered to be in quasiequilibrium. In the Supporting Material, we show, by varying c_A and k_A values, that oscillations occur only when the average A concentration is much larger than k_A. Thus, saturated degradation is a necessary (but not sufficient) condition for oscillations.

Onset of oscillations: integration with a Wnt gradient

In a growing embryo, new cells are added continuously to the posterior PSM. Older cells therefore have a relative movement toward the anterior PSM. It is known that there is a gradient of Wnt3a ligand across the embryo (2). If we assume that the gradient remains tied to the anterior end of the PSM, any given cell in the PSM effectively sees a slowly decreasing Wnt level with time. This provides a simple way of converting temporal periodicity to spatial periodicity required for precise placement of somites. Fig. 6 shows how oscillations look inside a cell with this kind of decreasing Wnt signal (modeled by a decreasing ν-value). When Wnt falls below a threshold level, of course, the oscillations cease. Before that, however, the oscillation amplitude steadily decreases, while the time period is quite robust.

The reason for this behavior can be explained mathematically using the stability analysis we did, described earlier, which enables us to examine the nature of the transition between oscillations and steady states. For the parameter ranges we have explored, we find that when a biologically feasible steady state (wherein all concentrations are positive and finite) exists, then it is unique. For some parameter values there is no feasible steady state—in this case some concentrations eventually become infinite. For the default parameters, we find that of the six eigenvalues, one is zero, three are real and negative, and two are complex-conjugated with positive real parts. Thus, the unique fixed point is unstable. For all the parameter variations we have studied, where the system changes from a steady state to an oscillating state, we find the onset of oscillations coincides with a Hopf bifurcation (see the Supporting Material). That is, at the point of onset, the real part of a complex conjugate pair of eigenvalues becomes positive, whereas the imaginary part is finite and nonzero. Hopf bifurcations indicate that the oscillations have a finite time period as soon as they occur, which is why the time period is so robust in the time series of Fig. 6, although the amplitude slowly increases as the system moves away from the Hopf bifurcation and deeper into the oscillating regime.

DISCUSSION

The important features of our model are:

1. It is based on a negative feedback loop within the canonical Wnt signaling pathway, which involves Axin2 and the destruction complex.
2. It exhibits oscillations in the expression of Wnt target genes such as Axin2.
3. The oscillation periods match experimental values for parameters in realistic domains.
4. The system incorporates Wnt signaling in a way that can account for the absence of oscillations in regions where Wnt concentration is low.
5. The system has the potential for interaction with the Notch signaling pathway in a way that can explain why Notch target genes oscillate out of phase to Wnt targets (see below).

What is necessary to produce the oscillations?

The oscillations arise from a combination of two features:

1. The core negative feedback loop visible in Fig. 1: a high concentration of free β-catenin promotes high concentrations of Axin2 mRNA and protein, which promotes the forming of the [GA] subcomplex and the destruction complex. The latter degrades β-catenin, leading to a reduced production of Axin2 and so on.
2. The binding of Axin2 at the LPR5/6 coreceptor results in saturated degradation of Axin2. The existence of an upper limit to the degradation rate means Axin2 is degraded relatively slowly when its concentration is large. This

produces an effective time delay, which is required to make a negative feedback loop oscillate (29).

The structure of the model, based on negative feedback plus a time delay due to saturated degradation, is remarkably similar to that of the regulatory circuit producing oscillations in the immune response regulator NF-κB in mammalian cells (29). The tumor suppressor gene, p53, along with its repressor Mdm2, also exhibits oscillations and has the same features (1). This design could thus be quite a ubiquitous way of generating ultradian oscillations in widely varying biological contexts.

Possible mechanism for interaction between Wnt and Notch pathways

In addition to its ability to phosphorylate and degrade β-catenin, GSK3β has also been shown to bind to the intracellular domain of the Notch receptor (N_{icd}) (30,31), which is a transcription factor in Notch signaling. This involvement of GSK3β in both Wnt and Notch signaling provides a simple mechanism which could explain the observation (2) that Wnt and Notch targets oscillate out of phase with each other. The argument would proceed as follows: When Axin2 levels are high, GSK3β is sequestered in the destruction complex or in [GA], leaving little to bind to N_{icd}. Vice versa, when Axin2 levels are low, GSK3β is free to sequester N_{icd}. If this results in an activation of N_{icd}, as is the case for some Notch receptors (31), then there would be an increased transcription of Notch target genes. That is, Notch targets would show a high expression when Wnt targets, like Axin2, have a low expression, and vice versa, i.e., the oscillations of Notch targets would be out of phase with respect to Wnt targets. This scenario, in which the two pathways (in a sense) compete for GSK3β, of course requires more experimental evidence.

Axin2 mutants

An important experimental observation that bears on this issue is that Axin2 deleted mice (Axin2^{-}/$^{-}$) have no dramatic segmentation phenotype (32). This suggests that Notch targets can still oscillate even in the absence of a Wnt oscillator such as the one proposed in this article. It has, however, been shown that Wnt signaling, at the level of β-catenin, is crucial for oscillations of both Wnt and Notch target genes (33). In loss of function mutants, neither Wnt nor Notch target genes oscillate; however, in stabilized (constant β-catenin level) mutants, only Wnt targets stop oscillating. This puts β-catenin upstream of all pathways and suggests for it a permissive rather than oscillatory role. Our proposed model could fit into this scenario, and can explain the observed oscillations in Wnt targets even if Notch targets have their own intrinsic oscillator. In Axin2^{-}/$^{-}$ mice, normal Axin, which is present in small concentrations, could resume its normal role from non-PSM tissue and supply the needed scaffolding for the destruction complex. This is sufficient for canonical Wnt signaling and for a permissive role of β-catenin.

SUPPORTING MATERIAL

Fourteen figures are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(09)05997-9.

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